‘The Regulation of Surface Responsive Genes in *Blumeria graminis f. sp. hordei*’

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Declaration of Originality

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**Natasha Cain:** - Subculture and screening of transformants.

**Issariya Chiram:** - Assessment of surface hydrophobicity.

**Oliver Cole:** - Screening of inducers and their affect on *Blumeria graminis* f. sp. *hordei* development.

**Nurul Ismail:** - Application of transformed *Magnaporthe oryzae* strains to barley and other surfaces, as well as the harvesting of *Blumeria graminis* f. sp. *hordei* RNA.

Additionally, the author was privileged to work alongside **Dr. Calin Andras** and **Dr. Maike Paramor** whose previous studies formed the basis for the PhD and whose efforts led to the cloning of many of the constructs used at stages within the project. Again, their inputs are noted with appreciation. The author is also grateful for the tremendous bioinformatic support of **Dr. Tim Burgis**. Apart from work that is noted as originating from the input of these people the author declares that all work contained herein as deriving from his efforts.

**Signed:**

(Timothy Simpson)
Abstract

Powdery mildew of barley is caused by the ascomycete pathogen *Blumeria graminis* f. sp. *hordei* (*Bgh*). *Bgh* is economically important throughout the world, causing crop losses varying between 5 to 20 % and in extreme cases as much as 60 %. *Bgh* is an obligate biotroph, relying on its host for growth and reproduction. This characteristic has hindered attempts to carry out biochemical and molecular biological analysis.

Previous work had highlighted differential gene expression during *Bgh* development on surfaces other than the host. Consequently, this thesis had three aims. The first attempted to elucidate the nature of this gene expression. Work listed within includes studies of *Bgh* morphological development on the host barley, wheat, cellulose membrane, and glass. Additional studies included the assessment of gene expression, via RT-qPCR, on glass surfaces enhanced with 1-hexacosonal (a synthetic C26 aldehyde known to spur *Bgh* development), 16-hydroxyhexadecanoic acid (a cutin monomer found within the barley leaf), as well as surfaces of differing hydrophobicity. Results collected reenforce the surface-dependent nature of gene regulation, and highlight how gene expression is determined by the integration of multiple signal inputs.

The second aim of this thesis was the transformation of *Bgh* utilising *Agrobacterium tumefaciens*. Efforts are discussed as are approaches for future work aimed at transforming this fungus.

The final aim of the thesis aimed to lay foundations for work involving the assessment of 5’-regulatory regions of genes showing clustered, and differential, expression on alternate surfaces. Utilising the phytopathogenic model fungus *Magnaporthe oryzae* (the causal agent of rice blast disease), 22 promoter regions were tested for their ability to drive GFP in this pathogen. 2 regions (for genes encoding a H4 histone and an aconitase) along with promoter regions selected for their conservation, were able to do so.
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Appressorial formation</td>
</tr>
<tr>
<td>AGT</td>
<td>Appressorial germ tube</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>Bgh</td>
<td><em>Blumeria graminis</em> f. sp. <em>hordei</em></td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serine albumin</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphatase</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>CMEG</td>
<td>‘Co-ordinate Mis-Expressed Gene’</td>
</tr>
<tr>
<td>COGEME</td>
<td>Consortium for the Functional Genomics of Microbial Eukaryotes</td>
</tr>
<tr>
<td>cv.</td>
<td>Cultivar</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>Double distilled water</td>
</tr>
<tr>
<td>DEPC</td>
<td>Diethylpyrocarbonate</td>
</tr>
<tr>
<td>dNTP</td>
<td>Deoxynucleotide triphosphate</td>
</tr>
<tr>
<td>d.p.i.</td>
<td>Days post-inoculation</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>Egst/App</td>
<td>Elongating secondary germ tube/Appressorium</td>
</tr>
<tr>
<td>EST</td>
<td>Expressed Sequence Tag</td>
</tr>
<tr>
<td>f. sp.</td>
<td><em>forma specialis</em></td>
</tr>
<tr>
<td>H</td>
<td>Haustorial formation</td>
</tr>
<tr>
<td>HHD</td>
<td>16-Hydroxyhexadecanoic acid</td>
</tr>
<tr>
<td>h.p.i.</td>
<td>Hours post-inoculation</td>
</tr>
<tr>
<td>GFP</td>
<td>Green fluorescent protein</td>
</tr>
<tr>
<td>LB</td>
<td>Lysogeny broth</td>
</tr>
<tr>
<td>Mb</td>
<td>Miscellaneous (branched germ tubes)</td>
</tr>
<tr>
<td>MCS</td>
<td>Multiple cloning site</td>
</tr>
<tr>
<td>MI</td>
<td>Miscellaneous (long germ tubes)</td>
</tr>
<tr>
<td>Mm</td>
<td>Miscellaneous (multiple germ tubes)</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>Ng</td>
<td>Non-germinated</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
</tbody>
</table>
PCR  Polymerase chain reaction
**Pers. Comm.**  Personal communication
PGT  Primary germ tube
PVP  Polyvinylpyrrolidone
REI  Relative Expression Index
RT-qPCR  Real-Time quantitative PCR
s.d.  Standard deviation
SDS  Sodium dodecyl sulphate
s.e.  Standard error
SGT  Secondary germ tube
spp  Species
TBE  Tris-borate/EDTA
Tf  Transcription factor
Tris  Tris(Hydroxymethyl)amino methane
TWA  Tap water agar
WGA  Wheat germ agglutinin
Chapter 1: General Introduction

1.1: The host: barley

The grass family (*Poaceae*) contain some of the most important cereal crops in use by man today (Matsuoka et al., 2002; Glémin and Battalion, 2009). Some 55 to 60 million years ago the *Poaceae* diverged into two clades (the BEP and PACCMA clades) with domesticated crops being found within each (Doust, 2007; Bolot et al., 2009; Glémin and Battalion, 2009). The BEP clade contains the subfamilies *Ehrartoideae* and *Pooideae*. The former contains the rices, whilst the latter contains crops such as oats (*Avena* sp.), wheats (*Triticum* sp.), rye (*Secale cereale*) and barley (*Hordeum vulgare ssp. vulgare* L.) (Glémin and Battalion, 2009). Within the *Pooideae*, *Avena* species belong to the tribe *Avenae*, which emerged 25 million years ago. In comparison, the genera of *Hordeum*, *Triticum* and *Secale* belong to the tribe *Triticaceae* which emerged 12 million years ago (Macfarlane, 1987; Wyand and Brown, 2003; Pourkheirandish and Komatsuda, 2007; Glémin and Battalion, 2009). Although the exact phylogenetic layout still being extensively debated, studies by Peterson et al., (2006) featuring analysis of sequence data of two nuclear genes, *DMC1* and *EF-G* and a plastid gene (*ndhF*) suggested that *Hordeum* may have monophyletic status within the *Triticaceae*. In comparison *Secale* sp. is often clustered with *Triticum* sp. (Kwahara, 2009).

Of the approximately 33 species found within the *Hordeum* genus, barley is the most economically important member (Blattner, 2009). One of the original crops of human agriculture, archaeological evidence points to the use of barley as a food crop as early as 8500 B.C. (Badr et al., 2000; Morrell and Clegg, 2007; Pourkheirandish and Komatsuda, 2007) (Figure 1-1). Although still being debated, analysis suggests the first domestication of barley from its wild progenitor, *Hordeum vulgare* ssp. *spontaneum* C. Koch, took place in the southern region of what has become known as the

**Figure 1-1: Barley (*Hordeum vulgare ssp. vulgare* L.).**

**Source:** Washington State University, Department of Crop and Soil Sciences [website](http://css.wsu.edu/research/crop_genetics/breeding/ullrich.htm). **Date accessed:** 2/9/2010
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‘Fertile Crescent’; an area spanning Israel, Jordan and parts of Turkey (Badr et al., 2000; Pourkheirandish and Komatsuda, 2007; Glémin and Battalion, 2009; Orabi et al., 2009).

Although similar in appearance to *H. spontaneum*, during domestication barley has slowly accrued traits that have allowed mass agricultural cultivation and human societal development in widespread geographic areas (Badr et al., 2000; Pourkheirandish and Komatsuda, 2007). Such traits included the development of a non-brittle rachis (where the head shattering mechanism of barley, featuring the segmentation of the grass spike, is reduced thereby allowing grain retention and easier harvesting), increased seed production, naked caryopsis (where the seed lacks adherence to the husk permitting easier separation during threshing), reduced dormancy (leading to a reduction in the temporary inability to germinate during favourable weather conditions), reduced vernalisation (permitting a reduced need for a period of low temperature before attaining a reproductive state) and finally photoperiod insensitivity (allowing modification of flowering time) (Kandemir et al., 2004; Taketa et al., 2004; Glémin and Battalion, 2009; Pourkheirandish and Komatsuda, 2007).

1.1.5: Current usage

![Figure 1-2: Worldwide barley production for 2008.](image)

**Colour code as follows:** White = Data not available; Pink = < 1000 metric tonnes; Yellow = 1000 to 99,999 metric tonnes; Purple = 100,000 to 999,999 metric tonnes; Beige = 1,000 000 to 9,999 999 metric tonnes; Green = ≥10,000 000 metric tonnes (based on figures released by the Food and Agricultural Organisation of the United Nations, via FAOSTAT website: http://faostat.fao.org/site/567/default.aspx#ancor. Map outline source: BlankWorldMap.org, 2010. Date accessed: 15/4/2010.)
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As of 2008, barley ranked fifth behind maize (Zea mays), wheat (Triticum spp.), rice (Oryzae sativa) and soybean (Glycine max) in world crop production at approximately 153 million metric tonnes (Food and Agricultural Organisation of the United Nations statistics division FAOSTAT; data for 2008; Baik and Ullrich, 2008) (Figure 1-2). Figures available for estimated cereal production within the United Kingdom during 2010 indicate 3,013,000 hectares were put aside for usage. Of these 1,939,000 hectares were used in wheat production (equating to 14,878,000 tonnes), 921,000 were used for barley production (5,252,000 tonnes), 124,000 were devoted to the growth of oats (685,000 tonnes) and the remaining 29,000 hectares were used for minor cereal production (including rye, providing 131,000 tonnes) (DEFRA, Department for Environment, Food and Rural Affairs, Cereals and Oilseed rape production estimate: 2010 Harvest United Kingdom- Final Results http://www.defra.gov.uk/evidence/statistics/foodfarm/food/cereals/cerealsoilseed.htm).

First used as human food in widespread parts of the world (Eastern and Northern Europe, North Africa, the Middle East and Asia), usage has changed as other cereal crops with better grain yields grew in economic prominence (studies listed in Baik and Ulrich, 2008). At the present time, although grown in many temperate climates, it is now primarily used in alcohol production (malting and whisky production) and livestock feed (either as grain, or to a limited extent, the straw). This equates to approximately 1/3rd and 2/3rd of the total world production respectively (Murphy, 2007). However due to the inherent ability of barley to allow better production than other cereal crops at high altitudes, high latitudes and areas of reduced water availability it still remains a principal food source in areas of extreme climates such as the Himalayas (Baik and Ulrich, 2008). As a prime example of crop utilisation, estimated use of barley within the United Kingdom (2009/2010) is split between ‘Human and Industrial Consumption’ (1,625,000 tonnes) and ‘Animal Feed’ consumption (3,321,000 tonnes). Only 204,000 tonnes are used utilised as source of seed or for other purposes (HGCA, Home Grown Cereals Authority Early Balance Sheet 2010, http://www.hgca.com/content.output/99/99/Markets/Markets/Supply%20and%20Demand.mspx)

1.2: The pathogen: barley powdery mildew

Powdery mildews are fungi of the family Erysiphaceae (Huckelhoven, 2005). These pathogens infect over 10,000 mono and dicotyledonous species of plants worldwide, causing
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heavy economic crop losses, probably surpassing those incurred by any other form of plant disease (Chaure et al., 2000; Agrios, 2005). Cereal crops are affected heavily as control is difficult, not cost effective and often hindered by the large spread of monocultures that exist in relatively close proximity.

*Blumeria graminis* is the species responsible for powdery mildew diseases upon the *Gramineae* (Muchembled et al., 2005) and due to its great agronomic impact, especially in Northern Europe it is highly studied (Pedersen et al., 2002). Belonging to the monotypic tribe *Blumerieae* *B. graminis* was classified into separate taxonomic divisions, each termed a *forma specialis* (f. sp.) (Marchal, 1902; Braun et al., 2002; Inuma et al. 2007). Such divisions reflected specific groupings that were thought to demonstrate infection of specific cereal genera, although later studies have suggested host ranges may be larger than just one single host genus (Marchal, 1902; Inuma et al., 2007). Eight *formae speciales* are classified, including those on wheat (f. sp. *tritici*), rye (f. sp. *secalis*), oats (f. sp. *avenae*) and four others that grow on wild grasses (f. sp. *dactylidis*, f. sp. *agropyri*, f. sp. *bromi* and f. sp. *poae*) (Oku et al., 1985; Wyand and Brown, 2003; Inuma et al., 2007). The best studied, however, is powdery mildew on barley, *Blumeria graminis* f. sp. *hordei* (formerly *Erysiphe graminis* f. sp. *hordei*) (Brown et al., 2001; Both and Spanu, 2004). For brevity in this work this pathogen will be referred to as *Bgh*.

1.2.1: Disease symptoms

*Bgh* is an obligate, biotrophic pathogen (Wright et al., 2000; Wright et al., 2002). Therefore, although it penetrates the epidermal cells of the host and absorbs nutrients via specialised organs it rarely kills the host cells (Panstruga and Schulze-Lefert, 2002; Huckelhoven, 2005). Its presence will, however, result in reduced growth and lower grain fill. Other symptoms include chlorosis at the centre of fungal colonies followed by a redevelopment of chlorophyll at later stages and increased host respiratory rate (Bushnell and Allen, 1962). Green pigment may also be

Figure 1-3: Powdery mildew infection upon barley.

A well progressed infection showing the characteristic powdery pustules diagnostic for this disease. Source: Talbot and Hamer (2000).
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retained around colonies on senescing leaves (Bushnell and Allen, 1961). If infections are heavy, and the host is young, the drain on photosynthates can be high. Poor root and stem development results, leading to lodging of the crops and subsequent grain loss. In severe cases such effects can lead to crop losses up to 40% (Chaure et al., 2000).

In the field, infections are often first observed as spots of white/grayish growth on young leaves which are opposite to chlorotic spots on the reverse side of the leaf. Although most common on upper leaf surfaces, infections will also occur on the abaxial surface and other organs including young shoots and stems (Agrios, 2005). Once the lifecycle has progressed organs may be completely covered with the white “powdery” growth normally associated with the sporulation stage of the lifecycle (Figure 1-3) (Agrios, 2005). Later in the season older colonies may contain gray/black spots as the sexual stage of the fungal lifecycle develops.

1.2.2: *Blumeria graminis* f. sp. *hordei* lifecycle and signals

As an ascomycete, there are two phases to *Bgh* reproduction: the sexual phase and the economically damaging asexual phase (Agrios, 2005). The sexual phase allows survival during non-optimal growth conditions and genetic recombination – both abilities are mediated via a structure known as the ‘cleistothecium’ and the spores that form within, the ‘ascospores’ (reviewed in Both and Spanu, 2004). The asexual phase (Figure 1-4) of the lifecycle, in comparison, centres on the production and spread of airborne spores, the ‘conidia’ (Both and Spanu, 2004). In Northern Europe this asexual cycle takes place, approximately, between October to the following July (Brown and Wolfe, 1990). This phase is not limited to one “permutation” per growing season so *Bgh* is considered a polycyclic pathogen. This characteristic results in the rapid spread of the pathogen, and means it continues to be one of very damaging, with approximately $380 million dollars being spent on fungicides to treat it (Hewitt, 1998; Rubiales et al., 2001). The asexual phase has been well studied, as described by both Bélanger et al., (2002) and Both and Spanu (2004), and is the focus of this study.
Lucas (2004) noted that for successful infection a pathogen must overcome multiple barriers by utilising tightly regulated processes that respond to both host and environmental cues. *Bgh* is no exception, and is thought to depend on multiple signals during the different stages of the asexual lifecycle (Hall et al., 1999).

### 1.2.3: Conidial germination

When airborne conidia land upon a compatible plant surface a well ordered developmental process follows ([Figure 1-4](#)). A conidium touches the wax platelets of the leaf surface via a number of distinct surface projections (Carver et al., 1999; Wright et al., 2000). An extracellular matrix (ECM) ([Figure 1-5](#)) is rapidly released approximately 20 seconds to 1 minute after spore deposition (Kunoh et al., 1988; Nicholson et al., 1988; Carver et al., 1999). Carver et al., proposed this exudate was released onto the surface of conidia before “flowing” via capillary force onto the substratum. What causes this release is still unclear – however, the same authors suggested it may be a response to a combination of physical and/or chemical cues.
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surface properties. This was based on the observation that the relative hydrophobicity of the contact surface may trigger ECM release (or at the very least easier visualisation). Glass treated to increase hydrophobicity showed greater ECM release than non-treated glass (chromic acid cleaned glass showed little to none). Evidence for the involvement of other factors include tests with conidia upon leaves without their epicuticular waxes that revealed little or no ECM release compared to conidia placed upon unaltered barley leaves (Carver et al., 1999). Interestingly, a less hydrophobic surface (cellulose) caused greater ECM release than on silanized plastic (a relatively more hydrophobic surface). This may be explained by the presence of cellulose breakdown products potentially released by the ECM itself or the greater availability of water.

ECM is believed to have roles in surface adherence and cell surface/host recognition (Meguro et al., 2001; Wright et al., 2002). Wright et al., (2002) noted at least two studies that show that the ECM lowers the hydrophobicity of the leaf concurrently with a decrease in spore hydrophobicity. Nicholson et al., (1993) suggested that this concurrent change is necessary for normal germling development. Studies have also shown the conidial ECM may contain esterases (released in 2 phases at 2 minutes and 15 minutes post-inoculation, at least on artificial surfaces) (Nicholson et al., 1988; Wright et al., 2002), cutinase (Pascholati et al., 1992), pectinases and cellulases (Suzuki et al., 1998) that may partially digest the barley cuticle. Likewise, hydrolytic enzyme activity is observed at 3 minutes post-deposition (Nielson et al., 2000). In support of this Nicholson et al., (1988) mention a re-evaluation of older studies looking at cuticle and epicuticular wax erosion, and suggest this erosion affects the underlying cuticular membrane (where cutin components reside). The first phase of release is not inhibited by cyclohexamide, unlike the second phase. This suggests initial release is passive, unlike the second phase release (Kunoh et al., 1990; Wright et al., 2002).

Notably, ECM release upon barley differs from that on certain artificial surfaces. The ECM released on the host is often very difficult to visualise, unlike on glass for example (where the ECM forms thick deposits as visible in Figure 1-5). During development on the host the ECM and is believed to just exist (or be part of) the tips of the conidial surface projections as it forms small deposits seen to eminate from these projections (Carver et al., 1999; Wright et al., 2002A,B). This difference in release behaviour leads some authors (for example Zhang et al., 2005) to suggest care should be taken when trying to understand the role of the ECM based on the use of artificial surfaces to gather evidence. With such differences in mind and
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since ECM release appears less on the host it is possible, as Both and Spanu (2004) speculate, that the ECM may not fully degrade the underlying cuticle (to aid penetration of the host), but merely provides stimuli (for example in the form of cutin degradation products) for proper germ tube emergence and subsequent appressorial development. Such an idea would fit with a hypothesis of sensing a surface by compound uptake, and would fit with the views of earlier authors, such as Carver et al., (1999), who having observed the limited amounts of ECM on the host compared to other surfaces suggested that it may offer a method of leaf perception. Inferential evidence for such a role in sensing was provided by Francis et al., (1996) who found that cutin monomer addition to glass slides can lead to increased proportions of later stages *Bgh* development (for example the increased formation of appressorial germ tubes) compared to those seen on untreated glass slides. Additionally, Nielsen et al., (2000) demonstrated the passive uptake of low molecular weight compounds (with properties potentially similar to cutin monomers) into a conidium by 30 minutes post-deposition. The greatest accumulation was present at points immediately adjacent to the substratum where the highest level of spore metabolism was taking place. That a similar matrix (although differing in constituent concentration) is produced by both germ tubes and appressoria (and also differs in amounts at different locations upon the germling during the developmental cycle) also supports the idea that the ECM may have roles in not just adhesion to, but also sensing of, the host (Carver et al., 1999; Both and Spanu, 2004).

With regards to spore metabolism at this time in their study of the expression of 2027 *Bgh* unigenes, Both et al., (2005a) observed the high expression of 3 enzymes involved in the breakdown of glycogen during early germination. This suggested this storage molecule was being broken down to fuel early post-emergence development. Lipids, like glycogen, also act as storage molecules in fungi. During germination expression of enzymes involved in fatty acid degradation increased during pre-penetration stages, before dropping post-penetration. This expression data (combined with Nile Red staining confirming the presence of large amounts of neutral lipids in conidia), led the authors to suggest the possibility that these storage molecules were being broken down to fuel early post-emergence development before accessing host nutrients.

In 2000 Talbot and Hamer noted that the nature of *Bgh* surface signal perception was very much a mystery. At this time this is still very much the case. However, during ECM production changes in the levels of cyclic AMP (cAMP) and a downstream cAMP-dependent
Protein Kinase A (PKA) have been observed (Hall and Gurr, 2000; Wright et al., 2000). Cyclic AMP based signal transduction pathways are believed to be involved in the development of many plant pathogenic fungi, for example the formation of appressoria in *Magnaporthe oryzae* (Xu and Hamer, 1996; Hall et al., 1999). Kinane et al., (2000) observed that in a classical cAMP pathway a cell surface receptor would respond to a stimulus, and by the mediating actions of GTP-binding proteins, lead to an activation of adenylyl cyclase. This enzyme would lead to the production of cAMP, which in turn would bind to the regulatory subunits of PKA. Consequently, this would allow the protein kinase to phosphorylate proteins and by doing so eventually lead to changes in gene expression. As further evidence of their involvement, when artificial adenylyl cyclase stimulators were added to non-germinated conidia germination was enhanced (Kinane et al., 2000). Therefore the presence of cAMP in this fungus at this stage is significant. Interestingly since cAMP levels are detectable 15 minutes after inoculation it is very likely, in the view of some authors, cAMP levels are controlling the emergence of the first germ tube (Kinane et al., 2000; Wright et al., 2000).

Other studies have also tried to discern the pathways controlling differentiation. Use of *Bgh* isolates resistant to qunioxyfen (a fungicide thought to perturb early signal transduction in fungi, leading to reduced germination and long germ tube formation) has also led to the discovery of a protein with a homology to GTPase-activating proteins (Wheeler et al., 2003; Lee et al., 2008). This discovery was based on the results of ‘differential display reverse transcription PCR’ which revealed the existence of a gene transcript which was present in wild-type (susceptible) *Bgh* isolates but which was absent (or significantly reduced) in the developing conidia of qunioxyfen-resistant *Bgh* isolates. This transcript was subsequently found to have an amino acid sequence homology to Ras-type GTPase activating proteins (with phylogenetic analysis suggesting its close alignment to a GTPase activator protein, BUD-2P, of *Saccharomyces cerevisiae*) (Wheeler et al., 2003). In wild-type isolates this gene has detectable levels of mRNA in conidia, but these disappear after germination (only to reappear during sporulation). As such this suggested to later authors that a decrease in the presence of GTPase-activating proteins (and an activation of Ras-type monomeric GTPases) is necessary for normal germination to take place (Both and Spanu, 2004).
1.2.4: The primary germ tube

In less than 2 hours after spore deposition a short (5 to 10 μm) ‘primary germ tube’ (PGT) emerges to contact the leaf surface (Kunoh et al., 1979) (Figure 1-6). Although more than one tube may develop, only that which contacts the leaf first will become the PGT. The others will terminate and are termed ‘subsidiary germ tubes’ (Wright et al., 2000; Wright et al., 2002). This tube, and its proposed function, is thought to be unique to Blumeria graminis (Aist and Bushnell, 1991; Hall et al., 1999; Glawe, 2008). Most germ tubes appear from the conidium in close proximity to the leaf at a site determined by 1 minute of contact with the surface (Wright et al., 2000). This was determined by Wright et al., using micromanipulation to roll conidia (inoculated on barley epidermis for set time periods) to study germ tube emergence. By rolling conidia 180° so the original point of spore contact was directly away from the epidermal surface the authors observed, using light microscopy, that approximately 84 % of the first formed germ tubes on control (non-manipulated) conidia merged close to the epidermis (and contacted it) whilst on rolled conidia 82 % of tubes failed to contact (and grew away from the surface). Furthermore the authors found that even if conidia were manipulated after only 1 minute of contact the site of emergence was still close to the site of original contact. This led Wright et al., to suggest that not only does such behaviour increase the chance of the tube finding the surface, but it also suggests the developmental process is well attuned to the surrounding environment employing complex signalling systems to do so (Wright et al., 2000). Authors such as Carver et al., (1999) and Nielsen et al., (2000) argue such targeting is evidence for the existence of early surface perception (perhaps as mediated by the conidial ECM). They note how again relative hydrophobicity of the surface (compared to the spore) plays a role in this germ tube emergence (with better targeting on more hydrophobic surfaces); although this author suggests such a requirement may be a continuation of its role in the release of ECM. Wright et al., (2000) define such responses to a greater degree suggesting two processes are at play: a non-specific response to contact and another, possibly to host signals, refining the emergence site/targeting. Arguments for this
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included the lower efficiency of primary germ tube contact on clean glass slides compared to host leaves where signal producing substrates are optimally abundant, and also the apparently normal development on hydrophilic cellulose membranes (Zhang et al., 2005).

The PGT (together with its accompanying ECM) may have roles in adherence, water uptake, and also in host recognition that permit later developmental stages (Pryce-Jones et al., 1999). The latter two may be mediated by the ‘cuticular peg’ that emerges from the PGT tip and breeches the cuticle to contact the plant wall. Again detection of host signals via uptake of anionic low molecular weight compounds may take place (Nielsen et al., 2000; Wright et al., 2000; Edwards, 2002). Suzuki et al., (1998) noted the release of an exudate at about 1 hour d.p.i. (from conidia upon cellulose membranes) which showed cellulose activity. This suggested to the authors it was released from the PGT (and at later time points from both the appressorial germ tubes and appressorium (Figure 1-4). This may aid both penetration by the cuticular peg (as well as the later stage appressorial peg) and also provide stimuli for later development. Thinner host cuticle under the PGT than surrounding areas corroborates this hypothesis (Kunoh et al., 1977).

In studies to discern the signal pathways of Bgh (Zhang et al., 2001) examined transcript levels for genes encoding Protein Kinase C (PKC, a serine threonine kinase). Two PKC genes have been identified in Bgh, pkc1 and pkc-like (Zhang et al., 2001). Transcript for pkc1 was present at all stages tested including in ungerminated conidia and those with appressoria. It reached a maximum abundance during the formation of the first germ tube. Transcript for pkc-like was only present during PGT formation. Not much is known about the role of this type of kinase during differentiation in plant pathogenic fungi, however they have been identified in differentiation in Neurospora crassa (studies in Lee et al., 2008). However findings by Lee et al. (2008) using the fungicide quinoxyfen, suggest that PKC may potentially have a role in controlling serine-esterase (cutinase) expression. Based on the assessment of relative transcript abundance of PKC-1 and a serine exterase gene, CUT1) it was observed that after quinoxyfen treatment PKC-1 transcript levels rose in the wild-type, whilst CUT1 gene expression was slightly reduced. This may suggest some form of role of PKC-1 in controlling serine-esterase (cutinase) expression, with PKC-1 being over expressed in the fungicide treated wildtype in an attempt to compensate for the action of the fungicide (which leads to the drop obsevredin the expression of CUT1).
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As with conidial ECM release, the characteristics sensed spur an increase in both cAMP and a flux in PKA signalling again suggesting a role for protein phosphorylation in governing further developmental processes (Kinane et al., 2000; Prats et al., 2008). Finally, this role in host sensing is supported by observed developmental termination should any germ tube fail to find a compatible host.

1.2.5: The appressorial germ tube

By 3-4 hours after initial deposition and after PGT formation with successful surface recognition a second germ tube (the ‘appressorial germ tube’, AGT) will emerge and elongate to approximately 40 μm (Wright et al., 2000; Rubiales et al., 2001). An ECM will be produced as it grows (Carver et al., 1995; Wright et al., 2002). By 9-10 hours post-deposition this septate germ tube has hooked and swollen to form an apical appressorial lobe/appressorium (APP) (Wright et al., 2000) (Figure 1-7). The lobe itself also produces abundant ECM which is believed to fix itself to the leaf during penetration (Wright et al., 2002). Just as in the case of the PGT the AGT must also contact an inductive surface for development to succeed. Failure to do so results in a hypha-like structure (Carver et al., 1999; Wright et al., 2000). By 15 h.p.i. a penetration peg develops beneath the appressorium, and attempts host penetration. Nielsen et al., (2000) noted that the failure of appressorial development on artificial surfaces (e.g. glass) means specific host factors are required. These may include substratum hydrophobicity (argued against by Francis et al., 1996), cutin monomers (noted specifically by Francis et al., 1996) and possibly cellulose breakdown products (studies listed in Nielsen et al., 2000). Studies utilising barley wax mutants (the ‘eceriferum’ mutants) led Yang and Ellingboe (1972) to believe physical features present in that layer were critical for development. However, later studies by Rubiales et al., (2001) involving the some of the same (as well as other) eceriferum mutant lines showed reduced Bgh appressorium formation on some but not all of the lines tested. This observation combined with results of Carver and Thomas (1990) (where Bgh development on cereal leaves with and without epicuticular waxes was similar in both cases) led Rubiales et al., to suggest wax chemistry rather than physical structure/appearance was
the key for correct *Bgh* development. Investigations by Tsuba et al., (2002) confirmed the wax layers involvement in both AGT emergence and APP formation. Such waxes are a mixture of long-chain fatty acids, fatty aldehydes, alcohols (primary and secondary), ketones and esters (Tsuba et al., 2002). The authors catalogued the percentage of appressorial germ tube emergence or the percentage of appressorium formation on *Bgh* conidia developing on polystyrene petri dishes coated with thin layer chromatography fractions of barley wax. These observations suggested the aldehyde fraction of the barley wax showed significantly higher levels of both AGT induction and APP formation compared to the other fractions (such as esters) assessed. Furthermore gas liquid chromatography showed that 66% of aldehydes present in barley wax were of carbon chain length 26. When appressorial differentiation was assessed on polystyrene petri dishes with aldehydes of different chain lengths, aldehydes of chain length 26 caused the greatest level of induction (and also in a dose dependent manner) (Tsuba et al., 2002). This inductive activity therefore appeared to depend upon both the quantity present but also chain length. Aldehydes of carbon length 26 were considered ideal, and appear to make up 66% of the aldehyde fraction (Tsuba et al., 2002). These authors suggest that alternate length aldehydes were actually inhibitors of germination, suggesting a bottleneck for development and a mechanism of pathogen specialisation. This links again to the idea of low molecular weight compound uptake as Tsuba et al., (2002) believe the aldehydes may be taken up as such. However, as formation of appressoria was less on the treated plastic surfaces tested, further host factors are required (Tsuba et al., 2002). As for when such uptake may occur it is unclear as both AGT and APP formation are stimulated. It is possible that in AGT formation the sensing is performed by the PGT, and for APP formation it is mediated by the elongated AGT. This would support the idea of the AGT being a distinct stage of development to the APP, as first suggested by Carver and Ingerson (1987). A further level of developmental regulation may have been observed by Iwamoto and co-workers (Iwamoto et al., 2002; Iwamoto et al., 2007). When barley cuticle was partially abraded (resulting in excess moisture supply on the leaf surface) enhanced appressorial collapse was experienced indicative of excess turgor pressure during development. When complete removal of the cuticle was performed no collapse was witnessed (although appressorium formation was still present and similar moisture conditions existed). This led Iwamoto et al., to conclude that only once appressorial penetration was begun does it prove vulnerable to excess moisture. This suggests the appressorium itself is also sensitive to signals.
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After germination of the conidia, an increase in the transcription levels of enzymes involved in glycolysis was observed (Both et al., 2005a). Significantly this expression reached a maximum at 8 h.p.i. then tailed off before rising again at 5 d.p.i. The authors suggested it is at these times the energy requirement is at its greatest: at 8 h.p.i. this equates to appressorial maturation and the initiation of turgor generation. The large quantities of lipids present in the conidia, suggested by Nile Red staining, also disappear during maturation. Both et al., (2005a) suggested that whilst neutral lipids are broken down during this stage of development polar lipids may be moved into appressorium (as is seen in Magnaporthe oryzae).

As with the PGT, studies to date (for example Hall and Gurr, 2000) implicated cyclic AMP-dependent Protein Kinase A signalling in the complete development of this germ tube. Hall and Gurr (2000) suggested, based on the action of exogenous cAMP and inhibitors, that high levels of PKA activity are required to initiate AGT differentiation (at approximately 4 h.p.i.) but that low levels are subsequently required for appressorial tube hooking and swelling. Additionally they suggested that some form of extra signalling may take place in a window between 2 and 8 h.p.i. This advanced the work of Hall et al., (1999) who noted that although exogenous cAMP can increase appressorial induction whilst on an inductive surface (although not to the same levels as seen during development on the host) it cannot do so on a non-inductive surface. This result suggested to the authors that there was a requirement for other signalling pathways to enable development of this critical structure. They concluded by noting that although cAMP was necessary for the induction of the AGT by itself it was not sufficient for full appressorial development. Later studies by Kinane et al., (2000) using cellulose with two differing levels of appressorium inductivity supported this view. Kinane et al., compared Bgh development on the host surface with development on these types of cellulose membrane. On one type the authors observed that frequencies of AGT formation and differentiation were similar to that seen during germling development on the host. The second cellulose type stimulated a lower degree of AGT and APP differentiation than the host. When cAMP or adenylyl cyclise stimulators were added to the substrate no increase in the proportion of AGTs that differentiated were observed (between 60 to 75 %, compared to 90 % on the more inductive cellulose). This was the case even though the level of AGT emergence itself increased. Together this evidence led Kinane et al., to speculate that a requirement for a separate stimulus exists in relation to appressorium formation, and that the cAMP pathway does not mediate that appressorial differentiation. Again together this evidence further re-enforced the notion that a higher degree of specificity of Bgh host
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recognition manifested itself in a more complex signal pathway than was seen in other pathogens, such as \textit{M. oryzae}.

As investigators suggested other forms of signalling pathways may be involved, attention was turned to the presence of a MAP kinase cascade (after the identification of two single copy \textit{Bgh} MAP kinase genes, \textit{mpk1} and \textit{mpk2}) (Zhang and Gurr, 2001). Zhang and Gurr produced degenerate PCR primers (to conserved domains found within MAP kinase sequences available at the time of publication) which produced amplicons available for sequence analysis. Blast analysis of the resultant data identified amplicons that were fragments of actual MAP kinase genes. These amplicons were then used for the design of PCR ‘walking primers’ and ‘step down PCR’ was performed which allowed full length genomic sequences to be determined (via DNA sequencing). Later two MAPKK genes, \textit{Bek1} and \textit{Bkk} were identified after sequence analysis (at the protein level using a BlastX algorithm) of expressed sequence tags (ESTs) and also after sequencing of transcripts found during profiling using ‘Serial Analysis of Gene Expression’ (SAGE) in libraries representing different stages of \textit{Bgh} development (Thomas et al., 2001; Thomas et al., 2002; and studies in Kinane and Oliver, 2000). As with cAMP signalling these cascades (based in the concept of three sequentially acting Mitogen-Activated Protein Kinase proteins) respond to extracellular stimuli, and act by phosphorylation on transcription factors. These factors would then affect gene expression (Zhang and Gurr, 2001; Kinane and Oliver, 2003). These cascades are widely conserved in eukaryotes, including filamentous fungi and have known roles in pathogenesis in plant pathogens, for example in the appressorium formation in \textit{Colletotrichum gloeosporioides} (Kim et al., 2000). Sequence analysis by Zhang and Gurr (2001) suggested that \textit{mpk1} showed homology, via encoded amino acid sequence, to the \textit{BMP1} MAPK of the necrotroph, \textit{Botrytis cinerea}. In the case of the second gene, \textit{mpk2}, amino acid sequence homology revealed similarity to \textit{MPS1}, a MAPK of \textit{M. oryzae} (whose disruption leads to appressorium formation, but with reduced pathogenicity). Furthermore RT-qPCR transcripts suggest \textit{mpk1} levels are elevated at the mature PGT and AGT stages unlike \textit{mpk2} whose levels increase during APP maturation (Zhang and Gurr, 2001). Work by Kinane and Oliver (2003) utilising inhibitors and activators suggested the MAPK pathway was not involved in PGT emergence, unlike cAMP (mentioned earlier), but instead solely transduces signals required for AGT elongation and most importantly APP formation. The authors manipulated MAPK activities using activators (such as sphingosine or Platelet Activating Factor-16) added to conidia developing on cellulose membrane known to induce less development, including APP
differentiation, than host surfaces. Additionally inhibitors (such as PD-98059) added to spores developing on a form of cellulose membrane known to show similar inductivity to the host surface). In both forms of experiment no effect on conidial germination was observed (when compared to controls). Furthermore when both the cAMP and MAPK pathways were activated (for example using cholrea or pertussis toxins to target the former, and sphingosine or PAF-16 to activate the latter) frequencies of germination were similar to those seen during stimulation solely by cAMP activators. As a consequence of these results Kinane and Oliver (2003) suggested that the MAPK pathway was believed not to have a role in PGT emergence, and that cAMP was the principal signal for germination. Evidence gathered by these same authors for the role of the MAPK pathway in AGT elongation (and APP formation) includes the observation that MAPK activity (as determined by phosphorylation assay) rose to a maximum prior to AGT and APP formation (between 2 and 8 h.p.i.). Also activators of the MAPK pathway which led to an increase in MAPK activity led to increased frequencies of AGT germination and APP differentiation. Similarly, when inhibitors of the MAPK pathway) were used frequencies of formation and differentiation decreased.

Additionally by employing bioactive lipids (including sphingosine) to activate MAPKs and to spur development Kinane and Oliver (2003) suggested the involvement of G-protein coupled receptors (the largest family of fungi transmembrane receptors) or related receptors (Bahn et al., 2007). According to the authors the bioactive lipids (such as sphingosine and PAF-16) used within their investigation may act via interaction with G-protein coupled receptors. This was based on evidence of sphogosine and PAF-16 related activation of MAP kinase-controlled events in G-protein dependent manners in mammalian systems gathered by Honda et al., (1994) and reviewed by Pyne and Pyne (2000). In the first study PAF acted via a cloned guinea pig PAF receptor (which exhibits the structural characteristics of the G-protein coupled receptor superfamily) which was expressed in Chinese hamster ovary. By utilising pertussis toxin (an inhibitor of the $G_{ai}$ subunit of the G-protein, which has an inhibitory effect on adenylyl cyclase) differential effects on PAF induced processes (such as the inhibition of cAMP accumulation and inositol phosphate production) occurred which suggested the PAF receptor coupled to ‘pertussis toxin’-sensitive and insensitive G-proteins within the ovary cells (with the toxin application permitting cAMP accumulation but not affecting inositol phosphate production) (Honda et al., 1994).
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In the case of the review by Pyne and Pyne (2000) sphingosine (in the form of sphingosine-1-phosphate) is identified as a polar sphingolipid metabolite, and they list five closely related G-protein coupled receptors of the ‘endothelial differentiation gene’ (EDG) family conforming to the typical topography for such receptors (i.e. the proteins contain 7 transmembrane spanning domains with amino acid sequence similarity to G-coupled protein families) (Studies listed in Pyne and Pyne, 2000). In the case of demonstrating that these receptors did also work through G-proteins, for example in the case of Edg-8, the authors (in that case, Im et al., 2000) utilised cell systems where ubiquitous endogenous responses to sphingosine were modest to minimal, and co-transfected these cells with DNA encoding the receptors and G-proteins. Sphingosine was observed to inhibit forskolin-dependent rises in cAMP when cells were transfected with Edg-8 DNA. However, by employing pertussis toxin again such an inhibition was alleviated, suggesting that sphingosine acted via G_{i/o}α proteins.

Since both PAF and sphingosine activated the MAPK pathway during treatment of Bgh conidia Kinane and Oliver (2003) imply that such receptors with similarities to the G-protein coupled receptors of mammalian cells may therefore be present in Bgh.

As such, this potential for G-protein coupled receptors being involved in the development of Bgh led Kinane and Oliver (2003) to note roles for such receptors in fungal development and lifecycles. Although they observe that no ‘classical’ type G-protein coupled receptors have been directly observed to have roles in ascomycete pathogenicity Kinane and Oliver point to the role of similar transmembrane receptor-like proteins that have. Focusing on PTH11, which DeZwaan et al. (1999) found mediated appressorium differentiation in Magnaporthe oryzae in response to inductive cues such as hydrophobicity and cutin monomers, and the fact that an orthologue had been found to exist in Bgh (Thomas et al., 2001), Kinane and Oliver suggested such a receptor could act as sense cutin monomers which Francis et al., (1996) had shown were capable of driving Bgh differentiation.

In order to determine how the cAMP and MAPK pathways interacted Kinane and Oliver (2003) employed effectors which targeted the cAMP and the MAPK cascades. Applied to conidia developing on cellulose membranes which were generally less inductive than the host surface their influence on both pathways was assessed. In the case of certain effectors (cholera and pertussis toxin) led to the activation of both the cAMP and MAP kinase pathways (as well as showing indistinguishable enhancement of germling development). As the authors noted in mammalian systems these toxins are known to target heterotrimeric G-
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protein subunits (studies listed in Kinane et al., 2003). This, combined with the knowledge of the existence of a G-protein subunit of mildew exhibiting a binding site for pertussis toxin, suggested to the authors several scenarios in which G-proteins lay upstream of both pathways (where a potential for convergence existed). Although such a convergence is the case in *Colletotrichum heterostrophus*, the authors noted this needed further elucidation in the case of *Bgh* would be necessary to determine the numbers of G-proteins involved and whether a sole protein may indeed act as a convergence point.

Recently Prats et al., (2008) discovered the presence of nitrogen oxide (NO) during appressorium maturation and secondary hyphae formation. Although they have yet to find a role for this molecule they note its inhibition of extra appressorial lobe formation and its potential link to cAMP signalling. As an example the authors point linkage of the two in mammals where NO generation is driven by cAMP signalling and leads to vascular homeostasis.

1.2.6: Penetration and the haustorium

![Image](image1.png)

Figure 1-8: A number of *B. graminis* f. sp. *hordei* haustoria present within barley epidermal cells. Example haustorium marked by arrow. Source: P. Spanu. Scale Bar = 10 μm approx.

Using a mix of enzymatic action (via cutinase and, possibly, cellulase) and mechanical pressure (reaching a maximum of 2-4 MPa) the appressorium forces an infection peg down through the plant cuticle into the epidermal cells below (Pryce-Jones et al., 1999; Talbot and
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Hamer, 2000; Iwamoto et al., 2007). If penetration fails, which is possible on a limited scale even on susceptible plants, then extra appressorial lobes form to re-attempt penetration (Eichmann and Huckelhoven, 2008; Prats et al., 2008). If successful in breeching the cell wall a root-like structure, the ‘haustorium’, develops (Figure 1-8) that although invaginating the cell membrane, leaves the cells fully intact (Huckelhoven, 2005). The host plasma membrane surrounds this structure at all times and is termed the ‘extra-haustorial membrane’ (Zhang et al., 2005). Between this membrane and the haustorial cell wall lies the ‘extra-haustorial matrix’ (Both and Spanu, 2004). This matrix is sealed off at the neck of the haustorium by a neckband thought to be derived from the remains of the papillae (Green et al., 2002).

Nutrients from the host cells are harvested via this nucleate haustorium (Schulze-Lefert and Vogel, 2000). Once fully active Bgh no longer needs to rely on the conidia as a source for energy (Eichmann and Huckelhoven, 2008). By 24 hours the haustoria are fully developed and the fungus begins to construct epiphytic mycelium on the leaf surface. This penetrates further epidermal cells and increases colonial spread (Both and Spanu, 2004; Seiffert and Schweizer, 2005).

In order for the fungus to develop haustoria and access the nutrients of the host, the biotroph must have developed, in the words of Panstruga and Schulze Lefert (2002) either ‘stealth mechanisms or subtle counter defences’ that permit the suppression of host defences and disrupt signalling pathways. However, due to the obligate nature of these fungi, how exactly they achieve this is not well understood (Eichmann and Huckelhoven, 2008).

Plant defences include ‘basal’ defences driven by the recognition of common ‘Pathogen-Associated Molecular Patterns’ (PAMPS) (Jones and Dangl, 2006). Such defences include the strengthening of cell walls (via the formation of cell wall appositions), the apoplastic accumulation of reactive oxygen species as well as cell death (Lipka et al., 2005; Zhang et al., 2005; Eichmann and Huckelhoven, 2008). In the case of race-specific host resistance (the next layer of defence “behind” basal defence mechanisms) defence revolved around the hypersensitive cell death response (Dangl and Jones, 2001).

As well as overcoming preformed defences, such as the cuticle, Bgh may actively suppress other basal defences rather than try to mask its PAMPS (Eichmann and Huckelhoven, 2008). Zhang et al., (2004) discovered that Bgh could excrete a catalase during penetration. This
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may explain the documented lack of reactive oxygen species build-up at sites of appressorial formation in susceptible plant-host interactions (studies in Zhang et al., 2005).

By successfully overcoming basal resistance there is also the implication of the action of microbial effectors within the host cell itself (Eichmann and Huckelhoven, 2008). It is these effectors, encoded by ‘avirulence’ (Avr) genes, which are the basis (or targets for) race-specific resistance, mediated by the ‘resistance’ (R) genes of the host. More than 20 barley powdery mildew Avr genes have been studied although how they perform their task is not so well known (Eichmann and Huckelhoven, 2008).

Numerous host proteins are thought to be involved in penetration and haustorial development in compatible infections. In the case of defence derived from a recessive loss of gene function (for example by mutation), the equivalent wild-type gene is thought to be a target for interference by the mildew. According to Huckelhoven (2005) some are negative regulators of host defences. The best example of which is the dominant Mlo gene. This gene encodes a 60 kDa protein with 7 transmembrane domains, similar to a G-protein coupled receptor (Buschges et al., 1997). Evidence suggests this susceptibility factor negatively regulates defences against Bgh, and so when in recessive form offers broad spectrum resistance (Schulze-Lefert and Vogel, 2000). Studies have shown that during resistant (non-compatible reactions) infection is aborted during penetration and no cell death results. Evidence suggests this susceptibility factor, when functional, may regulate syntaxin-dependent exocytosis. Other host proteins that may have roles in susceptible reactions are discussed in Eichmann and Huckelhoven (2008).

1.2.7: Epiphytic mycelia and sporulation

By 3-4 days post-inoculation (d.p.i.) the fungal colony can be detected by eye upon the leaf surface. At this stage perpendicular aerial structures form on the external vegetative hyphae (Glawe, 2008). Upon these ‘conidiophores’ (Figure 1-9) the colourless, uninucleate conidia develop in chains (Braun et al., 2002). A swollen basal “mother cell” will differentiate from the epicuticular hyphae (Schmitt et al., 2006). By a series of nuclear divisions and septations a new conidium will develop from the mother cell, eventually forming large chains of spores. This formation is ‘basauxic’ with each new conidiophore forming at the base of its predecessor (Glawe, 2008). Each conidiophore may survive for approximately 107 hours and produce
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approximately 30 conidia (Moriura et al., 2006). Finally after a restriction at the septum, a conidium may separate from the conidiophore (Schmitt et al., 2006). The asexual cycle then has the potential to begin again. Mechanisms for release are discussed in Glawe (2008), and can include wind or convection currents, electrostatic charge and leaf shaking. Although conidial dispersion is mainly over short distance, records have indicated barley powdery mildew infections in Denmark that were caused by spores originating in the United Kingdom, a journey of approximately 500 miles (Hermansen et al., 1978). Together this means that not only is disease transmission rapid but it also has the potential to be long-distance as well. The following days involve the expansion of the colony, whose lifetime may last approximately 460 hours in total (Moriura et al., 2006). It is these sporulating colonies that form the primary disease symptom.

It is during this stage of development that transcripts for enzymes regulating glycolysis again increase (especially at 5 d.p.i.): the period when external hyphal growth is well underway and energy is required to fuel sporulation (Both et al., 2005a). Glycogen synthesis, as well as neutral and polar lipid formation, also increases, no doubt to prepare nascent conidia to begin the infection cycle afresh. Expression profiles of tRNA synthases, the enzymes that create tRNAs required for protein synthesis, although increasing throughout development, also showed a marked surge post-penetration (Both et al., 2005a). This would allow increased protein synthesis during epiphytic mycelial development and especially sporulation. Simultaneously as these changes to metabolism of the fungus are occurring, in order to maintain growth and expansion the barley powdery mildew must alter the metabolism of the host to its benefit.

As noted by Both and Spanu (2004), its close relative wheat powdery mildew (B. graminis f. sp. tritici) causes a significant change in the photosynthetic sink-source relationship within the host plant that leads to carbohydrate and starch accumulation. It does this by triggering an
acid invertase activity (Both and Spanu, 2004). The pathogen also affects leaf photosynthesis as shown by a reduction in Rubisco and enzymes involved in the Calvin Cycle and also a loss in chlorophyll (Akhkha et al., 2003; reviewed by Both and Spanu, 2004). This latter point results in the plant compensating for the loss of carbohydrate production. Increasing respiration and the activity of the pentose phosphate pathway has been observed as has the diverting further of nutrients to the area and hence to the fungus (Akhkha et al., 2003; Both and Spanu, 2004). Uptake also appears to be active and carrier mediated (Sutton et al., 2007). H$^+$-ATPases have increased transcription during haustorial formation and are believed to create the electrochemical potential needed for nutrient transfer to the mildew (Both et al., 2005b). To date, detailed knowledge of the nutrients actually utilised by the powdery mildews is still unclear (Sutton et al., 2007), although studies suggest glucose is the main carbon source transferred to the pathogen, at least in the case of mildew of wheat (B. graminis f. sp. tritici, Sutton et al., 1999). In Blumeria it may be the case that sucrose is the main sugar taken up as two sucrose transporters have been observed in the genome (Spanu and Kämper, 2010).

Further evidence of the mastery of the host by Bgh may be the ‘green island’ effect (Figure 1-10) or ‘green bionissia’, acknowledging the fact that in this situation both host and pathogen cells are alive (Walters et al., 2008). This phenomenon refers to the maintained green colour (due to chlorophyll retention or recuperation) in immediate areas underneath and surrounding colonies on senescing leaves that has been noted as far back as 1897 by Von Tubeuf (von Tubeuf, 1897; Schulze-Lefert and Vogel, 2000; Hucklehoven, 2005). As speculated by Schulze-Lefert and Vogel (2000) host cell death may be one way of cutting off the nutrient supply to a biotroph, so suppression of this defence seems intuitive for such a parasite. Maintenance of the host tissue has been believed to allow longer lasting sporulation by the pathogen. However this has been disputed as merely being a side effect of early
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attempts to ensure diversion of nutrients to the infection site, rather than attempts to prolong infection itself (Walters et al., 2008). Therefore, although the characteristic is only apparent during the later stages of leaf life, it may be a result of interactions early on in the infection process (Walters et al., 2008).

1.3: Co-ordinate Mis-Expressed Genes (‘CMEGs’)

This lifecycle and its symptoms suggest complex signal transduction systems exist within the fungus to allow the “hijacking” of the plants nutrients for its own uses. More work is needed to elucidate these mechanisms, but what is known has been reviewed by (Both and Spanu (2004) and Eichmann and Huckelhoven (2008). ‘Expressed Sequence Tag’ (EST) sequencing of cDNA libraries (derived from the mRNA of conidia germinating on different surfaces) and microarray analysis began to define gene transcript expression dynamics present during different early stages of Bgh growth (Thomas et al., 2001; Both et al., 2005b).

In a key study, Both et al., (2005b) studied the transcript profiles of 2027 Bgh genes during infection. RNA samples were collected from fresh ungerminated conidia as well as at stages throughout development (4, 8 and 15 h.p.i.) to the stage of fresh sporulation at 5 days post-inoculation. At 3 and 5 d.p.i., both epiphytic mycelia and RNA from infected epidermis were collected. Two-colour microarrays were performed, comparing each collected stage to a general reference sample made up from a mixture of all samples. The authors observed a large scale shift in gene expression between pre- and post- penetration Bgh life stages (thought to be representing a change from the use of endogenous reserves to reliance on host nutrients). Furthermore the authors make particular note of a small group of genes that were up-regulated pre-penetration. These genes followed the expression profile of the Bgh homolog to Cap20, with mRNA transcript rising to a peak at 8 h.p.i. before decreasing at 15 h.p.i.. Cap20 is a Colletotrichum gloeosporioides gene known to accumulate during appressorial formation (Hwang et al., 1995). Further analysis suggested many of the clustering Bgh genes showed homology to the pathogenicity genes of other fungi. This led Both et al., (2005b) to suggest that these genes may be responsible for virulence and pathogenicity in Bgh.

This study spurred further work, where apart from examining gene expression during infection on the host, expression was monitored during germination on non-host surfaces. Carried out by Dr. Maike Paramor (née Both) the surfaces chosen, apart from the host,
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included glass (considered a “non-inductive” surface due to its ability to cause solely the formation of multiple, small germ tubes), cellulose (“semi-inductive” due to a limited ability to stimulate appressorial formation) and wheat (a fellow member of the *Gramineae* where all development stages bar actual penetration are witnessed) (Both, 2005). On each surface a time course of 0, 4, 8 and 16 h.p.i. was performed. This led to the identification of certain transcripts with grouped expression at set times during that development and their alternate expression on surfaces other than the host (e.g. Figure 1-11). This expression clustering suggests the presence of common *cis-* and *trans-* regulatory elements within genes that permit co-ordination with one another during the highly synchronous developmental programme of *Bgh*. The alternate expression also suggests these genes are responding (or not as may be the case) to a lack of development stimulating signals presented by the non-host surfaces. Due to this behaviour such genes have been designated ‘Co-ordinate Mis-Expressed Genes’ and have become candidates for further study.

![Figure 1-11: Grouped relative expression levels of ESTs from a microarray study conducted by Both et al., 2005;](image)

**REI:** log2 transformed signal intensity. RNA from each sample was compared to RNA from a mixture of all samples) of the *Blumeria graminis* EST clone C01417(red) and similarly expressed transcripts (grey) on different surfaces. **Source:** Dr. Maike Paramor

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General Introduction

**1.4: Green fluorescent protein**

Over the last 15 years the use of fluorescent proteins has energised the study of living cells (Freitag et al., 2004). Green fluorescent protein (GFP) is a 238 amino acid, 27 kDa protein which absorbs light at a maxima of 395 and 475 nm, whilst emitting it at a single maximum of 508 nm (Lorang et al., 2001; Czymmek et al., 2002). The gene encoding this protein was cloned by Prasher et al., (1992) from the jellyfish *Aequora victoria*. A landmark study by Chalfie et al., (1994) demonstrated that GFP would still fluoresce in other organisms, opening the way for its employment as a reporter gene (Gold et al., 2001).

Unlike previous reporter genes, for example *Escherichia coli* β-glucoronidase system, GFP can be used to visualise activities in cells without the need for fixation or the use of co-factors or exogenous substrates (Gold et al., 2001). This is an important advantage as both plant and fungi have diverse cell walls which are serious obstacles to the addition of cofactors etc or the use of antibodies to localise proteins (Czymmek et al., 2002). It has therefore assumed the important role of detailing gene expression in living cells.

As a general rule wild-type GFP is stable and active in fungi, although mutant alleles which have overcome transcript processing problems and codon usage issues tend to hold prominence (Hein and Tsien, 1996; Gold et al., 2001).

GFP has been used to study gene expression by transcriptional fusion, as well as to investigate sub-cellular localisation of proteins, e.g. defining the expression of endopolygalaturonase genes in *Colletotrichum lindemuthianum* (Dumas et al., 1999). The latter feature is available as GFP doesn’t appear to interfere with protein folding (in general) even if fused to the N or C protein terminal (Gold et al., 2001). Furthermore GFP doesn’t appear to affect the host cell itself.

When GFP is matched with laser scanning confocal microscopy a powerful research tool is created. Unlike in normal light microscopy, laser scanning confocal microscopy uses focused laser light to illuminate the specimen. With better resolution than normal light microscopy, the main advantage of this technique is the ability to non-destructively analyse living cells, especially when the cells in question may be deep within a host (Gold et al., 2001).
General Aims

At the current time defences against *B. graminis* f. sp. *hordei*, although utilising host resistance, centre around the judicious use of mildewcides (Gilbert et al., 2009). Unfortunately, *Bgh* displays an ability to rapidly overcome many resistance genes as well as to develop tolerance to fungicides (Chaure et al., 2000; Thomas et al., 2001). As a result it is necessary to develop a greater understanding of the infection cycle, in particular early development and interaction with the host, so that more efficient methods of control may be established.

Although attempts have been made to grow this fungus *in vitro* (separate from the host), for example by Arabi and Jawhar (2002), its lifecycle as an obligate, biotrophic fungus means this is impossible. Cultivation is necessary when trying to transform the pathogen, which is the basis for advanced genetic analysis. As such, although transformation has been attempted (e.g. Chaure et al., 2000), molecular studies have been severely hindered as methods of stable transformation have proven unworkable. Consequently, this led Talbot and Hamer (2000) to describe barley powdery mildew as ‘something of a nightmare for molecular geneticists’. This still rings true today as the individual signalling pathways and networks which link surface perception to the genes involved within the differentiation programme are still poorly understood. What is known is either cytological in nature or, in the case of molecular pathways, very fragmentary. Even so, most of what is known about powdery mildews in general has been based on this species (Glawe, 2008).

Therefore this thesis has 2 distinct aims. In the first half of the thesis, studies are made to contribute to the existing knowledge of *Bgh* infection. Building on previous studies which identified a modulation of gene expression depending on the underlying substrata germination, studies are undertaken that try to discern the regulation of these surface responsive genes (the ‘CMEGs’).

The second aim of the investigation is to develop a successful transformation procedure of barley powdery mildew. In addition to this, trials are also made regarding the use of a related fungus, *Magnaporthe oryzae*, as a heterologous test bed for genetic analysis of *Bgh* elements.
General Aims

This organism may act as a “surrogate” whilst methods of direct transformation of barley powdery mildew are refined.
Chapter 2: Materials and Methods (General)

2.1: Plant growth conditions

Barley (cv. Golden Promise) seedlings for long term stocks were grown (≈ 20 per pot) in 5 inch pots at 20 °C and 30 % humidity with 16/8 hour light/dark cycle in a Sanyo Fitotron growth room. Light Intensity was 56 µmol m$^{-2}$ s$^{-1}$ (at 1.2 metres). Plants were infected 7 days after sowing (the single leaf stage). Plants required for infection were kept isolated in a plant growth suite (Unigrow, UK) at 23 °C (daylight) and 20 °C (night) with 50/60 % humidity respectively (Light intensity of 120 µmol m$^{-2}$ s$^{-1}$) or a ‘Grodome’ Greenhouse suite (Unigrow, UK) 23 °C (daylight) and 18 °C (night) with ambient humidity. Light Intensity was ≥ 100 µmol m$^{-2}$ s$^{-1}$. Wheat (cv. Riband) seedlings were also grown in the same conditions as required.

Seed was provided by the National Institute of Agricultural Botany (NIAB, Cambridge, UK), by Professor Paul Schulze-Lefert (Max Planck Institute for Plant Breeding Research, Cologne, Germany) and by Eric Simpson (Masstock Arable [UK] Ltd, Throws Farm Arable Development Centre, Stebbing, Essex). Levington Professional Growth Medium (F2+S) was used for all plant growth. The Insecticides Intercept 70WG (Scotts) and Exemptor (Scotts) were added as required and as according to the instructions of the manufacturer.

2.2: *Blumeria graminis* f. sp. *hordei* isolate storage conditions and infection procedure

*Bgh* isolate DH14 was a gift from Professor James Brown (John Innes Centre) (received in 2006), although it was originally isolated during 1976 by D. W. Holloman (1981) investigating resistance in *Bgh* populations in the UK to the fungicide ethirimol. For long-term storage sporulating colonies were stored on barley (cv. Golden Promise) (susceptible) in a isolation pot for 8-12 weeks at 4 °C. Preceding experimentation *Bgh* isolate DH14 was inoculated on non-treated 7-day-old barley seedlings and allowed to progress to sporulation.
Material and Methods (General)

(for inoculation stock) in a growth room at 25 °C and 50 % humidity with 16/8 hour light/dark cycle. Light Intensity was 56 µmol m² s⁻¹. To reduce air turbulence, and unintended dispersal of conidia, all infected plants were placed in a perspex isolation tank (Figure 2-1). Infected barley directly used for experimentation was capped with a perspex isolation top (Figure 2-2) to maintain humidity and reduce cross infection. To infect surfaces, 1 to 2 week old infected plants with heavy sporulation were blown 24 hours before sprinkling of the target substrates to ensure fresh conidiation. Plants were gently agitated over the target surface, in a still air room, to give a conidial concentration of approximately 150 spores/cm².

Figure 2-2: This picture shows infected barley seedlings with Bgh isolate DH14 contained with an isolation pot cover (19 cm X 8 cm).

To minimise contamination and to maintain humidity these pots could be sealed with adhesive tape.

2.3: Fungal staining protocol

2.3.1: Trypan blue stain

Infected leaves were stained for fungus by boiling them for 5 to 8 min in 0.1 % trypan blue (BDH Gurr®) in ethanolic lactophenol (1 vol ethanol: 3.35 vol lactophenol, NBS Biologicals Ltd). The leaves were then de-stained in chloral hydrate (2 g/ml, BDH GPR®) for several days. This solution was changed until the leaf material was transparent. Observation was then permissible.
Material and Methods (General)

2.4: Microscopy and photography

Stained fungal structures and spores were viewed under x10, x40 bright field microscopy using ‘Letiz® Diaplan’ microscope. Epifluorescence microscopy was performed on ‘Carl Zeiss® Axioskop 2 plus®, microscope with HAL-100 fitting, with 50 W HBO mercury lamp and filters (FITC filters) (A: BP485/20, FT510, LP515; B: BP450-490, FT510, LP515; C) BP450-490, FT510). Bright field and fluorescent images were captured on ‘Carl Zeiss® Axioskop 2 Plus®, microscope with ‘Carl Zeiss® Axiocam Fitting’ camera. Images were viewed using ‘Axiovision’ (version 3.1.21) (Carl Zeiss) software for PC with ‘Carl Zeiss® AxioCam-HR’, (version 5.05.10) and ‘TWAIN’, (version 1.0) add-ons. Light Photography was performed with a Canon® ‘Powershot Pro1’ and also a Nikon® D-100 with 55 mm Nikkor Macro Lens.

Time-lapse photography utilised an Ortholux II (Letiz Wetzlar) microscope and a Nikon coolpix 990 (with Nikon MC-EUI timing adapter). All spores and substrate samples were placed on a glass slide (Super premium microscope slide, VWR International) and suspended in a 9 cm petri dish containing water to maintain high humidity during the film duration. Substrates used include barley leaf, wheat leaf, glass slide and cellulose membrane (Medicell International Ltd.). Both barley and wheat leaves were dissected to create a window of epidermal tissue to permit microscopy (Figure 2-3). This technique was also used for viewing of Magnaporthe oryzae spore development.
Figure 2-3: Creation of barley epidermal windows.

Two shallow incisions were made on the adaxial leaf surface. The epidermal surface flap was folded back until surface disconnection had reached the second incision site. Flap was then removed. Abaxial epidermis remained, and acted as a site for fungal spore deposition. Leaves were placed adaxial side down on 1.5% TWA agar (Appendix A, 9.1.1) or ddH2O to retain hydration.

All images were processed using ‘Adobe® Photoshop® Version 6.0 and Adobe® Photoshop® Version CS3’ and ‘Adobe® Image Ready® Version 3.0’.

2.5: Competent *Escherichia coli* cell preparation

All vectors were propagated using ‘One shot® Omni-max™ 2 T1 Phage Resistant’ *E. coli* cells (Invitrogen™) as hosts. The preparation of competent cells followed a protocol by Inoue et al., (1990) with modifications. To summarise, 250 ml SOB (Appendix A, 9.1.2) in 1 L flask was inoculated and incubated at 19 °C with vigorous shaking (approx. 200 rpm) until the OD₆₀₀ = 0.5. The culture was placed on ice for 10 min then cells were pelleted by centrifugation at 3220 x g for 10 minutes at 4 °C. Cells were re-suspended in 80 ml of ice-cold TB solution (Appendix A, 9.1.2) and stored on ice for 10 min, followed by centrifugation at 3220 x g for 10 min at 4 °C. Cells were then re-suspended in 20 ml of ice-cold TB solution and 1.4 ml of dimethyl sulfoxide (previously stored overnight at -20 °C before use). Cells were aliquoted into sterile, pre-chilled microcentrifuge tubes and stored at -
80 °C until required. The required *E. coli* strain cultured on LB agar with selective antibiotics ([Appendix A, 9.1.2](#)) at 37 °C overnight.

2.6: *E. coli* transformation protocol

All transformations used ‘One shot® Omni-max™ 2 T1 Phage Resistant’ *E. coli* cells (Invitrogen™). 0.01–100 ng DNA (10 μl ligation mixture) was gently mixed with 100 μl ‘One shot® Omni-max™ 2 T1 Phage Resistant’ *E. coli* cells. After incubation on ice for 30 min, the cells were heat shocked for 45 s at 42 °C. Cells were then incubated on ice for approximately 2 min. 250 μl SOC medium ([Appendix A, 9.1.2](#)) was then added to cells and the suspension was incubated at 37 °C with shaking (120 rpm) for 1 hr. This cell suspension was then pelleted by centrifugation at 1800 x g, re-suspended in 100 μl and then plated upon LB-antibiotic agar ([Appendix A, 9.1.2](#)) plates. Plates were incubated at 37 °C until transformed colonies appeared.

2.7: Miniprep plasmid purification

Plasmids were purified from 6 ml LB-antibiotic cultures grown overnight at 37 °C with agitation using ‘Qiagen™ Miniprep Spin’ following the protocol of the manufacturer. Antibiotic concentration added is listed in [Appendix A, 9.1.2](#). The extra washing step with PB buffer was performed.

2.8: DNA concentration measurement

For quantification the OD$_{260}$ of DNA samples was measured on a Beckman Coulter™ DU® 640 spectrophotometer. The formula used: concentration of DNA in sample (µg/ml) = A$_{260}$ x 50 µg/ml x dilution factor. Purity of DNA was also gauged by calculation of A$_{260}$/A$_{280}$ ratio.

2.9: Agarose gel imaging

1 % and 2 % (w/v) agarose gels (run at 80 V for 3/4 hour in TBE buffer [Tris-Borate 45 mM, EDTA 1 mM pH 8]) and stained with Sybr® Safe DNA Stain (1 μl/50 ml of 1:10, 000 mix) were used to visualise DNA at different stages of the cloning process. Samples were mixed with loading buffer (0.4 % orange G, 0.03 % bromophenol blue, 0.03 % xylene cyanol FF, 15 % Ficoll® 400, 10 mM Tris-HCl [pH 7.5] and 50 mM EDTA [pH 8.0]). The ‘1kb DNA ladder’ (0.25-10 kb; Promega®), the ‘100 bp’ ladder (Promega®), Fermentas FastRuler™
Material and Methods (General)

Low Range DNA ladder ready-to-use (Fermentas Lifesciences) and Fermentas GeneRuler™ 1Kb ladder (Fermentas Lifesciences) were used to gauge molecular weight. 5 μl of ladder was loaded per gel lane. Agarose gels were visualised on a ‘Gel Doc-2000’ (BioRad™) gel imager augmented with a ‘Safe Imager™’ (Invitrogen) Blue light unit. Images were captured using ‘PD Quest’ software (The Discovery Series, Version 7.31, Biorad Laboratories, Inc).

2.10: DNA precipitation

To allow re-suspension of DNA in alternate volumes DNA samples were precipitated as follows: 0.3 M of sodium acetate (pH 5.2) was added followed by 0.7 volumes of isopropanol. The solution was then incubated at 4 °C or -20 °C for 30 min or overnight respectively. Following this the sample was centrifuged at 15,000 x g at 4 °C for 30 min. The supernatant was then decanted without disturbing the pellet. The pellet was then washed with 200 μl 70 % ethanol and then centrifuged at 15,000 x g for 15 min at 4 °C. The supernatant was decanted and the pellet air dried for 10 min. Finally the pellet was re-dissolved in the appropriate buffer (e.g. Elution Buffer, TE, or ddH₂O).

2.11: RNA extraction

Based on the protocol of Chomczynski and Sacchi (1987). Isolated fungal material was ground in liquid N₂. All labware was washed in 8 g/L NaOH and 1 % SDS solution, rinsed in excess ddH₂O and then pre-cooled before use in liquid N₂. 5 ml 4 M GTC (Appendix A, 9.1.2) was added, followed by 5 ml saturated phenol (pH 4.5), 42 μl β-Mercaptoethanol (2-Mercaptoethanol, Sigma) and 30 μl 50 % PVP (Sigma-Aldrich®) to the material in 50 ml polypropylene tube (Sterilin). The mixture was vortexed for 5 min to ensure viscosity. 0.2 volumes of chloroform were added and mixed. The solution was then centrifuged at 3220 g for 15 min. Subsequently, the top phase was added to an equal volume of chloroform (BDH AnalAr Normapur©), mixed and then centrifuged again for 15 min at 3220 g. The top phase was then added to 3M sodium acetate (Sigma-Aldrich®), pH 5.2 (final concentration of 0.3M) and precipitated with an equal amount of iso-propanol (propan-2-ol, BDH AnalAr®). This mixture was then left at -20 °C for 30 min or longer. After incubation the mixture was incubated and centrifuged at 3220 x g for 25 min. Subsequently the supernatant was discarded and the pellet was air-dried for 10 min. The pellet was then re-suspended in 100 μl DEPC water (Appendix A, 9.1.2).
A modified Protocol was followed for later samples. The material was ground in liquid N\(_2\) 6 to 6.5 ml GTC/Phenol-β-Mercaptoethanol (Appendix A, 9.1.2) per 50 ml tube of material (~2 g of material corresponding to one plant pot or set of glass slides, etc). The mix was vortexed for 5 min. 0.2 volumes of chloroform was added, then mixed by vortexing. The sample was then centrifuged at 3220 x g for 25 min. The top phase was transferred and added to an equal volume of chloroform before mixing and centrifugation at 3220 x g for 25 min. The top phase was collected, 3 M sodium acetate pH 5.2 was added to a 0.3 M final concentration and precipitated with an equal volume of iso-propanol. The sample was left overnight at -20 °C, before pelleting by centrifugation at 3220 x g for 30 min. Iso-propanol was decanted and the sample was centrifuged at 3220 x g for 10 min before pellet was air dried (approximately 10 min). The pellet was then re-suspended in 100 µl DEPC water.

2.12: Purification of RNA using AGENCOURT® RNA CLEAN™ KIT

The usage of this system followed the protocol of the manufacturer. Briefly, before use the Agencourt® RNAClean solution™ (Agencourt® Bioscience Corporation, now part of Beckman Coulter, Inc) was re-suspended by shaking. To 100 µl of RNA solution 180 µl ‘RNAClean solution’ was added (1.8 x target volume = RNAClean solution volume to be added). This reaction solution was mixed by pipetting 10 times and placed into the Agencourt® SPRIPlate® 96R magnet plate for 10 min to separate the beads from solution. Subsequently, the cleared solution was aspirated from the beads. 200 µl 70 % ethanol was then added to the reaction and incubated for 30 s before aspiration. This was repeated for a total of 3 times. The reaction was air-dried for 10 min before addition of 100 µl DEPC RNAse free water. This was then mixed be pipetting 10 times, before placement back on the Agencourt® SPRIPlate® 96R magnet plate and an incubation of 10 min. The bead-less solution was removed and stored at -20 °C before use.

2.13: cDNA synthesis using superscript™ III reverse transcriptase

This procedure followed the protocol of the manufacturer (Invitrogen™). Contained within a 13 µl total volume were: 1 µl 10 mM dNTP Mix (10 mM each dATP, dCTP, dGTP and dTTP), desired amount of RNA template (in DEPC water) and 250 ng Random primers. This mixture was heated for 5 min at 65 °C and then incubated at on ice for at least 1 minute. To the mix was added: 4 µl 5X First-Strand Buffer, 1 µl 0.1 M DTT, 1 µl RNaseOUT™ Recombinant RNAse Inhibitor, 1 µl of SuperScript™ III Reverse Transcriptase (200 units/
μl). After mixing the solution was then incubated at 25 °C for 5 min. After this, the solution was incubated at 50 °C for 60 min followed by heat inactivation at 70 °C for 15 min. The cDNA was then diluted in TE buffer (10 mM Tris [tris(hydroxymethyl)aminomethane], pH 7.5; 1 mM EDTA [ethylenediaminetetraacetic acid]) to act as template for RT-qPCR analysis. All incubation steps utilised the PTC-200™ Peltier thermocycler (MJ Research Inc.).

500 ng of RNA was used as a template for cDNA production. cDNA template was then diluted 1 in 15 for use. 100 ng of RNA was used as a template for cDNA production (Chapter 4 and Chapter 6). Template was then diluted 1 in 15 for use and then 1 in 20 for use, respectively.

**2.14: Report creation**

Graphs were compiled with SigmaPlot® for Windows Version 10.0 (Build 10.0.0.54, 2006, Systat Software, Inc.).
Chapter 3: Characterising *Blumeria graminis f. sp. hordei* Germination Behaviour

### 3.1: Overview

Powdery mildews are biotrophic pathogens whose infections of the host are limited to the epidermis and who use haustorial complexes to collect host nutrients (Sutton et al., 2007). After detaching from a conidiophore, a *Bgh* spore is capable of beginning the asexual lifecycle again (Noir et al., 2009). On landing spores will first secrete varying amounts of extracellular matrix that aid attachment to the surface. Germination often follows, leading in some cases to the formation of an appressorium. In order for this stage to be reached, a number of developmental steps must occur, each of which appear to require their own particular suite of chemical and physical cues, presented by the underlying substrata. Complete development is only seen on the host, and by removing such signals from pathogenic fungi can lead to reduced infection frequencies and developmental errors (Wynn, 1981).

### 3.2: Aims and objectives

To allow familiarisation with *Blumeria graminis f. sp. hordei* and to begin an assessment of the effect of different stimuli on the germination program of this pathogen, the development of *Bgh* on the host was characterised. *Bgh* isolate DH14, originally isolated by Holloman (1981), was used. This isolate shows sensitivity to fungicides such as ethirimol and tridimenol and was selected due to its use documented in Chapter 6 (Wyand and Brown, 2005) and due to its use in sequencing the *Bgh* genome (Spanu et al., 2010). Future work will involve both analysing the effect of stimuli on gene expression and also of the regulatory sequences of genes demonstrating alternate behaviours on different surfaces. Therefore developmental behaviour was also characterised on wheat, glass and cellulose.
Characterising *B. graminis* Germination

### 3.3: Materials and methods

#### 3.3.1: Fungal culture and plant growth conditions

Fungal cultures were stored as noted in section 2.2. Barley (cv. Golden Promise) seedlings were grown (≈100 per pot) as noted in section 2.1. Plants were used after 7 days growth. Wheat (cv. Riband) seedlings were also grown in the same conditions.

#### 3.3.2: *Blumeria graminis* f. sp. *hordei* development study

Four surfaces, barley, wheat, glass (Super Premium Slides, VWR International) and cellulose (Medicell International Ltd.) were utilised as substrates to analyse development at 4, 8 and 16 hour time points post-inoculation. At each time point 8 samples (glass slides or sections of cellulose/primary barley leaf/primary wheat leaf) from each of 3 trays or pots (24 samples in total) had the germination state of 50 *Bgh* spores assessed on them. Surfaces were infected as noted in section 2.2 with a spore coverage of 180 /mm$^2$ approximately, and incubated in light (56 μmol m$^{-2}$ s$^{-1}$) at 20 °C and 30 % humidity in a Sanyo Fitotron growth room. The primary leaf (Zadoks growth stage 11) was defined as shown in Figure 3-1. Both glass slides and cellulose were contained within plastic trays (23.5 x 23.5 cm) with plastic lids to maintain humidity. Moist paper towelling was used as required to ensure high humidity and prevent spore dessication. Cellulose hydration and high humidity was maintained by the placing of cellulose on tap water agar. Only swollen spores indicating maintenance of water relations over the experimental period were counted. Additionally, only single spores were counted to reduce the possibility of growth inhibition due to crowding. Spores were classified according to eight criteria (Table 1).

![Figure 3-1: The first fully emerged barley leaf.](image)

Equivalent to Zadok growth stage 11. Modified from Source: Poole (2005)
Characterising *B. graminis* Germination

<table>
<thead>
<tr>
<th>Class Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ng</td>
<td>Non-germinated</td>
</tr>
<tr>
<td>PGT:</td>
<td>Emergence of one germ tube</td>
</tr>
<tr>
<td>PGT/SGT:</td>
<td>Emergence of 2 germ tubes</td>
</tr>
<tr>
<td>PGT/SGT/A:</td>
<td>Emergence of both primary and secondary germ tubes with the latter assuming hooking characteristic of the appressorial stage of development.</td>
</tr>
<tr>
<td>PGT/SGT/A/H:</td>
<td>All germ tubes are present and appressorial formation has led to penetration and subsequent haustoria formation.</td>
</tr>
<tr>
<td>Mm (Miscellaneous: multiple)</td>
<td>More than 2 germ tubes</td>
</tr>
<tr>
<td>Mb (Miscellaneous: branched)</td>
<td>Germ tubes present show branching</td>
</tr>
<tr>
<td>Mi (Miscellaneous: long)</td>
<td>Germ tubes present are longer than perceived average developmental parameters</td>
</tr>
</tbody>
</table>

Table 1: Spore germination categories.

Spore status for pre-penetration and early post-penetration events was assessed. Three miscellaneous categories showing abnormal development were observed. If spores demonstrated characteristics of these three classes they were assessed as follows: MB>Ml>Mm. This was based on the assumption that more complex processes had taken place to cause branching.

3.3.3: Fungal staining protocol

3.3.3.1 Trypan blue stain

Infected leaves were stained as noted in section 2.3

3.3.3.2 Wheat germ agglutinin alexa 488 Stain

Leaves were incubated on methanol until colour was lost and then cleared by incubation over night in chloral hydrate (2 g/ml). Leaves were subsequently incubated in PBS to remove chloral hydrate from tissue. PBS solution was changed at least twice (Karpovich-Tate et al., 1998). The fungal structures were labelled with wheat germ agglutinin (WGA), coupled with
Characterising \textit{B. graminis} Germination

Alexa-488, by incubating the leaf tissue with 1 μl/ml of WGA Stain stock solution (10 mg/ml, Molecular Probes, Leiden, The Netherlands). After $\frac{1}{2}$ hr incubation microscopic observation was done (section 2.4).

\subsection*{3.3.4: Preparation of cellulose}
Cellulose strips (Cuprophan, Medicell International Ltd.) were boiled in H$_2$O, 3 times for 10 min and then autoclaved for 15 min at 120 °C. Cellulose strips were then placed on 1.5 \% TWA plates (23.5 cm x 23.5 cm) with sealable lids to maintain humidity.

\subsection*{3.3.5: Statistical analysis}
Spore counts were analysed using ‘R: A language and environment for statistical computing version 2.5.0’ (http://www.R-project.org). Count data were transformed by square root and modelled using three-way, two-way and one-way ANOVA, with inbuilt error terms to compensate for spatial pseudo-replication. Percentages were calculated via the equation \[(average \ number \ of \ spores) / (total \ number \ of \ spores)\]*100. The ‘average number’ represents the average number of spores per replicate at the ‘pot or tray’ level. ‘Total number of spores’ represents the average number of spores (400) counted for all germination categories at the selected time point (4, 8, 16 h.p.i.). Standard deviation was calculated according to the following equation:

\[ \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}} \]

Where $x$ is the sample mean average and $n$ is the sample size. Standard error was calculated by dividing the standard deviation by the square root of the number of replicates.

\subsection*{3.3.6: Microscopy}
Microscopy was performed as described in section 2.4.
3.4: Results

3.4.1: Development familiarisation

![Diagram of B. graminis germination stages](image)

**Figure 3-2: Developmental cycle of barley powdery mildew on barley.**


To observe the developmental progression of *Bgh* on barley throughout the asexual developmental cycle leaves were infected with spores of the *Bgh* isolate DH14. At time points relevant to development (0, 4, 8, 16 h.p.i., 3 and 5 d.p.i.) leaves were stained with Wheat Germ Agglutinin (WGA) to allow observation of development. As noted in the literature the infection starts with a conidium landing on a barley leaf surface (**Figure 3-2A**). By 4 h.p.i. conidia with primary germ tubes were visible (**Figure 3-2B**) as well as others where a second germ tube had begun to emerge (**Figure 3-2C**). By 8 h.p.i. many of these
Characterising *B. graminis* Germination

Secondary germ tubes had elongated, hooked and swollen to form the appressorium ([Figure 3-2D](#)). By 16 h.p.i. haustorial formation was present ([Figure 3-2E](#)). By 3 d.p.i. epiphytic mycelium had begun to form ([Figure 3-2F](#)). This supports the formation of epiphytic mycelia growth across the leaf surface and results in the completion of the lifecycle by the production of the airborne spores on conidiophores, which are visible by 5 d.p.i. ([Figure 3-2G](#)).

### 3.4.2: Germination profile on barley

A three-way ANOVA reveals that the germination states of *Bgh* spores on barley differs significantly from the other surfaces tested (wheat, cellulose and glass) over the time points selected (4, 8, 16 h.p.i.) (P < 0.001). Additionally, there is an interaction between surface type and time that affects the relative proportion of the germination states upon those surfaces (P < 0.001) ([Appendix B, 9.2.1](#)).

A profile of the growth stages reached by *Bgh* at three time points (4, 8, 16 h.p.i) on barley is shown in [Figure 3-3](#). At 4 h.p.i. the majority (an average of 264 out of 400, equating to 66.16 % ± 6.19 % s.d.) of spores assessed remained non-germinated. Germlings with a primary germ tube (PGT), second germ tube (SGT) and appressorial (A) stages of development were also observed. Conidia with solely a primary germ tube formed the minority of these germinated spores (17, 4.43 % ± 0.66 % s.d.). The majority of germinated spores (69, 17.4 % ± 2.75 % s.d.) had matured to produce a second germ tube. This class average was significantly larger than the two other stages of germinated conidia following normal morphological development (P < 0.001, n = 3 for both PGT and A classes respectively) ([Appendix B 9.2.1.2](#) and [9.2.1.3](#)). At 4 h.p.i. an average of 44 conidia (11 % ± 1.8 % s.d.) had begun to show preliminary indications of advanced appressorial formation (including both swelling and hooking of germ tube terminus) ([Figure 3-2](#) and [Figure 3-3](#)). Additionally, a small number of spores were spotted undergoing multiple germ tube formation.
Characterising *B. graminis* Germination

![Graph profiling development of Bgh on barley at 4, 8 and 16 hours post-inoculation.](image)

**Figure 3-3:** A graph profiling development of *Bgh* on barley at 4, 8 and 16 hours post-inoculation.

- **Pgt** = Primary (1) germ tube; **Sgt** = Secondary (2) germ tubes; **A** = Appressoria formation (with symptomatic hooking and swelling); **H** = Formation of haustoria; **Mb** = Branched germ tubes; **Ml** = Long germ tubes; **Mm** = More than 2 germ tubes. The final three classes are considered aberrant germination states. Levels of non-germinated conidia at 4 h.p.i. = 265 ± 14 s.e.; 8 h.p.i. = 243 ± 3 s.e. 16 h.p.i. = 216 ± 12 s.e. approximate to the nearest spore. (Error bars = ± standard error, n = 3. Graph constructed from average of 400 spores, 3 independent means of 8 sub-replicates)

As time progressed (8 and 16 h.p.i) the stages of development achieved by *Bgh* also progressed. Numbers of non-germinated conidia decrease at 8 and 16 h.p.i. At 8 h.p.i. significantly more spores achieved secondary germ tube status than primary germ tube status (P < 0.01, n = 3, **Appendix B 9.2.1.4**). By 8 h.p.i. the numbers of conidia undergoing appressorial formation was significantly larger (P < 0.001, n = 3, **Appendix B 9.2.1.5** and **9.2.1.6**) than those that had attained primary and secondary germ tube status. Both observations may be explained by normal developmental stage progression of the fungus. At 16 h.p.i. the PGT to SGT trend observed at 8 h.p.i. is reversed. This may be explained by the delayed germination of physiologically less-fit spores, combined with developmental progression of fitter spores to later stages of development. The average proportion of
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apressoria decreases (although not quite significantly, $P = 0.05467$, $n = 3$, Appendix B 9.2.1.7) to approximately 94 (23.58 % ± 4.78 % s.d., 16 h.p.i.) from 147 (equating to 36.8 % ± 6 % s.d., at 8 h.p.i.) as haustorial formation occurs. At both time points the number of spores accorded to the aberrant “multiple” germ tube class were reduced compared to 4 hr. In summary, the behaviour of *Bgh* isolate DH14 isolate appears to follow expected growth behaviour upon the host.

3.4.3: Germination profile on wheat

![Graph profiling development of Bgh on wheat at 4, 8 and 16 hours post-inoculation.](image)

*Pgt* = Primary (1) germ tube; *Sgt* = Secondary (2) germ tubes; *A* = Appressoria formation (with symptomatic hooking and swelling); *H* = Formation of haustoria; *Mb* = Branched germ tubes; *Ml* = Long germ tubes; *Mm* = More than 2 germ tubes. The final three classes are considered aberrant germination states. Levels of non-germinated spores at 4 h.p.i. = 309 ± 10 s.e.; 8 h.p.i. = 298 ± 7 s.e.; 16 h.p.i. = 279 ± 12 s.e. approximate to the nearest spore. (Error bars = ± standard error, $n = 3$. Graph constructed from average of 400 spores, 3 independent means of 8 sub-replicates).

The profile for development at 4, 8 and 16 hours on wheat is shown in Figure 3-4. At all time points fewer conidia germinated upon this surface in comparison to the natural host barley (for example at 16 h.p.i. $P = < 0.001$, $n = 3$, Appendix B 9.2.1.8). As time increases fewer of the germinated conidia are observed at the early stages of development (i.e. formation of the
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primary germ tube/second germ tube) whilst more attain formation of appressoria. By 16 h.p.i. development the majority of germinated conidia have reached appressorial formation (Figure 3-4 and Figure 3-5). This observed average (103 conidia, 25.9 % ± 4.3 % s.d.) is very similar to those observed on barley (94 conidia, 23.58 % ± 4.78 % s.d.) (No significant difference at α = 0.5, Appendix B 9.2.1.9). This may be due to a decrease in barley of conidia with solely this developmental state as many progress to begin haustorial formation. Only rarely was the formation of haustoria observed on wheat and this was significantly less than that observed for barley (P = < 0.001, n = 3, Appendix B 9.2.1.10).

3.4.4: Germination profile on glass

On glass (Figure 3-5 and Figure 3-6) fungal germination profiles appear fundamentally different from that on barley. At all time points significantly fewer spores germinated compared to those on barley (for example at 4 h.p.i. P < 0.05, n = 3, Appendix B 9.2.2.1) as the average number of non-germinated conidia for barley is approximately 264 (66.16 % ± 6.19 % s.d.) whilst for glass it is 317 spores (79.4 % ± 5.57 % s.d.). Although spores reached secondary germ tube status only one spore formed an appressoria at 4 h.p.i. Notably spores with primary germ tubes were significantly more numerous than on barley at most time points (for example at 8 hours P < 0.001, n = 3, Appendix B 9.2.2.2) where the barley average is approximately 8 (1.91 % ± 0.94 % s.d.) compared to approximately 43 (10.66 % ± 7.28 % s.d.) on glass. 16 h.p.i. (Appendix B 9.2.2.3) is an exception. Conversely, spores with secondary germ tubes and appressoria were found in greater numbers on barley at all time points.

At all time points the majority of germinated spores were of the primary germ tube types (for example at 16 hours, p ≤ 0.001, n = 3, Appendix B 9.2.2.4, when compared to the secondary germ tube form). At 4 and 8 h.p.i. spores representing most developmental stages observed were not significantly different from each other. This was not the case with the 16 hour time point where each recorded class differed significantly with the equivalent class at earlier time points. Fewer spores germinated (P < 0.05, n = 3, Appendix B 9.2.2.5), leading to fewer attaining primary (P < 0.001, n = 3, Appendix B 9.2.2.6) and secondary germ tube status (P < 0.05, n = 3, Appendix B 9.2.2.7). Small numbers of spores were symptomatic of aberrant germination with the formation of multiple germ tubes, or with branched germ tubes. Similarly germ tubes were observed that were longer than expected. It appears glass causes
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lower germination of spores, with greater suspension of growth at earlier developmental stages.

**Figure 3-5: Extent of Bgh Development on different surfaces.**

A) Germ tube emergence from *Bgh* developing on cellulose at 4 h.p.i.  
B) Germ tube emergence on cellulose at 8 h.p.i. with a subsidiary germ tube (black arrow) shown growing above the focal plane. Since no contact will be made with the underlying surface, the elongation of a such germ tube ceases, leading to the outgrowth of other germ tubes.  
C) Emergence of primary germ tubes, and secondary germ tubes with appressorial hooking from spores upon barley at 8 h.p.i.  
D) Emergence of primary germ tube, appressorial germ tube with hooking from a conidium upon wheat at 16 h.p.i. Note the re-attempted penetration generally associated with failure of initial penetration attempt (marked by arrow).  
E) Germination of multiple germ tubes (arrow) from a spore developing upon wheat. In this case one or more of the germ tubes may terminate growth as subsidiary germ tubes.  
F) and  
G) Light microscopy of barley powdery mildew spore upon glass at 8 h.p.i. Germination with branched or long germ tubes. **Scale Bars** = 20 μm approx.
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*Blumeria graminis* f. sp. *hordei* Germination Behaviour on Glass

![Graph profiling development of Bgh on glass at 4, 8 and 16 hours post inoculation.](image)

**Figure 3-6:** A graph profiling development of *Bgh* on glass at 4, 8 and 16 hours post inoculation.

**Pgt** = Primary (1) germ tube; **Sgt** = Secondary (2) germ tubes; **A** = Appressoria formation (with symptomatic hooking and swelling); **H** = Formation of haustoria; **Mb** = Branched germ tubes; **Ml** = Long germ tubes; **Mm** = More than 2 germ tubes. The final three classes are considered aberrant germination states. Levels of non-germinated spores at 4 h.p.i. = 318 ± 13 s.e.; 8 h.p.i. = 332 ± 13 s.e.; 16 h.p.i. = 376 ± 3 s.e. approximate to the nearest spore. (Error bars = ± standard error, n=3. Graph constructed from average of 400 spores, 3 independent means of 8 sub-replicates)

### 3.4.5: Germination profile on cellulose

*Bgh* germination upon cellulose membranes is shown in **Figure 3-5** and **Figure 3-7**. Activity upon this surface appeared similar to that upon glass. Lower germination was encountered than upon barley, although the same at each time point as on glass (e.g. **Appendix B 9.2.3.1 and 9.2.3.2**; expect 16 h.p.i. (**Appendix B 9.2.3.3**). Of those that germinated the majority developed just one primary germ tube, and for most time points (the exception being 16 h.p.i.) although similar to glass (**Appendix B: 9.2.3.4 to 9.2.3.6**) it appeared that significantly more reached this status than on wheat (**Appendix B: 9.2.3.7 to 9.2.3.9**). In comparison to glass, however, appressorial formation was observed at alter time points (although rarely at only 4 spores) although to a significantly lesser degree than on both barley and wheat at all.
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time points. No haustoria were observed. Just as for glass-based development this was expected as penetration of the fungus required for this stage is impossible upon this surface.

![Blumeria graminis f. sp. hordei Germination Behaviour on Cellulose](image)

Figure 3-7: A graph profiling development of *Bgh* on cellulose at 4, 8 and 16 hours post-inoculation.

**Pgt** = Primary (1) germ tube; **Sgt** = Secondary (2) germ tubes; **A** = Appressoria formation (with symptomatic hooking and swelling); **H** = Formation of haustoria; **Mb** = Branched germ tubes; **Ml** = Long germ tubes; **Mm** = More than 2 germ tubes. The final three classes are considered aberrant germination states. Levels of non-germinated spores at 4 h.p.i. = 298 ± 4 s.e.; 8 h.p.i. = 306 ± 10 s.e.; 16 h.p.i. = 289 ± 12 s.e. approximate to the nearest spore. (Error bars = ± standard error, n = 3. Graph constructed from average of 400 spores, 3 independent means of 8 sub-replicates).
3.5: Discussion

To assess the morphological effect on germination of different surfaces, and to form the basis for future work, a basic characterisation of *Bgh* development on different surfaces was made. To summarise, at all time points when on barley, the fungus followed expected developmental behaviour in accordance with known descriptions (Both and Spanu, 2004). A small number of spores with more than two germ tubes were spotted, representing a departure from expected development. These numbers decreased with time. An explanation may be the initial failure by some conidia to recognise the surface as conducive to growth, or the movement of the spore after initial deposition resulting in a failure of PGT/surface contact. In comparison to development on barley, all other surfaces permitted varying stages of development combined with lesser rates of germination.

Development on wheat followed the same pattern as on barley and appeared to match what is known about germination behaviour of *forma specialis* on inappropriate hosts (Olesen et al., 2003). These results suggest most of the signals thought to induce each stage of *Bgh* pre-penetration development are found at similarly optimal concentrations on wheat. Of significance was the heavily reduced formation of haustoria (which in other studies observing the progression of *Bgh* infection on wheat was apparently absent altogether) (Olesen et al., 2003). It is possible that wheat basal defence responses are preventing penetration by many appressoria. Although not actively assessed, papillae formation by wheat was observed during the study. This defence formation is thought by some authors to be the major form of resistance to *forma specialis* infection attempting an infection on a non-host (Olesen et al., 2003). Furthermore, spores that formed appressoria often formed a “node” or enlargement on the side of existing appressoria that is indicative of re-attempted penetration (Figure 3-5). No distinction was made during the study between “lobed”, swollen or “hooked” appressoria although each, may represent different stages within appressorial formation. Therefore, it is possible other factors including reduced appressorial maturation were affecting penetration. As noted by Iwamoto et al., (2002; 2007) the thorough removal of barley cuticle, although allowing AGT and later stages of appressorial germ tube formation, did not permit penetration due to a failure to initiate turgor generation within the appressoria. Iwamoto et al., suggested that a signal derived from the leaf stimulates the final act of pre-penetration *Bgh*
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development. If this is true such a signal may not exist in optimal conditions on the wheat leaf surface and a reduction in frequency of attempted penetration by *Bgh* may result.

Development on barley and wheat, compared to that on glass (where primary germ tubes and secondary germ tubes were present) or cellulose (where, rarely, appressorial germ tubes were present) confirms that signals sensed by the PGT are not the same as those sensed by the AGT/APP (Zhang et al., 2005). What these are remain to be fully elucidated, although the inevitable lack on cellulose or glass of the C_{26} aldehydes Tsuba et al., (2002) noted as being a highly inductive component of the barley cuticle suggest at least one critical signal missing for successful development.

On glass the majority of germlings appeared to stop at the PGT stage of germination. A second germ tube may not necessarily indicate a higher stage of development. Many may have been subsidiary germ tubes that failed to contact the surface (Wright et al., 2000; Wright et al., 2002). Later hydrophobicity experiments (Chapter 4) suggested the glass slides as used in this original characterisation were coated with an unknown substance and highly hydrophilic. Nicholson et al., (1993) suggest a concomitant drop between surface and spore hydrophobicity is necessary to permit normal development. Carver et al., (1999) note how increased targeting of the PGT occurs on more hydrophobic surfaces. Additionally Zabka et al., (2008) have recently shown on glass slides treated with varying amounts of aldehyde that greater development is shown on those slides whose treatment generated increased hydrophobicity. Consequently, it may be inferred that an unknown substance on these non-pre-cleaned slides either i) acted as a direct inhibitor of later stages of germination, and/or ii) resulted in a drop of hydrophobicity that led to reduced ECM release and a negative impact on development. Indeed, later studies within this investigation and others suggest it is possible to observe significant appressorial formation on glass when cleaned (Francis et al., 1996; Zabka, 2008). Therefore cutin monomers etc, although being sufficient for germination, are not absolutely necessary for germination on glass as other factors, potentially physical in nature, such as hydrophobicity, may also influence germination to an extent. Unfortunately, the effect of this unknown substance on previous work determining expression levels of CMEGs upon glass (by Dr. Maike Paramor) is unknown and offers potential complications for future work.
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A truer indicator of development is the formation of an appressorial germ tube or appressorium, as demonstrated on cellulose. This is interesting as past work has shown a greater release of ECM on cellulose (with a contact angle of 27˚) to glass (contact angle of 38˚) (Wright et al., 2002). This formation may result from the presence of cellulose breakdown products under the action of ECM-contained cellulases (Wright et al., 2002). Interestingly, more conidia on cellulose formed “aberrant germination states” in comparison to the other surfaces. This may be an indicator of the surface being an imperfect inducer on some level, for example hydrophobicity, microporocity, or because of potential over-stimulation by cellulose breakdown products. Consequently, although permitting later germination stages it is most likely signals required for early germination are incomplete. For example while the PGT was induced, a lower efficiency of targeting of the PGT to the surface may occur in comparison to that seen on the host (Wright et al., 2000). This would result in multiple “subsidiary germ tubes” (MM). Failure to stop the elongation of the PGT would explain the presence of elongated germ tubes (ML). Yamaoka et al., (2006) suggest elongation termination is necessary to allow AGT formation, and showed that by removing the coleoptile surface PGT length increased and AGT emergence was delayed. Again this suggests host factors are required and their lack may also explain the formation of branched germ tubes (Mb).

In this chapter the differential response of *Bgh* to diverse surfaces has been characterised. It is clear that *Bgh* shows distinct developmental behaviour on the host, with truncated development on surfaces other than the host. In the next chapter this work is progressed to discern the germination behaviour of this mildew in response to selected stimuli. Attempts to relate the signals to effects on gene expression are discussed therein.
Chapter 4: Germination on Modified Surfaces

4.1: Introduction
Germination of a fungal spore may be viewed as the most critical step in pathogenesis (Seong et al., 2008). *Bgh* is an ascomycete pathogen that invades its host via an appressorium. The surface recognition process required to reach this level of development is a complex process requiring multiple signals in the form of both physical properties, such as hydrophobicity, and cuticle derived compounds such as cutin monomers and cellulose (Hall et al., 1999).

4.1.5: The plant cuticle
Before reaching the nutrients of a plant, a pathogen must overcome the outer layers of the host, the first of which is the cuticle (Walters, 2006) This covering has multiple roles for the plant including preventing water loss, and defence (Liu, 2006; Koch et al., 2008).

In contrast to the plant cell wall (containing cellulose, hemicelluloses and lignin) the plant cuticle is made of cutin (a polymer containing fatty acid derivatives) and is coated with waxes (Wisniewska et al., 2003; Jetter et al., 2006; Chassot et al., 2007). Although a direct barrier to assault, pathogens have evolved to recognise signals derived from the cuticle to spur certain stages of infection (Chassot et al., 2007). The components of the cuticle are synthesised by the underlying epidermal cells and based on their properties the cuticle can be defined as hydrophobic (Heredia, 2003; Bargel et al., 2006).

Polysaccharides and pectins link this extraneous coverage to the plant cell wall below (Wisniewska et al., 2003; Bargel et al., 2006). Although variable in structure, depending on the species involved, the cuticle can be generalised into a structure of 2 layers; the ‘cuticle proper’ (on top of which lie the ‘epicuticular waxes’) and the cuticle layer (Bargel et al., 2006) (Figure 4-1). It is important to note however that to a great extent the structure of the cuticle is unknown (Koch and Ensikat, 2008).
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Figure 4-1: Simplified cross section of the plant cuticle.

Theorised position of constituents shown. The ‘cuticle proper’ is believed to be made from alternating layers of wax and cutin. The cuticle layer thickness and exact composition depends on the species being considered. Pectin is not labelled as its evidence as a discrete layer only exists for some species (Koch and Ensikat, 2008). Cutan is a second bio-macromolecule, composed of ether-linked long chain alkyl moieties. Present in fossilised cuticles it is thought to be present in some extant species (Bargel et al., 2006). Modified from: Bargel et al., (2006).

Cutin is the main component of the cuticle (forming 40 to 80 % of the total structure) (Heredia, 2003). Cutin itself is a bio-polyester of C_{16} and C_{18} esterified fatty acids, hydroxylated and epoxyhydroxylated with the ratio between the two being species specific (Heredia, 2003; Bargel et al., 2006). Major examples of C_{16} cutins include 9- or 10,16-dihydroxyhexadecanoic acid and 16-hydroxyhexadecanoic acid (Heredia, 2003). An example of the C_{18} monomer family would be 9,10,18-trihydroxy octadecanoic acid. The cutin monomers are often cross-linked by ester bonds, which can be broken by esterases (cutinases) (Muller and Riederer, 2005). Depending on the location of the surface in question other constituents may be present including dicarboxylic acids and glycerol compounds (Bargel et al., 2006). Work by Francis et al., (1996) has demonstrated the potential for the induction of *Bgh* germ tubes by cutin monomers. Two synthetic cutin monomers (cis-9,10-epoxy-18-hydroxy-stearic acid and 8,16-dihydroxy-palmitic acid, equivalent to those present in the barley leaf) were added to glass slides and on doing so caused an increase in the percentage of AGT formation by *Bgh* developing on these slides compared to the control.
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Present in the cuticle (as ‘intracuticular wax’) aliphatic or aromatic lipids are most visible in their epicuticular forms when present as complex 3-D structures (Bargel et al., 2006). These epicuticular waxes are the interface between the plant and the wider environment (Koch et al., 2006). In general waxes are a complex mixture of long chain aliphatics, such as alkanes, alkenes, esters of fatty acids and primary alcohols, free fatty acids, alcohols, aldehydes, ketones and cyclic compound such as terpenoids and phenolic compounds (Heredia, 2003; Wisniewska et al., 2003). These complex molecules can have varying lengths of hydrocarbon backbone (between 20 to 40 carbon atoms approximately) (Muller and Riederer, 2005). For many plants, the composition of the epicuticular waxes can differ from the intracuticular and as with cutin the exact make-up of the waxes is species specific (Jetter et al., 2000; Bargel et al., 2006). As noted in section 1.2.5 Tsuba et al., (2002) confirmed the involvement of wax layer components in the emergence of both the Bgh AGT and the development of the APP. In their investigations the authors observed the percentage of emergence/formation of Bgh appressorial structures developing on polystyrene petri dishes coated with different fractions of barley wax. The results of these tests suggested the aldehyde fraction in particular was extremely inductive for both AGT emergence and APP formation. Additionally, trialling of aldehydes of different chain length suggested those with a backbone of 26 carbons induced the highest proportion of AGT and APP (Tsuba et al., 2002).

Epicuticular waxes often have a major component or class and may form structures which protrude from the epicuticular layer itself (‘epicuticular wax crystals’) (Jetter et al., 2000; Bargel et al., 2006). These wax crystals may have various shapes (determined by their constituents) which may include platelets, ribbons and tubules (Barthlott et al., 1998; Gniwotta et al., 2005). These crystals are also believed to have the ability to self assemble (Koch and Ensikat, 2008). In the case of Hordeum vulgare, in a study by Baum et al., (1989) the authors found in barley grown to anthesis, that epicuticular wax crystals were present as filaments. Additionally they note the waxes presented an average alcohol content of 40 to 60 % (the authors monitored for hexacosanol) and a hydroxyl-ß-diketone (in this case 25-hydroxyhentriacontane-14, 16-dione) was present. They also note that other studies by Mikkelsen (1979) which found other ß-diketones (such as triacontan-16,18-dione; nanacosan-12,14-dione and nanacosan-14,16-dione). Furthermore they note that in eceriferum mutants waxes may be present as plates rather than filaments (Rubiales et al., 2001). It should be noted however that Baum et al., (1989) did not differentiate between internal and external waxes (and it must also be remembered that wax content can alter with the age of the plant).
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4.1.6: Hydrophobicity

One of the primary roles of the cuticle is that of water repellence. This makes the surface of the plant harder to colonise by microbes due to a reduction in the leeching of plant nutrients and also because free water is minimised (Bargel et al., 2006). The hydrophobicity of a surface is assessed by the contact angle that a water droplet makes with the surface in question (Figure 4-2) (Lee et al., 2006). For example the more hydrophobic a surface, the more the droplet assumes a spherical shape (resulting in a higher contact angle) and the lower the area of contact between surface and droplet. For detailed descriptions regarding the thermodynamics involved and equations used to model the interactions between the liquid, surface and surrounding vapour the reader is referred to Koch et al., (2008). Suffice to say the wettability of a surface depends on the equilibrium of energy required to maximise the liquid surface area and the energy gained by adsorping to the surface below.

In general surfaces with a contact angle (Figure 4-2) of less than 90° are called ‘wettable’ (or ‘hydrophilic’) and those above 90° are considered ‘non-wettable’ (or ‘hydrophobic’) (Bargel et al., 2006). Koch et al., (2008) go further, defining superhydrophilic surfaces as one which presented a contact angle as less than 10°. In contrast an especially hydrophobic surface, with a contact angle in excess of 150°, can be classified as ‘superhydrophobic’ (Lee et al., 2006; Koch et al., 2008).

As a rule, the hydrophobicity of a surface depends not just on its chemical properties but its geometrical properties (Lee et al., 2006). Rough plant surfaces can give larger contact angles than would be true of a surface of similar hydrophobicity, but smoother nature (Wisniewska et al., 2003). The roughness of a plant surface can be decided by the presence of features such as cuticular folding, the presence/structure of epicuticular waxes and any plant microstructures (e.g. trichomes) (Bargel et al., 2006). Two major theories exist in regards to surface roughness increasing the contact angle. The first revolves around rough surfaces presenting an increased surface area to a droplet. If complete wettability is assumed this greater surface area will lead to greater repellence (or attraction) compared to a comparable flat surface (Wenzel, 1936). The second theory regards the possibility of an increase in contact angle due to the water “sitting” on trapped air (Cassie, 1944).
Investigations have also suggested a role for hydrophobicity in *Bgh* development. Carver et al., (1999) observed how surfaces which were treated to increase hydrophobicity showed a greater level of ECM release in comparison to control surfaces. Additionally, more recent studies by Zabka et al., (2008) demonstrated an inductive effect of artificial surfaces that were free of plant wax components but were significantly hydrophobic.

### 4.2: Aims and objectives

As a consequence of earlier work which demonstrated the effects on *Bgh* isolate DH14 growth of different germination surfaces, efforts were made to define the nature of the stimuli received by the fungus during early development. Previous work by Dr. Maike Paramor has shown the existence of genes with distinct expression patterns during germination on the host and other surfaces, especially on glass. The effect of these stimuli on the expression of these genes during *Bgh* isolate DH14 development on glass and other surfaces at 4 h.p.i. was investigated in the belief that such information would elucidate role of the stimuli, the gene and its clusters role in development, and potentially their role in *Bgh* pre-penetration
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pathogenicity. As a result the position of *Bgh* as a model organism for powdery mildews would be consolidated and the identification of targets for new fungicides would be permitted.

After a general screen of a number of barley cuticle components (Figure 4-3), glass slides were enhanced with the barley cutin monomer 16-hydroxyhexadecanoic acid, 1-hexacosanal (a synthetic C\textsubscript{26} aldehyde similar in properties to a C\textsubscript{26} aldehyde present within the epicuticular wax fraction of the barley leaf surface (Tsuba et al., 2002) and also a rough cuticle extraction derived from barley leaves. These additions were believed to emulate signals encountered by *Bgh* on the barley leaf surface and would hopefully cause development on the glass slides to resemble that found on the host. Several small development characterisation studies were carried out followed by the employment of quantitative real time PCR to determine whether these differential gene expression patterns could be altered to match those encountered during germination on barley. Additionally, analysis of surface hydrophobicity on enhanced glass slides, abaxial and adaxial barley and wheat leaf surfaces, hydrophilic and hydrophobic gelbond (a polyester film used for supporting polyacrylamide gels) was carried out to define the exact nature of the signal affecting CMEG expression.

![Compounds applied to glass slides.](image)

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4.3: Materials and methods

4.3.1: Stimuli screen

*(performed by Oliver Cole under supervision, methods are quoted from undergraduate report)*

*Ether extract of cuticular components*

The primary and secondary leaves of 300, 14-day-old uninfected barley plants were incubated for 1 minute in anhydrous diethyl ether (Iwamoto et al., 2002). The final solution (200 ml) formed two phases and the lower aqueous phase was discarded.

*Synthesis of 1-hexacosanal*

(Performed by Cassim Ashraff, Department of Chemistry, Imperial College London)

After attempts to synthesise hexacosanal using the method of Tsuba et al., (2002) proved unsuccessful the method of Corey and Suggs (1975) was used. To summarise, 1-hexacosanol (Sigma Aldrich) was oxidised by pyridinium chlorochromate (Sigma Aldrich) with acetone in dichloromethane (dried over calcium chloride) as a solvent. The reaction was stirred continuously for 3 hours under an argon atmosphere. The hexacosanal product was purified using column chromatography, with the identity and purity (recorded as 95 %) assessed by both thin layer chromatography and NMR.

*Treatment of glass slides*

Glass multi-well slides (containing 6 x 0.385 cm² wells) were treated with 50 μl of stimuli solution (cuticular extract, 1-dodecanol, 16-hydroxyhexadecanoic acid, hexacosanoic acid, 1-hexacosanal, 1-hexacosanol), applied by positive displacement pipette to three wells on each slide. The solvent was allowed to evaporate, before more solution was applied as required. Cuticular extract equivalent to 1.5 leaves per cm² containing cuticular components (equivalent to about 1.5 leaves cm²), 16-hydroxyhexadecanoic acid and 1-dodecanol (equivalent to 0.385 μg cm² corresponding to work performed by Francis et al., 1996) and 0.5 μg cm² of C26 compounds (hexacosanoic acid, 1-hexacosanal, 1-hexacosanol, corresponding with findings of Tsuba et al., 2002) were applied to each well. 1-dodecanol and 16-hydroxyhexadecanoic acid were dissolved in diethyl ether, while C26 compounds (1-hexacosanol, 1-hexacosanal, hexacosanoic acid) were dissolved in dichloromethane.
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Inoculation of treated slides
Plastic trays (272 mm x 272 mm) with lids were lined with moistened paper towelling to maintain humidity. Treated glass slides were placed directly onto the moistened paper towelling. Mature *Hordeum vulgare* cv. Golden Promise with widespread infection, having been shaken 24 hours before, was then shaken over the treated slides to ensure inoculation with fresh conidia. Samples were then incubated in light (56 µmol m$^{-2}$ s$^{-1}$) at 20 ºC and 30 % humidity in a Sanyo Fitotron growth room for 24 hours before visual assessment for growth stages.

Assessment of developmental stage
24 hours after inoculation, 8 slides per treatment had 150 conidia randomly assessed (50 per well, 3 wells in total) according to the classifications in **Table 2**, using a Leitz Diaplan microscope at 400x magnification. Glass slides were observed directly (without a cover slip).

4.3.2: Cleaning of glass slides
Glass Slides (Super Premium Microscope Slides, VWR) were left for approximately 10 min in a mixture of Chromic and Sulphuric Acid (VWR International). After thorough washing in distilled water the slides were then air-dried.

4.3.3: Aldehyde, cutin monomer and cuticular extract application to glass slides
The primary leaf of 7-day-old uninfected barley plants were incubated for 2 x 5 minute intervals in anhydrous diethyl ether (Iwamoto et al., 2002). The 16-hydroxyhexadecanoic acid was dissolved with diethyl ether (BDH) to produce a final concentration of 1 mg/ml. 3 ml of the mixture or of diethyl ether were then applied to clean glass slides using Microman® Positive Displacement Pipettes (Gilson), resulting in a 0.015 mg/cm$^2$. Similar procedure was followed for the coating of slides with aldehyde (to a final concentration of 0.5 µg/cm$^2$) and cuticular extract (to a final concentration of 1 cm leaf surface/cm$^2$). The mixture was spread using a moulded glass pipette. Slides were then freeze dried for 15 min or longer. Slides were left for 1 day before use.
## Germination on Modified Surfaces

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<tbody>
<tr>
<td>Ng:</td>
<td>Non-germinated</td>
</tr>
<tr>
<td>PGT:</td>
<td>Emergence of one germ tube</td>
</tr>
<tr>
<td>PGT/SGT:</td>
<td>Emergence of 2 germ tubes</td>
</tr>
<tr>
<td>ESGT/App:</td>
<td>Emergence of both primary and secondary germ tubes with the latter assuming some characteristics of a fully formed appressorial germ tube.</td>
</tr>
<tr>
<td>PGT/SGT/A:</td>
<td>Emergence of both primary and secondary germ tubes with the latter assuming hooking characteristic of the appressorial stage of development.</td>
</tr>
<tr>
<td>PGT/SGT/A/H:</td>
<td>All germ tubes are present and appressorial formation has led to penetration and subsequent haustoria formation.</td>
</tr>
<tr>
<td>Mm (Miscellaneous: multiple)</td>
<td>More than 2 germ tubes</td>
</tr>
</tbody>
</table>

Table 2: Spore germination categories used in the ‘Stimuli Screen’. Spore status for pre-penetration and early post-penetration events was assessed. One miscellaneous category, more than 2 germ tubes, showing abnormal development was classified.
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4.3.4: Cutin treatment studies

Studies were conducted to discern the effect of cutin monomer (16-hydroxyhexadecanoic acid, Fluka Ltd.) addition to glass slides on germling development. In the first non-chromic acid cleaned glass slides were treated with diethyl ether, diethyl ether/cutin monomer, or left untreated. 10 slides in each of 4 trays had development categorised for 80 spores per slide after 16 h.p.i. In the second test all slides were pre-cleaned with chromic acid and then similarly treated. 3 slides (in each of 9 trays) were assessed for development of 100 spores after 16 h.p.i. Germlings were graded according to classifications as listed in Chapter 3, Table 1.

4.3.5: Developmental behaviour at 4 and 16 h.p.i. on host, treated and artificial surfaces

In both studies 3 batches of 3 independent replicates (either humidity regulated trays in the case of modified slides or gelbond surfaces, or pots in the case of plant surfaces) had 30 spores assessed for each of 3 slides/gelbond segments or 3 leaves contained therein. Surfaces assessed include 7-day-old primary barley and wheat leaves, slides left un-cleaned, slides pre-cleaned with chromic acid, slides treated with cutin monomer, aldehyde, and cuticular extract, segments of hydrophobic and hydrophilic gelbond. Fresh Bgh spores (infected plants were blown 12 hours previously) were sprinkled to an approximate density of 150 spores per cm². Single, intact spores were assessed according to the classification scheme first mentioned in Chapter 3, Table 1.

4.3.6: Analysis of surface hydrophobicity

Droplet diameter was measured using a method modified from Hill et al., (1980). Surfaces to be tested were placed flat on a level table as verified by a spirit measure. 20 μl of 1 % aqueous crystal violet solution was dropped onto the surface from a height of 10 mm. The droplet was then photographed in profile. 5 droplets each were applied to each of 5 surface replicates. Surfaces tested via this method include the hydrophilic and hydrophobic sides of GelBond® (Lonza Rockland inc.), the adaxial and abaxial side of barley leaves, slides left untreated, slides cleaned with chromic acid, slides treated with 0.015 mg/cm² 16-hydroxyhexadecanoic acid and slides treated with ether solvent. Advancing contact angles were measured on a Kruss ‘Easy Drop’ goniometer with ‘teli ccd’ camera at 20 °C and 33.5 % humidity. 10 μl drops of sterile double-distilled water were applied to surface. 5 drops per replicate to each of 5 replicates per surface were assessed. Statistical analysis was performed
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utilising 1-Factor ANOVA. Homogeneity of variances was assessed utilising the Levene statistic and where homogeneity was not assured a Games-Howell post-hoc analysis was used. Where homogeneity was assured a post-hoc Tukey Test was employed. As a further check a Welch Test was performed to support the conclusions of the Games-Howell test. All analysis was performed utilising ‘PASW statistics 18, release 18.0.0 (Jul 30, 2009) SPSS inc.’

4.3.7: Statistical Analysis

Spore counts were analysed using ‘R: A language and environment for statistical computing version 2.5.0’ (http://www.R-project.org). Count data were transformed by square root and modelled using one-way ANOVA, with inbuilt error terms to compensate for spatial pseudo-replication. Percentages were calculated via the equation [(average number of spores) / (total number of spores)]*100. The ‘average number’ represents the average number of spores per replicate at the ‘pot or tray’ level. ‘Total number of spores’ represents the average number of spores counted for all germination categories. Standard deviation was calculated according to the following equation:

\[ \sqrt{\frac{\sum (x-\bar{x})^2}{n-1}} \]

Where x is the sample mean average and n is the sample size. Standard error was calculated by dividing the standard deviation by the square root of the number of replicates.

4.3.8: Photography and droplet analysis

Images were viewed using Axiovision (version 3.1.21) software for PC with ‘Carl Zeiss AxioCam-HR’, (version 5.05.10) and ‘TWAIN’, (version 1.0) add-ons. Light photography was performed with a Canon ‘Powershot Pro1’ and also a Nikon D-100 with 55 mm Nikkor Macro Lens.

Droplet diameter analysis was performed with ‘TINA, version 2.10g’ (Raytek Scientific Ltd. (Raytest GmbH). Advancing contact angles of droplets were assessed using ‘Drop Shape Analysis [DSA]’ version 1.80.1.2 [HS] for Windows 9/NT/2000 (Kruss© 1997-2002). The profile of the sessile drop in the region of the baseline is fitted to the rational function (y = a + bx + cx^{0.5} + d/lnx + e/x^{2}). This allows the advancing contact angle to be determined.
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4.3.9: Isolation of fungal material
7 to 10-day-old barley leaves, 7 to 10-day-old wheat leaves, non-cleaned glass slides, glass slides cleaned with chromic acid, cleaned slides treated with 0.015 mg/cm² 16-hydroxyhexadecanoic acid, cleaned slides treated with 0.5 µg/cm² 1-hexacosanal, slides treated with barley cuticular extract equivalent to 1 cm² leaf surface/cm², hydrophobic and hydrophilic side of gelbond were sprinkled with Bgh spores at a density of 100 to 150 spores/cm². After 4 h.p.i. the surfaces were dipped in 5 % cellulose acetate in acetone. After drying the cellulose acetate was stripped off and stored at -80 °C until RNA extraction.

4.3.10: cDNA synthesis using superscript™ III reverse transcriptase
500 ng of RNA was used as a template for cDNA production. This procedure followed the protocol of the manufacturer (Invitrogen™). Briefly, contained within a 13 µl total volume were: 1 µl 10 MM dNTP Mix (10 MM each dATP, dCTP, dGTP and dTTP), 500 ng RNA template (within DEPC water) and 250 ng Random primers. This mixture was heated for 5 min at 65°C and then incubated at on ice for at least 1 minute. To the mix was added: 4 µl 5X First-Strand Buffer, 1 µl 0.1M DTT, 1 µl RNaseOUT™ Recombinant RNAse Inhibitor, 1 µl of SuperScript™ III Reverse Transcriptase (200 units/ µl). After mixing the solution was then incubated at 25 °C for 5 min. After this, the solution was incubated at 50 °C for 60 min followed by heat inactivation at 70 °C for 15 min. The cDNA was then diluted 1 in 15 in TE buffer (10 mM Tris, pH 7.5; 1 mM EDTA) to act as template for qPCR analysis. All incubation steps utilised the PTC-200™ Peltier thermocycler (MJ Research Inc.).

4.3.11: RT-qPCR Analysis
'Using the analysis program ‘Expressionist™ Analyst Pro 2.0.36 (Genedata, Basel, Switzerland)’ expression profiles of genes gathered during the original microarray analysis were filtered by gene variance and valid value proportions to find genes that had the most constant expression values across all conditions (i.e. all 4 surfaces used in the original study). These genes were candidates for control genes and the NADH ubiquinone oxioreductase was selected. Welch Tests were employed to analyse the gene responses at 4 h.p.i. on barley and glass. Genes with the statistically most differential expression between both conditions were selected. PCR was then used to select genes with primer sets that would successfully amplify.'
### Table 3: Primers used for differential gene expression analysis at 4 h.p.i.

<table>
<thead>
<tr>
<th>EST Clone Library Identifier</th>
<th>CMEG Cluster Leader</th>
<th>Putative CMEG Function</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>D00972</td>
<td>N/A</td>
<td>Unknown</td>
<td>GTG GAG GCC ATC TTT TTA CAA A</td>
<td>CAG GCC AAC GGT CTA TCA TGC</td>
</tr>
<tr>
<td>D00658</td>
<td>C00998</td>
<td>Glycosyltransferase</td>
<td>GTC ACC GGA CGG AAC ACA GA</td>
<td>CCG CGA TGA ATT TTT GAT GA</td>
</tr>
<tr>
<td>C00009</td>
<td>N/A</td>
<td>MAPK Interacting Protein</td>
<td>TCC GGC CAA ACT GGA AAC TC</td>
<td>CTC ATT CCG GAT GGC ACT GG</td>
</tr>
<tr>
<td>D00881</td>
<td>Cluster Leader</td>
<td>α-1,6-mannosyltransferase</td>
<td>GCC TCA GAA GCC CGG AAT TT</td>
<td>GCC CTC CCG ACT CCA TAA C</td>
</tr>
<tr>
<td>D00944</td>
<td>N/A</td>
<td>Unknown</td>
<td>GCC GAG AGG CAG ACC CAT TG</td>
<td>TCG CAT CGA AAT GCT CGA AA</td>
</tr>
<tr>
<td>PS11B04</td>
<td>C01417; D00154</td>
<td>calcium+/hydrogen+ exchanger</td>
<td>ATG GCC AAT CGT CTT GGG A</td>
<td>CCA TGG TCT CAA ATG TTT CAA AGT G</td>
</tr>
<tr>
<td>C00482</td>
<td>N/A</td>
<td>1,3-β-glucanase</td>
<td>CGC CAA CGA GGC GGA ACT AT</td>
<td>CCA GGT CAT GGA ACC CTT T</td>
</tr>
<tr>
<td>C01244</td>
<td>C00206; D00154</td>
<td>Transporter (related to PHO87)</td>
<td>CAT CGT TCC GTA GCC AAC AGT G</td>
<td>ATG GGT TGC GCT CTC ACC TG</td>
</tr>
<tr>
<td>C01417</td>
<td>Cluster Leader</td>
<td>Unknown</td>
<td>GCC CAA CGA GGC GGA ACT AT</td>
<td>CCG GGC TCG GAA TAA CAC TG</td>
</tr>
<tr>
<td>C01157</td>
<td>C01417; D00154</td>
<td>protein disulphide isomiserase</td>
<td>TGC CGA CAA GGT TGG GGT TTG</td>
<td>GCT GGA GCA GTC ACC CTT C</td>
</tr>
<tr>
<td>C00148</td>
<td>N/A</td>
<td>1,3-β-glucanase</td>
<td>TGC CAT CCC GTC ACA GAC AA</td>
<td>GCC CTT GGA TTC GGG AAA AG</td>
</tr>
<tr>
<td>D00189</td>
<td>Cluster Leader</td>
<td>Unknown</td>
<td>GGA CAT CAC CGG CAC CTG TT</td>
<td>TGA CAC GGG TTC TGG GAT CA</td>
</tr>
<tr>
<td>D00471</td>
<td>D00933</td>
<td>Predicted protein</td>
<td>GAA CCG CTT CCG GAA CAC AC</td>
<td>AGA ATC ACG CGT CGC AC</td>
</tr>
<tr>
<td>C00506</td>
<td>C00988</td>
<td>Mitochondrial glycerol-3-phosphate</td>
<td>CTT CGC AGG TCA GCC GAG ACC A</td>
<td>ACC CGG TAG CAG CG GAA GAG</td>
</tr>
<tr>
<td>C00563</td>
<td>C00206; D00154</td>
<td>Histone H3</td>
<td>CCG GTC CTCCAA ACG TAT CC</td>
<td>AAA TCC GAC TTG CGC TGC CA</td>
</tr>
<tr>
<td>C00606</td>
<td>Cluster leader</td>
<td>Unknown</td>
<td>CGA CGT CAC CGC GAT CAA TA</td>
<td>CGC TGT AGA TGC TCC GTG CGA A</td>
</tr>
<tr>
<td>D01260</td>
<td>N/A</td>
<td>Adenosylhomocysteinase</td>
<td>GCC TTG ACG TGA GCC AAG AG</td>
<td>CCG GGC CAT TCT TCT GCC</td>
</tr>
<tr>
<td>Control</td>
<td>N/A</td>
<td>NADH-ubiquinone oxidoreductase</td>
<td>GCC CTG CCC AGG CTA TTA CC</td>
<td>CTC GTC GTT CCG TGA CTCA</td>
</tr>
</tbody>
</table>

Genes assessed during development at 4 h.p.i. on different surfaces are listed according to their EST Clone Library Identifier, the name of the EST used during the original microarray analysis, hosted on the Phytopathogenic Fungi and Oomycete EST Database by COGEME (Consortium for the Functional Genomics of Microbial Eukaryotes at [http://cogeme.ex.ac.uk/](http://cogeme.ex.ac.uk/)). Where applicable their relation to CMEG clusters (genes with similar expression profiles during development on the host and other surfaces at 4, 8, 16 h.p.i.) is shown by listing the EST providing the most representative expression profile of the cluster. Where a gene has a profile upon which other genes have their profile categorised/clustered it is labelled a ‘cluster leader’. In certain cases the expression profile is not associated with a cluster (‘N/A’). Expression profiles are shown in Figure 4-13 to 4-30. ‘Putative Function’ is based on information contained with ‘The Phytopathogenic Fungi and Oomycete EST Database’ by COGEME.

Since no attempts were to be made to compare the Relative Expression Index (REI, i.e. the ratio of test gene and reference gene expression) of one test gene with that of another, primer binding efficiencies were not determined. Instead primer efficiencies of both the test and
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Reference genes were assumed to be 1, i.e. that binding was 100% efficient. Primers for both test and reference genes were supplied in excess and experiments were conducted such that the same primer ‘master mix’ was used in all reactions for all surfaces tested. Similarly all reactions for a test gene (across all 8 surfaces) were conducted within the same RT-qPCR run. As a consequence any inefficiency in primer binding for either the test gene, or the control/reference gene, would remain constant across the surfaces tested. In turn this would mean any alterations in expression of the test gene (due to the different surfaces tested) would still be detected and any conclusions based on these alterations would be valid.

RT-qPCR analysis followed the protocols of the manufacturer (Invitrogen™) of the Platinum® Quantitative PCR SuperMix-UDG with ROX dye. All reactions were performed upon an ABI systems 7500 real time PCR system. 20 μl reactions were carried out. The mix was as follows: 10 μl Platinum® Quantitative PCR SuperMix-UDG with ROX. Forward Primer (10 μM) 0.4 μl, Reverse Primer (10 μM) 0.4 μl, ROX fluorogenic probe (0.04 μl) and Sterile HPLC grade water (BDH) to total volume inclusive of 4 μl cDNA template. All analyses were carried out using the Applied Bio-systems Fast System SDS software (Version 1.4) and Microsoft Excel®. All primers were manufactured by Sigma®-Genosys Ltd. Primers used are shown in Table 3.

The PCR amplification conditions were: 20 s at 95 °C, then 40 repetitions of 3 s at 95 °C and 30 s at 60 °C. This was followed by a dissociation cycle: 95 °C for 15 s, 60 °C for 20 s, 95 °C for 1.5 s and 60 °C for 15 s. Following amplification threshold detection parameters (cycle threshold limit) were adjusted manually to optimise results. Data was logged (base 2) and quantities calculated by \(2^{-(Ct-20)}\). Ratios were calculated by Quantity (Target)/Quantity (Reference). Statistical analysis was performed utilising 1-Factor ANOVA. Homogeneity of variances was assessed utilising the Levene statistic and where homogeneity was not assured a Games-Howell post-hoc analysis was used. Where homogeneity was assured a post-hoc Tukey Test was employed. As a further check a Welch Test was performed to support the conclusions of the Games-Howell test. All analysis was performed utilising ‘PASW statistics 18, release 18.0.0 (Jul 30, 2009) SPSS inc.’
4.3.12: Bioinformatic Analysis

Performed with the aid of Dr. Tim Burgis (Imperial Colelge London). Genes that displayed significant alterations in expression on exposure to stimuli were selected for further bioinformatic analysis. Unisequences (Appendix C: 9.7.1-9.7.18) were derived from microarray data for Bgh hosted by the COGEME phytopathogenic Fungi and Oomycete EST database (http://cogeme.ex.ac.uk/). Unisequences are available by following links for the relevant EST Library Clone Identifier. Unisequences were then blasted (utilising the ‘BlastN’ function) against the Bgh genome (June 2007 assembly, using the ‘Genome v3: Contigs’ setting) on the BluGen website (https://www.blugen.org/). This identified the gene most similar to the unisequence which could then be viewed using the ‘Gbrowse’ (Generic Genomic Browser) function available on the BluGen website. Using the ‘Apollo’ Genome Annotation Curation Tool function the full nucleotide and protein sequences could be then accessed via the annotated gene model (if one existed). Nucleotide sequences were re-blasted using BlastN against the genome to ascertain gene copy number and against the nucleotide collection (nr/nt) (hosted on NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm function. Predicted protein sequences were aligned to indicate similarity using the ClustalW2 alignment software (http://align.genome.jp/). Automatic site settings were used alongside the ‘slow alignment’ setting. EST and protein sequences were analysed using ‘ORF Predictor’ software (http://proteomics.ysu.edu/tools/OrfPredictor.html), and ‘InterProScan’ software (http://www.ebi.ac.uk/Tools/pfa/iprscan/). Protein weights were calculated using software hosted on http://www.sciencegateway.org/tools/proteinmw.htm. Protein sequences were blasted against the ‘non-redundant protein database’ (NCBI) utilising the ‘BlastP’ function to ascertain species distribution. Neighbour joining trees were drawn utilising an in-built function of http://align.genome.jp/.
### 4.4: Results

For the benefit of the reader the following results are linked to their relevant protocols and classifications used to categorise spore germination in the following table:

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Materials and Methods Section</th>
<th>Spore Germination Classification</th>
<th>Result Figures</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Stimuli Screen’</td>
<td>4.3.1</td>
<td>Table 2 (page 79)</td>
<td>Figure 4-4</td>
</tr>
<tr>
<td>‘Characterisation of <em>Blumeria graminis</em> f. sp. hordei upon cutin-monomer treated glass slides’</td>
<td>4.3.2 - 4..3.4</td>
<td>Table 1 (Page 58)</td>
<td>Figure 4-6; Figure 4-7</td>
</tr>
<tr>
<td>‘Developmental behaviour at 4 h.p.i. on the host and alternate surfaces’</td>
<td>4.3.3; 4.3.5</td>
<td>Table 1 (Page 58)</td>
<td>Figure 4-8</td>
</tr>
<tr>
<td>‘Developmental behaviour at 16 h.p.i. on treated and artificial surfaces’</td>
<td>4.3.3; 4.3.5</td>
<td>Table 1 (Page 58)</td>
<td>Figure 4-9</td>
</tr>
<tr>
<td>‘Analysis of surface hydrophobicity’</td>
<td>4.3.6; 4.3.7</td>
<td>N/A</td>
<td>Figure 4-12</td>
</tr>
<tr>
<td>‘Effect of stimuli on genes differentially expressed at 4 h.p.i.’</td>
<td>4.3.8-4.3.10</td>
<td>N/A</td>
<td>Figure 4-13 to Figure 4-30</td>
</tr>
</tbody>
</table>

*Table 4: Experimental studies featured within Chapter 4.*

Relevant materials and methods sections pertaining to each experiment are listed, as is the relevant spore categorisation scheme if applicable, and also figures summarising results.

#### 4.4.1: Stimuli screen

As a prelude to later work involving the assessment of gene expression after addition of stimuli, a small study focusing on the germination profile of *Bgh* on treated glass slides was performed by Oliver Cole, a student under the guidance of the author. Treatments included barley leaf cuticle extract, an aliphatic alcohol of chain length 12 (1-dodecanol), a cutin monomer (16-hydroxyhexadecanoic acid, a C\textsubscript{16} compound) and C\textsubscript{26} compounds (including 1-hexacosanal, a synthetic C\textsubscript{26} aldehyde and related compounds including the primary alcohol 1-hexacosanol and hexacosanoic acid) either present, or with equivalents, within the barley leaf surface. Slide wells were treated by allowing solution droplets to evaporate in situ. Concentrations used are as listed in section 4.3.1. According to earlier studies, Chapter 3, the most advanced stage of development encountered during germination on glass was primarily the formation of a second germ tube. Therefore, if these additives did stimulate development, an increase in the formation of secondary germ tubes and later developmental structures such as appressoria should be apparent, especially by 24 h.p.i. The developmental profile (according to the germination categorisation scheme in
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Table 2) of Bgh exposed to these treated substrates is shown in Figure 4-4.

![Graph profiling development of Bgh on treated glass surfaces (utilising welled slides) at 24 h.p.i.](image)

**Figure 4-4:** A graph profiling development of Bgh on treated glass surfaces (utilising welled slides) at 24 h.p.i.

Ng = Non germinated; Pgt = Primary (1) germ tube; Sgt = Secondary (2) germ tubes; Esqt/App = Elongating Second Germ Tube, App = Appressoria Formation (symptomatic hooking and swelling); Haus = Formation of haustoria; Mm = More than 2 germ tubes. (Error bars = ± standard error, n=8. Graph constructed from percentages based on averages of 150 spores, derived from 8 independent means of 3 sub-replicates).

Two-way ANOVA analysis confirmed that germination behaviour does differ on the different surfaces after this incubation period compared to glass coated with the solvents di-ethyl ether and dichloromethane (considered the control treatments for extract/monomer compounds and aldehydes respectively). As with the studies shown in Chapter 3 relative proportion of the different developmental classes depends on the surface substrate which the conidia have germinated on (P < 0.001, n = 8 Appendix C, 9.3.1.1, a and b.).
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As with earlier tests performed on glass no haustoria development was visible or expected for any of the treatments, although as suggested by studies in Chapter 3 haustorial formation would be well underway by 24 h.p.i. in barley. When compared to their respective control treatments utilising the solvents di-ethyl ether and dichloromethane, it is clear different substances had significant effects, although not always to advance development. In the case of 1-dodecanol, an aliphatic alcohol, its most significant effect appeared to be the reduction of germination, with on average 79.75 % (± 3.98 % s.d.) of conidia failing to germinate compared to approximately 47.5 % (± 5.77 % s.d.) on the di-ethyl ether control (Appendix C 9.3.1.2). In analogous similarity, hexacosanoic acid treatment resulted in approximately 77 % (± 4.4 % s.d.) of spores failing to germinate (compared to the dichloromethane control where only 50 % failed to germinate) (significant at α = 0.001, n = 8, Appendix C 9.3.1.3). As a consequence of poor germination, in the cases of both treatments, all other developmental classes were reduced when compared with their control treatment counterparts (as shown in Figure 4-4). While some appressorial formation was present during dodecanol treatment, possibly due to a residual effect of the di-ethyl ether used as a solvent for the monomer, no appressorial formation was seen for the C_{26} compound, hexacosanoic acid. Similar statements could be made regarding the behaviour of the other C_{26} compound, 1-hexacosanol, although this did spur greater a primary germ tube formation (31.08 % ± 3.16 %) compared to its counterpart hexacosanoic acid (19 % ± 3.5 %) (P < 0.001, n = 8, Appendix 9.3.1.4).

In an attempt to create a form of positive control, a treatment consisting of barley leaf cuticle extracted using di-ethyl ether was utilised (in the fashion of Iwamoto et al., 2002). It was thought that of all substances tested this extract stood the best chance of appressorial formation. Unexpectedly, although development of an equal number of PGT and SGT occurred, when compared to the di-ether control (analysis indicated a P value = 0.08459 and P = 0.3317 respectively meaning no significant difference at α = 0.05, Appendix C 9.3.1.5 and 9.3.1.6), the extract did not stimulate large numbers of appressoria (Appendix C 9.3.1.7). In fact analysis indicated at P < 0.001 that the control treatment spurred greater appressorial development (Appendix C 9.3.1.8) suggesting that this treatment was actually inhibitory in nature.
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As shown in Figure 4-4 two additives, were shown to promote the development of appressoria (classified as Esgt/App, “elongating secondary germ tube/appressorium”, and App, for mature appressorium) as compared to their respective control treatments. On average the cutin monomer 16-hydroxyhexadecanoic acid promoted significantly more spores (11.75 % ± 3.49 % s.d.) with elongating secondary germ tubes with appressorial characteristics, and on average (9.5 % ± 3.47 % s.d), as fully matured appressoria compared to slides treated with the solvent di-ethyl ether (approximately 4 % and 1.3 % respectively). In both cases P < 0.001, n = 8 (Appendix C 9.3.1.9 and 9.3.1.10). In similar fashion, when compared to slides treated with its solvent, dichloromethane, the aldehyde 1-hexacosanal stimulated significantly more total appressorial development (on average approximately 10.1 % spores compared to dichloromethane at 1 %) (Appendix C 9.3.1.11 and 9.2.1.12).

As a direct consequence of this study, future work would seek to test the effect of 16-hydroxyhexadecanoic acid and 1-hexacosanal on gene expression. Additionally, the difficulty in differentiating between developing and fully mature appressorium, classification reverted back to that used in Chapter 3 (Table 1) as opposed to that shown in table 2.

4.4.2: Characterisation of Blumeria graminis f. sp. hordei upon cutin-monomer treated glass slides

As the initial screen involved glass multiwall slides with plastic surrounds, signal enhancement studies were carried out on simple glass slides of the type utilised in Chapter 3: This would ensure spores would encounter just the signal added and permit easier harvesting of conidial RNA in preparation for quantitative RT-qPCR. As the supply of 1-hexacosanal was limited, two growth studies were carried out to assess the effect on Bgh development of treating glass slides (by the pipetting and spreading of solutions) with 0.015 mg/cm² of the cutin monomer, 16-hydroxyhexadecanoic acid, (Figure 4-5; Table 4). For both studies two-way
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ANOVA suggested there was an interaction between Bgh germination state and the surface (P < 0.01, n = 4 and n = 9 respectively) (Appendix C 9.3.2 – 9.3.3).

The first, featuring the use of glass slides not pre-cleaned with chromic acid, is shown in Figure 4-6. The developmental profile after 16 hours incubation for non-treated glass slides and those treated with the ether solvent appear to follow that of the earlier study shown in Chapter 3, Figure 3.4 showing development on glass slides at 16 h.p.i. Only a small number of spores inoculated on slides (4) or slides treated with ether (16) reach the appressorial stage of development out of 3200 assessed for each surface during the study.

**Figure 4-6:** A graph profiling development of Bgh on treated glass surfaces at 16 hours post-inoculation.

\[ \text{Pgt = Primary (1) germ tube; Sgt = Secondary (2) germ tubes; App = Appressoria Formation (symptomatic hooking and swelling); H = Formation of haustoria; Mb = Branched germ tubes; Ml = Long germ tubes; Mm = More than 2 germ tubes. The final three classes are considered aberrant germination states. For ease of viewing non-germinated conidia are not included in the graph but equated to 85.6 \% \pm 2.7 \% s.e. (16-hydroxyhexadecanoic acid), 79.65 \% \pm 3.4 \% s.e. (ether treated glass), 79.7\% \pm 2.8\% s.e. (non-cleaned glass) (Error bars = \pm standard error, n = 4. Graph constructed from percentages based on average of 800 spores, 4 independent means of 10 sub-replicates).} \]

The formation of a secondary germ tube represents the furthest developmental stage reached, although at each time point the majority of germlings cease development at the primary germ tube stage. Interestingly, it appears that ether treatment of the slides reduces the number of
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secondary germ tube forming conidia compared to non-ether treated glass (P = < 0.01, Appendix C 9.3.2.1). Addition of the cutin monomer to slides stimulated the formation of germ tubes with appressorial characteristics. This addition causes a very low percentage of appressorial development (approximately 3.2 % ± 3 % s.d.) compared to approximately 23 % for barley of assessed spores at the same time point. Additionally, it appears that although this structure is formed differences in length and thickness are apparent when compared visually with appressoria formed on barley as appressoria are often longer and thinner (Figure 4-5).

The apparent statistically significant reduction (at α = 0.01) in the SGT stage for slides treated with diethyl ether or cutin monomer/ether solution compared to glass slide controls suggested a potential cleaning effect of the solvent. The glass slides used both for studies listed in Chapter 3 and here are polished and although contact was made with the manufacturer commercial rules prevented disclosure of the polish constituents. Therefore a smaller germination study was carried out after cleaning of the glass slides with chromic acid (Figure 4-7). Chromic acid treatment clearly reduced the difference in spore number at PGT and SGT (e.g. Appendix C 9.3.3.1) stages between monomer treatment and ether treatment compared to non treated glass. Furthermore, pre-cleaning accentuated the difference in appressorial formation between surfaces to significant levels (monomer-treated glass to non-treated glass and ether treated glass, both significant, P ≤ 0.001, Appendix 9.3.3.2 and 9.3.3.3) although formation compared to the same time point on barley is still lower at approximately 7 % (± 2.8 % s.d.). In conclusion these studies suggested the best chance of causing a discernable effect on CMEG gene expression would be found utilising the cutin monomer 16-hydroxyhexadecanoic acid, and furthermore to ensure the best possible effect, slides should be vigorously cleaned with chromic acid solution before use to remove polish.
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Figure 4-7: A graph profiling development of Bgh on cleaned, treated glass surfaces at 16 hours post-inoculation.

Pgt = Primary (1) germ tube; Sgt = Secondary (2) germ tubes; App = Appressoria formation (symptomatic hooking and swelling); H = Formation of haustoria; Mb = Branched germ tubes; Ml = Long germ tubes; Mm = More than 2 germ tubes. The final three classes are considered aberrant germination states. For ease of viewing non-germinated spores are not included in this image but equated to 59.2 % ± 4.2 % s.e. (16-hydroxyhexadecanoic acid), 65.6 % ± 4.2% s.e. (ether treated glass), 57.8 % ± 2.5 % s.e. (chromic acid cleaned glass). (Error bars = ± standard error, n = 9, Graph constructed from percentages based on average of 300 spores, 9 independent means of 3 sub-replicates).

4.4.3: Developmental behaviour at 4 h.p.i on the host and alternate surfaces.

Previous analysis conducted by Dr. Maike Paramor, a number of genes (listed in 4.3.12) appeared to show a difference in expression on non-inductive surfaces when compared to expression on the host at 4 h.p.i. Investigations were made to discover if compounds added to glass slides, or surfaces differing in hydrophobicity, could cause differential expression to resemble that present when germinating on the host. As an accompaniment to these studies of gene expression at 4 h.p.i., a study was performed to visualise the germination behaviour under these treatments at 4 h.p.i. (Table 4). The germination profiles generated are shown in Figure 4-8 and as with previous studies a two-way ANOVA indicates that there is a significance difference between surfaces, with those surfaces affecting the relative proportions of the different germination stages (Appendix c, 9.3.4).
Figure 4-8: A graph profiling development of *Bgh* on alternate surfaces at 4 hours post-inoculation.

**Blumeria graminis f. sp. hordei** Germination Behaviour on Alternate Surfaces at 4 h.p.i.

- **Pgt** = Primary (1) germ tube; **Sgt** = Secondary (2) germ tubes; **App** = Appressoria formation (symptomatic hooking and swelling); **H** = Formation of haustoria; **Mb** = Branched germ tubes; **Ml** = Long germ tubes; **Mm** = More than 2 germ tubes. The final three classes are considered aberrant germination states. For clarity non-germinated conidia are not displayed but equated to 51.7 % ± 4.5 % s.e. (barley), 62.8 % ± 3.9 % s.e. (wheat), 64.7 % ± 3.4 % s.e. (cuticular extract), 73.9 % ± 2.2 % s.e. (16-hydroxyhexadecanoic acid), 53.5 % ± 4.6 % s.e. (1-hexacosanal), 83.6 % ± 2.7 % s.e. (cleaned glass), 90.9 % ± 1.9 % s.e. (non-cleaned glass), 85.8 % ± 2.39 % s.e. (hydrophobic gelbond), 76.5 % ± 2.8 % s.e. (hydrophilic gelbond). (Error bars = ± standard error, n = 9. Graph constructed from percentages based on averages of 90 spores, 9 independent means of 3 sub-replicates).

As expected from previous studies (listed in **Chapter 3**), plant surfaces showed the most advanced levels of development at this time and this is reflected in the germination rates and morphological states observed. Approximately 48.3 % of all spores assessed on barley germinated (and achieved different stages of development). A lower although not significantly different number germinated during development on wheat (approximately 37.2 %) (**Appendix C 9.3.4.2**).
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Of all slides treated with plant-derived/plant mimicking compounds slides treated with 1-hexacosanal showed the highest rate of germination at this time point (approximately 46.6 %) which proved to be statistically similar as to that as found on barley and wheat (Appendix C 9.3.4.1). Surprisingly this proved to be significantly higher than that stimulated by the cutin monomer (26.1 %) and cuticular extract (35.4 %) (at P < 0.01 and P < 0.001 respectively) (Appendix C, 9.3.4.3 and 9.3.4.4). All of these germination rates were significantly larger than those seen on pre-cleaned (16.5 %) or non-cleaned glass (9.1 %) (at P < 0.05).

Of the two surfaces included in this 4 h.p.i. study that had known levels of hydrophobicity the hydrophilic side of gelbond demonstrated a significantly higher rate of germination (at an average of 23.5 %) than its hydrophobic counterpart (14.2 %).

At this time primary germ tube formation of the plant surfaces appears lower than on glass surfaces treated with host-mimicking compounds (although this appears in favour of later developmental stages, for example appressorial growth). One of the first noticeable effects of these treatments as portrayed in Figure 4-8 is the increase in primary germ tube formation by the 1-hexacosanal treatment (an average of 29.8 % ± 9.4 % s.d. of assessed conidia). This is significantly more than as generated on chromic acid-cleaned untreated glass slides (an average of 11.7 % ± 4.2 % s.d.), (P < 0.001, n = 9, Appendix C 9.3.4.5). Furthermore it is also significantly higher than all other germination surfaces. This includes the slides treated with both the cutin monomer, 16-hydroxyhexadecanoic acid and the cuticle extract. Both of these treated surfaces show increased PGT formation compared to cleaned glass controls. This clearly indicates an inductive effect. On average the cuticle extract induced approximately 21.1 % ± 4.6 % s.d. of all spores and 16-hydroxyhexadecanoic acid (an average at 19.5 % ± 6.48 % s.d.), when compared to cleaned glass controls (both significantly different at P < 0.01, n = 9, Appendix C 9.3.4.6 and 9.3.4.7). As expected from earlier studies, development, at least for this stage, on glass slides not pre-cleaned with chromic acid is the lowest of surfaces tested.

Surprisingly, the formation of the first germ tube on the hydrophobic side of gelbond is lower than that seen on hydrophilic gelbond (at an average of 15.8 % compared to 10.98 %, P = 0.02358, at α = 0.05, n = 9, Appendix C, 9.3.4.8).
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The development of the second germ tube appears to follow very similar trends, at least regarding artificial surfaces. For this germ tube, unlike with the primary germ tube, barley and wheat appeared to induce the highest proportion of this morphological stage, with barley being the optimal inducer as expected. Of the treated glass surfaces 1-hexacosanal (with an average of 11.85 % ± 4.4 % s.d.) again induces the formation of the most tubes of this class by all artificial surfaces (including those of varying hydrophobicity). Interestingly at this time point the numbers generated appear very similar to the number generated by wheat (11.48 % ± 3.9 % s.d.), not significantly different at P < 0.05, Appendix C 9.3.4.9) and also on barley (16.29 % ± 2.9 % s.d.), with P = 0.0547, n = 9, Appendix C 9.3.4.10).

The next treatment to induce the most secondary germ tubes is the cuticle extract (which, at an average induction of 10.3 % ± 3.2 % s.d. is found to be significantly less than the amount induced on barley, with a P-value = 0.01518, α = 0.05, n = 9, Appendix C 9.3.4.9), although not different to that shown by wheat (P-value = 0.7218, Appendix C 9.3.4.10).

In comparison to the formation of primary germ tubes where similar numbers of tube were formed on slides treated with the cuticular extract and with the cutin monomer, this was not the case with the secondary germ tube. Treatment with 16-hydroxyhexadecanoic acid only induced on average 4.69 % (± 1.8 % s.d.) at this stage, which were comparable to the numbers induced by hydrophilic gelbond (4.81 % ± 1.69 % s.d.) (P-value = 0.9957, Appendix C 9.3.4.13).

As with the earlier developmental stage, SGT induction by the hydrophobic side of gelbond (1.6 % ± 1.19 % s.d.) appears lower in comparison to all other surfaces, bar the un-cleaned glass slides (1.72 % ± 4.2 % s.d.).

On artificial surfaces the optimal sign of induction is that of the formation of appressorial germ tubes. It is apparent that by this time point we see the formation of tubes illustrating characteristics of this advanced stage, i.e. swelling and hooking, on the plant surfaces. In comparison the only artificial surfaces to induce appressorial formation of any note were glass slides treated with the cuticular extract (0.37 % ± 0.3 % s.d.) and 1-hexacosanal (0.7 % ± 0.64 % s.d.). At this time point these do not approach the number seen on either wheat or barley.
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During this test it was also apparent that treatment on slides, and other artificial surfaces, were also inducing spore morphologies which were previously classed as aberrant, i.e. the formation of branched germ tubes, more than two germ tubes and also the formation of germ tubes that appeared longer than those expected even for germ tubes destined to form appressorial germ tubes. To focus on the aberrant class showing the most activity, as with both the formation of primary and secondary germ tubes the aldehyde, 1-hexacosanal and the cuticular extract were the cause of the greatest formation of multiple germ tubes (at 3.95 % and 2.96 % respectively).

To summarise these results, at 4 h.p.i. artificial surfaces such as aldehyde treated slides, or slides treated with a cuticular extract appeared to offer the highest possibility of induction both at earlier developmental stages, but most importantly of appressorial germ tubes. Furthermore, the use of 16-hydroxyhexadecanoic acid by appearing to encourage primary germ tube growth at this stage may offer a distinct effect on gene expression compared to the aldehyde or cuticular extract.

4.4.4: Developmental behaviour at 16 h.p.i on treated and artificial surfaces

As a final indicator of inducer performance, which would allow comparisons with previous studies, the developmental behaviour for all artificial surfaces was studied at 16 h.p.i. (Table 4). Barley and wheat were not included in this study as they had already been assessed for development at this time in studies presented in Chapter 3. To summarise, in that study by 16 h.p.i. approximately 23.5 % and 25.9 % of all spores monitored for barley and wheat, respectively, formed appressorial structures. Additionally, 15.4 % and 1.08 % of spores on barley and wheat had begun to form haustoria by that time point: the most advanced form of structure visible at this time. The haustorium, as indicated by studies in Chapter 3, is not formed on artificial surfaces.

After the increased incubation time germination rates for all artificial surfaces, bar the aldehyde, appeared to improve. Surprisingly in this study, spores developing on all glass surfaces whether pre-cleaned and treated or left untouched seemed to show a germination rate of 35 to 40 % although in some cases variation could be relatively large. Interestingly, the hydrophilic gelbond showed a very high level of germination at approximately 58 %. This is
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significantly different from its hydrophobic counterpart, which stimulated an average germination of 26.2\% (P < 0.001, Appendix C 9.3.5).

Figure 4-9: A graph profiling development of Bgh on alternate surfaces at 16 hours post-inoculation.

Pgt = Primary (1) germ tube; Sgt = Secondary (2) germ tubes; App = Appressoria formation (symptomatic hooking and swelling); H = Formation of haustoria; Mb = Branched germ tubes; Ml = Long germ tubes; Mm = More than 2 germ tubes. The final three classes are considered aberrant germination states. For clarity non-germinated spores are not displayed in this image but equated to 62.17\% ± 9\% s.e. (cuticular extract), 63.2\% ± 6.4\% s.e. (1-hexacosanal), 64.5\% ± 2.4\% s.e. (16-hydroxyhexadecanoic acid), 63.7\% ± 5.1\% s.e. (cleaned glass), 64.7\% ± 10.1\% s.e. (non-cleaned glass), 73.7\% ± 1.1\% s.e (hydrophobic gelbond), 41.9\% ± 3.8\% s.e. (hydrophilic gelbond). (Error bars = ± standard error, n = 9. Graph constructed from percentages based on average of 90 spores, 9 independent means of 3 sub-replicates).

The germination profiles of spores developing on these surfaces at 16 h.p.i. is shown in Figure 4-9. As with other studies, the proportions of developmental class depends on the substrate the conidia are germinating on (P < 0.001, Appendix C 9.3.5).
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By 16 h.p.i. the most numerous class of development induced by slides cleaned with chromic acid, treated with the monomer 16-hydroxyhexadecanoic acid, and also the hydrophilic side of gelbond, is that of the formation of primary germ tubes (in all cases \( P < 0.001 \), Appendix C 9.3.5.1 to 3). Although both the cleaned glass and glass treated with monomer may induce the secondary germ tube and also induce appressorial formation, it is the formation of the first germ tube that is most numerous germination status observed. Both induce similar percentages of spores to form a primary germ tube: cleaned glass (22.09 % \( \pm 3.0 \) % s.d.) and the monomer treated glass (21.25 % \( \pm 1.13 \) % s.d.).

At this time point the best indicator of developmental induction on artificial surfaces is the formation of appressorial germ tubes. The most striking feature of the germination profiles in Figure 4-9 and in similar fashion to the behaviour seen at 4 h.p.i, is the level of appressorial induction after 1-hexacosanal treatment of glass slides (18.27 % \( \pm 8.9 \) % s.d.). This is larger than all other germination surfaces featured in this study. It is also significantly larger than the proportion formed on the negative control slides treated with chromic acid, 2.59 % \( \pm 0.42 \) % s.e., \( P < 0.001 \), Appendix C 9.3.5.4).

The next strongest inducer of appressorial formation was the cuticle extract, leading to an average of 8.03 % (\( \pm 4.71 \) % s.d.) of assessed conidia reaching the appressorial stage, which is again significantly more than those induced by the chromic acid cleaned slides, with a P-value = 0.0001957, \( \alpha = 0.001 \), \( n = 9 \), Appendix C 9.3.5.5). Such appressorial induction was not different to the level of induction of secondary germ tubes by the cuticular extract (P-value = 0.789, Appendix C 9.3.5.6).

In this study at 16 h.p.i. 16-hydroxyhexadecanoic acid only led to an appressorial induction of 3.75 %, approximately half of that seen in earlier studies and similar to that induced on the hydrophobic side of gelbond (3.7 % \( \pm 0.9 \) % s.d.) (P-value = 0.6422, at \( \alpha = 0.05 \)) (Appendix C 9.3.5.7). This level of AGT induction proved to be significantly smaller than the level of secondary germ tubes induced (\( P < 0.01 \), Appendix 9.3.5.8). Both the monomer and the hydrophobic side of gelbond induced appressorium development of spores to a level that was not significantly different from that induced by chromic acid cleaned glass slides (2.59 % \( \pm 3.69 \) % s.d.) at \( \alpha = 0.05 \) (16-hydroxyhexadecanoic acid: P-value = 0.6; Hydrophobic Gelbond = P-value = 0.3, Appendix C 9.3.5.9 and 9.3.5.10).
At this time point classes of aberrant development are readily apparent. Noticeably there are more spores with more than two germ tubes than in the other two unsuccessful development classes. The percentage of spores induced by hydrophilic gelbond (15.25 % ± 9.5 % s.d.) appears very similar to the proportion of appressoria induced by the aldehyde (not significantly different with a P-value = 0.5303) (Appendix C 9.3.5.11). The next highest level of aberrant development in this class was located on slides that had not been pre-treated with chromic acid and consequently were still polished.

In contrast, the primary inducer of spores that showed a germ tube longer than expected was the rough cuticular extract (with an average induction of 6.3 % ± 4.49 % s.d.) and this was significantly different larger when compared to the number that developed on pre-cleaned glass slides, (average of 0.24 %, P-value = 0.02975 at α = 0.05) (Appendix C 9.3.5.12).

Of final note is the induction of spores showing branching of germ tubes, with the primary substrates causing their induction in this study being the hydrophobic and hydrophilic sides of gelbond (not significantly different with a P-value = 0.1574) (Appendix C 9.3.5.13).

To summarise the results for both the 4 h.p.i. and 16 h.p.i. studies (Table 4), of all surfaces tested the aldehyde 1-hexacosanal caused significantly higher amounts of appressorial formation than the glass slide control. As a consequence of this alteration of morphology, the 1-hexacosanal offers a distinct possibility of causing distinguishable modulation of gene expression during development on glass. Other treatments such as the cutin monomer induce varying levels of other stages of development, including in the case of gelbond surfaces, numbers of spores with aberrant morphological classes. These too offer the potential for the visible modulation of expression.

4.4.5: Analysis of surface hydrophobicity
Several surfaces employed for the re-modulation of gene expression were assessed for their relative hydrophobicity. These included glass slides that were not pre-cleaned before use with chromic acid, pre-cleaned glass slides treated with ether solvent, pre-cleaned glass slides treated with 0.015 mg/cm² 16-hydroxyhexadecanoic acid cutin monomer, pre-cleaned slides treated with 0.5 µg/cm² 1-hexacosanal, pre-cleaned slides treated with a cuticle extract equivalent to 1 cm² leaf surface/cm² slide surface, both hydrophobic and hydrophilic surfaces
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of gelbond and the adaxial and abaxial surfaces of both barley and wheat primary leaves. Two methods (Table 4) were used the first of which involved measuring the diameter of 19 μl crystal violet droplets on the substrates (Figure 4-10). The second, performed at a later time during the investigation, involved measuring the advancing contact angle of 20 μl water droplets using a goniometer (Figure 4-11). Wheat leaf surfaces, cuticle extract- and 1-hexacosanal-treated slides were only assessed via goniometer. However both methods delivered similar results with ANOVA analysis combined with Tukey or Games Howell ‘Post-Hoc’ multiple comparisons at α = 0.05 (n = 5) (Appendix C 9.4 and 9.5) was employed to indicate statistical significance.

Figure 4-10: Relative hydrophobicity of treated surfaces.

Droplets of crystal violet applied to the barley adaxial surface (A), polished glass slides not pre-cleaned with chromic acid (B), glass slides pre-cleaned with chromic acid (C), chromic acid cleaned glass slides treated with ether (D), chromic acid glass slides treated with cutin (E). Hydrophilic side of Gelbond (F). Hydrophobic side of Gelbond (G). Droplet volume is 19 μl. Scale bar = 3.5 mm
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As shown in Figure 4-12, surfaces could be putatively divided into 2 groups, those with contact angles less than 30° (or droplet diameters larger than 7 mm on average) and more hydrophobic surfaces with angles of 80° or greater (or droplet diameters smaller than 6 mm). Plant surfaces showed the greatest hydrophobicity with contact angles in excess of 120°, or in the case of barley surfaces, droplet diameters less than 4 mm on average. The upper (adaxial, 130.53° ± 4.35° s.d.) and lower (abaxial, 132.258° ± 3.52° s.d.) surfaces of barley leaves, although not statistically different from one another, or from either wheat leaf surface (adaxial = 127.818° ± 5.56° s.d.; abaxial = 129.49° ± 6.29° s.d.), were significantly more hydrophobic than all other surfaces tested (P < 0.01, n = 5, Appendix C 9.4 and 9.5). Either of the two methods employed suggested that the hydrophobic surface of Gelbond (contact angle of 79.95° ± 0.54° s.d.) was significantly less than the plant surfaces but also greater.
than either its hydrophilic Gelbond counterpart, the non-cleaned slides, the pre-cleaned glass slides, or those cleaned and then treated with stimulatory compounds.

![Figure 4-12: Relative surface hydrophobicity of surfaces tested.](image)

**A)** Hydrophobicity as indicated by the average diameter of drops upon surface. The cutin monomer treatment featured addition of 16-hydroxyhexadecanoic acid, whilst the aldehyde was 1-hexacosanal. Error Bars = ± standard error, N = 5 with 5 droplets averaged per surface replicate.

**B)** Hydrophobicity as indicated by the average contact angle on substrate surface. The cutin monomer treatment featured addition of 16-hydroxyhexadecanoic acid, whilst the aldehyde was 1-hexacosanal. Error Bars = ± standard error, N = 5 with 5 droplets averaged per surface replicate.

Slides, either solely pre-treated with chromic acid or additionally treated with cuticle-derived components, appeared to group together with angles of approximately 20°. Glass slides that were not pre-cleaned before use showed (as indicated by larger droplet diameter, 10.32 mm ± 0.42 mm s.d.,) significantly lower hydrophobicity in comparison to all other surfaces. Analysis of contact angles supported this conclusion for the majority of slide treatments (non pre-cleaned slides had an angle of 7.48° ± 1.57° s.d.) except for those treated with extract or pre-cleaned with chromic acid (an average contact angle of 18.62° ± 6.73° s.d.). This, however, appeared to be due to the intrinsic variation encountered in their specific treatments. Non-cleaned slides were significantly (P <0.05, n = 5) more hydrophilic than the hydrophilic side of Gelbond (16.86° ± 0.9° s.d., which in itself was not significantly different in hydrophobicity when compared to pre-cleaned and treated slides).

Treatment of the cleaned slides, either with the cuticle extract (25.03° ± 7.33° s.d.), the cutin monomer (21.572° ± 4.87°/ 6.81 mm ± 0.17 mm s.d.), or the cuticular aldehyde (20.684° ±
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4.59° s.d.), did not appear to significantly alter the hydrophobicity of the slides compared to slides pre-cleaned with chromic acid treated (18.62° ± 6.73°/ 7.27 mm ± 0.245 mm s.d.) or those treated with the ether solvent as a control (7.28 mm ± 0.312 mm s.d.). These results are summarised in Figure 4-12 with the analysis available in Appendix C 9.4 and 9.5. In conclusion this result provides further evidence that the untreated slides had a coating which could affect Bgh germination behaviour, possibly by providing an infection court of severely reduced hydrophobicity. Furthermore these results suggest that treatments added to the slide did not affect hydrophobicity, but would merely provide chemical stimuli.

4.4.6: Effect of stimuli on genes differentially expressed at 4 h.p.i.

By utilising the data gathered from a previous microarray study which examined expression profiles of 2027 ESTs during germination on 4 different surfaces, and by comparing expression between 4 h.p.i. barley and 4 h.p.i. glass samples, 18 ESTs were identified (by Dr. Maike Paramor) as being differentially expressed between these two stages. As a result RNA was extracted from Bgh spores inoculated on the following surfaces at 4 h.p.i. (chromic acid cleaned glass slides, non-cleaned glass slides, 16-hydroxyhexadecanoic acid treated slides, 1-hexacosanal treated glass slides, cleaned slides treated with a cuticular extract, hydrophobic gelbond, hydrophilic gelbond, wheat and the host, barley). cDNA was then synthesised and RT-qPCR performed utilising primers for these 18 ESTs (Table 4).

During this study these ESTs are referred to by their library identifier code. Expression was measured against the Bgh NADH-ubiquinone oxidoreductase, which represents a constitutively expressed control according to the microarray study (Dr. Maike Paramor, pers. comm). The ratio of the signal returned by the sample versus the reference gene was log₂ transformed. It is this value which is referred to as the ‘Relative Expression Index’.

Expressed Sequence Tag Clone C00148

This EST encodes a 1, 3-ß Glucanase. The expression profile is shown in Figure 4-13. This profile was constructed by Dr. M. Paramor based on microarray data. All microarray data is relative to a universal standard assembled by combining equal amounts of RNA from all developmental stages assessed during that experiment (same as in Both et al., 2005). As can be seen from that data at 4 h.p.i. abundance of RNA for the EST is higher during Bgh development on barley, and on wheat, than when germination is occurring on glass. A similar trend is observed when visually comparing the expression during germination on barley,
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wheat and on non-treated glass (Figure 4-13) as collected by RT-qPCR. However this trend was not confirmed during analysis by 1-WAY ANOVA followed by post-hoc testing (Appendix C 9.6). This revealed that not only did expression during wheat-based germination, germination on all treated glass slides and artificial surfaces differing in hydrophobicity, not significantly differ from the host at $\alpha = 0.05$, but that it also did not significantly differ from expression induced by either the chromic acid cleaned glass or the non-cleaned glass control. This includes the expression levels assessed during development on hydrophilic gelbond which, visually, appeared to show the most deviation from that experienced on the host. Therefore it appears that in the case of this gene, conclusions as to the varying effects of the treatment on expression are not possible.

Figure 4-13: A) Comparison of RNA abundance of EST clone C00148 relative to the NADH-ubiquinone oxidoreductase in $Bgh$ spores developing on 9 different surfaces at 4 h.p.i.

This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of the EST Clone C00148 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

**Expressed Sequence Tag Clone D00189**

The gene this clone relates to encodes an unknown gene product. Relative expression as displayed by the microarray generated data (as shown in Figure 4-14b) suggested that expression during pre-penetration germination on both the host, wheat and glass slides are similar at 4 h.p.i. As a form of negative control for this gene expression analysis, assessment was made to see if different surfaces could induce a deviation away from that previously
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observed on the surfaces, or from each other. Expression is shown in Figure 4-14a. Statistical analysis suggested gene expression is similar during germination on both barley and non-cleaned glass slides (no significant difference at $\alpha = 0.05$). Furthermore all other surfaces demonstrated expression that was not significantly different from either the host on the non-cleaned glass slides. Interestingly there was a significant difference at $\alpha = 0.05$ between expression on slides treated with the aldehyde (1-hexacosanal, where lower expression was encountered) and slides cleaned chromic acid (Appendix C 9.6). Similarly, there was a significant difference between gene expression from spores developing on aldehyde treated slides and hydrophillic gelbond (where a higher level of expression was encountered). Interestingly, gene expression was close to achieving significance on the hydrophobic side of gelbond and on slides treated with the aldehyde.

![Figure 4-14: A) Comparison of RNA abundance of EST clone D00189 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i.](image)

This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone D00189 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

**Expressed Sequence Tag Clone C00563**

This transcript encodes a H3 histone involved in nucleic acid structure. The expression profile is shown in Figure 4-15a. As can be seen from that data at 4 h.p.i. expression appears 2 fold higher on barley and wheat, than during development on glass. When assessed by RT-
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qPCR (Figure 4-15b), the general trend appears similar with plant surfaces showing a higher expression than glass slides. Statistical analysis again reveals that EST expression on non-cleaned slides is not different from that on barley (Appendix C 9.6). Cleaning of slides with chromic acid reduces the variance encountered when sampling from the slides but still expression levels are not significantly different from that on barley. As is the case with previous expression analysis, although in some cases approaching statistical significance, all other surfaces are not different from barley or from each other.

Figure 4-15: A) Comparison of RNA abundance of EST clone C00563 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i.

This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone C00563 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

Expressed Sequence Tag Clone D00471

This gene encodes a predicted protein with no known function. The expression profile as assessed by microarray and RT-qPCR is shown in Figure 4-16. At 4 h.p.i. expression appears approximately a fold higher on wheat and glass than that seen on barley (Figure 4-16b). The data collected in this study (and shown in Figure 4-16a) suggests no differential expression (at $\alpha = 0.05$) occurred on wheat, all treated glass slides and artificial surfaces differing in hydrophobicity, when compared to expression during germination on the host, but they also did not significantly differ from either the chromic acid cleaned glass or the non-cleaned glass control. This includes the expression levels assessed on the cutin monomer treated
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slides, where expression visually suggests a decrease in expression. Therefore it appears that in the case of this EST, conclusions as to the varying effects of the treatment are not possible.

![Graph of Relative Expression of EST Clone D00471 at 4 h.p.i. on Different Surfaces](image)

Figure 4-16: A) Comparison of RNA abundance of EST clone D00471 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i.

This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone D00471 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

**Expressed Sequence Tag Clone C00606**

This gene encodes an unknown protein. The expression profile is shown in Figure 4-17. As can be seen from the microarray data at 4 h.p.i. the abundance of RNA is higher on glass than on barley and wheat (which show very similar expression levels to one another). Visually, a trend appears to be present with plant surfaces and slides treated with the monomer and cuticular extract, inducing decreased expression compared to non-treated slides. However analysis only confirmed significantly lower gene expression (at \( a = 0.05 \)) in Bgh on wheat when compared to that on glass slides left un-treated with chromic acid (Appendix C 9.6).
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Figure 4-17: A) Comparison of RNA abundance EST clone C00606 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i. This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone C00606 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

Expressed Sequence Tag Clone C00506

This transcript encodes a mitochondrial glycerol-3-phosphate dehydrogenase. The expression profile collected both during the original microarray study and in this present study is shown in Figure 4-18. As can be seen expression appears approximately 2 fold higher for developing spores on barley and wheat, than spores on glass at 4 h.p.i. When the RT-qPCR results were analysed, however, this was not confirmed. Analysis suggested that all spores developing on treated slides showed expression that was significantly different from one another. Unfortunately, a lack of functioning independent replicates for the cutin monomer treated slides means that statistical analysis could not be carried out for this substance. However, the plotting of the values observed indicate that induced expression lies in close proximity to values for those slides treated with plant extracts suggesting a similar result in regards to lower expression would be seen (Appendix C 9.6).
Germination on Modified Surfaces

Figure 4-18: A) Comparison of RNA abundance of EST clone C00506 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i.

This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone C00506. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’) and glass (‘G’) slides (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

Expressed Sequence Tag Clone C00658

This gene was believed to encode a glycosyltransferase. Microarray profiles (Figure 4-19b) suggested that expression was higher in germlings at 4 h.p.i. on barley and wheat, than spores on glass. Although a slight visual trend is shown, this has not held up to analysis (Appendix C 9.6). Again no deviation in gene expression from that of the host or un-treated glass was detected for any surface. Although data was incomplete regarding expression on the hydrophobic gelbond and cutin-monomer treated slides the data collected suggested that expression would not show any difference from any of the other surfaces. Analysis did reveal that that at α = 0.05 there was significantly higher expression in spores developing on hydrophilic gelbond compared to those developing on slides treated with cuticular extract. This is displayed in Figure 4-19a.
Germination on Modified Surfaces

Figure 4-19: A) Comparison of RNA abundance of EST clone C00658 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i. This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone C00658 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

Expressed Sequence Tag Clone D00972

Another gene of unknown function, the expression profile for this gene is shown in Figure 4-20. As can be seen from the microarray data at 4 h.p.i. the expression on glass was believed to be 1 fold higher than that seen on during infection of barley (with expression on wheat being half-way between the respective values). Such a difference was not detected by RT-qPCR and the expression on treated glass slides or gelbond surfaces failed to show any detectable modulation of this gene expression (Appendix C 9.6). If visual trends are to be emphasised in this experiment it appears that wheat may actually be showing a lower expression than barley. However a lack of resolution hinders this interpretation.
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Figure 4-20: A) Comparison of RNA abundance of EST clone D00972 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i. This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone D00972 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley ('B'), wheat ('W') and glass slides ('G') (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

Expressed Sequence Tag Clone D00944

With an unknown function, the expression profile of this EST is shown in Figure 4-21b. Microarray data suggested that expression would be higher during germination on barley and wheat, than on untreated glass. In Figure 4-21a, at 4 h.p.i. a similar trend, supported by statistical analysis is seen at this time point (Appendix C 9.6). Expression on barley is significantly higher than that observed on non-treated glass at $\alpha = 0.05$. All other surfaces, excluding the cutin monomer where replication was not sufficient to allow statistical analysis (but appeared to suggest higher expression than seen in barley-stimulated development), appeared to induce gene expression that lay between both surfaces, but were not significantly different from either. 1-hexacosonal treated slides, whilst still being non-significantly different to either pre-cleaned or non-treated glass slides, came close to inducing significantly different in gene expression when compared to barley. Therefore although treatments or artificial surfaces may modulate gene expression it did not appear that any, by themselves caused enough of a modulation of gene expression to be significantly different from any other.
Germination on Modified Surfaces

Figure 4-21: A) Comparison of RNA abundance of EST clone D00944 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i. This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone D00944 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

**Expressed Sequence Tag Clone D00881**

This gene encodes a α-1,6-mannosyltransferase. The expression profile is shown in Figure 4-22b. Data suggested than expression at 4 h.p.i. should be higher during development on glass than on barley and wheat (which itself should show expression that is moderately higher than that seen on barley). RT-qPCR (Figure 4-22a) failed to confirm this trend although it did suggest significantly higher expression in spores germinating on cleaned glass slides and both hydrophilic and hydrophobic sides of gelbond compared to wheat (at α = 0.05) (Appendix C 9.6). Slides that hadn’t been pre-cleaned with chromic acid also had a higher level of expression that was nearly, but not quite, significant in comparison to that on wheat. Although not significantly different from the cleaned glass the visual trends suggest some form of repression in slides treated with either 1-hexacosanal, cutin monomer and cuticle extract and wheat as gene expression appears uniformly reduced in these samples.
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Figure 4-22: A) Comparison of RNA abundance of EST clone D00881 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i.

This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone D00881 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

Expressed Sequence Tag Clone D01260

This transcript encodes an adenosylhomocysteinase. The expression profile is shown in Figure 4-23b. As seen from the microarray data the expression on glass at 4 h.p.i appears to be 1-fold higher than on barley and wheat. Statistical analysis of the RT-qPCR data (Figure 4-23a), suggested no surface induced expression differing from either that of the host or from either form of the glass slides (Appendix C 9.6). As with previous EST it appears that conclusions as to the varying effects of the treatment are not possible.
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Figure 4-23: A) Comparison of RNA abundance of EST clone D01260 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i. This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone D01260 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

**Expressed Sequence Tag Clone C00009**

This gene encodes a MAPK Interacting protein. As can be seen from that data at 4 h.p.i. abundance of RNA for the gene for which the EST relates to is 3 fold higher in barley than when germination is occurring on glass (Figure 4-24a). Expression during development on barley is also 1.5 fold higher than that seen on wheat. This difference between spores developing on barley and glass was not confirmed by RT-qPCR (Figure 4-24b) (Appendix C 9.6). Failure of all replicates of the cutin monomer to replicate with this gene meant its expression could not be assessed. Similarly because certain replicates would not amplify with regards to the aldehyde and the cuticle extract the values were not included in the statistical analysis. However plotting of their available values suggested expression by spores on that surface may lie in a similar vicinity to that seen in spores developing on wheat.
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Figure 4-24: A) Comparison of RNA abundance of the EST clone C00009 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i.

This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone C0009 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

Interestingly there was a significantly higher expression for this EST on barley than on wheat at $\alpha = 0.05$, seemingly confirming the microarray data in this instance. Furthermore, the expression seen in spores germinating on both sides of gelbond and on slides either cleaned with chromic acid or left un-treated was significantly higher than on that seen on the wheat at $\alpha = 0.05$ (Appendix C 9.6).

**Expressed Sequence Tag Clone C00482**

This gene relates to encodes a 1, 3-ß glucanase. The expression profile is shown in Figure 4-25a. In this case although untreated glass provided the basis for an expression level equal to that found on barley (and wheat, hence contradicting the microarray data presented in Figure 4-25b), treatment of slides with 16-hydroxyhexadecanoic acid led to a 3 fold decrease in gene expression. This decrease was significantly different at $\alpha = 0.05$ (Appendix C 9.6). Statistical analysis suggested that although expression was lower than that seen for either cleaned glass or non-cleaned glass, both types of gelbond and aldehyde treated glass, it was not significantly different at $\alpha = 0.05$. 

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**Figure 4-25:** A) Comparison of RNA abundance of EST clone C00482 relative to the NADH-ubiquinone oxidoreductase in *Bgh* spores developing on 9 different surfaces at 4 h.p.i.

This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. **B) Relative Expression of EST Clone C00482 RNA as assessed by microarray analysis and as expressed relative to a universal standard.** RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’), and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). **Error Bars = A) Standard error, N= 3 B) Standard deviation**

**Expressed Sequence Tag Clone C01244**

This gene encodes a transporter, potentially related to the PHO87 phosphate transporter protein. The expression profile is shown in Figure 4-26b. As can be seen from that data at 4 h.p.i. abundance of transcript is a fold higher for spores on barley and wheat than on glass. Statistical analysis failed to define any difference in gene expression present between the surfaces for this EST (Appendix C 9.6). Although the full number of replicates weren’t present, the available data suggested that that the cutin monomer would lead to expression levels similar to those present in spores developing on non-cleaned glass.
Figure 4-26: A) Comparison of RNA abundance of EST clone C01244 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i. This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone C01244 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

Expressed Sequence Tag Clone C01417

This gene has an unknown function. The expression profile is shown in Figure 4-27. As can be seen from that data at 4 h.p.i. abundance of RNA for this EST is higher in approximately 5-fold higher on barley than when germination is occurring on glass. Wheat-based germination shows a similar profile to that of barley (Figure 4-27b). The RT-qPCR data supported this trend with expression on glass (either chromic acid treated or left untreated) being significantly less at $\alpha = 0.05$ than on barley (Figure 4-27a). Furthermore all artificial surfaces appeared to provide expression levels that were significantly less than on the barley at $\alpha = 0.05$ (but not wheat) (Appendix C 9.6). In the case of untreated glass slides (either pre-cleaned or not) and also those treated with cuticular extract expression was significantly less than that stimulated by wheat at $\alpha = 0.05$. In conclusion it would seem in the case of this EST treatments either chemical or physiochemical in nature failed to lead to host-like expression on artificial surfaces.
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Figure 4-27: A) Comparison of RNA abundance of EST clone C01417 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i. This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression EST Clone C01417 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

Expressed Sequence Tag Clone C00059

The related gene (a cPKA gene) encodes a catalytic subunit for the cAMP-dependent protein kinase A. The expression profile is shown in Figure 4-28b. Expression for development at 4 h.p.i. is a fold higher during development on barley and wheat, than during germination occurring on non-cleaned, untreated glass. Visually although expression levels appear lower in non-treated glass than barley this could not be confirmed by statistical analysis (Figure 4-28a) (Appendix C 9.6). No germination surface led to a significantly different level of expression.
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Figure 4-28: A) Comparison of RNA abundance of EST clone C00059 relative to the NADH-ubiquinone oxidoreductase in *Bgh* spores developing on 9 different surfaces at 4 h.p.i. This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone C00059 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

**Expressed Sequence Tag Clone PS11B04**

This transcript is thought to encode a calcium ion transporter. The expression profile is shown in Figure 4-29b. Showing similar expression profiles, RNA abundance during development on both plant surfaces is approximately 3-fold higher than when germination is occurring on glass. As with many of the previous tests expression this difference could not be confirmed by RT-qPCR (Figure 4-29a). Although treatments of slides with 1-hexacosanal suggested an increase in gene expression on slides treated with this substance when compared to non treated controls this was not supported by statistical analysis (Appendix C 9.6).
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Figure 4-29: A) Comparison of RNA abundance of EST clone PS11B04 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i. This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone PS11B04 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

Expressed Sequence Tag Clone C01157

This transcript encodes a protein disulfide isomerase. The expression profile is shown in Figure 4-30b. The treatments and artificial surfaces failed to reveal a significant induction of any gene expression either from each other or from non-treated glass slides or the host barley (Figure 4-30a) (Appendix C 9.6). A slight visual trend is seen with gene expression during development on barley appearing slightly higher than that of glass, but this could not be confirmed statistically.

In conclusion, germination screens suggested different surfaces had alternate effects on gene expression (as visualised by different germination/developmental patterns) with some compounds such as 1-hexacosanal treated slides inducing significant levels of appressorial formation. However, these effects were not so easily discerned when assessing expression levels of ESTs with distinct expression profiles at 4 h.p.i.
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Figure 4-30: A) Comparison of RNA abundance of EST clone C01157 relative to the NADH-ubiquinone oxidoreductase in Bgh spores developing on 9 different surfaces at 4 h.p.i. This comparison was made by RT-qPCR. HHD = 16-hydroxyhexadecanoic acid, cutin monomer. B) Relative Expression of EST Clone C01157 RNA as assessed by microarray analysis and as expressed relative to a universal standard. RNA abundance was studied at 0, 4, 8, 16 h.p.i. on barley (‘B’), wheat (‘W’) and glass slides (‘G’) (Microarray data provided by Dr. Maike Paramor). Error Bars = A) Standard error, N= 3 B) Standard deviation

4.4.7: Bioinformatics.

Of the genes investigated by RT-qPCR eight showed some form of significant response in expression during spore development after exposure to different stimuli. As a consequence attempts were made to confirm i) the function of the genes in question, ii) the copy number present in the Bgh genome (i.e. the existence of paralogs), and iii) the existence and relatedness of orthologs (i.e. species distribution). EST sequences, predicted protein sequences, protein signature recognition results produced by InterProScan software, lineage (taxonomy) reports and neighbour joining trees are displayed in Appendix C: 9.7 and 9.8 and on the accompanying CD.

EST Clone Library Identifier D00189.

BlastN nucleotide analysis indicated that there was only one copy of this gene within the Bgh genome. No accurate model existed for this gene and so attempts were made to create one. It
was not clear either what frame the EST was in or which start/stop codon were correct and so difficulties were encountered that could not be resolved. However the largest nucleotide sequence attainable was run on the ‘nucleotide database (nr/nt)’ hosted on NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) utilising the BlastN function there-in. No significant hits were encountered so attempts were made to blast the protein sequence against the ‘non-redundant protein sequences’ database also hosted on NCBI. A weak hit was present indicating a very low similarity to a putative TonB-dependent iron outer membrane transporter (E-value = 3.2). As a consequence no function could be determined with certainty. Furthermore with the evidence available at the time of writing, this gene appears to be an ‘Orphan’ (an open reading frame of unknown function) as no orthologs could be determined.

**EST Clone Library Identifier C00606.**

Similar to EST D00189, this gene appears to have only one copy within the \textit{Bgh} genome. Attempts to identify gene function by scanning both the nucleotide and protein sequence databases on NCBI (which were derived from attempts to generate a gene model) did not suggest the presence of orthologs or confirm the function of this gene product.

**EST Clone Library Identifier D00944.**

As with the previous genes (D00189, C00606) that showed a significant response to the stimuli tested here this gene exists as a single copy within the \textit{Bgh} genome. Encoding a 330 amino protein predicted to weigh 38.19 kilodaltons, neither BlastP analysis on NCBI nor InterProScan motif recognition algorithms could identify a function for this protein or homologues existing in other species. However a signal peptide was identified comprising amino acids 1-16 (\textit{Appendix C: 9.8}).

**EST Clone Library Identifier C01417.**

A single copy within the \textit{Bgh} genome a with a predicted product of 14.5 kilodaltons (148 amino acids). Analysis using InterProScan (protein signature recognition) software (\textit{Appendix C: 9.8}) suggested the sequence contained a signal peptide (amino acids 1-17) as well as one transmembrane region (20 amino acids in length, positions 5-25). Together these characteristics suggest that the protein is membrane-associated (and potentially secreted). BlastP analysis suggests this protein shows similarity to several hypothetical proteins (15
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with E-values ranging from \(1 \times 10^{-16}\) to \(1 \times 10^{-5}\) all of unknown functions found in 12 different species of ascomycete fungi. These fungi species are found within the taxon *Leotiomyceta* (which contains filamentous fungi). Based on neighbour joining tree analysis (visible on the accompanying CD, this *Bgh* protein clusters close to a clade of proteins from species including *Glomerella graminicola* (anamorph *Colletotrichum graminicola* – a non-obligate biotrophic fungus of the *Glomerellaceae* family known to cause plant disease, specifically anthracnose of cereal species; Behr et al., 2010) and a protein from the hemibiotroph *Magnaporthe oryzae*. Both of these fungi belong to the class *Sordorimycetes* (of the *Glomerellaceae* and *Magnaporthaceae* fungal families respectively). Other proteins within this clade were found within the model fungal species *Neurospora crassa, Sordaria macrospora* (Engh et al., 2010) and *Chaetomium globosum* (a soft rot fungi involved in wood degradation; Popescu et al., 2011) and *Verticillium albo-atrum*. This analysis is displayed in the tree diagram found on the accompanying CD to this thesis, as is the clustalw alignment upon which the tree is based. Other pathogenic fungi species with homologous proteins include *Pyrenophora teres f. teres, Pyrenophora tritici-repentis, Leptosphaeria maculans, Phaeosphaeria nodorum* and the necrotophic plant pathogens *Sclerotinia sclerotiorum* and *Botryotinia fuckeliana* (anamorph *Botrytis cinera*) (Schamber et al., 2010; Mei et al., 2011).

**EST Clone Library Identifier C00482.**

A single copy gene encoding a 47.45 kilodalton protein of 426 residues BlastP analysis utilising the NCBI ‘non-redundant protein sequence’ database matched this protein with a *Bgh* protein previously defined by Zhang and Gurr (E-value = 0.0, submitted directly to NCBI, accession number: Q96V64) as a glucan 1,3-beta-glucosidase. This identity was further supported by InterProScan protein recognition software utilising HMMPfam prediction search algorithms to compare the sequence against member databases. The sequence identified a motif characteristic of cellulases between residues 81 to 315, with a catalytic core region of a glycoside hydrolase between residues 29 to 417. BlastP analysis indicated homologous gene products present within both ascomycete (46 to be specific) and a small number (6) of basidiomycete members of the fungal kingdom *Dikarya* (E-values ranging from \(9 \times 10^{-16}\) to \(1 \times 10^{-9}\)). Basidiomycete species with homologous products include *Agaricus bisporus* (the common mushroom), and the biotrophic pathogens *Ustilago maydis* (a disease of maize) and *Sporisorium reilianum*, both members of the *Ustilaginaceae* family (Schirawski et al., 2010). Proteins with homology from these organisms include exo-1,3-beta-
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glucanses and members of the glycoside hydrolase protein family. The 46 ascomycete species are too numerous to list here but also include proteins identified as 1, 3 beta-glucanases or 1,3-beta glucosidases (species and protein identities are listed in the lineage report found on the accompanying CD). These species include 18 members of the class *Saccharomycetes* (specifically of the families *Saccharomycetaceae*, *Metschnikowia*ceae, *Wickerhamomyces*ceae, *Dipodascaceae* and *Debaryomyces*aceae) as well as *Tuber melanoporum* (French truffle, a member of the *Tuberaceae* family, of the class *Pezizomycetes*). The majority of fungal species were members of the taxon *Leotiomyceta* (part of the subphylum *Pezizomycota*) including the *Pleosporaceae*, *Leptosphaeriaceae*, *Phaeosphaeriaceae* (all families of the class *Dothideomycetes*) and the *Arthrodermataceae*, *Ajellomycetaceae*, *Trichocomaceae* families (of the class *Eurotiomycetes*). According to neighbour joining tree analysis these taxon members also included two species with proteins of the highest homology, the necrotrophs *Sclerotinia sclerotiorum* and *Botryotinia fuckeliana* (both belonging to the *Sclerotiniaceae* a member family of the class *Leotimycetes* which contain the fungal family *Erysiphaceae*, itself containing barley powdery mildew). The proteins of these fungi are listed as a glucan 1,3-beta-glucosidase in the case of *S. sclerotiorum* and a hypothetical protein in the case of *B. fuckeliana*. Other proteins (both hypothetical and proteins listed as glucan 1,3-beta-glucosidase precursors, listed in the sequence report on the accompanying CD) with lower homology include those of the ascomycete plant pathogens *Pyrenophora teres f. teres* (causal agent of net blotch on barley; Liu et al., 2011) and *Pyrenophora tritici-repentis* (causing red smudge of wheat; Bouras et al., 2010), both members of the family *Pleosporaceae*, of the taxon *Pleosporineae*). Other fungi with proteins of close homology include *Leptosphaeria maculans* (causal organism of black leg of crucifers; Light et al., 2011) and *Phaeosphaeria nodorum* (synonym *Stagnospora nodorum*, a blotch disease of wheat; Mebrate et al., 2001) – two other members of the *Pleosporineae* (of the *Leptosphaeriaceae* and *Phaeosphaeriaceae* families).

**EST Clone Library Identifier C00009.**

Encoding a 33.93 kilodalton protein of 217 amino acids this gene is found as a single copy within the *Bgh* genome. BlastP analysis suggested it showed similarity to MAPK interacting proteins. The protein sequence contained a low complexity region (indicative of a transmembrane region between amino acids 198-216) and a signal peptide (amino acids 1-18) that suggested it was membrane bound or secreted. BlastP and InterProScan analysis utilising
HMMpfam prediction search algorithms suggested that the protein showed similarity to the Pfam domain ‘Drmip_Hesp’ (between amino acid 30-103), a family involved in fungal fruiting body formation and in host attack (Szeto et al., 2007) (Appendix C: 9.8). BlastP analysis (NCBI) suggested homology to 62 proteins from other organisms including 32 species of ascomycete fungi and a monocot (Zea mays) with E-values ranging from $4 \times 10^{-27}$ to $9 \times 10^{-04}$. All fungal species are identified as belonging to the subphylum Pezizomycotina or more specifically the taxon Sordariomyceta. Member families include Glomerellaceae, Nectriaceae, Clavicipitaceae, Magnaporthaceae, Ophiostomataceae, Chaetomiaceae (all families of the class Sordariomycetes), Sclerotiniaceae (of the class Leotiomycetes), Onygenaceae, Ajellomycetaceae, Arthrodermataceae, Trichocomaceae, Leptosphaeriaceae (all families of the class Eurotiomycetes), and the Phaeosphaeriaceae (both of the class Dothideomycetes). Homologous proteins from these organisms include extracellular matrix proteins and hypothetical proteins with no formally annotated function. Based on neighbour joining tree analysis of all these sequences, this Bgh protein clusters with predicted/hypothetical proteins from Sclerotinia sclerotiorum and Botryotinia fuckeliana, while also showing close relationship to proteins from Glomerella graminicola (family: Glomerellaceae) and Verticillium albo-atrum (family: incertae sedis) both of the order Hypocreales, of the class Sordariomycetes. Fusarium oxysporum f. sp. lycopersici (a soilborne plant pathogen of the family Nectriaceae), which causes fusarium wilts of tomato (Escobosa et al., 2010), is phylogenetically clustered near to the clade containing the predicted Bgh protein (the tree is found on the accompanying CD) and contains a protein predicted to be an extracellular matrix protein precursor. Therefore this protein may show some characteristics of a secreted protein.

**EST Clone Library Identifier D00881.**

Another single copy gene within the Bgh genome that encodes a predicted product of 369 amino acids (41.38 kilodaltons). BlastP analysis suggested this protein was a glycosyltransferase. Analysis utilising InterProScan (Appendix C:9.8) confirmed this by indicating the presence of a sugar binding motif between residues 116-198, but also suggested that this protein contained a signal peptide (amino acids 1-24) as well a predicted transmembrane region (amino acids 7-27). Together these characteristics could suggest this protein is potentially membrane bound or secreted. As with other gene products mentioned previously the protein sequence suggested that this gene product functioned as a
glycosyltransferase when analysed using the BlastP function on NCBI. The organisms with homologous proteins were all members of the ascomycete fungi (E-values = $2e^{-126}$ to $3e^{-26}$) (species and protein identities found on the accompanying CD). These proteins include those annotated as being mannosyltransferase-like proteins, glycosyltransferases, or initiation-specific alpha-1,6-mannosyltransferases. Two species (Schizosaccharomyces pombe and Schizosaccharomyces japonicus) were members of the family Schizosaccharomycetaceae (of the class Schizosaccharomycetes, subphylum Taphrinomycotina). 19 species were members of the subphylum Pezizomycotina. These included members of the families Phaeosphaeriaceae, Leptosphaeriaceae, Onygenaceae, Ajellomycetaceae, Arthrodermataceae, Trichocomaceae and Tuberaceae. The 14 other fungal species were classed as members of the Leotiomyceta [(including members of the families Sclerotiniaceae (of the Leotimycetes), Sordariaceae (order: Sordariales), Chaetomiaceae, Lasiosphaeriaceae, Magnaporthaceae, Ophiostomataceae, Glomerellaceae, Nectriaceae and Clavicipitaceae (all of the Sordariomycetes)]. Interestingly as with other gene products studied in this investigation the homologous proteins (although with no predicted function) that clustered most closely during neighbour joining analysis originated in Sclerotinia sclerotiorum and Botryotinia fuckeliana of the Sclerotiniaceae family (class members, alongside Bgh, of the Leotimycetes). Other organisms with proteins clustering close to this Bgh protein include species of the Leotiomyceta (or more particularly the Sordariomyceta): Glomerella graminicola (with a protein containing a glycosyltransferase sugar-binding region), Verticillium albo-atrum (initiation-specific alpha-1,6-mannosyltransferase), Magnaporthe oryzae, Grommannia clavigera (a beetle-associated fungal pathogen of conifers, alpha-mannosyltransferases; Wang et al., 2010), Neurospora crassa, Sordaria macrospora, Chaetomium globosum, Podospora anserina, Gibberella zeae (anamorph Fusarium graminearum, a necrotrophic pathogen of wheat, barley and maize; Desmond et al., 2008; Wong et al., 2011), Nectria haematococca (synonym: Fusarium solani; Su et al., 2010), Metarhizium anisopliae and Metarhizium acridum (both entomopathogenic fungi with initiation-specific alpha-1,6-mannosyltransferases, Leng et al., 2011; Paula et al., 2011).

**EST Clone Library Identifier D00658.**

BlastN nucleotide analysis suggested that there were three identical copies of this gene within the Bgh genome (all with E-values of 0), suggesting the existence of a paralogous family based on gene multiplication (BluGen annotations: bghT007426000001001;
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bgh00800_mRNA; bght008100000001001). A closely related gene was also found (with an E-value of 2.3 e\(^{-96}\), BluGen annotation: bgh00799_mRNA) (Appendix C:9.8). Two of the three copies had existing gene models whilst one required the formulation of a new gene model. The resulting gene models indicated the existence of four proteins of 123, 210, 228 and 144 residues respectively (the ClustalW alignment is shown in Figure 4-31).

Alignment of the protein sequences (corresponding to the four gene products) revealed a long stretch of identity corresponding to the overlapping regions of sequence. The similarity of sequences for BghT007426000001001, Bgh00800 and BghT008100000001001 was 100 % (both for amino acid identity and property). Notably the sequence of Bgh00799 contained an arginine instead of a glycine (which was found in all other sequences found in all other sequences at position 135). The primary difference between these sequences however was in length, with truncations found at both the N and C termini (Figure 4-31). The highly conserved nature of the sequences, combined with the incomplete stage of Bgh annotation, may point to these sequences actually being encoded by the same gene (but found at this stage on overlapping genome contigs). As such it is possible that this gene may have a lower copy number than suggested by BlastN analysis.

Analysis of the sequences using InterProScan suggested the first protein (19.08 kilodaltons in size) contained a glycosyltransferase motif (residues 1-120) and was a member of a product of a nucleotide diphosphate sugar transferase superfamily (Appendix C: 9.8). The second gene product (estimated at 25.28 kilodaltons) contains a similar motif (amino acids 9-174),
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according to HMMPfam sequence recognition algorithms, whilst the final 23.05 kilodalton protein contains a glycosyltransferase motif (amino acids 3-148). The closely related gene had a predicted protein sequence of 228 amino-acids (equating to a product of 15.99 kilodaltons). The glycosyltransferase signature motif as determined by HMMPanther recognition algorithms of InterScanPro lay between amino acids 2-141. BlastP analysis of the ‘non-redundant protein sequences’ database of NCBI suggested this gene product showed homology to proteins from ascomycetes (49 species), basidiomycetes (5 species) as well as from other members of the Eukaryota from the kindom Animalia (including lancelets, amphibians, rodents, insects, nematodes, sea urchins, and even-toed ungulates (E-values= 9e$^{-58}$ to 2e$^{-41}$). These species are too numerous to be listed here but are found on the accompanying CD alongside the taxonomy lineages of all sequences found during the BlastP analysis. Of the ascomycete fungal species present these include members of the Sclerotiniacea, Hypocreaceae, Clavicipitaceae, Nectriaceae, Glomerellaceae, Magnaporthaceae, Lasiosphaeriaceae, Ophiostomataceae, Pleosporaceae, Leptosphaeriaceae, Ajellomycetaceae, Anthrodermataceae, Onygenaceae, Trichocomaceae, and Tuberaceae. As noted with previous gene products this Bgh protein clustered most closely with Sclerotinia sclerotiorum and Botryotinia fuckeliana. In the case of S. sclerotiorum the protein was identified as a mannose phospho-dolichol synthase. Furthermore the proteins showing greatest homology (based on neighbour joining analysis found on the accompanying CD) all belonged to fungal species of the Sordariomyceta.

In conclusion of the eight genes that underwent bioinformatic analysis 7 were conclusively shown to exist as a single copy within the genome. Another may exist with more than one paralogs, although as noted at this stage this requires further elucidation. Of the genes tested four of them had identifiable functions which will need further testing for confirmation. One (EST C00482) encodes a glucan 1,3-β glucanase. Another (EST C00009) shows a motif characteristic of a MAPK interacting protein. Two others have characteristics indicating their functions as glycosyltransferases. Searches for homologous proteins within other organisms suggested that three proteins contained no known counterparts. All other proteins had homologs within varying numbers of ascomycete species, including organisms that were necrotrophs or hemibiotrophs.
4.5: Discussion

The aim of this chapter was to elucidate the surface dependent nature of gene regulation in *Bgh*. It has been suggested in the previous chapter that differential gene expression (as visualised by different developmental states) occurs during germination on surfaces other than the host. Therefore investigations (Table 4) featured within this chapter aimed to firstly discern the physical effects of selected stimuli (chosen to mimic signals present on the host) on *Bgh* development. This was followed by investigations discerning any modulation of gene expression of selected differentially expressed genes caused by these stimuli. It was hoped that compounds may induce expression to match that seen on the host proving conclusively the role of that stimulus in regulating gene expression.

The behaviour of two compounds in particular (a barley leaf cutin monomer, 16-hydroxyhexadecanoic acid and a barley cuticle aldehyde, 1-hexacosanal) showed, in different studies, potential as inducers by advancing development on glass slides to the appressorial stage. This was further than encountered originally during developmental studies on glass slides in Chapter 3. These compounds were employed alongside a rough barley cuticular extraction and two surfaces offering known levels of hydrophobicity to attempt the induction of selected genes shown to have differential expression at 4 h.p.i. The results encountered are discussed below.

4.5.1: Stimuli screen

In the first preliminary screen a number of compounds (Table 4; Figure 4-3) were tested to define their relative abilities to enhance development on glass slides (Figure 4-4: A graph profiling development of *Bgh* on treated glass surfaces (utilising welled slides) at 24 h.p.i.

Ng = Non germinated; Pgt = Primary (1) germ tube; Sgt = Secondary (2) germ tubes; Esgt/App = Elongating Second Germ Tube, App = Appressoria Formation (symptomatic hooking and swelling); Haus = Formation of haustoria; Mm = More than 2 germ tubes. (Error bars = ± standard error, n=8. Graph constructed from percentages based on averages of 150 spores, derived from 8 independent means of 3 sub-replicates). Those that induced the more advanced stages of development when compared to untreated slides would be selected for further analysis. These stimuli included compounds known to reside within the barley leaf (in the case of 1-hexacosanal, 1-hexacosanol and 16-hydroxyhexadecanoic acid), aliphatic alcohols of known chain length (1-dodecanol) and precursors/related compounds to the
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aldehyde 1-hexosanal (hexacosanoic acid) (Espelie et al., 1979; Reynhardt and Riederer, 1994; Tsuba et al., 2002). Solutions of known concentration were applied by droplet to wells of a slide where evaporation of the solvent was allowed to take place. Spore germination was then assessed.

As a result of this study several compounds were eliminated from further investigations. These included 1-dodecanol and 1-hexacosanol (C₁₂ and C₂₆ aliphatic alcohols respectively). Additionally work with hexacosanoic acid was similarly terminated. All three compounds either induced less germination compared to other stimuli or failed to induce significant numbers of appressoria (the furthest level of development expected on artificial surfaces). The results encountered with the aliphatic alcohols were not unexpected. In their work Tsuba et al., (2002) note that the thin layer chromatography fraction of barley wax equating to primary alcohols led to a combined AGT/APP induction of 1.3 % (in comparison to the fraction associated with aldehydes that led to a combined induction of 50 % approximately) after 24 h.p.i. Initially this is surprising as, according to studies by Reynhardt and Riederer (1994) and Richardson et al., (2005) n-alkanols equate to up to 87 % of the barley leaf wax complement (with C₂₆ compounds forming the majority of this at approximately 82 %). However, as suggested by Jetter et al., (2000) the epicuticular wax layer may act very much as a cloak, covering up more specific host-related intracuticular contents, in a more generic covering of aliphatic compounds. Therefore Bgh may have evolved to rely on the less common aldehydes as more specific signals of host presence.

In the case of 1-dodecanol (Figure 4-3) the compound leading to the lowest germination rates of all compounds tested) the case may be more straightforward. Reynhardt and Riederer (1994) suggest that the majority of barley wax constituents (approximately 90 %) have chain lengths from C₂₀ to C₃₄. The remaining 10 % have chain lengths from C₃₉ to C₅₀. Although, as a caveat, it must be noted their study does utilise barley leaves from the 3-leaf growth stage, and cuticular wax composition may change with age, no note is made of any primary alcohols present within the barley leaf cuticle with a chain length under C₂₀. Bearing in mind Tsuba et al., (2002) suggested that aldehydes of chain lengths other than C₂₆ may act as inhibitors to development it is possible that a similar effect is caused by this “alien” chain length primary alcohol that may not be found to any negligible degree in the barley epidermis.

When considering hexacosanoic acid, an alkanic acid of C₂₆, the lack of appressorial induction may be solely decided by its lack of presence within the barley leaf. Reynhardt and
Riederer (1994) observe that C\textsubscript{26} alkanoic acids may form merely 0.7 \% (by mass) of the cuticular waxes. Although the fungus may detect “rare” components of the leaf to ensure correct host location, the rarity of compounds such as of alkanoic acids of chain length C\textsubscript{26} in the barley leaf does not necessarily mean that they are relied on to ensure advanced development.

The two most successful inducers of appressoria were 16-hydroxyhexadecanoic acid and 1-hexacosanal. It was decided of all stimuli tested these two compounds may show the most easily discerned effect on gene expression. As a result, these compounds were taken forward for further study and their relative effects on development are discussed later.

Although a useful study as it informed later studies there were drawbacks. As each well was surrounded by a plastic edging, the attempted spreading of compounds evenly across the wells was not possible. Therefore as solvent evaporated from the compounds tested, ‘rings’ of compound formed within the selected wells. Thus, this aggregation meant that compound distribution was not uniform and may very well have impeded spore development. This may well have been the case when the under performance of the cuticle extract is considered. If all components from the leaf cuticle had the potential to be removed during the extraction (as suggested by the removal of coleoptile cuticle by Iwamoto et al., 2002; 2007) and then applied to the well accordingly, advanced germination stages should have been expected, especially by 24 h.p.i. However, as very few germ tubes with either AGT or full appressorial characteristics were seen it may be that signals were presented at a non-optimal concentration. Another potential explanation for the unexpected lack of induction by the extract may be that the solvent (and dipping procedure) used may have led to compounds inhibitory to the development of the mildew being extracted from the inside leaf spaces. An uneven presentation of these compounds on the slide well may have led to their accumulation at inhibitory concentrations. Combined with this the extraction via dipping of selected barley leaves was limited to 1 minute in length. This may not have been enough to fully remove all components from the leaf surface meaning certain key components of the leaf surface were not presented to the developing fungus, therefore affecting its development.

4.5.2: Characterisation of \textit{Blumeria graminis} f. sp. \textit{hordei} upon cutin-monomer treated glass slides.

Previous investigations in \textbf{Chapter 3} and in microarray studies conducted by Dr. M. Paramor, utilised slides without the multi-well setup as used in the previous general stimuli
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screen. Therefore to ensure uniformity of technique attempts were made to optimise the coating of these slides in preparation for later expression modulation studies (Table 4). To summarise these results for this study: firstly (as eluded to in the discussion of Chapter 3) it was discovered that a polish was present on these slides (Figure 4-6). This polish could inhibit later stages of development (limiting development to primary germ tube formation, with lower levels of secondary germ tube development and lower levels still of appressorial formation). After cleaning with chromic acid a low level of appressorial formation could be induced (providing similar results to Francis et al., (1996), a study where cutin monomer effect on development was also observed) (Figure 4-7). Even though the manufacturer had been contacted no information regarding this polish could be ascertained. Therefore as it may show unknown reactivity for added compounds, and had a demonstrable effect on germling development, further work focused on application of compounds after this substance had been removed by chromic acid washing.

The second result, already suggested by the general screen, was that the addition of the cutin monomer, 16-hydroxyhexadecanoic acid (Figure 4-3), to glass slides induced the formation of appressorial germ tubes. This supported the earlier findings of the general stimuli screen. This formation is not unexpected as similar work has been performed previously (Francis et al., 1996). Interestingly, formation of the appressorial germ tubes was not observed to the same extent as seen on barley. In later studies appressorial formation equalled 7% compared to approximately 23% in barley at the same time point. Interestingly, appressorial formation does not equal that seen in the study of Francis et al., (1996) although the concentrations used in both their study and this were similar. Both monomers used by Francis et al., induced approximately 29 to 33% appressorial formation amongst germinated conidia (in this study the largest appressorial formation equates to 19.5% of germinated conidia). The monomers, cis-9,10-epoxy-18-hydroxy-stearic acid and 8,16-dihydroxy-palmitic acid, used by Francis et al., (1996) make up 33 and 20%, respectively, of the aromatic cutin monomer composition of the barley leaf (according to Espelie et al., 1979). In comparison previous studies have indicated that 16-hydroxyhexadecanoic acid forms only 1% of the aliphatic cutin monomers complement (Espelie et al., 1979). Ideally all three monomers would be used in expression studies. However, attempts to attain these more prevalent monomers were unsuccessful meaning tests had to rely on the less common, but easier to attain, 16-hydroxyhexadecanoic acid.
The larger presence in the leaf of the monomers used by Francis et al., coupled with their greater inducement of appressoria, suggests there may be greater emphasis placed on certain monomers as signals than others, potentially due to actual availability in the leaf cuticle. There are several complicating factors to this conclusion. Firstly, the hydrophobicity of the glass slides used in the 1996 study was gauged at being approximately 41 degrees which is higher than glass slides used in these experiments. Combined with the longer incubation times this hydrophobicity may generate appressorial levels that are not solely due to the action of the monomers by themselves. Authors have noted an increased chance of germ tubes contacting the surface on more hydrophobic surfaces, which may then lead to later stages of development (Carver et al., 1999; Nielsen et al., 2000). In the study presented here the intended concentration of 16-hydroxyhexadecanoic acid lay within limits assessed by Francis et al., as non-toxic. However this study may have been hampered by an inability to prepare a uniform distribution across the slide surface using the droplet/spreading method. This may result in intrinsic variation of the compound on the slide, leading to poor germination in some cases. As a side-note, the 16-hydroxyhexadecanoic acid-induced appressorial structures appear to differ in morphology (being longer and sometimes thicker) to those normally observed developing on barley. This, again, suggests that other signals (for example, cellulose breakdown products, Pryce Jones et al., 1999) have effects that cumulatively act to “fine-tune” AGT development.

4.5.3: Developmental behaviour at 4 h.p.i. and 16 h.p.i. on treated surfaces

Previous studies had shown the ability of 16-hydroxyhexadecanoic acid and 1-hexacosanal as stimuli with the capability of causing significantly higher levels of appressoria on glass than would be encountered on untreated glass slides. To accompany the testing of their effect on gene expression two more observational studies were completed (Table 4). One was carried out at 4 h.p.i. (Figure 4-8) the same time as which gene expression on both the host and glass slides were seen to differ (Dr. M. Paramor). This would provide morphological data to support gene expression analysis at 4 h.p.i. A second test would also be performed at 16 h.p.i (Figure 4-9). This would use the same conditions as both the 4 h.p.i. developmental study and the gene expression analysis tests (also at 4 h.p.i.). The aim of this 16 h.p.i. test would be to give a truer indicator of differential morphological development (that may not be readily apparent at 4 h.p.i.). In both the 4 h.p.i. and the 16 h.p.i. morphological tests, and also the 4 h.p.i. expression analysis other stimuli as well as 16-hydroxyhexadecanoic acid and 1-
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hexacosanal would be employed. These would include slides treated with a barley cuticle extract, hydrophilic and hydrophobic surfaces of gelbond, wheat and the host, barley.

Although both developmental studies at 4 h.p.i. (Figure 4-8) and 16 h.p.i. (Figure 4-9) are not directly comparable as they were performed at different stages of experimentation, it is possible to make some general statements regarding trends of development over the two time periods. As with previous developmental studies as time increased larger numbers of later developmental stages were encountered for the surfaces tested. Furthermore in the case of stimuli-treated slides this behaviour was often different (and more advanced) than the behaviour on slides not pre-cleaned with chromic acid. This non-cleaned type of slide had been used in the original expression studies by Dr. M. Paramor so it seemed reasonable that they be retained for assessment of treatment effectiveness, even if part of the developmental behaviour associated with them could be explained by polish. A more accurate form of negative control for treated slides was the employment of slides pre-cleaned with chromic acid. In many cases treatment with stimuli caused more advanced development compared to these pre-cleaned slides as well.

Although not necessarily full matured appressoria, the tubes classified as AGT at 4 h.p.i. had begun to show elongation and swelling. These structures were most common on the plant surfaces. This was not unexpected. As seen previously in Chapter 3 (Figure 3-3; Figure 3-4) and as demonstrated again in this chapter as part of the study of development at 4 h.p.i. (Figure 4-8), wheat may also induce high levels of appressorial development, but not equal to that seen in barley. Reasons for this may include some form of inhibition that limits later stages of development. The most prevalent aldehyde present in the wheat leaf surface is octacosanal (a C28 compound) with C26 and C30 aldehydes being a minor component of the aldehyde fraction (Reisige et al., 2006). Although it was suggested by Tsuba et al., (2002) that aldehydes of alternate chain length could act as inhibitors, all aldehydes of specific chain lengths contained in wheat have similar counterparts within the aldehyde fraction of the barley epicuticular waxes (Tsuba et al., 2002; Reisige et al., 2006). Therefore in this case the potential for inhibition seems unlikely, although a lack of inducing C26 aldehydes would go someway to explaining the imperfect induction. Additionally, work by Kosman (2003) suggests that the C16 fatty acid components of the cutin monomer are greater in proportion to the C18-type monomers. Again non-optimal presentation of these monomers, although
allowing advanced development of appressoria, may not support host-like levels of development.

Interestingly, according to chemical analysis as performed by Koch et al., (2006) the most prevalent primary alcohol present in the wheat cultivar ‘Naturstar’ was octacosan-1-ol (a C\textsubscript{28} compound prevalent at 89 \%, whilst the C\textsubscript{26} alcohols were only present at 3.5 \%). According to some authors this C\textsubscript{28} alcohol has the capacity to induce appressorium formation in \textit{Bgh} (Reisige et al., 2006). Analysis by Reynhardt and Riederer (1994) and Richardson et al., (2005) suggest that similar C\textsubscript{28} alcohols make up only 1 \% of the barley wax fraction. If this was the case the prevalence of the alcohol in wheat would suggest it had a source of greater induction than barley, although numbers in this study do not seem to support this. Maybe this alcohol is a minor signal in regards to appressorial formation on barley. Further work needs to elucidate this.

According to Feng et al., (2009), of the compounds tested for their ability to induce germination and appressorium induction of the wheat specific \textit{forma specialis}, \textit{B. graminis} f. \textit{sp. tritici}, it was the fatty alkane of the wheat leaf wax fraction which led to most induction. These alkanes (C\textsubscript{12}, C\textsubscript{22}, C\textsubscript{24} and C\textsubscript{25}) do not appear to be present within the barley leaf epicuticular wax fraction (Reynhardt and Riederer, 1994). This would add support for the theory that these obligate pathogens may place at least some of their stimuli-requirements on compounds that are specific to the host plant, or at least rare in others, to ensure host specificity and correct development.

These results for the plant surfaces are not unexpected. Both barley and wheat leaves contain multiple signal combinations that \textit{Bgh} uses to spur its development. Attempts were made to mimic these signal combinations by treating glass slides with a barley cuticle extract. By comparing both the 4 h.p.i. and 16 h.p.i. morphological studies (\textbf{Figure 4-8, Figure 4-9}) (it is apparent that as time increases that rates of secondary germ tube and appressorial induction increase. Furthermore as length of incubation increases and development progresses spores with earlier forms of development decrease, just as on the plant surfaces. In this study, to improve on the technique used in the earlier general screen leaf dipping time in the extraction solvent was extended and repeated. It was hoped that by doing more cuticular components would be harvested. However appressorial formation as induced by the extract is still well below that seen during development on barley and wheat, although significantly larger than
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seen on the either pre-cleaned or non cleaned glass slides. This has been seen before by different authors (Tsuba et al., 2002; Zabka, 2008). It seems therefore that there are extra signals present in the leaf not extracted by solvent which account for the extra development on the plants surfaces. Primary and secondary plant metabolites may be transported through the cuticle. Harder to discover than the permanently present counterparts these compounds may also act as signals during development and may act as extra sources for induction that lead to higher levels of development on the host plant and its wheat relative.

Jetter et al., (2000) suggest only the outermost layers of the cuticle would be relevant for host recognition by fungi (with the epicuticular wax layers taking priority for initial induction as it is that layer upon which the fungus lands). This would suggest the mixing of epicuticular and intra-cuticular layers (as occurred during extraction) may detrimentally affect a very synchronous developmental program, even if qualitatively both layers were very similar. Another reason for lower induction was the potential for the solvent to access internal leaf spaces, leading the extract to “present” inhibitory compounds to the fungus. Jetter et al., (2000) found this not to be the case with leaves of Prunus laurocerasus but this may be a problem when considering barley leaves. The spurious presentation of signals and inhibitors may lead to confused development, as evidenced by a high number of longer than average germ tubes and the number of spores with more than two tubes present on slides treated with extract at 16 h.p.i.

Other slides in this study were then treated with individual components of the cuticle. It was hoped that these individual stimuli would lead to differences in gene expression that could be directly attributed to that presented signal. On plant surfaces, and with the cuticular extract, this would not be not the case.

Of all of the individual treatments to glass slides, the main stimuli seen to induce development and hence affect gene expression, was 1-hexacosanal, an aldehyde. This induction was significantly greater than that seen on slides treated with the cuticular extract. According to Zabka et al., (2008) the aldehyde fraction of barley cultivar ‘bonus’ secondary leaves form approximately 6 % of the wax complement. At 4 h.p.i. large numbers of both the primary and secondary germ tubes were stimulated, in both cases more than seen on untreated glass slides (Figure 4-8). By 16 h.p.i. many of these spores had developed appressoria, which supports the findings of both Tsuba et al., (2002) and Zabka et al., (2008)
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(Figure 4-9). During this study 0.5 µg cm\(^{-2}\) was used based on the findings of Tsuba et al., (2002) that suggested concentrations higher than this led to a decreased frequency of appressorial formation. As noted by Tsuba et al., (2002) this compound may be taken up by the conidia. This builds on the suggestion of Nielsen et al., (2000) who reported the uptake of low molecular weight compounds by the conidia. The aldehyde also has the chance for uptake via the PGT. At this time point it is hard to say whether solely the conidium was responsible for the induction or if it was a combination of uptake by both the conidium and germ tube. To find this out further studies would be needed to compare numbers of primary germ tubes at 30 to 60 minutes, with the expectation that if the conidium was also sensing the aldehyde then numbers would be significantly higher than controls.

Glass slides treated with the cutin monomer, 16-hydroxyhexadecanoic acid, behaved as has been described previously, showing an ability to induce higher levels of advanced development (PGT, SGT) compared to glass slide controls. The 4 h.p.i screen (Figure 4-8) appeared to be following expected trends with this compound inducing higher levels of primary germ tubes when compared to those induced by the untreated slides. However, during the 16 h.p.i. screen development enhancement (at this time point normally appressorial germ tubes are apparent) seemed muted resembling that of cleaned glass slides. This contradicts the earlier testing on clean and non cleaned glass slides. Such behaviour may be explained by an experimental error during the application of monomer to the slides, combined with non-uniform distribution of the sample during application.

Alongside glass surfaces presenting varying stimuli, both sides of gelbond (hydrophilic and hydrophobic) were used as surfaces providing known levels of hydrophobicity. Studies by Carver et al., (1999) and Wright et al., (2000) suggested surfaces with higher hydrophobicity permit greater ECM release and also better germ tube to surface contact. Although the extra ECM release suggests a response to a non-optimal surface this result would suggest that the spore is responding to this property and altering gene expression accordingly. However, as can be seen from the profiles for 4 h.p.i and 16 h.p.i. (Figure 4-8, Figure 4-9), in this experiment the hydrophilic gelbond seemed to provide a more inductive surface than its hydrophobic counterpart. At both time points, germination, primary germ tube development and secondary germ tube development was higher on the hydrophilic gelbond than its opposite. Furthermore the induction of these stages often appear higher on the hydrophilic gelbond than as seen on the chromic acid treated glass slides. This latter factor would suggest
that the response seen in this experiment is not solely due to the spore encountering physical contact: which some authors (e.g. Wright et al., 2000) believe is partly responsible for germ tube emergence.

Although the level of hydrophobicity is higher on the hydrophobic side of gelbond compared to glass slides it is noticeable that developmental stages are often lower than (or equal to, in the case of appressoria at 16 h.p.i., (Figure 4-9) those seen for un-treated glass slides. In this instance it would suggest that increased hydrophobicity does not play as important a role in development as previous authors have suggested. However, the presence of significant numbers of spores with branched or long germ tubes at 16 h.p.i. for both sides of gelbond suggested the presence of some form of inhibitor/interfering compound on the surface of the gelbond.

In summary, although different compounds and surfaces induced different proportions of Bgh development, none of the incubation regimes caused development to match that seen on the host. This was expected and supports the theory that the fungus must sense a combination of signals (or ‘Host Associated Surface Patterns’) to complement development (Reisige et al., 2006). This differential morphological response to these stimuli may make it easier to understand the role of each in the surface dependent nature of gene expression. Wheat leaves induce high levels of advanced development because of their apparent surface stimuli complement. A barley cuticular extract although also inducing appressorial development did not match the abilities of the host. This suggests the presence of signals in the living host leaf that cannot be extracted by the solvent. Also individual cuticular components such as cutin monomers can be themselves induce developmental progression. In the case of one signal in particular, 1-hexacosanal, may even cause induction of appressoria to levels higher than the cuticular extract. This demonstrates the importance of this stimulus in influencing Bgh gene expression.

### 4.5.4: Analysis of surface hydrophobicity

To better understand the nature of the stimuli presented in the previously discussed studies to Bgh, and their affects on development, attempts were made to measure the hydrophobicity of surfaces upon which the fungus was incubated (Table 4). In this study two methods were used to discern hydrophobicity. A simple, but robust, technique featured the measuring of
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crystal violet droplet diameter (modified from methods used by Hill et al., 1980; Selabuurlage et al., 1991) (Figure 4-10), whilst the other focused on the now more common technique of measuring advancing contact angles of water droplets placed on substrates (Figure 4-11). Although performed at different stages of the experimentation both techniques supported the majority of conclusions of the other for the surfaces involved. In the two cases where such support was not provided (extract treated slides and pre-cleaned slides compared to non pre-cleaned slides) it appeared that intrinsic variation (due to technical variation of application/treatment to slides) was the cause.

Hydrophobicity is an effect of chemical properties and geometrical structural components (Lee et al., 2006). To summarise the results seen (Figure 4-12), all plant surfaces were very hydrophobic (i.e. they showed contact angles above 90°). The so-called hydrophobic side of gelbond, although showing greater hydrophobicity than other surfaces is still classed as hydrophilic with a contact angle under 90°. All treated slides and the hydrophilic side of gelbond were very hydrophilic (classed as having contact angles below 90°, Koch et al., 2008) showing contact angles in the region of 20°. Glass slides that had not been pre-cleaned had contact angles which were super-hydrophilic in nature (< 20°, Koch et al., 2008).

It is unsurprising that plant surfaces display such hydrophobicity. Not only is it as a result of cuticular wax components but also leaf cell architecture. These hydrophobic properties allow the leaf to reduce free water on the surface, allow surface cleaning and provide a more inhospitable environment to phytopathogens (Muller and Riederer, 2005). However, it appears that pathogens have evolved to respond to this property and may use it as a stimulus for growth.

Regarding the assessment of both leaf surfaces from the barley cultivar ‘Golden Promise’, the technique focused on placing water droplets (or crystal violet droplets) either side of the leaf midrib with care being taken not to abut the main vein. This followed observations of Wisniewska et al., (2003) who observed that areas alongside veins could give smaller contact angles. The results (Figure 4-12) for the barley primary leaf seem to match well the measurements gathered by Wisniewska et al., of 129° and 118° for the adaxial and abaxial leaf surface: although the authors themselves stipulate that their leaves were taken from barley straw (of unknown cultivar) after harvesting and wax content can change with age. Data for 7-day-old Golden Promise leaves collected by Francis et al., (1996) presented a
smaller contact angle of 104°, although experimental details for that investigation are sparse. Data collected by Zabka et al., (2008) for leaves from a different cultivar, ‘Bonus’, suggested contact angles of approximately 140° were not that far removed from the readings recorded for these leaves, approximately 130°. As hydrophobicity is a factor of both chemical and structural facets this difference may be due to differing cultivar architecture and wax crystal morphology.

During this study wheat (cultivar ‘Riband’) presented a very similar level of hydrophobicity to barley (Figure 4-12). This would suggest that behavioural differences, at least with initial contact before ECM-mediated degradation of the surface, would probably be down to a chemical stimulus rather than a physical one.

Slides were also treated with a barley cuticular extract. It was hoped that this extract would allow the enhancement of glass slides with many of the signals present on the barley leaf surface. Earlier work had showed an ability to induce appressorial formation by 16 h.p.i., suggesting that it did possess such inductive stimuli (Figure 4-9). However, measurements of the hydrophobicity of cuticular extract treated slides suggested, surprisingly, that the slides did not differ from those that were only pre-cleaned with chromic acid. They were therefore very hydrophilic in comparison to either of the barley leaf surfaces. Consequently, it appears that the signals provided by this extract would solely be biological in nature and that enhanced hydrophobicity would not be one of them. This may explain why, although inducing appressoria, the levels do not match that of the host.

Although some authors (for example Neinhuis et al., 2001) note that wax crystals are self-assembling (Koch et al., 2006) were not able to attain wax platelet formation on glass slides, rather irregular layers and aggregates were formed. Furthermore in that study, slides were left for 3 days before use to allow for full solvent evaporation and crystallisation. In the morphological and gene expression studies presented in this thesis, wax structure resembling that on the leaf surface is unlikely to be present after reapplication of the extract to the slide as the disordered layering of the applied compound would not form the ordered base some authors recommend as necessary for full wax re-crystallisation. Furthermore, Koch et al., (2006) pointedly state that a non-polar substrate with a crystalline structure is optimal for wax platelet formation and that glass slides are polar surfaces. Combined with a lack of micro-structures and architecture as present on the barley leaf epidermal cells, as well as the
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disordered presence of compounds from within the intracuticular layer, these factors may explain the lack of change seen regarding the hydrophobicity of cuticular extract treated slides. Furthermore this lack of hydrophobicity, combined with the disordered signals mentioned earlier, would go some way to explaining both the reduced appressorial induction and the presence of germ tubes that appear longer than expected or spores with more than two germ tubes which indicates that contact may not have been made with the surface below.

Interestingly, both the addition of the 16-hydroxyhexadecanoic acid and the aldehyde, 1-hexacosanal, to glass slides also did not significantly alter hydrophobicity in comparison to glass slide controls or to each other. Both induce appressorial development, although in these studies 1-hexacosanal appears the greater inducer, even more so than a barley cuticular extract. It is possible that further alteration of the compounds when applied to the slide by the ECM did not occur as both were already in their monomeric form. If this was the case hydrophobicity could remain the same after conidial germination and any induction of hydrophobicity would be based on a purely chemical structure of the signal rather than changes in hydrophobicity.

Although not seen in this study (Figure 4-12), possibly due to the lower concentration used, Zabka et al., (2008) found that different concentrations of 1-hexacosanal could alter hydrophobicity. This would suggest the aldehyde offers both a signal based on its chemical structure and one on its hydrophobic nature. Unlike in studies of Zabka et al., (2008) where 0.15 μg cm⁻² led to a contact angle of 83°, in this study 0.5 μg cm⁻² of 1-hexacosanal treated slides exhibited far lower contact angles of approximately 20°. Experimental procedure may explain this discrepancy. Apart from their use of silanized-slides (i.e. slides treated to increase hydrophobicity to roughly 40°) the authors waited 14 days before usage of the slides (to allow full evaporation of the solvent). In this study slides were utilised after 1 night evaporation time. The extra time employed by Zabka et al., as well as their use of spraying rather than evaporation/spreading, may have allowed better re-crystallisation and contributed to the increased hydrophobicity. The results of Zabka et al., (2008) suggest the aldehyde offers both a signal based on its chemical structure and one on its hydrophobic properties. This would explain role of 1-hexacosanal as such a powerful inducer of development. As support to this the aldehyde seems to be present in both the barley intra- and the epicuticular wax layer, a surface ‘with hydrophobicity as its most important physiochemical property’
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(Zabka et al., 2008). If correct their results would also suggest that in this current study the aldehyde was not employed to its full effect.

Zabka et al., (2008) note that with contact angles (induced by 1-hexacosanal) of less than 80°, after 24 hour incubation, there was a reduction in germination to only 34 % (whilst contact angles higher than 80° led to approximately 75 % germination), being similar to control slides. In this experiment a germination rate of 38 % after 16 hours incubation was seen, similar to the prediction of Zabka et al., although this may have increased somewhat with an equivalent incubation time. However, the results presented in this study seem to contradict the statement of Zabka et al., in the regard that ‘at contact angles smaller than 80° neither germination nor app formation is stimulated by hexacosanal’. At 4 h.p.i. germination was significantly different to that seen on cleaned glass slides (although not by 16 h.p.i.) and furthermore significant appressorial induction was also seen to be induced at 16 h.p.i.

As with the application of 1-hexacosanal, the addition of 16-hydroxyhexadecanoic acid did not alter the hydrophobicity of the glass slides significantly. This monomer may not be present in the epicuticular wax layer, primarily residing within the cuticle itself Bargel et al., (2006). Combined with its naturally low presence within the host, the signal it provides may just depend on its chemical structure, rather than any hydrophobic properties, and its potential for uptake by the developing germling. If so this would also support Nielsen et al., (2000) who suggested that both the conidium and the PGT may uptake low molecular weight anionic compounds, similar to cutin monomers. This lack of hydrophobicity, yet also the ability to increase the formation of primary and secondary germ tubes, would add evidence to the observations of Wright et al., (2000) who argued PGT surface targeting had both a non-specific and a specific nature to it, the latter being based on host specific signals that refined targeting.

In comparison to plant surfaces, the presentation of a hydrophilic surface, such as the very hydrophilic nature of the un-cleaned glass, and the hydrophilic gelbond, may lead to incomplete or aberrant development. Such hydrophilicity could explain the large number of spores with more than two germ tubes. Previous authors such as Carver et al., (1999) and Nielsen et al. (2000) have noticed reduced surface contact of germ tubes on substrata which are less hydrophobic than others. This reduced contact would lead to turn would lead to further germ tube emergence as the fungus attempts to detect the surface.
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The cleaned glass used in this study was approximately 10° more hydrophilic than slides used in a study, released after the cessation of experimentation of this work, by Zabka et al., (2008). That study also featured observations of *Blumeria* development at different hydrophobicities. Interestingly, germination rates were nearly identical even though that study had a longer incubation time of 24 hours. From these results it could be speculated that that there is a set limit of germination (and later stages of development) based solely on levels of hydrophobicity. Zabka et al., demonstrated that very high levels of hydrophobicity (94° and 111°) on variants of the tetrafluoroethylene copolymer surfaces could stimulate 59 % and 66 % germination respectively (with greater appressorial differentiation). Although the probability of the fungus gaining other signals from these surfaces is unknown, future work could focus on the use of glass slides with varying degrees of silanization (to create a gradient of hydrophobicity) to see if this germination rate increases linearly or is in discrete steps, which although unlikely may suggest alternate gene regulation systems activating. Furthermore, as noted by Zabka et al., (2008) themselves, testing of artificial surfaces providing contact angles greater than 111° would be useful to see just how close germination rates get to matching that seen on the host surfaces. This would then determine how much reliance the germinating spores put on the chemical characteristics of stimuli rather than physical characteristics.

To recap, this experiment suggested all glass slides, whether treated with combinations of stimuli or with individual components, did not seem to present increased hydrophobicity to the fungus. In comparison plant surfaces, and to a degree the hydrophobic side of gelbond, offered the additional stimuli of increased hydrophobicity.

4.5.5: Effect of stimuli on gene differentially expressed at 4 h.p.i.

Previous studies had shown that different stimuli in the form of cuticle components or surfaces presenting different levels of hydrophobicity could induce varying levels of morphological development (for example Figure 4-8 and Figure 4-9). This development will be the result of alterations of gene expression. As such these previous studies have highlighted the surface-dependent nature of gene regulation in barley powdery mildew. At this stage the signal transduction pathways that lead to these alternate levels of expression are not well understood. As a result this study (Table 4) aimed to determine what affect these
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signals may have had on expression of specific genes seen to be differentially expressed at 4 h.p.i. It was hoped that growth on surfaces with specific hydrophobicity or biological stimuli will then cause a recognisable governance of expression. Ideally, this stimulation would then lead genes to mimic expression levels commonly associated with germination on barley and define specific regulatory signals.

To recap, by 4 h.p.i. during development on barley and wheat, a primary germ tube will have emerged (sensed the underlying surface) and in many cases a secondary germ tube, which will go on to become an appressorial germ tube, will begin to emerge (Figure 3-3, Figure 3-4, Figure 4-8). On glass (specifically non-cleaned, non-treated glass) at this time point the majority of spores, if they have germinated will have formed primary germ tubes, and in some cases a second germ tube. Often these germ tubes fail to contact the surface and sense inducing compounds. They therefore fail to develop appressorial characteristics and remain similar in appearance to the primary germ tube, resulting in the spore to attempt further germ tube formation. A similar statement could be made about growth on hydrophobic and hydrophilic gelbond. All of these surfaces would provide mechano-sensory input on spore landing. However differences exist including levels of hydrophobicity and the presence (or lack) of cuticle located compounds.

In this investigation out of the 18 genes assessed, 8 had functions which were not initially identifiable (two genes had their functions identified during the course of this investigation). Of the 12 with identifiable functions, their identity/roles included encoding 1,3-ß glucanases (potentially involved in cell wall modification/development; Kim et al., 2001), H3 histones (used in nucleic acid structure; Graessle et al., 2001), an α-1,6-mannosyltransferase (involved in modifying cell wall proteins; Bates et al., 2006), an adenosylhomocysteinase (involved in amino acid metabolism), membrane-localised transporters, a catalytic sub-unit for protein kinase A, a mitochondrial glycerol-3-phosphate dehydrogenase (an important component of carbohydrate and lipid metabolic processes), a glycolsyltransferase possibly involved in protein regulation, a MAPK interacting protein (potentially involved in morphological development, Szeto et al., 2007), and a protein disulphide isomerase (involved in protein folding in the endoplasmic reticulum).

According to previous microarray studies, it is apparent for the majority of genes analysed (which had a known identity/function), that at 4 h.p.i. expression levels were higher during
germination on plant surfaces than observed during development on glass. These included genes involved in cell wall modification, proteins with roles related to oxidative phosphorylation, proteins with similarities to transporters, proteins involved in cAMP signalling and enzymes required to aid protein folding. For those genes of unknown function, 3 had raised expression during development on plant surfaces. Both RT-qPCR and microarray analysis do not account for post-translational processing of mRNA and so how much protein is actually activated, and when, remains unknown. However, as these genes are clearly more active on surfaces where development may reach its most complex state, it suggests the spores are preparing not just for appressorial formation, but appressorial maturation and beyond. Such development requires conidial wall modification, as well as preparing for the formation of a separate nucleus in the second, septate germ tube. This second tube needs to become robust as, during maturation, it will undergo large osmotic stresses during penetration. Hall and Gurr (2000) observed that cAMP-dependent PKA signalling was required for the initial development of this germ tube and so enhanced expression is seen on surfaces where an appressorial germ tube is more likely to emerge.

It is notable that in many cases, for example as seen in the expression of EST C00148 (Figure 4-13), that expression of genes in wheat (as assessed by microarray analysis) although similar to that seen induced by barley, is not identical. This supports the morphological observations that suggest that wheat, although inducing many of the pre-penetration stages of Bgh development that barley induces, does so at lower levels. This lesser induction may be due to an imperfect stimulation of the genes controlling development. With the most notable developmental difference between these two surfaces being penetration and haustorial formation, at approximately 16 h.p.i. it would be at this time that differences would become more pronounced; for example as seen in the expression profile of the CMEG related to D01260 (encoding an adenosylhomocysteinase). With hydrophobicity of primary leaf surfaces being statistically equal, it would suggest that this differential expression may be based on the imperfect chemical stimulus rather than a physiochemical property. Furthermore this imperfect induction would suggest gene expression, at least for many of the genes assessed in this study, may respond in a “quantitative/additive fashion” rather than simply “on/off”. This may mean that the simple presentation of any amount of one signal is not enough to cause expression to match that at 4 h.p.i. on the host. Rather, in the case of genes, for example in the case of EST D00972 (Figure 4-20), it is a certain amount of stimulus or a combination of separate stimuli which must be met before expression levels
mimic the host. Consequently interpreting the contributions of signals to gene regulation may be difficult, especially if their contribution is minor.

Previous microarray data suggested in the case of untreated glass, that 2 of these differentially expressed genes of known function (encoding an α-1,6-mannosyltransferase and an adenosylhomocysteinase), showed lower expression in germlings on the host (and wheat) than on glass. The first is involved transferring an α-D-mannosyl residue to lipid-linked oligosaccharides found in cell surface mannoproteins (in fungi such as Candida albicans, Bates et al., 2006), whilst the second is involved in converting S-adenosylhomocysteine to homocysteine, an amino acid. Additionally, of the genes of unknown function 3 were up-regulated on the glass surface. These may be an indicator of spores trying to re-synthesise key products of the ECM, known to be released in greater quantity on artificial surfaces (Zhang et al., 2005), whilst also “re-enforcing” the interface between the conidial wall and the surface in order to ensure adhesion to a very hydrophilic surface and, possibly, sensing. Also they may indicate re-synthesising of proteins required for new, hopefully successful, germ tube emergence. The lack of induction of other genes would suggest that their functions are either not required at this time during development, or that enzyme/protein stores present in conidia at this time are sufficient to deal with requirements at this developmental stage.

However, when RT-qPCR data collected during this study was analysed to discern significant differences, multiple returns (although suggesting visual trends) showed no significant difference in expression from that encountered during development on either barley or non-treated glass slides (of the type used in previous microarray work). This was very problematic as discerning the effects of the incubation regimes with any statistical rigour became very difficult.

Three ESTs (ESTs D00944, C0009, C01417, Figure 4-21, Figure 4-24, Figure 4-27 respectively) did provide returns resembling microarray data. Analysis of EST D00994 (Figure 4-21) suggested that barley induced significantly more expression of this gene than the non-cleaned glass. All other surfaces induced similar activity on both the plant and glass surfaces. This may suggest that this gene is downstream of a convergence point in the signalling pathways. Therefore multiple different signals (possibly based on hydrophobicity and cuticle products) may lead to its activation. Although not statistically proven, visual
trends suggest that this gene is not necessarily stimulated by the aldehyde 1-hexacosanal, but more by the presence of the cutin monomer (since it appeared expression on cutin monomer treated slides was higher than that seen on slides treated with the aldehyde). If this is the case, it may explain why the expression in spores developing on the aldehyde treated slides was less (but not significantly so) than seen in spores developing on barley (where cutin monomers are at their optimum concentration for induction). As for a function for this gene product, a 330 amino acid protein predicted to weigh 38.19 kilodaltons, no homologous proteins or specific motifs (only a signal peptide implying translocation or potential secretion) could be detected. Further work, in the form of gene disruption (discussed with alternatives in later chapters) or proteomics experiments including overexpression of this gene product in bacteria and structural analysis would be needed to determine the potential role of this protein in Bgh development.

RT-qPCR data for EST C00009 (Figure 4-24) suggested that rather than being regulated by hydrophobicity (where both barley and wheat appear identical), the gene is instead responding to chemical stimuli. The fact that wheat-based development shows reduced expression may suggest that some form of wheat defence is leading to gene repression or that part of the wheat cuticle is responsible. The suggestion that 1-hexacosanal may lead to a similar reduction implies aldehydes in the epicuticular wax may be involved, although the higher expression in barley (where 1-hexacosanal is also present) could suggest some compound is lifting that repression during development on the host. The host-like induction shown during germling development on non-clean and cleaned glass, as well as both sides of gelbond, do hinder this interpretation since no barley derived compounds exist on these surfaces. As such, further work in the form of slides treated with a greater range of stimuli (possibly derived from the wheat cuticle) needs to be performed to elucidate this result. Furthermore, this level of host-like induction seen on artificial surfaces also calls into question the validity of the reference gene chosen for this work (discussed below).

BlastP analysis suggested the gene product showed similarity to a MAPK interacting protein (Szeto et al., 2007), and contained a transmembrane region and a signal peptide implying that it may be membrane bound. Consequently, it is tempting to speculate that this protein has some role in the Bgh outer membrane, where it interacts with the MAPK signalling pathway. Components of the MAPK signalling pathway may assemble into complexes which are coordinated by regulatory proteins such as scaffold proteins (Whitmarsh, 2006). It may be that
this protein helps regulate the activity and behaviour of a MAPK pathway in some fashion by helping subcellular localisation or by transducing signals from stimuli receptors. However, what exactly that MAPK pathway could be controlling, and what the protein interacts with, is unknown at this time. Nonetheless as noted in the introduction to this thesis, Kinane and Oliver (2003) suggest that the MAPK pathway may have a role in AGT elongation (and APP formation) based on the observation that MAPK activity (as determined by phosphorylation assay) rose to a maximum prior to AGT and APP formation (between 2 and 8 h.p.i.). Notably the microarray data suggest that a maximum in EST C00009 expression is seen at 4 h.p.i., the time when MAPK activity would be at its highest, and hence when the need for regulatory proteins would also be high. A yeast 2-hybrid screen utilising the products of, for instance, the Bgh mpk1 and mpk2 MAPK genes (mentioned in section 1.2.5) could be carried out to see if proteins directly interacted with the gene product of EST C00009. If the traditional yeast two hybrid screen is not an option, due to the potential for this interacting protein being membrane bound, a split-ubiquitin yeast two-hybrid analysis may be considered (Stagljar et al., 1998). From this first step consecutive experiments could aim to investigate the interacting partners of those proteins that were isolated and delineate the regulatory role of the protein in Bgh development.

It would be interesting to see where the RNA transcript was localised during development (for example near the site of germ tube emergence or in the developing germ tube). This would go further in confirming the role of this interacting protein in germ tube development. In their study of the Le.DRMIP MAPK interacting protein in Lentinula edodes, Szeto et al., (2007) used RNA-RNA in situ hybridisation (via ultra-thin cryosectioning and antisense probes) to localise the transcript within different developmental stages of the fungus. Although the author knows of no similar attempt in Bgh, it is possible the fixation process could be adapted for the Bgh germling and prove useful not just for the localisation of this transcript but others.

In the last of the ESTs where differences between the host and glass-based development was provable (EST clone C01417, Figure 4-27), all artificial surfaces induced significantly lower expression than barley (although wheat did not). Furthermore, in the case of untreated glass slides (either pre-cleaned or not) and those treated with cuticular extract expression was significantly less than that stimulated by wheat. In conclusion it would seem in the case of this EST treatments either chemical or physiochemical in nature failed to lead to host-like
expression on artificial surfaces. Additionally the hydrophobic side of gelbond was also significantly different from barley. Although some form of inhibitor present may be biasing results, it is also possible that hydrophobicity is not a cue for the stimulation of this gene. Furthermore the lack of stimulation by both extract and aldehyde (and potentially cutin monomer) suggested there is some unknown stimulus (maybe a primary or secondary metabolite) or C₁₈ cutin monomer present in the living cuticle that is stimulating growth. Although no catalytic domains of known function could be identified within this protein a potential signal peptide as well as a transmembrane region were. Together these characteristics could suggest that the protein was membrane-associated (and potentially secreted). Unlike other genes of unknown function analysed within this thesis, homologous proteins within other organisms were discovered. In particular two appressoria forming organisms contain proteins that clustered with this Bgh protein during neighbour joining phylogeny analysis were Colletotrichum graminicola and Magnaporthe oryzae (Sexton et al., 2006). These fungi are relatively easy (when compared to an obligate biotroph such as Bgh) to store, transform and perform gene disruption within (Munch et al., 2011, and see later within this thesis). Furthermore the C. graminicola strain M1.001 has had its genome sequenced, as has M. oryzae. (http://www.broadinstitute.org/annotation/genome/colletotrichum_group/MultiHome.html; date accessed: 25/3/2011). Together these fungi provide an avenue for future work aimed at ascertaining what function this Bgh protein may have during the early developmental cycle. Ideally experiments would firstly focus on the disruption of the homologous gene within either of these organisms. The resultant phenotype could be ascertained potentially giving a clue as to this proteins role during infection. Following successful disruption, attempts could then be made to complement these mutants with the homologous protein from Bgh. Hopefully a wild-type phenotype would be restored. If so these organisms would then be suitable for over-expression studies or in situ localisation studies that would offer further clues to its role and other proteins it interacts with.

Several ESTs provided RT-qPCR data that suggested differences between mRNA abundance on surfaces other than barley, wheat and non-cleaned glass. These included EST D00189, C00606, D00658, D00881 and C00482 (Figure 4-14, Figure 4-17, Figure 4-19, Figure 4-22, Figure 4-25 respectively). The gene relating to EST D00189 suggested a significantly higher gene expression on chromic acid treated glass slides in comparison to that of spores developing on cleaned slides treated with the aldehyde 1-hexacosanal. Similarly, there was a
significant difference between gene expression from spores developing on aldehyde-treated slides and hydrophilic gelbond (where a higher level of expression was encountered). In addition to this, higher gene expression (albeit not significantly higher) was obtained in spores germinating on the hydrophobic side of gelbond compared to slides treated with the aldehyde. Since both the treated and cleaned glass slides do not differ significantly in hydrophobicity these results suggest that the presence of the aldehyde is the cause of this differential expression (rather than hydrophobicity). Such a conclusion is supported by the behaviour of spores on the hydrophobic side of gelbond. This side is significantly greater in hydrophobicity than either the cleaned slides or its hydrophilic counterpart and yet spores developing on this surface do not differ in expression from either. Visual trends suggested higher expression on cleaned slides and the hydrophilic gelbond surface when compared to barley and wheat (although this was not statistically proven). Both plant surfaces have a higher hydrophobicity than all other surfaces as well as acting as a source of cutin monomers and epicuticular waxes. So again it is possible that the aldehyde causes repression. Although indicating this gene exists as a single copy within the genome bioinformatic analysis failed to reveal any information regarding function, either directly through the analysis of the predicted protein sequence with motif-specific algorithms (InterProScan) or by comparison with known proteins from other organisms. In many cases of unknown gene function disruption followed by phenotypic analysis and complementation would be the obvious choice for analysis. However a lack of organisms with similar homology, combined with the obligacy of \textit{Bgh} means that transformation is not possible, and a different approach must be undertaken. As mentioned previously a yeast-hybrid screen where this protein acts as a bait for detecting interactors may be the way forward.

EST C00606 (\textbf{Figure 4-17}) shows significantly lower expression during development on wheat than it does on non-cleaned glass. This matches trends suggested by the microarray data. With higher resolution a similar difference between the glass and barley may have been confirmed. Visible trends suggest that slides treated with cutin monomers and the cuticle extract have a lower expression than other surfaces suggesting, although needing further work to confirm, that cutin monomers may be the cause of this lower expression. As with EST D00189 this single copy gene encodes a protein whose function was not discernable using bioinformatic analysis. As such no obvious situation can be created that links this gene product to \textit{Bgh} development.
The expression of the product of EST D00881 (a glycosyltransferase with similarities to an α-1,6-mannosyltransferase, Figure 4-22) suggests that during development on wheat lower expression is encountered than seen on cleaned glass and both sides of gelbond. Visual trends, furthermore, suggest that slides treated with extract, aldehyde and cutin monomer may also lead to reduced expression (as seen on barley in the microarray analysis data and as hinted at visually in the data collected during these experiments). That may suggest this gene is downstream from independent receptors that sense aldehydes and cutin monomers, which lead to its reduced expression. In silico analysis using InterProScan combined with the discovery of homologous proteins from other species annotated as α-1,6-mannosyltransferases adds further evidence to this gene product being involved in protein glycosylation (and hence control). As noted earlier some authors (e.g. Bates et al., 2006) have suggested a role for α-1,6-mannosyltransferases in modifying cell wall proteins. The potential presence of both a transmembrane region and a signal peptide would suggest that this protein localises to the outer membrane, and may indeed have such a role in cell wall protein modification. This would need confirming experimentally for example by localisation analysis using fluorescent tags in transformable organisms with homologous proteins (for example M. oryzae). The microarray data suggests higher expression on glass than wheat surfaces (with increasing expression as the incubation time increases, Figure 4-22). Focusing on the RT-qPCR data gathered during this project it could be envisaged that this protein (by being reduced in expression on the wheat, and with a suggestive reduction on other plant surfaces) is no longer exerting control on other proteins. From this two scenarios could be envisaged. Firstly, when present on surfaces exhibiting no host-like stimuli, this product activates other proteins that allow the further breakdown of internal stores in the spore to promote growth of, for example, a primary germ tube. Alternatively, it no longer activates proteins required for the very early stages of development (for example primary germ tube specific ECM production) since later stages of development are now underway.

The expression of EST C00482 (Figure 4-25) although not showing differences between barley and non-cleaned glass, showed a significant reduction of expression on slides treated with 16-hydroxyhexadecanoic acid. Similarly the cuticle extract treated slides seemed to show a slight depression, although not significant. This would suggest that 16-hydroxyhexadecanoic acid by itself leads to the repression of this gene which may only be lifted by the presence of other stimuli such as aldehydes or increased hydrophobicity. This data does not match the microarray data where higher expression is seen in spores developing
on barley and wheat compared to that of glass. Blasting the protein sequence against known sequences present within the NCBI database indentified an identical *Bgh* protein, previously annotated (in 2000) as a exo-β-1,3-glucanase or a β-1,3-glucosidase, as well as showing high homology to proteins from other organisms including exo-1,3-beta-glucanses and members of glycoside hydrolase families. This direct match to the previously identified *Bgh* protein is slightly at odds with the results suggested by this more recent, but admittedly more cursory, bioinformatic analysis that suggested it contained a motif identified as a cellulase. Cellulose is a β-1,4-glucan, as is cellobiose another β-1,4-glucan, meaning this *Bgh* protein is possibly better termed a β-1,4-glucanase. β-glucanases aid in the metabolism of β-glucan (polymers consisting of glucose subunits). Exo-hydrolases catalyze the hydrolysis of the glucan chain by cleaving subunits from the non-reducing end of the polymer, leading to the release of glucose (Martin et al., 2007). Cellulose is one of the main constituents of the plant cell wall (although barley also contains β-1,3-1,6-glucans, Martin et al., 2007 and studies therein). Additionally, the resultant breakdown product may also act as a nutrient source for developing fungi (Zhou et al., 2008). Even if this enzyme turned out not to be a β-1,4-glucanase, but a β-1,3-glucanase, it may still have roles in degrading the barley cell wall. Alternatively this enzyme may be used to modify the *Bgh* cell wall in preparation for germ tube emergence or elongation.

Inspection of the microarray data suggests maximum expression occurs at 8 h.p.i. when appressoria form and penetration is underway. Therefore as other authors have suggested (e.g. Pryce Jones et al., 1999 who utilized immunolocalisation studies and suggested cellulases may be released from the primary and appressorial germ tubes), enzymes such as cellulases may be involved in this penetration, alongside mechanical force. Why expression of such an enzyme would be inhibited by the presence of cutin monomers, is a mystery although it could reveal some form of negative feedback whereby the spore senses when cuticle degradation is sufficient for penetration and ceases production of costly and unnecessary protein production. Interestingly no motif was identified as a signal peptide so further work would be needed to be sure this protein was secreted or associated with the cell wall. Future work could focus on attempts to use immunolocalisation to detect the presence of this enzyme around the tip of the appressorial germ tube which would prove if this protein is secreted or not. Additionally, sequence data by itself is not sufficient proof that the gene encodes an enzymatically active gene product (Phalip et al, 2009). Attempts must be made to express such a gene in other recipient organisms, for example *Saccharomyces cerevisiae* or
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the filamentous fungus Aspergillus oryzae (used in studies by Esteban et al., 1999 and Ooi et al., 2009 respectively). Such an action allows overexpression, purification from culture filtrates (if secreted) and analysis of enzymatic activity (for example incubation with different glucan substrates) (Ooi et al., 2009). The homology analysis suggested that a glucan 1,3-β-glucosidase of Sclerotinia sclerotiorum clusters closely with the Bgh protein. As this fungus is amenable to transformation then future experiments focusing on this enzyme could aim to complement cellulase mutants in S. sclerotiorum. Caution would have to be applied to any results gathered however as S. sclerotiorum is a necrotroph unlike Bgh meaning statements regarding the cellulases role in the Bgh lifecycle would have to be tempered (Hegedus et al., 2003).

Analysis of D00658 (Figure 4-19) demonstrated there was significantly higher expression of this gene in spores developing on hydrophilic gelbond compared to slides treated with cuticular extract. Variations amongst expression on other surfaces failed to reveal any other significant changes in expression (although visually higher expression was suggested in spores developing on barley compared to untreated glass). Since both the slides treated with cuticle extract and hydrophilic gelbond do not differ in hydrophobicity some compound found within the cuticular extract maybe leading to a repression in expression. The microarray data suggests an increase in expression on plant surfaces at 4 h.p.i. compared to glass surfaces. Analysis of the protein sequence using InterproScan suggested that the protein was a glycosyltransferase and part of a nucleotide diphosphate sugar transferase superfamily. As noted by Maeda et al., (2008), glycosylation is necessary for correct protein folding, sorting and function. Some of the glycosylation process (where monosaccharides are transferred onto proteins by glycosyltransferases) takes place in the endoplasmic reticulum and two types of sugar donor may be involved (Maeda et al., 2008). These donors include lipid-linked sugars and, potentially more relevant for this gene, nucleotide-linked sugars. As such this gene product may catalyze the transfer of the glycosyl moiety from an activated nucleotide-diphospho-sugar donor to an acceptor (Charnock and Davies, 1999). Alternatively the clustering of this Bgh protein with a homologous protein from S. sclerotiorum (identified as a mannose phospho-dolichol synthase, potentially involved in the synthesis of dolichol-phospho-mannose) may suggest a potential role for the Bgh protein in lipid-linked sugar donor molecule synthesis (Maeda et al., 2008). Its up regulation at 4 h.p.i on plant surfaces indicates an important role in aiding protein folding and function during growth these surfaces and hints at the mobilisation of proteins necessary for the morphological changes
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observed during the pre-penetration stages of *Bgh* development. The lack of similar expression on glass (as seen in the microarray analysis, Figure 4-19) suggests its upregulation is only necessary for the more advanced stages of development for example the formation of the appressorial germ tube and that this gene must inevitably be downstream of receptors or signalling pathways that integrate the signals received by the developing spore.

Unlike the other genes featured within this study, nucleotide blast analysis suggested more than one copy of this gene within the *Bgh* genome (and hence the existence of a paralogous family based on gene multiplication). However analysis of the predicted protein sequences illustrated a very high homology (99% to 100% identity). Although protein sequences were not complete with N and C terminal deletions (possibly due to sequencing error combined with incorrect gene model annotation) this high level of similarity suggests two scenarios. Firstly, these sequences (either all or some) are actually derived from the same gene, and that because the blast search was run using the current genome assembly (version 3: Contigs) the search is detecting a gene from overlapping contigs (i.e. false positives). This interpretation may be supported by the knowledge that 63% of the *Bgh* genome is believed to be transposable elements (Spanu et al., 2010). These elements may contribute to keeping gene numbers low – except for those believed to be essential for biotrophy (Spanu et al., 2010).

The second scenario focuses on this last point. Although gene numbers are low for a genome of this size (5845 annotated genes for a genome size of 120 Mb), paralogs of genes are not unknown with large numbers of copies (greater than 1350) known for atypical avirulence genes (Spanu et al., 2010). Consequently if this gene number did exist then it would highlight a gene with a key, if not essential, role in *Bgh* parasitism. It should therefore be a gene of interest for future work. When genome annotation is complete (and the genome is fully assembled) it would be useful to run the nucleotide blast scan again to determine which of these scenarios is true. Also annotation will reach a stage where it will be possible to find homologues of proteins by directly searching with the known protein sequences. This may be more useful as a method of determining homology as the protein is the functional unit of action, not the gene.

As a short aside the studies presented herein used un-rooted neighbour joining tree analysis as an indicator of species distribution and homology. This method reconstructs a phylogenetic tree from evolutionary distance data and was considered by its originators to be more efficient than other methods, for example the standard maximum-parsimony algorithm, that
produce single parsimonious trees (Saito and Nei, 1987). It is computationally quick but does assume phylogenetic divergence is mirrored by sequence divergence. As a consequence if sequences are poorly aligned then tree topologies are affected. (ftp://statgen.ncsu.edu/pub/thorne/molevoclass/Oct5Atchley756.pdf; date accessed 24/3/2011). It has been used in other studies for example Zhou et al., (2008) as a form of basic form of phylogenetic analysis. In this study it was primarily used as a method for identifying species with proteins showing greatest homology to the Bgh gene product and also for suggesting alternate hosts for future functional genomic studies. For these purposes it appears to be useful as with the majority of genes tested closely related species (for example members of the Sclerotiniaceae, a sister family to the Erysiphaceae that contains barley powdery mildew) were more closely clustered than species that belong to more diverse phylogenetic groups. If the studied proteins had homologs within other fungal species (and in some cases members of the animalia) these included fungi that are considered biotrophs, necrotrophs or hemibiotrophs. In some cases however no homologs could be identified, although it is to be envisaged that as genome sequencing of fungi continues these cases would decrease. Of course the gene set analysed was just a very small fraction of the gene number identified so far in Bgh (5845, Spanu et al., 2010) so no conclusions can be accurately made from this fact. However to build on a theme presented by Yoder and Turgeon (2001) it could be speculated that a biotroph may contain genes that are commonly found in non-obligate biotrophs and necrotrophs, as well as having genes that are more specific for its own lifecycle. The genes that did not have homologs within other organisms may therefore become a point of focus for future work as they would be prime candidates for Bgh pathogenicity/lifestyle “enablers”.

Even though these experiments have allowed some conclusions to be drawn there were several drawbacks to the experimentation used in this section of the thesis. As noted earlier many of these conclusions were hampered by a lack of RT-qPCR resolution (as shown by large the variation seen within the samples). Potential reasons for this include spore samples themselves showing intrinsic variation in RNA levels and the coating of slides was manually performed (by droplet evaporation/spreading). This meant, that although unintended, a non-uniform substrate layer could have been presented to the fungus leading to a variation in expression. Furthermore, although experiments were performed during the same time period it was physically impossible to spore inoculate the surfaces simultaneously. Therefore, although two week old plants (with 7 to 10-day-old infections) were blown 12 hours
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preceding inoculation, some form of colony aging may have led to spores of varying fitness. If future work features a repeat of this study, attempts should be made to nullify both of these points. These should include moving to the spray application of slides (if logistics permit), the use of more uniform ages of colony and the employment of higher replication to increase resolution.

Another reason relates to the original collection of samples during the microarray studies (Both, 2005; Both et al., 2005). In these two studies spores were sprinkled onto glass slides in a plastic (non airtight) tray and incubated without an additional source of moisture. In the growth chambers used in both studies humidity reaches approximately 37% at 25 °C. This lack of humidity often lends itself to spore desiccation, although spores are rich in water themselves (Glawe, 2008). By 16 h.p.i., many spores are no longer full and intact, often appearing shrivelled with only the hardiest appearing to survive. Although this thesis focused on gene expression at 4 h.p.i. it is possible that even at such an early time period spores assessed by Both et al., were beginning to experience osmotic stress, combined with the stress inherent with germinating on a surface other than the host. This stress may have led to differential gene expression (in the two original studies of 2005) that is not seen, or at least not as emphasised, in cases where high levels of humidity are maintained (as attempted in this current investigation). Such a maintenance of humidity may therefore nullify some of the expression differences visible during germination on the host and on glass.

Combined with this potential reduction in difference, the analytical/normalisation reference is also different between the 2005 studies and this current study. In the microarray work the reference was based on a combined RNA sample taken from all developmental stages at all time points for all samples. In this experiment, the reference was not as comprehensive being based solely on the activity of the NADH-ubiquinone oxidoreductase. This gene was selected from the microarray study for its relatively homogenous expression across all surfaces at all the studied time points. However, although one of the better members of its class, spikes and deviations are still visible (Appendix C: 9.9). Also its behaviour on glass, although detected as statistically similar, appears to show a visible increase in expression as time increases during development. Therefore, this gene does not offer the same degree of uniform homogeneity as seen with the universal reference from the microarray studies and may lead to complications when trying to compare expression from different surfaces. A prime example of this is the response of EST C00009 (Figure 4-24), where tests detected
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differences between expression in spores germinating on barley and wheat (as suggested by the microarray) but not on glass.

4.5.6: Future work and conclusions

In the studies of developmental behaviour displayed thus far (Figure 4-8, Figure 4-9), no distinction was made to the use of either side of the leaves of both barley and wheat. In the case of the barley leaf, Zabka et al., (2008) noted that the intra and epicuticular waxes of the adaxial and abaxial side of secondary leaves showed no qualitative or quantitative differences. Furthermore hydrophobicity appears identical for both sides of the leaves concerned. This would suggest that germination behaviour would be the same on both sides of the leaf. However, a study by Russell et al., (1975) suggested there is a behavioural difference with slightly higher germination being seen on the adaxial leaf surface of primary leaves (specifically towards the leaf base). This would suggest either some form of structural difference of the leaf itself or the microclimate found in that region of the plant. Although attempts were made to confirm this, data not shown, further work needs to be carried out in this regard. Also it should be combined with studies determining whether cutin monomers differ between either side of the leaf. For wheat, if it is to be used as an extra source of discernment regarding alternate gene expression then efforts should be made to ascertain both the germination behaviour and make-up of both sides of the leaf. Other future work could include ‘data-mining’ the recently annotated Bgh genomic sequence for transmembrane receptors and signal transduction pathway components. By comparing those found to those in other fungi it may be possible to build a better picture regarding the sensing of surface stimuli and how exactly these lead to the regulation of gene expression.

To conclude, in this chapter it was demonstrated that processes leading to varying levels of germination and development are stimulated by surfaces which provide chemical or physical signals, yet with frequencies differing to that of the host but also wheat (a close relative). Furthermore, although inconclusive, results suggest that gene expression, as stimulated by surface-based signals is not an “on/off” affair but appears cumulative in nature. Several signals of different types may be required for full expression as seen on the host. Examples may include a gene linked to EST D00944, where all surfaces bar untreated glass slides provided some level of induction. Additionally several of these genes were shown to encode products with identifiable function which included those with potentially important roles in
key eukaryotic processes such as protein modification and signalling. The functions of others could not be identified suggesting the presence of ‘Orphans’ (open reading frames of unknown function). This study also showed that the majority of the genes analysed with bioinformatic methods appeared as single copy genes within the genome. Also when homology to proteins of other organisms was demonstrated it appeared that homology was not limited to organisms of either the same taxon or obligate biotrophs as non-obligate biotrophs, necrotrophs and hemibiotrophs were detected.

Why does \textit{Bgh} rely on host signals to regulate the induction of genes? Due to evolutionary fitness a pathogen may not have lost entire metabolic pathways (the favoured “auxotrophy principle”) but only the dependency to regulate or initiate the pathways (Spanu, 2006). This initiation (or rather multiple initiation events at set stages) would then depend on the host derived signals for activation at set times. This would explain the creation of the different development states on glass or cellulose that are precursors to the final pre-penetration state, the appressorium, and why attainment of that state is rare. To support this argument a non-obligate pathogen such as \textit{Magnaporthe oryzae} may not have such regimented requirements, as demonstrated by its ability to form appressoria on multiple hard surfaces with the minimal of input.

In this first half of this thesis, morphological observations were made regarding development on different surfaces. Attempts were made to discern the signals that were governing the expression of genes known to show alternate expression on substrates other than the host. In the second half of this thesis, attempts to make \textit{Bgh} amenable to genetic manipulation and attempts to utilise \textit{Magnaporthe oryzae} as a heterologous host for the future analysis of regulatory elements controlling CMEGs are documented.
Chapter 5: Towards the *Agrobacterium*-mediated Transformation of *Blumeria graminis* f. sp. *hordei*

5.1: Introduction

As more sequence data becomes available for plant pathogenic fungi, including barley powdery mildew, the requirement for processes that permit functional genomic analysis similarly increases. Transformation of fungi was reported by Mishra et al. (1973) and Mishra and Tatum (1973) in *Neurospora crassa*, who described the conversion of inositol requiring mutants (*inl*) to *inl*\(^+\) by the incubation of them with wild type unfractionated DNA and calcium chloride (Mishra, 1991; Mach, 2004). At present a number of techniques exist which permit the transformation of fungi and enable subsequent analysis. Examples include the electroporation of protoplasts or their chemical treatment with calcium chloride and polyethylene glycol, the use of which was first reported in the filamentous fungi *N. crassa* by Case et al., (1979) and *Aspergiullus nidulans* by Tilburn et al., (1983) (de Groot et al., 1998; Covert et al., 2001). Drawbacks to these techniques include their time consuming nature and the difficulty of adaption to previously untransformed species (Covert et al., 2001). Alternatively, a relatively recent technique, in the form of *Agrobacterium tumefaciens*-mediated transformation, has come to prominence.

5.1.1: *Agrobacterium tumefaciens*

*Agrobacterium tumefaciens* is a soil dwelling gram-negative bacterium which induces ‘crown galls’ or tumours on plants, but is now better known as a tool of genetic engineering (Michielse et al., 2005; Weld et al., 2006; Dafny-Yelin et al., 2008). This bacterium contains the ‘Tumour Inducing’ (‘Ti’) plasmid, which houses a region termed the ‘T-DNA’ (‘Transferred DNA’; Michielse et al., 2004). The ‘T-DNA’ is incorporated into the genome of the target plant cell during the tumorigenesis process (de Groot et al., 1998). This transfer depends on another section of the Ti-plasmid, designated the ‘vir’ region (Bundock et al., 1995; Lee and Bostock, 2006).

During tumorigenesis the VirA gene product senses phenolic compounds, such as acetosyringone, released by plant wounds, and by utilising VirG initiates transcription of the
rest of the vir loci. The products of these genes (specifically VirD2 in conjunction with VirD1) cause the excision of a linear, single-stranded T-DNA segment (the ‘T-strand’) via the nicking of border sequences (24 to 26 bp imperfect direct repeats) found either side of the T-DNA (de Groot et al., 1998; Michielse et al., 2004; Lee and Bostock, 2006). This process is enhanced by the action of the VirC protein binding to an enhancer sequence that lies adjacent to the right border (Toro et al., 1988). VirC may therefore affect host range (Bundock et al., 1995). Subsequently, the VirD2 protein binds to the 5’-end of the T-DNA (Bundock et al., 1995). This protein, containing C-terminal domain nuclear localisation signals, mediates the transfer of the T-DNA into the nucleus of the target plant cell (Bundock et al., 1995). The ‘T-strand’ is transferred as a single stranded DNA molecule to the host cell via a type IV secretion system, the ‘T-Pilus’ consisting of the virB operon and virD4 gene products (Bundock et al., 1995; Michielse et al., 2004; Michielse et al., 2005). Independently of this strand but still using the same secretion system other Vir proteins (VirE2, VirE3, VirF and VirD5) are also exported into the host cytoplasm. When the T-strand enters the cytoplasm it is coated in VirE2 proteins, which protects it from nucleases (Citovsky et al., 1989; Michielse et al., 2005). This mature T-complex now enters the nucleus via a nuclear pore with the help of both bacterial and host factors where it is integrated into the genome. For more information readers are referred to Gelvin (2010). Although needing elucidation, this integration may involve the conversion of T-strands into double stranded intermediates. These intermediates are thought to anneal in ‘double stranded breaks’ in the host genome by host repair mechanisms (Lacroix et al., 2006; Lee and Bostock, 2006; Citovsky et al., 2007). Host range may also be modified by the VirF and VirH proteins, although their precise function is unknown (Bundock et al., 1995).

Advantageously, recent experiments have shown it is possible to have T-DNA transfer to other kingdoms apart from plants (Lacroix et al., 2006). Bundock et al., (1995) showed that transfer was possible to Saccharomyces cerevisiae, whilst other studies suggested mycoparasites and filamentous fungi (e.g. Aspergillus awamori) from ascomycetes, basidiomycetes and zygomycetes could be transformed as well (Bundock et al., 1995; de Groot et al., 1998; Dunn-Coleman and Wang, 1998). For an extensive, if dated, list the reader is referred to Michielse et al., (2005).

Importantly many of the Agrobacterium proteins required for transfer to plants are still required, as is the presence of a vir gene inducer such as acetosyringone (Covert et al., 2001).
Mutations in certain vir genes led to attenuated virulence/ transformation of non-plant hosts within Agrobacterium strains (Lacroix et al., 2006). Other similarities with plant targeted transfer include truncations seen at one of the T-DNA borders (where integrated T-DNA and host DNA meet) (Lacroix et al., 2006). These examples lead Lacroix et al., (2006) to suggest that the DNA transfer process is, to a certain degree, the same in plants and other eukaryotic organisms.

Even though this may be the case there are some notable differences in the integration process between different hosts. In plants integration often occurs by illegitimate recombination at random sites, even if the T-DNA shows extensive sequence homology to the plant genome (Lee and Bostock, 2006). In fungi, especially if the T-DNA shows sequence similarity to the genome, integration may be via homologous recombination (Weld et al., 2006). If the T-DNA does lack sequence homology then integration is probably due to illegitimate recombination, resulting in a low copy number and random insertion (Dunn-Coleman and Wang, 1998; Lacroix et al., 2006). Bundock et al., (1995) suggest host proteins or lack of them may be the primary cause of this difference.
Agrobacterium-mediated Transformation

To transform fungi, the *A. tumefaciens* DNA-transferring system has been modified (Figure 5-1). A ‘binary system’ is used whereby the bacterium contains an attenuated Ti-plasmid (containing solely the *vir* gene system) and the binary vector containing the T-DNA (Michielse et al., 2008).

The use of *Agrobacterium*-mediated transformation provides a number of advantages over other systems. Firstly it avoids the need for protoplast preparation. As shown by de Groot et al., (1998) *Agrobacterium* may transform intact conidia and hyphal tissue as well as fungal protoplasts, with efficiencies for example 600 times that of those experienced using polyethylene glycol transformation methods (Zhang et al., 2008). Furthermore, its ease of handling, less labour intensive nature and reproducibility, are often superior to such characteristics shown by other transformation techniques (for example biolistic transformation). Additionally, with refinement it is possible to have a high percentage of stable, single T-DNA insertions (Zhang et al., 2008). Together these facts suggest this system may be ideal for insertional mutagenesis as well as specific gene targeting in fungi (Lacroix et al., 2006; Weld et al., 2006).

### 5.1.2: Demethylation inhibitor fungicides

Transformation systems require a method of selection that permits the differentiating between the cells that are untransformed and the rarer, transformed cells (Todd and Tague, 2001; Chung et al., 2002). In the case of phytopathogenic fungi their successful transformation was delayed because of difficulties in creating such a selection system (Panaccione et al., 1988). Panaccione et al., suggest such hindrances may have included the rarity of auxotrophic mutant strains that could be complemented by the required metabolic genes of the wild-type, as had been used in earlier cases of transformation (Mishra and Tatum, 1973; Panaccione et al., 1988, Wu, 2003). An effective system which overcomes such a requirement involves the use of dominant genes offering a directly selectable phenotype (Panaccione et al., 1988; Honda et al., 2000).

In the case of obligate pathogens such a selection system has the added complexities of needing to be applied *in planta* (ideally working before the pathogen has successfully penetrated the host) and having to allow selection through a pathogen lifecycle on a living host (Matthews, 2000; Wirsel et al., 2004). It has been the case that commonly used selection
Agrobacterium-mediated Transformation

systems (for example the hygB gene system of E. coli which offers resistance to the protein inhibitor hygromycin B) may also affect the plant host (Waldron et al., 1985; Punt et al., 1987; Dai et al., 2003; Wirsel et al., 2004). As a consequence there is the requirement that such “collateral” inhibition not occur, and depending on the pathogen involved, certain pesticides such as commercially available fungicides may offer that capability. Depending on the chemical chosen the advantages of use compared to systems based on dominant drug resistance markers or other selective agents such as hygromycin B may include ready availability, affordable costs and lower toxicity to humans (Ahuja et al., 2008). Other advantages include the ability for systemic fungicides to be applied as a prophylactic seed treatment, thereby protecting young seedlings (often used for laboratory experiments) during emergence and removing issues regarding timing of fungicide application and good coverage of the host (Matthews, 2000). Practical disadvantages can exist with the fact that with some fungicides the modes of action are not be fully understood. This leaves them vulnerable to potential legislation that may impede use (for example EU council directive 91/414/EEC, the Sustainable Use Directive, which aimed to remove from use older pesticides) (http://www.knowledgescotland.org/briefings.php?id=99. Date accessed: 9/2/2011).

There are a variety of methods to combat powdery mildew, but one that has potential for laboratory (especially transformation) use is that of fungicides that target critical fungal processes such as sterol biosynthesis (Leroux, 2003). Sterol biosynthesis is targeted by the ‘Demethylation Inhibitor’ fungicides (‘DMIs’, ‘azoles’) (Baldwin and Corran, 1995; Maffi et al., 1995; Ma et al., 2006). Used in both medicine and agriculture, due to a target spectrum which includes ascomycetes, basidiomycetes and deuteromycetes, the DMIs have been used to treat barley powdery mildew infections since the 1970s (Delye et al., 1997; Muchembled et al., 2000). One member of the DMIs is triadimenol (marketed as Baytan® produced by Bayer) (Figure 5.2) (Delye et al., 1997).
A triazole, triadimenol is the active ingredient of the fungicide formulation, Baytan (Huston et al., 1998). Its mode of action was first elucidated by Buchenauer, (1978). Triadimenol primarily targets the enzyme cytochrome P450 sterol 14α-demethylase, a protein of the endoplasmic reticulum, and binds to the enzymes active site (Delye et al., 1997; Blatter et al., 1998; Marichal et al., 1999). The azoles have curative and eradicative properties as well as being systemic protectants (Delye et al., 1997). As such they are the most important fungicides of their class (Tsuda et al., 2004). Modified from: Senior et al., (1995).

The DMIs deactivate lanosterol 14α-demethylase (a cytochrome P450 dependent mono-oxygenase involved in C-14 sterol demethylation and encoded by the CYP51 gene) by binding to the haem iron of the cofactor cytochrome P450 (Blatter et al., 1998; Leroux, 2003; Waterman and Lepesheva, 2005). It is this interaction which determines selectivity although in high concentrations the azoles may inhibit other cytochrome P450s (including those of plants) (Joseph Horne et al., 1995). Some azoles such as fluconazole will affect the Δ22 desaturase as well the CYP51 protein (Sanglard et al., 2003).

This binding inhibits the demethylation at carbon 14 of lanosterol and/or other precursors to ergosta-5,24(24‘)-dien-3β-ol (24-methylene cholesterol), the predominant sterol of Bgh (Figure 5-2) (Loeffler et al., 1992; Mysyakina and Funtikova, 2007). In other members of the Pezizomycotina ergosterol is the predominant sterol (Weete et al., 2010). Although sterols in the membrane are often in a mixture featuring the major sterol and related intermediates (in Bgh episterol and eburicol), a reduction of 24-methylene cholesterol, combined with and accumulation of C14-methylated precursors is thought to lead to membrane disruption and electrolyte leakage (Loeffler et al., 1992; Hewitt, 1998). Sterol depletion will lead to changes in membrane fluidity, affecting the activity of membrane-bound enzymes (e.g. those involved in chitin synthesis and those in the mitochondria) and a loss of a “sparkling hormonal function” (i.e. the initiation of growth by very small amounts of a specific sterols) (Lorenz et al., 1989; Marichal et al., 1999; Lupetti et al., 2002; Mysakina and Funtikova et al., 2007). Cumulatively these effects lead to severely hindered growth and even fungal death (Hewitt, 1998; Marichal et al., 1999; Lupetti et al., 2002). If growth does occur then colonies developing before fungicide application will sustain morphological malformations including
enhanced mycelial branching, irregular wall thickening and organelle disorganisation (Kang et al., 2001). Cytoplasmic degeneration with increased vesicular formation is also apparent (Maffi et al., 1995; Mangin-Peyrard and Pepin, 1996). Since the main target of DMIs is sterol biosynthesis, early development post-germination is not thought to be affected as spores rely on internal stores (Hewitt, 1998). The majority of the effect is seen during the inhibition of haustorial formation (Mangin-Peyrard and Pepin, 1996; Damgaard and Nielsen, 1999).

Figure 5-3: The sterol biosynthesis pathway of barley powdery mildew.

The genetics and biochemistry of the pathway in Bgh are little known and as stated by Mysyakina and Funtikova (2007) the particular complement of sterols can depend on fungal growth stage. This diagram is modified from (Weete et al. 2010) and is based on evidence provided by a study of conidial sterol complement by Loeffler et al., (1992). In fungi, sterol synthesis occurs de novo from acetyl-CoA (Hewitt, 1998). Biosynthesis is associated with membranes and the actual pathway depends on the fungal species concerned (Alcazar-Fuoli et al., 2008; Yang at al 2008). Downstream of the cyclisation of the precursor, squalene 2,3-epoxide, CYP51 catalyses a rate-limiting reaction where the 14 α-methyl group is removed from lanosterol as a formic acid moiety (after first being converted to an alcohol, followed by an aldehyde) (Waterman and Lepesheva, 2005). Lanosterol is a branch point in the synthesis pathway (Alcazar-Fuoli et al., 2008). Although both branches meet again at the production of fecosterol, one route is based on the sequential demethylation of intermediates via the precursor zymosterol, whilst the other proceeds via eburicol (produced after a methylation and itself followed by demethylation). Apart from 24-methylene cholesterol, Loeffler et al., (1992) noted trace amounts of eburicol and episterol. This would suggest that the biosynthesis proceeds via the eburicol route. Either way CYP51 has a vital role. After formation of fecosterol, and episterol, the process may again branch via 5-dehydroepisterol, which leads onto 24-methylene cholesterol (the predominant sterol of Blumeria graminis), and also in some fungal taxa, 24-methyl cholesterol. Alternatively, as shown in the diagram, the process may lead to ergosterol production or 24-methyl-cholesterol by other routes. Red Triangle = CYP51 catalysed reaction. Blue Arrow = multiple synthesis reactions. Red Arrow = single synthesis reaction. Black narrow arrow = Predicted Bgh sterol biosynthesis pathway.
5.1.3: Demethylation inhibitor resistance

Ma and Michailides (2005) define fungicide resistance ‘as a stable inheritable adjustment by a fungus to a fungicide, resulting in reduced sensitivity of the fungus to the fungicide.’ Due to mass usage and the site-specific nature of DMI action, resistance within plant pathogens such as Bgh has grown (Delye et al., 1997; Ma and Michailides, 2005). Resistance occurs via a number of mechanisms, although many have been discerned in laboratory mutants (especially clinical isolates) rather than field isolates (Delye et al., 1997; Karaoglanidis et al., 2003; Bean et al., 2009). Mechanisms observed include detoxification or efflux of the DMI in the case of Aspergillus fumigates using ABC (ATP-binding cassette transporters) (Leroux, 2003; Ferrina et al., 2005; Leroux et al., 2007) or a lack of activation of the compound itself (Hewitt, 1998; Ma and Michailides, 2005). The use of alternative sterols is also a possibility (studies in Mysyakina and Funtikova, 2007). In the case of triadimenol, deposition in the vacuole of resistant strains of Ustilago avenae (loose smut of oats) provides resistance (Hewitt, 1998). However the most common form of resistance is a modification of the target protein (Cools et al., 2006). This may include increased enzyme expression or as seen in Candida species and Bgh a change in the affinity of the target site of the fungicide due to point mutations (Marichal et al., 1999; Delye et al., 1998; Hanamoto et al., 2000).

As noted by Wyand and Brown (2005) the genetic basis for DMI resistance within pathogens including Bgh is quite complex. DMIs are subject to ‘Quantitative /Polygenic’ resistance (i.e. that resulting from a mutation of a number of genes) and ‘Qualitative/Major’ gene resistance. The former resistance reflects the multitude of resistance mechanisms against the DMIs, and takes the form of a slow decrease in fungicide ability, dependent upon the accumulated number of genes present within the organism itself (JosephHorne and Hollomon, 1997; Hutson et al., 1998).

In the case of triadimenol major gene resistance (often as a result of a mutation in a single gene) or oligogenic (relying on low number of resistance genes) has been observed in Bgh (Holloman et al., 1984; Brown et al., 1992; Blatter et al., 1998). Major gene resistance (not necessarily at the same locus) is thought to be an underlying cause of resistance that is regularly found within three of the highest categories of resistance of mutants studied (Blatter et al., 1998; Cools et al., 2006). Analysis by Delye et al., (1997) has revealed a specific point mutation in codon 136 of certain Bgh isolates’ single copy of the CYP51 gene (Albertini et
A similar mutation is also observed in highly resistant isolates of grape powdery mildew and *Penicillium italicum* (Delye et al., 1997; Leroux., 2005; Maetal et al., 2005). In the latter case, it is never associated with low resistance phenotypes thereby leading Delye et al., (1997) to suggest the mutation may be enough to provide a substantial level of resistance. Subsequently, Wyand and Brown (2005) showed that although correlated with high resistance Bgh phenotypes, this mutation was also found in an isolate with low resistance. Therefore solely by itself the mutation was only thought to convey low resistance. Further work located a second mutation, K147Q, which was solely located in high resistant isolates (but only those with the Y136F mutation present).

The Y136F substitution results from a nucleotide A-T change at position 458 (Delye et al., 1998) and results in the substitution of the amino acid phenylalanine for tyrosine in region CR-2, one of four regions implicated as part of the CYP51 substrate recognition site (Aoyama et al., 1996; Delye et al., 1997). This substitution effectively equates to the structural loss of a hydroxyl group and is thought to enhance the hydrophobicity of the active site without a significant change in enzyme conformation (Delye et al., 1997). Delye et al., (1997) postulates that this leads to a loss of enzyme affinity for the inhibitor (a hydrophobic molecule) without a detrimental effect on sterol biosynthesis. Also such mutations in the substrate binding site could result in a perturbation in the interaction points between theazole and the haem due to a subsequent movement in tertiary structure (Marichal et al., 1999; Lupetti et al., 2002). Wyand and Brown (2005) echo this thought, although they note that it has yet to be tested. The K147Q mutation causes a substitution of glutamine for lysine and occurs within the B’ helix of the active site of the enzyme (Wyand and Brown, 2005). This mutation may reduce the net positive charge of the helix, preventing azole binding due to reduced attraction (Wyand and Brown, 2005).
5.2: Aims and objectives

As sequencing of the genome has finished (www.blugen.org, Spanu et al., 2010) the need for a stable and reproducible method of transformation, permitting functional genomic analysis, is high. *B. graminis f. sp. hordei* is an obligate biotroph with an inability to grow in axenic culture. This characteristic severely hinders molecular studies as common methods of stable transformation, for example based upon the isolation of protoplasts, are either very difficult or not possible. In 1995 transformation was achieved by Christiansen et al., (1995), although this was only transient. Again, in 2000, transformation was achieved by Chaure et al., (2000) using biolistic transformation. The pathogen was transformed with an allele of a β-tubulin gene (permitting resistance to the benzimidazole fungicide, benomyl) and also the *bar* gene from *Streptomyces hygroscopicus* (providing resistance to the herbicide Bialaphos, a phosphinothricin analog). Unfortunately, although stable, this transformation could not be reproduced.

As a result this study had one primary objective. In order to allow the testing of gene expression and regulation within *Bgh*, attempts were made to develop an *Agrobacterium tumefaciens*-mediated transformation system. This method utilised a triadimenol-selection system combined with a visual selective marker (GFP). Triadimenol is the key fungicidal component of the commercially available compound Baytan®. The procedure was tested on *Blumeria graminis* f. sp. *hordei* isolate (DH14) which is considered highly sensitive to triadimenol (Wyand and Brown, 2005). In accordance with this an *Agrobacterium* binary transformation vector was completed featuring the *CYP51* coding sequence from *Bgh* isolate CC146 (containing both the Y136F and K147Q mutations) that permits high resistance to triadimenol (Wyand and Brown, 2005).

If completed successfully, this system would allow genetic studies such as mutational analysis and visual marking of gene expression, thereby permitting information gathering on the function of a gene during development. As a result the role of *Bgh* as a model organism for powdery mildews would be consolidated and the identification of targets for new control methods may be permitted.
5.3: Materials and methods

This project continues work begun by the author and Dr. Pietro Spanu. For *Blumeria graminis* f. sp. *hordei* transformation vectors used include pCambia-eGFP-bar-TaqII (a binary transformation vector containing eGFP acting as a marker for transformation procedures, but also containing a *Bar* gene encoding resistance to the fungicide bialaphos) and pCambia-CYP51-2 (a binary transformation vector containing eGFP acting as a visual marker for transformation, but also containing a *CYP51* gene encoding resistance to the fungicide triadimenol) (Figure 5-4) (Appendix D). All vectors contain promoter sequences from the *Blumeria graminis* f. sp. *hordei* histone gene H3, and terminator sequences from the aquaporin gene, *aqu1*. Both vectors were adapted and modified in previous work by both the author and Dr. P. Spanu (Dr. P. Spanu, Pers. Comm) (Appendix D: Chapter 5 (Towards *Agrobacterium*-mediated Transformation)). A cloning strategy for the creation of pCambia-CYP51-2 is shown below (Figure 5-5).

![Figure 5-4: Plasmids used during the cloning and during *Agrobacterium*-mediated transformation.](image)

Restriction digestion sites are shown. A) Plasmid pCambia-eGFP-Bar-TaqII. This binary transformation vector has a pCambia vector-0380 with left (LB) and right (RB) *Agrobacterium* T-DNA border sequences. A kanamycin selection marker is present within the backbone (6811 bp). B) Plasmid pCambia-CYP51-2. Diagram shows insertion point of promoter-CYP51-terminator cassette. Cassette originates from pCambia-CYP51-TaqII (a plasmid containing a H3 promoter/CYP51/Taq cassette in a pCambia-0380 backbone). CYP51 = 1693 bp; eGFP = 900 bp; H3 = *Blumeria graminis* f. sp. *hordei* histone H3 promoter, 1289 bp; Taq = *Blumeria graminis* f. sp. *hordei* aquaporin terminator sequence, 480 bp.
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Figure 5-5: Creation of Agrobacterium transformation vector pCambia-CYP51-2.

Step 1 (A) EcoRI digestion of pCambia-eGFP-Bar-TaqII to release the H3 promoter/bar gene/aquaporin terminator cassette. The H3 promoter/eGFP/aquaporin terminator cassette remains. Step 2 (A) The linearised vector is dephosphorylated with Shrimp Alkaline Phosphatase. Step 1 (B) High Fidelity PCR amplification of H3 promoter/CYP51/aquaporin terminator using pCambia-CYP51-taqII as a template. Step 2 (B) Purification of PCR product followed by EcoRI digestion to produce complimentary ends. This step is followed by Ligation of the digested PCR product with the processed pCambia-eGFP-Bar-TaqII to create pCambia-CYP51-2.

5.3.1: PCR amplification of CYP51 cassette from pCambia-CYP51-TaqII

All PCRs were performed using a PTC-200™ Peltier thermocycler (MJ Research Inc.). All oligonucleotide primers were synthesised by Sigma®-Genosys Ltd. The High Fidelity ‘Phusion’ DNA Polymerase’ (Finnzymes) was used for all reactions. Reaction volumes used were 50 μl and followed the guidelines of the manufacturer. 100 ng template (pCambia-CYP51-TaqII) (or 2 μl water for negative controls) was used per 50 μl reaction volume. Primers used include H3-not (5’-ATA GCG GCC GCG AATTCT CGG ACG TAT TAC AAG TCT CGT TG-3’) and Taq-Rev-Eco (5’-TAT AGA ATTCGC TAC TCT AAG AGC AAG GAA ATG ATT AG-3’). Annealing temperature was at 60 °C and extension time was 1 min (15 s per Kb).
5.3.2 EcoRI digestion of CYP51 cassette
Preceding the digestion the amplified CYP51 cassette was purified using ‘QIAquick® PCR Purification Kit’ (Qiagen) following the protocol of the manufacturer. The amplified CYP51 cassette was then digested with EcoRI (New England Biolabs) performed in 100 μl utilising the recommended buffer of the manufacturer to create complimentary ends on the insert. Mixture was then purified using ‘QIAquick® PCR Purification Kit’ (Qiagen) and quantified according to section 2.8.

5.3.2: Preparation of pCambia-eGFP-Bar-TaqII and ligation with CYP51 cassette
pCambia-eGFP-Bar-TaqII was digested with EcoRI (New England Biolabs) using recommended buffer of the manufacturer. The plasmid was then dephosphorylated using Shrimp Alkaline Phosphatase (Roche) (according to protocols of the manufacturer), followed by heat inactivation at 65 °C for 15 min, and purified using ‘QIAquick® PCR Purification Kit’ (Qiagen). The ligation of the CYP51 insert to digested plasmid used a 4:1 insert to plasmid molar ratio and utilised T4 DNA Ligase (New England Biolabs) following recommendations of the manufacturer. Incubation was at 16 °C overnight. Both plasmid and Insert concentrations were gauged as noted in Section 2.8.

5.3.3: Detection of successful insertion into plasmids via microprep and restriction digestion
To show insert presence in pCambia-Bar-eGFP-TaqII potentially transformed bacterial colonies were transferred by pipette tip to 20 μl ‘1X Cracking Solution’ (Appendix D: Chapter 5 (Towards Agrobacterium-mediated Transformation)). This solution was subsequently placed on ice. 3 μl ‘Loading buffer’ was then added. Solution was then heated to 70 °C for 5 min 25 on PTC-200™ Peltier thermocycler and was then centrifuged at 3000 rpm (2415 x g) for 5 min. 20 μl of upper layer of microprep solution was then transferred to 0.8 % agarose gel for visualisation. If successful integration was indicated by higher migrating bands, miniprep preparations were made from the potentially successful transformations to allow restriction digestion utilising EcoRI, NdeI, BamHI and HindIII (New England Biolabs) for pCambia-CYP51-2. Products were then visualised upon an agarose gel and recorded as required.
5.3.4: Sequencing

All plasmids were prepared for sequencing by growing 5 ml overnight cultures (12 to 16 hrs) on selective medium, LB cultures followed by plasmid purification utilising the QIAprep® Miniprep Spin (Qiagen). The recommendations of manufacturer were followed and elution was carried out using 50 μl HPLC Grade Water (BDH). Sequencing reactions contained 1 μg of DNA and 12.8 pmole of primer. Volume made up to 12 μl with sterile water. Purified plasmids preparations were sent to ‘Advanced biotechnology centre (ABC)’, Imperial College London and run on an Applied Biosystems 3100 capillary sequencer. Primers utilised as shown in Table 5 and appendix D:6 Sequences were analysed and aligned using ‘Chromas Lite (Version 2.1)’ by Tecnelysium Pty Ltd and ‘BioEdit version 7.0.0’ (Hall, 1999). ‘Chromas Lite’ Freeware available from http://technelysium.com.au/chromas_lie.html. Bioedit freeware available from http://www.mbio.ncsu.edu/BioEdit/bioedit.html. Sequences were identified using internet BLAST facilities available on http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi.

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</tr>
<tr>
<td>‘cc146seq1B’</td>
<td>5’-GGC TAG TGG AAT TAT AAG TTT AT-3’</td>
<td>54.2</td>
</tr>
<tr>
<td>‘146Seq2’</td>
<td>5’-TCG GTG GCT CAT TTG G-3’</td>
<td>62.0</td>
</tr>
<tr>
<td>‘146Seq3’</td>
<td>5’-GCC GAA GAA ATT TAT ACG-3’</td>
<td>54.8</td>
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<td>‘146Seq4’</td>
<td>5’-GCG AGT TTT GTA TCA TGA CC-3’</td>
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<td>‘146Seq5’</td>
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<td>61.2</td>
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<tr>
<td>‘146Seq6’</td>
<td>5’-CAA CGG TGC AAT TAG TTA C-3’</td>
<td>55.6</td>
</tr>
</tbody>
</table>

Table 5: PCR primers used to sequence CYP51 genes.

5.3.5: Software

Sequence alignment was performed using Clustal_W (Chenna et al. 2003; available at: http://www.ebi.ac.uk/clustalw/). Determination of restriction sites was performed using Webcutter 2.0 (Available at: http://www.firstmarket.com/cutter/cut2.html). Sequences were translated using ExPASy (available at http://www.expasy.org/). Sequence highlights by GeneDoc version # 2.6.02 (available at http://www.psc.edu/biomed/genedoc/gddl.htm).
5.3.6: **Triadimenol treatment of cv. Golden promise seeds**
Triadimenol fungicide was applied as Baytan™ flowable seed dressing (Bayer Crop Science) to barley cv. Golden Promise at rates of 1 ml/kg. All treatment was performed by Prof. James Brown (John Innes Centre).

5.3.7: **Evaluation of triadimenol tolerance**
To determine the influence of Baytan seed-treatment on the sensitive isolate DH14, heavily infected plants containing the isolate were shaken over 6-day-old Baytan™ treated barley plants (1st true leaf stage) to cause infection. 3 pots of approximately 50 Baytan-treated seedlings and 3 pots of approximately 50 non-treated seedlings were infected per experiment. All inoculation was carried out in a sterile ‘Microflow Peroxide Advanced BioSafety Cabinet- Class 2’ with airflow turned off. Plants were then capped and sealed with transparent isolation covers and placed back in the 25 °C growth room. After 1 week the leaves of the target plant were inspected for colony presence. Plants were then monitored for an additional 2 weeks. At 7 d.p.i. light and epifluorescence microscopy was performed on selected leaves from each pot to determine the stage of mildew development. A minimum of 100 spores were observed per leaf.

5.3.8: **Competent *Agrobacterium tumefaciens* cell creation**
The strains used in this investigation include LBA1100, EHA 105, LBA 4404 pBBR 1MCS5-virGN54D (Rifr, Gmr), LBA 4404 pTiAch5(T1+Tr-DNA) (gifts of Dr. C.B. Michielse, Swammerdam Institute for Life Sciences and Professor J. Memelink, Institute of Biology, Leiden University). To produce competent cells a pre-culture of the strain in LB media was grown for approximately 6 hours at 30 °C. 100 μl was then removed and added to 100 ml LB media + 0.1 % glucose and grown at 30 °C until an OD600 of 1-1.5 was reached. After incubation on ice the cells were then centrifuged at 4000 rpm and the pellet was washed three times in 10 ml of 1 mM HEPES (pH 7.0). Subsequently the pellet was washed in 10 ml 10 % glycerol. Following this the pellet was re-suspended in 750 μl of 10 % glycerol then stored as 45 μl aliquots at -80 °C until needed.
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5.3.9: **Transformation of Agrobacterium tumefaciens strains**

100 ng of plasmid (in 3 μl H₂O) was added to 45 μl of cells and added to a 0.2 cm electroporation cuvette (Bio-rad). These cells were then electroporated by pulsing for 5 ms at 12.5 Kv/cm using a Gene Pulser II electroporator (Bio-rad). 1 ml SOC solution was added and the cells were incubated at 30 °C for 1.5 hrs before plating on selective media.

5.3.10: **Agrobacterium tumefaciens-mediated transformation of Blumeria graminis f. sp. Hordei**

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**Figure 5-6: Flow diagram and time line of Agrobacterium-mediated transformation protocol of Blumeria graminis f. sp. hordei.**

Day 1: Plant susceptible barley. Day 7: Barley primary leaf is well developed. Infect with Blumeria graminis f. sp. hordei. Day ≤7 + n: allow required level of colony development (from 3 to 6 days [n]). Day (7+n)-1: The day before attempted transformation begin Agrobacterium strain cultivation. Day (7+n): Place Agrobacterium in induction media. Allow development until OD₆₀⁰ is 0.3 to 0.5. Dip Infected leaved and incubate. Day ≤ (7+n)+x: Allow sporulation (3 to 4 days [x]) then infect target plants. Day [(7+n)+x]+7 = 7 days after infection check plants.

Susceptible barley (cv. Golden Promise) plants were inoculated with *Blumeria graminis* f. sp. *hordei* and infection was allowed to develop from 3 to 6 days 25 °C and 30 % (50 %) humidity with 16/8 hour light/dark cycle in a Sanyo Fitotron growth room. The day before
transformation (Figure 5-6) (at required *Blumeria* infection level), selected *Agrobacterium* strains were cultured overnight in 5 to 10 mls selective media at 30 °C. These cultures were used as an inoculum in Induction media (final volume 50 mls) (Appendix D: Chapter 5 (Towards *Agrobacterium*-mediated Transformation)) to create an OD$_{600} = 0.15$. The culture was incubated with shaking at 30 °C until an OD$_{600}$ of 0.3 to 0.5. 1 μl/ml Silwett L77 (Union Carbide Chemcials and Plastics [Europe] S.A.) was then added to the Induction media. Infected leaves were then cut from the plants and dipped into the solution. After short incubation times (up to 5 minutes) leaves were extracted and gently dried. Leaves were then placed upon phytadvert (1.5 %) contained within transparent incubation boxes (Figure 5-7) for 3 to 4 days and permitted to sporulate at 25 (and later 25 °C). In early experiments the phytadvert was infiltrated with 1 % benzimidazole (Fluka). In later experiments this phytadvert was augmented with acetosyringone (Sigma Aldrich) (1-(4-Hydroxy-3,5-37dimethoxyphenyl)-ethanone, 200 μm final concentration). After sporulation potentially transformed *Blumeria graminis* f. sp. *hordei* was inoculated upon both Baytan (triadimenol) treated- and susceptible- barley (cv. Golden Promise) (independent plants of each type for each *Agrobacterium* strain were used). After 7 days of incubation visual inspection of inoculated plants was performed.

*Figure 5-7: Infection incubation box.*

Infected barley leaves, previously dipped in *Agrobacterium tumefaciens* transformation solutions are allowed to develop until sporulation. Spores are then sprinkled over target plants.
5.3.11: Inspection of *Blumeria graminis* f. sp. *hordei* transformation status

Potentially transformed *Blumeria graminis* colonies were allowed to sporulate and spores were sprinkled over both Baytan-treated and non-treated leaves to bulk up stock for microscopic analysis. Due to the difficulty with staining the leaf and retaining active GFP visual microscopy was attempted on non-cleared leaves. All colonies were identified under normal light then interrogated via Blue-light. As a control similarly aged colonies of non-transformed *Blumeria graminis* were also visualised in a similar fashion. Additionally leaves were cleared and stained to observe via epifluorescence microscopy the status of conidial development upon leaves where colonies had failed to materialise. Samples were viewed from a minimum of three independent replicates.
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5.4: Results

In preparation for this work an Agrobacterium-binary vector designed to incorporate a gene providing resistance to triadimenol was completed. This vector, pCAMBIA-CYP51-2, (Figure 5-4) also contains an eGFP gene to permit visual detection during gene activation. Both the triadimenol resistance gene, CYP51 (originating from isolate CC146) and the eGFP gene have a Blumeria graminis f. sp. hordei histone promoter with aquaporin terminator. This means both genes should be expressed simultaneously.

Previous work had called into question the viability of the selection mediated by triadimenol on the normally sensitive isolate, DH14. This work suggested that both the identity of powdery mildew stocks and barley seed treatments used were suspect. As such on the receipt of new seed and fungal stocks simple tests were performed to ascertain the efficacy of the selective agent and the suitability of DH14 as a receptor strain. Spores of isolate DH14 were sprinkled on barley (treated with 1 ml/kg Baytan, recommended by Professor James Brown, John Innes Centre, Norwich, UK) and non-treated barley.

Figure 5-8: The effect of Triadimenol on 7 day Blumeria graminis f. sp. hordei infections.

A) Non-treated barley plant demonstrating visible powdery mildew infection symptoms in the form of powdery pustules (inset). B) Baytan-treated plant with leaves demonstrating no visible infection symptoms (inset).
One week after inoculation leaves were inspected by microscopy. As shown in Figure 5-8 the susceptibility of the stock of DH14 to Baytan at the selection level utilised was clearly demonstrated. No visible colonies were seen on treated leaves, unlike on non-treated leaves where mildew development appeared to conform to morphological expectations. Growth was allowed to continue and after three weeks post-inoculation colonies were still not visible on Baytan-treated plants.

Figure 5-9: The effect of Triadimenol on *Blumeria graminis f. sp. hordei* asexual development.

A+B) 7 days post-inoculation spores on non-treated leaves have progressed through the life cycle and reached sporulation. Conidiophores are present and hyphal spread across the upper epidermis is apparent. C+D) Spores on Baytan-treated leaves. Development appears to have halted at, or just following, initial penetration. No conidiophores are present and external hyphae, if present, are limited. D) Composite image of fungal structures at different focal planes. Haustorium formation (*) is limited as is hyphal development. Fungal structures stained using wheat germ agglutinin stain (section 3.3.3.2) Scale bar: A+C) = 150 μm approx; Scale Bar: B) = 80 μm approx; Scale Bar: D) = 40 μm approx

Inspection of inoculation zones on treated leaves at 7 d.p.i., via leaf clearing and fungal staining, suggested many of spores that had germinated halted during their lifecycle during the early phases of penetration (Figure 5-9). If penetration occurred, as demonstrated by the formation of mycelia and haustoria, development was limited. Haustoria development often
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appeared incomplete. Structures were smaller, both with less digitation and also the appearance of a disturbed/uneven membrane surface (Figure 5-10). This is in direct contradiction to mildew development on the control leaves where haustoria were numerous, and fully-developed, supporting the formation of dense hyphal nets and heavy sporulation. In conclusion this efficacy assay indicated that the triadimenol levels present prevented wild-type DH14 from completing its lifecycle.

Figure 5-10: Haustoria formation on Baytan-treated leaves.

A) Haustoria formation by Blumeria graminis f. sp. hordei on non-treated barley. Fungal structures stained by wheat germ agglutinin alexa-488 B) Haustoria formation by germlings developing under triadimenol treatment. Haustoria display reduced digitation and perturbed surface formation. Leaf cleared by boiling in methanol and incubating in chloral hydrate. Scale Bar = 10 μm approx

Multiple attempts have been made to transform Blumeria graminis f. sp. hordei isolate DH14 with a gene encoding resistance to triadimenol which originated from the Baytan-resistant isolate CC146. These trials attempted to utilise Agrobacterium tumefaciens as a delivery method for plasmids containing this gene. Early trial experiments featuring a number of studies with LBA1100 containing the plasmids pCAMBIA-eGFP-Bar-TaqII or pCAMBIA-CYP51-2 as well as non-transformed LBA1100 took place at 25 ºC. No successful infection took place on treated triadimenol treated plants for all treatment regimes. This includes Blumeria graminis f. sp. hordei treated with Agrobacterium containing the plasmid conferring resistance to triadimenol (pCambia-CYP51-2). A lack of infection on indicator
plants was expected for infected-leaves dipped within solutions (section 5.3.10) containing *Agrobacterium* with no plasmid (a negative -ve control) and also the plasmid pCambia-eGFP-Bar-Taq II which confers resistance to the fungicide Bialaphos, rather than Triadimenol (also a form of negative control). However, a failure of colony growth originating from infected leaves dipped within solutions containing *Agrobacterium* containing pCAMBIA-CYP51-2 suggested that transformation was unsuccessful. Infection did take place during the majority of experiments on plants that were not treated with the selection pressure. This indicated the survival of *Bgh* during the procedure, which includes the dipping of leaves in *Agrobacterium*-containing solution.

Other attempts took place involving other strains including the hypervirulent LBA 4404 pBBR 1MCS5-virGN54D and have been conducted at 20°C. All such attempts are listed in Table 6, 7. The majority of these attempts have failed to produce colonies on triadimenol treated plants, whilst still permitting infection on non-treated plants. However, during one experiment (Table 6) featuring a triadimenol-treated indicator plant sprinkled with heavily sporulating leaves (dipped originally within a solution of LBA 4404 pBBR 1MCS5-virGN54D containing pCAMBIA-CYP51-2), 1 colony was seen 1.5 weeks after inoculation on a secondary leaf. This colony is shown in Figure 5-11A and its survival on a triadimenol treated plant indicated potential transformation. Inspection via microscopy featuring UV light revealed interesting results. It should be noted that microscopy was performed without the leaf undergoing fixing and staining as this would have adversely affected the colony. This caused difficulty when attempting to overlay water above the colony in question and hence problems in focusing.

Attempts were made to interrogate the pathogen with blue light to discern if it incorporated the GFP gene. As shown in Figure 5-11 B-F, a number of fluorescent spores originating from the colony were spotted in several stages of germination. Not all spores fluoresced (as seen in Figure 5-11B) and not all fluoresced to the same degree. Also spotted was the
### Table 6: Agrobacterium-mediated transformation of *Blumeria graminis* f. sp. *hordei* trials.

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<td>LBA1100-CYP51</td>
<td>0.176</td>
<td>0.235</td>
<td>3 days</td>
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<td>LBA1100-eGFP</td>
<td>0.192</td>
<td>0.271</td>
<td>3 days</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5 (2 days at 4 degrees)</td>
<td>25 °C</td>
<td>LBA1100</td>
<td>0.139</td>
<td>0.311</td>
<td>6 days</td>
<td>Mostly infection of non-treated control plants. However, 1 colony spotted on triadimeno treated plants inoculated with spores treated with LBA4404-PBBR-CYP51.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LBA1100-CYP51</td>
<td>0.151</td>
<td>0.394</td>
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<td>LBA1100-eGFP</td>
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</tr>
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<td>6 days</td>
<td>20 °C</td>
<td>LBA 1100</td>
<td>0.214</td>
<td>0.33</td>
<td>5 days</td>
<td>Mostly infection of non-treated control plants. However, 1 colony spotted on triadimeno treated plants inoculated with spores treated with LBA4404-PBBR-CYP51.</td>
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<td></td>
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<td>LBA 1100-CYP51</td>
<td>0.173</td>
<td>0.354</td>
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<td>LBA 1100-eGFP</td>
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<td></td>
<td>LBA 4404-PBBR</td>
<td>0.199</td>
<td>0.307</td>
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<td></td>
<td></td>
<td>LBA 4404-PBBR CYP51</td>
<td>0.117</td>
<td>0.191</td>
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<td>LBA 4404-PBBR-eGFP</td>
<td>0.113</td>
<td>0.19</td>
<td>5 days</td>
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**Agrobacterium-mediated Transformation**

<table>
<thead>
<tr>
<th>Attempt</th>
<th>Source Infection Age</th>
<th>Incubation Temperature</th>
<th>Agrobacterium Culture Type</th>
<th>Starting (OD_{600})</th>
<th>Finishing (OD_{600})</th>
<th>Incubation Time</th>
<th>Result/ Notes</th>
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</thead>
<tbody>
<tr>
<td>8</td>
<td>3-4 days</td>
<td>20 °C</td>
<td>LBA-1100</td>
<td>0.141</td>
<td>0.634</td>
<td>4 days</td>
<td>Infection of all non-treated control plants only.</td>
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<td></td>
<td></td>
<td></td>
<td>LBA1100-CYP51</td>
<td>0.122</td>
<td>0.622</td>
<td>4 days</td>
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<td></td>
<td></td>
<td></td>
<td>LBA4404-PBBR</td>
<td>0.15</td>
<td>0.232</td>
<td>4 days</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>LBA440-PBBR CYP51</td>
<td>0.172</td>
<td>0.203</td>
<td>4 days</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3-4 days</td>
<td>20 °C</td>
<td>LBA1100</td>
<td>0.086</td>
<td>0.8</td>
<td>4 days</td>
<td>Infection of all non-treated control plants only.</td>
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<td>LBA1100-CYP51</td>
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<td>BA 4404-PBBR</td>
<td>0.043</td>
<td>0.707</td>
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<td>LBA 440-PBBR CYP51</td>
<td>0.024</td>
<td>0.692</td>
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<td>10</td>
<td>4-5 days</td>
<td>20 °C</td>
<td>LBA-1100</td>
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<td>0.774</td>
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<td>0.198</td>
<td>0.601</td>
<td>4 days</td>
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<td>EHA404</td>
<td>0.154</td>
<td>0.504</td>
<td>4 days</td>
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<td>EHA404-CYP51</td>
<td>0.157</td>
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<td>4 days</td>
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<tr>
<td>11</td>
<td>4 days</td>
<td>20 °C</td>
<td>LBA1100</td>
<td>0.092</td>
<td>0.718</td>
<td>5 days</td>
<td>Infection of all non-treated control plants only.</td>
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<td>LBA1100-CYP51</td>
<td>0.07</td>
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<td>LBA4404-PBBR</td>
<td>0.075</td>
<td>0.363</td>
<td>5 days</td>
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<td></td>
<td></td>
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<td>LBA440-PBBR CYP51</td>
<td>0.011</td>
<td>0.019</td>
<td>5 days</td>
<td></td>
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<tr>
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<td></td>
<td></td>
<td>EHA105</td>
<td>0.033</td>
<td>0.178</td>
<td>5 days</td>
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<td>EHA105-CYP51</td>
<td>0.016</td>
<td>0.045</td>
<td>5 days</td>
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</table>

**Table 7: Agrobacterium-mediated transformation of Blumeria graminis f. sp. hordei trials.**

‘Source Infection Age’ indicates the age of the infection on leaves dipped in Agrobacterium cultures. ‘Incubation Temperature’ indicates the temperature at which Blumeria graminis infected leaves were incubated after dipping. ‘Agrobacterium Culture Type’ indicates the strain of Agrobacterium used. –eGFP indicates use of GFP containing plasmid pCambia-eGFP-Bar-TaqII; -CYP51 indicates use of pCAMBIA-CYP51-2. Finishing \(OD_{600}\) indicates the optical density of Agrobacterium-strain containing solutions at which infected leaves were dipped.
Agrobacterium-mediated Transformation

fluorescence of epiphytic mycelia within the colony perimeter. Conversely, no conidiophores were observed to fluoresce. Observation of colonies of the same age originating from *Blumeria graminis* f. sp. *hordei* dipped within solutions of LBA1100 (the negative control) failed to highlight conidia that were fluorescing to the same magnitude throughout their structure (Figure 5-11G), although some showed a slight glow near their outer wall. Interestingly, epiphytic mycelia within these colonies was observed to fluoresce (and possibly to the same degree as those observed within the transformed colony), although observations also revealed hyphae that were not fluorescing.

Attempts were made to propagate this colony (Figure 5-11A) by the sprinkling of conidia on non-treated and treated leaves. However, although infection succeeded upon plants not treated with Baytan, limited infection was seen upon treated leaves. Attempts to further propagate this fungus upon treated leaves failed to result in infection. This colony therefore appeared to lose its ability to grow on triadimenol treated plants and it was subsequently lost to experimentation.
Agrobacterium-mediated Transformation

**Figure 5-11: Potential transformation of *Blumeria graminis* f. sp. *hordei.*

The possible transformation of Barley Powdery mildew via *Agrobacterium* mediated transformation (utilising strain LBA 4404 pBBR 1MCS5-virGN54D). A) *Blumeria graminis* f. sp. *hordei* colony on a triadimenol treated leaf. B and C) Spores originating from colony in A. One, indicated by arrow, appears not to fluoresce under UV light. The fluorescing spore structures include the conidial body, primary germ tube, appressorial germ tube and appressorium). D, E, F) Other fluorescing conidia showing a reduction of fluorescence in the appressorial germ tube. G) Spore originating from a colony taken from a control infection (*Agrobacterium tumefaciens* strain LBA1100 dipping solution). No fluorescence is visible within conidial body, or primary germ tube (indicated by arrow). **Scale Bar:** A-F = 40 μm, G = 15 μm
Agrobacterium-mediated Transformation

5.5: Discussion and future work

As bluntly stated by Takken et al., (2004): ‘to prove the function of a gene identified by sequence (homology)… complementation analysis is inevitable’. In order for this to be possible it is first necessary to have an operational fungal transformation system to implement the functional genomics testing required. In the course of this chapter attempts at developing such a system utilising *Agrobacterium tumefaciens* for *Blumeria graminis* f. sp. *hordei* were described. Although the system has been used successfully with many filamentous fungi its application to new species is far from simple (Covert et al., 2001). This has also proven to be the case with *Bgh*. Although in one case transformation may have been achieved (as shown by the presence of fluorescence, although its nature was not categorically proved as being due to eGFP) this was not reproducible and experiments were stopped shortly after.

When selecting and optimising a transformation system for a fungus there are several questions which must be addressed. These include: i) what selection system should be used and how should it be employed; ii) what advantages does this system have over contemporary techniques; iii) what fungal tissue is optimal to use as the basis for transformation.

Fang et al., (2004) observed that a pre-requisite for efficient genetic transformation of an organism is a good selection system for transformants. It is highly desirable that no background level of infection is encountered. Therefore the testing of DH14 sensitivity to triadimenol was important. Multiple tests (including trial runs and operational use, Figure 5-8; Figure 5-9) have indicated that the selection holds, even during heavy spore inoculation, meaning that during these experiments there was no background level of infection to consider. Examination revealed limited growth of the sensitive *Bgh* isolate which never reached a stage that would permit sporulation. Most conidia observed failed to form haustoria (if they did this was limited, Figure 5-10). This is supported by Smolka and Wolf (1986) who assessed a variety of triazole fungicides (similar to triadimenol) and concluded they terminated growth at the haustoria developmental stage. They observed the formation of haustoria with fewer protrusions than normally associated with mature haustoria and those present were fewer and shorter. In similar tests ManginPeyrard and Pepin (1996) mention that papillae number was not affected, leading to them to believe that penetration resistance was not affected (therefore suggesting a plant-mediated resistance to penetration was not being
Agrobacterium-mediated Transformation

enhanced). However, Smolka and Wolf (1986) noted a plant defence reaction (possibly a post-infection defence) which featured an altered extra-haustorial membrane, followed by the encapsulation of still developing haustoria with a plant-derived material including callose and polysaccharides. This encapsulation would be complete by 8 days, the time at which colonies are normally visible to the eye. Although they believe it would not terminate growth by itself, as this occurs before encapsulation is complete, they believed it formed some role (be that precautionary) in defence of the plant. Although caution should be used when extrapolating results especially as DMIs do sometimes have different activities even with the same mode of action (Kang et al., 2001), it seems triadimenol may have had similar effects; with the initiation of potential encapsulation contributing to the rough uneven appearance of the haustoria present.

If the primary time point for the fungicide effect is during haustorial development such a time point seems reasonable. Spore germination is primarily thought to rely on internal reserves. As the haustorium develops the fungus would first gain most access to the fungicide (even if it had a translaminar effect) as it starts to begin harvesting of nutrients and water from the host. Smolka and Wolf (1986) mention specifically that encapsulation was seen around developing rather than developed haustoria. This may be due to the developing fungus being unable to “mask” their presence from the host. Blatter et al., (1998) note that resistance to this fungicide is located at one locus in CC146 and further research links mutations in the CYP51 gene to resistance (Wyand and Brown, 2005). Therefore although the mode of action for triadimenol is not fully understood, as Schmitt et al., (2006) observe an agrochemical can be developed and sold without a full understanding of the mode of action, it does appear this fungicide primarily affects sterol synthesis. By doing so membrane development would be perturbed. This in turn could seriously affect both the membrane-bound enzyme activity and transport capability of a developing haustorium. The reduction in sterols with an accumulation of intermediates and the activity of enzymes such as β-1,3-glucanase can lead to irregular thickening of fungal cell structures (thereby explaining the appearance of haustoria in this study) (Kang et al., 2001). Whilst simultaneously causing a reduction in fungal growth, due to an inability to take up nutrients, the developing fungus would also lack the ability to transport fungal proteins (such as avirulence proteins) or signal molecules into the host. These molecules would normally be able to subvert the signal transduction pathways of the host and repress any defence reactions. Therefore, in this scenario the host would now be able to recognise a damaged foe and initiate this form of encapsulation defence. In
developed haustoria, formed before the fungicide was applied, it is possible what mechanisms used to defend against this encapsulation reaction are already in place by the time the fungicide has begun to have an effect, resulting preventing encapsulation.

As the selection appeared viable attempts were made at the transformation of *Blumeria graminis* isolate DH14. As noted, the majority of attempts, with three strains of *Agrobacterium tumefaciens* at two temperatures were unsuccessful. Although spores of the fungus appeared to survive the treatment process (shown by subsequent infection on non-treated control plants) in the majority of experiments a failure to infect triadimenol treated plants was the case. Interestingly, a glimpse of possible transformation was offered in one experiment utilising the hypervirulent strain LBA4404-PBBR (Figure 5-11). In this case one single colony appeared to survive the first round of selection, thereby appearing to have been transformed with pCambia-CYP51-2. In spite of this the colony failed to spread during later attempts at inoculation on treated leaves. Additionally microscopy painted a confused picture. Although certain structures were seen to fluoresce to a greater degree than similar structures from non-transformed colonies, other structures within the same colony failed to fluoresce at all. This leads to a number of possibilities. Firstly, the H3 promoter chosen for both the CYP51 and the eGFP is not uniform in its activity throughout the lifecycle of the fungus. If so then this will have drawbacks when trying to select for transformed fungus. Secondly, although incorporation into the fungus occurred it was only transitory in nature. This would not be the first time such an event has occurred with *Bgh*. Similar behaviour was seen during transformation attempts by Christiansen et al., (1995) and Chaure et al., (2000).

Wyand and Brown (2005) identified two mutations in the CYP51 gene coding region of the CC146 isolate that resulted in high resistance, however no analysis was performed of the promoter region. It is possible that apart from these mutations over-expression of the gene is also a feature resulting in high resistance in this strain. Therefore, future work should focus on trying to identify any repeated elements or “over-drive” sequences present in the upstream sequences of the CC146 CYP51 gene. Such sequences may, for example, be similar to those present in studies by Hamamoto et al., (2000) (who found a 126 bp tandem repeat sequence acting as transcriptional enhancer in DMI-resistant isolates of *Penicillium digitatum*) or Mellado et al., (2007) (who discovered a duplicated 34 bp sequence in the promoter of *Aspergillus fumigates* isolates resistant to azoles plays a role in their resistance). Even if no such sequences are found (and utilised to enhance CYP51 expression) future efforts should
include the use of the CC146 CYP51 promoter sequences (or at least as much as can be identified of the proximal promoter region as possible) in an attempt to cause levels of expression normally found in CC146 in the spores of sensitive isolates. Such a usage of the CC146 promoter may also remove a potential problem encountered, unintentionally, with the vector design. As can be seen in Figure 5-4 both the CC146 allele of CYP51 and GFP genes are bordered by sequences of the H3 histone promoter and an aquaporin terminator, which were chosen based on their ability to permit uniform expression of both the selective and screenable markers during the lifecycle of the fungus. Given that the same sequence is used either side of the genes within the same plasmid construct, there is a chance that homologous recombination within the vector (rather than integration with the host) may have occurred. Potentially this recombination may have resulted in the loss of the selectable marker and hence any chance of resistance to triadimenol. As a consequence the likelihood of successful transformation of the host (which for reasons mentioned later may already be difficult to achieve with the transformation system chosen) may have been further reduced.

In order to reduce the likelihood of transitory incorporation the selection should be applied as soon as possible. Although the original host plants to the sensitive DH14 cannot be treated with triadimenol it may be possible to capitalise on the vapour action of triazoles. By placing treated filter papers (in the fashion of Maffi et al., 1995) in close proximity to the dipped leaves the pesticide may act to select against non-transformed colonies. Although the author is unaware if triadimenol has a vapour action it is known that another triazole, triadimefon, has such an action (Maffi et al., 1995). Fortuitously the main breakdown product of this fungicide is triadimenol and so resistance may be solely provided by the CYP51 gene already in use by the vectors employed in this study. In studies listed by Maffi et al., (1995) they note that although germination is delayed, possibly highlighting another mode of action for triazoles or maybe the low level of in situ sterol production present in early-germination stage conidia, the main effect is observed during haustoria development.

At the time of testing it was not possible to analyse the wavelength of the fluorescence emitted by the potential transformed fungus. If further transformants are achieved in future efforts must be made to ensure any fluorescence present is due to eGFP and not autofluorescence. In the sole case found in this study, non-fluorescent spores were lying in close proximity to spores. This would suggest that they originated from a non-transformed colony, but more importantly any fluorescence visualised was not due to autofluorescence.
In the case of Agrobacterium-mediated transformation there are a number of distinct advantages when compared to techniques such as polyethylene glycol mediated transformation of protoplasts. The technique does not require expensive enzymes whose protoplasting ability may vary between batches and is significantly less laborious (Combier et al., 2003). Protoplasting and electroporation, require to an extent, that aseptic techniques be followed. This permits the growth and repair of potentially fragile selected fungal material on sterile media laced with selective agents. This is not possible with Bgh as it would be impossible to truly sterilise both the plants and inoculum. However, as Agrobacterium functions in nature alongside other microbes to form “crown galls” it is likely the bacterium can overcome any impedence they may engender.

In Agrobacterium-mediated transformation the T-DNA is present as a single strand, not as a double stranded structure as in other techniques. Furthermore, the translocation of the vir operon proteins into the host cell means that the T-strand has extra defence against the action of nucleases (in the case of VirE2), as well as targeting to the nucleus. Both are characteristics which are not present in other transformation techniques and probably account for stable transformation efficiencies that are orders of magnitudes higher than protoplasting techniques (de Groot et al., 1998). Apart from the option of homologous recombination with increased T-DNA sequence homology as well as the ability to permit random T-DNA integration (advantageous both to targeted gene disruption as well as general mutagenesis screens respectively), the partial conservation of the T-DNA border sites means the isolation and identification of integration sites by methods such as ‘Thermal Asymmetric Interlaced (Tail) PCR may be utilised. These reasons cause Agrobacterium-mediated transformation to recommend itself over other techniques.

The primary advantage, and the factor which above all, which dictated the selection of this system in this instance was that several forms of fungal starting material, e.g. spores and hyphae, have been used as the basis for transformation (de Groot et al., 1998). As with previous work (e.g. Chaure et al., 2000 who trialled biolistic transformation on developing colonies) efforts in this study also focused on transforming young colonies with the hope that either the colony itself (i.e. the conidiophore mother cell) or the spores themselves would be transformed. Seven days post-initial infection generous numbers of freshly developing colonies and conidia may be formed under lab conditions, essentially meaning there is no
Agrobacterium-mediated Transformation

limit to the starting material available for transformation. If transformed either directly or via developing from a transformed mother colony, a transformed spore would need only an uninfected leaf for it to begin an entirely new infection. It was hoped that, apart from the application of a selection method, post-transformation would be relatively input free. Unfortunately, in order to achieve this infection both colonies and spores need to be intact and undamaged to maintain development on the host and to permit infection of new hosts. This rules out the use of protoplast based methods that are part of the most common methods of transforming fungi. It may also be a contributing factor in the failure to develop a successful system during this work.

During the transformation process leaves were dipped, and co-cultivated with the bacterium, in an induction medium laced with acetosyringone. Chowdhury et al., (2003) list studies which demonstrate that water and aqueous media cause severe damage to conidia, in some cases leading to a 90% reduction in infectivity. Reasons for this could be the osmotic strain placed on the spore which leads to damage to membranes and alterations of internal concentrations. Also this medium may have inadvertently interfered with the ECM coverage of the spore. This matrix is critical during the very early stages of transformation in providing signals that firstly aid primary germ tube emergence and then spur later stages of development. If this is the case then by affecting the ECM coating we will have decreased the chances of developing successful infection of spores by removing its ability to accurately sense the substrata on which it had landed. If spore penetration ability is modelled on a normalised bell curve with half the spores being, on average, less able to overcome penetration resistance (even by susceptible genotypes) of the barley, then such an action may significantly reduce the chances of an infection by a successfully transformed spore. This would explain why the indications of transformation (for example in this study colonies on triadimenol treated leaves and fluorescent spores) were so rare. During this study no attempt was made to systematically analyse spore development on triadimenol leaves that had been sprinkled with the spores from “dipped” colonies. If work is to be continued on this method then efforts should be made to discern both the stage at which the majority of spore germination terminates and importantly the percentage of primary germ tubes which make contact with the underlying leaf surface. It may be necessary to find the correct co-incubation/dipping time to minimise any damage to the ECM, if such a proposition is feasible. However, this must be balanced carefully with the need for transformation itself. In
certain studies listed by Michielse (2005) the longer the co-cultivation time the higher the efficiency of transformation (and chance for multiple integration).

Instead of dipping the infected leaves the spraying of a bacterial/induction solution may be trialled. This means some control is had over the amount of aqueous solution in contact with the colonies. It could be applied whilst the leaves are still attached, meaning they are not any undue stress caused by their removal from the plant. Once a leaf surface has been wetted any surplus liquid runs off, which can lead to a depletion of the liquid originally left on the leaf (Matthews, 2000). Therefore the finer droplets of an aerosol may equate to an excellent coverage of the target with a smaller likely-hood of droplets rolling off the hydrophobic leaf surface (although “run-off” may to some degree be minimised by the use of surfactants). Furthermore the surface area of smaller droplets increases significantly when the droplets are under 50 µm in diameter (i.e. fine-coarse aerosol sprays). This results in an increased rate of evaporation, possibly minimising damage to the spores whilst still leaving them in proximity to the bacterium.

Other areas of technique, as listed by Michielse et al., (2004), could also offer room for optimisation, or at the very least an indicator of the limits of this technique. Concentrations of the vir gene inducer, acetosyringone, although similar to concentrations used in other studies may be altered as can the choice of A. tumefaciens strain. Other areas of optimisation are not practicable with Bgh. These include finding the most optimal ratio of bacterial cells to target material. In this case, although bacteria concentration could be measured, the number of spores present on a developing Bgh could not be. In future work attempts should be made to at least form some type of conservative estimate of the number spores on a colony of a certain age at a certain size- and from there estimations could be used to gauge an optimal ratio of conidia to Agrobacterium. Temperature of co-incubation should also be considered. Combier et al., (2003) found that the highest efficiency of transformation was at temperatures most optimal for their organism of study. In this study although dipped leaves were placed at 25 °C after dipping had occurred, during the dipping itself they were at room temperature (approximately 20 °C).

Bearing all this in mind, note should be taken of the fact that the efficiency of Agrobacterium-mediated transformation of fungi is lower than that of plants (Lacroix et al., 2006). Reasons for this lower efficiency could centre around an inability of the bacterium to
Agrobacterium-mediated Transformation

coop-fugal host factors as it would plant host equivalents to achieve its aim. As an example Citovsky et al., (2007), although debated by Gelvin (2010), highlights the role of VirE2 (a protein that enters the nucleus of plant cells in a complex with a basic-zipper-protein called VIP1). In non-host systems it appears that VIP1 has yet to be found, meaning the bacterium then has to rely on its own Vir proteins to provide some form of redundant back-up. In this case VirE3 can duplicate some of functions of VIP1 and promote VirE2 nuclear import (studies in Citovsky et al., 2007). This may highlight the limit to which this system may be utilised and also how difficult it can be to apply the system to fungi even without the added hindrance of spore damage due to water contact.

In conclusion although ultimately unsuccessful, these trials were encouraging in that, even if transformation was indeed transitory, it did occur. They have also provided avenues for progression. Future work, possibly in the laboratory of Prof. James Brown (John Innes Centre, Norwich) will aim to replicate this result and if needs be attention may be turned to trying to alter the T-DNA border sequences within the vector to increase recombination (Lacroix et al., 2006). Should transformation succeed then tests with Southern Blotting may occur to illustrate just how exactly the T-DNA has incorporated itself. Nevertheless, the problem of trying to isolate a single transformant from a colony of mixed genotypes (more than one conidia may be transformed) will prove quite a challenging one.

Such a transformation would allow investigators to delve further into the pathosystem of this critically important fungus by utilising such techniques as T-DNA insertional mutagenesis, T-DNA activation tagging and targeted gene disruption which have proven so useful in the study of plants.

Since the transformation of Bgh has once again not been possible, in the final chapter the testing of an alternative host for future investigations involving the Bgh regulatory elements are discussed.
Chapter 6: CMEG 5’-regulatory region driven GFP expression in *Magnaporthe oryzae*

6.1: Gene transcription and regulation

The precise expression of protein coding genes is vital to allow the correct development and differentiation of cells as well as to allow reaction to environmental or host signals (Szutorisz et al., 2005; Reid et al., 2009). Eukaryotic gene transcription, the first stage of expression, is the main point at which regulation of expression occurs (Wasserman and Sandelin, 2004). Transcription regulation is multi-faceted requiring *cis*-acting elements and *trans*-acting factors which bind to these *cis*-elements (Yu et al., 2006). Factors controlling transcription may include sequence specific DNA binding factors, chromatin regulators and the general transcription machinery (and their regulators) (Venters and Pugh, 2009). Via these elaborate control mechanisms genes may be expressed in a highly defined and temporal manner as well as in co-ordination with other genes (Pedersen et al., 1999). For an in-depth review the reader is referred to Venters and Pugh (2009).

There are multiple stages to eukaryotic RNA synthesis. These include initiation, promoter clearance, elongation and termination (Szutorisz et al., 2005). Initiation requires the RNA polymerase to interact with the DNA at the promoter of the gene of interest and is an important control point (Szutorisz et al., 2005; Pollard and Earnshaw, 2008). The promoter may be defined as a set of nucleotides indicating the start of RNA synthesis (Alberts et al., 2002). Promoter sequences for RNA polymerase II (encoding mRNAs) are usually situated upstream of the transcriptional start site (TSS) (Pedersen et al., 1999; Kornberg, 2007). RNA polymerase cannot perform its function by itself - instead additional factors known as ‘General Transcription Factors’ (GTF) are required (Pedersen et al., 1999). These GTFs, for example TFIID, will by recognising short specific sequences in the promoter region, for example the TATA Box, bind to the DNA and begin to form the pre-initiation complex with RNA polymerase II (Alberts et al., 2002; Pollard and Earnshaw, 2008). This formation may be aided by the presence of the ‘mediator’ complex which reversibly binds the RNA polymerase II (and with other factors forms the RNA Polymerase II Holoenzyme). The mediator helps the polymerase “find” the promoter by interacting with gene regulatory
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proteins called transcriptional activators (Alberts et al., 2002; Pollard and Earnshaw, 2008). Additionally chromatin modifying enzymes may also be involved. After forming the transcription initiation complex, a GTF (TFIIH) containing a DNA helicase subunit (that on hydrolysing ATP) will cause the unwinding of the DNA to be transcribed. After a series of conformational changes the RNA polymerase will proceed from the promoter and begin transcription (Alberts et al., 2002).

The minimal, or core promoter, is the set of sequences sufficient for the assembly of the pre-initiation complex (Pedersen et al., 1999; Szutorisz et al., 2005). However there is not just 1 class of minimal promoter (Pedersen et al., 1999). Some contain the previously mentioned TATA-box (with the consensus TATAAAA), whilst others have contained another element known as the ‘initiator’ near the ‘transcription start site’ (TSS) (Smale, 1997), possibly accompanying another element 30 bp downstream of the TSS (the ‘Downstream Promoter Element’, DPE) (Burke and Kadonaga, 1997). Additionally, some core promoter appear to lack all known core promoter elements (Juven-Gershon et al., 2008). There are often high numbers of sequences with similarities to these elements spread throughout the genome (e.g. studies by Prestridge and Burks (1993) who demonstrated 1 predicted TATA-box every 120 bp. Since, as Pedersen et al., 1999, note that promiscuous transcription does not take place other factors, besides core elements, must aid in the control of transcription.

Specifically in the case of RNA polymerase II those elements which are found 30 to 200 bp upstream of the TSS are termed promoter proximal elements (Szutorisz et al., 2005). Others, known as distal elements (known as enhancers which increase transcription, and silencers which repress it) may be even further away (Pedersen et al., 1999; Szutorisz et al., 2005; Abeel et al., 2008; Pollard and Earnshaw, 2008). These elements, up to several thousand bp away from the promoter, may exert effects independently of their position or orientation (Brasset and Vaury, 2005). It is at these sites that transcriptional regulatory proteins (such as transcription factors) may bind and allow precise control over gene expression (Pedersen et al., 1999; Brasset and Vaury, 2005).

Downstream of the receptors that respond to external stimuli, and at the end of many signal transduction pathways, transcription factors (TFs) form the last step in the signal transduction pathways that mediate the cells responses to stimuli (Pollard, 2008; Hu, 2010). These factors are the elementary units of the transcription regulatory mechanism (Park et al., 2002; Reid et
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al., 2009). TFs may bind to the basal machinery directly or may act in concert with co-
activator or co-repressors (Pollard and Earnshaw, 2008). Unlike GTFs, TFs may number in
the thousands, all capable of binding different regulatory elements (Pedersen et al., 1999).
Looping of the DNA is thought to bring the TF close to its site of action when binding
elements far from the promoter (Pollard and Earnshaw, 2008) (Figure 6-1).

![Figure 6-1: Components of transcriptional regulation.](image)

Transcription factors (TFs) bind to specific sites (transcription-factor binding sites) that are either proximal or
distal to a transcription start site. Sets of TFs can operate in functional cis-regulatory modules (CRMs) to
achieve specific regulatory properties. Interactions between bound TFs and co-factors stabilize the transcription-
initiation machinery to enable gene expression. The regulation that is conferred by sequence-specific binding
TFs is highly dependent on the three dimensional structure of chromatin (Source: Wasserman and Sandelin,
2004).

*Cis*-regulatory regions often contain binding sites for several TFs, with genes showing their
own pattern of sites, each of which may bind several types of TF (Pedersen et al., 1999;
Kristiansson et al., 2009). Additionally, promoters may contain multiple copies of the same
TF binding sight (Paixao and Azevedo, 2009). Transcription binding sequences
(‘transcription factor binding sites’) are usually 5 to 25 bp in length and may be found
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upstream of the TSS, in the 5’-UTR, and downstream of it (Butler and Kadonaga, 2002; Chowdhary et al., 2006). They are often found in clusters (known as *cis*-regulatory modules, CRMs) (Reid et al., 2009). TFs do not work in isolation to one another, often requiring a combinatorial presence (or absence) to regulate the expression of the genes in question (Reid et al., 2009). However as cellular processes often require the co-ordination of genes it is thought that sets of TFs are often active on these co-expressed genes (Reid et al., 2009). Transcription factors themselves are under such expression control and so transcriptional cascades and feedback loops also form a level of regulation (Pedersen et al., 1999). It is because of this modular nature of control regions that the particular spatial and temporal gene expression requirements of the cell are met (Pedersen et al., 1999).

As well as transcription factors, chromatin structure can also influence gene expression with tightly bound heterochromatin preventing the access of transcription factors to their binding sites (Narlikar et al., 2002; Alvarez et al., 2010). Therefore its unfolding is an important part of gene regulation (Pedersen et al., 1999; Narlikar et al., 2002).

One of the first steps in identifying the role of a gene includes trying to identify regions housing control elements (Wasserman and Sandelin, 2004). As highlighted core promoters can be varied. They may also contain regions of unknown function with many transcription factor binding sites (often short and varying in nature) to choose from (Park et al., 2002; Juven-Gershon et al., 2008; Kristiansson et al., 2009). This makes the determination of regulatory regions difficult and an often imprecise task needing lengthy computational analysis. Although promoter predictors are numerous the lack of distinctive features can give many false positives or negatives (Muller et al., 2007). The initial step in the identification of promoter regions is often the identification of the transcriptional start site (TSS) (Chowdhary et al., 2006). This may allow the identification of any potential primary regulatory elements (such as TF binding sites) within this proximal promoter region (Chowdhary et al., 2006). If knowledge regarding *cis*-regulatory elements is lacking then phylogenetic footprinting may be used (Wasserman and Sandelin, 2004). This process is underpinned by the hypothesis that random mutations occur more frequently in regions without specific function which aren’t necessary for gene expression. By comparing orthologous sequences, it is possible to locate areas of evolutionary conserved sequence (Wasserman and Sandelin, 2004).
6.2: *Magnaporthe oryzae*- A heterologous expression host

Rice Blast disease is caused by the filamentous ascomycete *Magnaporthe oryzae*, formerly *M. grisea* (Herbert) Barr (Couch and Kohn, 2002; Khang et al., 2005; Liu et al., 2006). This fungus is classed within the *Sordariomycetes*, a close neighbour of the class *Leotiomycetes* (containing the family *Erysiphaeaceae*) (Robbertse et al., 2006). This disease also causes high crop losses (up to 33%) and is considered the most devastating rice disease (Talbot, 2003; Xue et al., 2004). Unlike *Bgh*, *M. oryzae* may survive on over 50 hosts, including barley and wheat (Valent and Chumley, 1991).

![Figure 6-2: Magnaporthe oryzae lifecycle on rice.](image)

A) Conidia are aerially dispersed from conidiophores. B) Conidia adhere to the host surface. C) Conidia germinate and produce a hyphal filament. D) Formation of the appressorium with concomitant autophagy of conidia and germ tube. E) Formation of penetration peg and invasion of host tissue. Movement into surrounding cells appears to be specifically at plasmodesmata clusters, ‘pit fields’. Invasive hyphae have a membrane cap believed to function in protein secretion into the host cell. **Source:** (Ebbole, 2007)

In close similarity to *Bgh*, infection begins when an asexual spore lands upon the host leaf surface (Figure 6-2). A preformed mucilage (the ‘spore tip mucilage’) is released upon contact, after spore hydration and aids adhesion to the leaf (Hamer and Talbot, 1998; Gerbeaud et al., 2001). By 0.5 to 2 h.p.i. a short, polarised germ tube emerges from the multicelled conidium (Lu et al., 2005; Tongen et al., 2006). The tube is unbranched and is surrounded by an ECM providing adhesive and protective qualities (Jelitto et al., 1994; Xue...
CMEG 5’-Regulatory Region driven GFP expression in *M. oryzae* et al., 2004). By 4 h.p.i. elongation ceases and the germ tube swells at its tip, flattens and alters direction in a process known as ‘germ tube hooking’, similar to the behaviour of the AGT of *Bgh* (Bourett and Howard, 1990). Following this process a dome-shaped appressorium is formed and without this structure infection fails (Choi et al., 1998; Kang et al., 1999). By 4 to 8 h.p.i. melanisation of the appressorium begins, and with a build-up of intracellular glycerol levels, turgor generation is permitted. Storage products are transported to the appressorium from the conidia and germ tube, which after isolation, undergo collapse (Tongen et al., 2006). By 24 h.p.i. an appressorial pore ring has formed and it is from here the penetration peg emerges (Lu et al., 2005). This appressorium can generate a turgor pressure of up to 8.0 MPa (Howard et al., 1991), and at its peak (occurring between 24 to 31 h.p.i.) an infection peg is thrust down through the plant cuticle into the tissue below. This penetration may also be aided by enzymatic digestion of the leaf surface (Tongen et al., 2006). Host colonisation by hyphal growth then occurs. The initial hypha (often ≈31 h.p.i) differentiates into a number of branched hyphae that spread rapidly into surrounding epidermal cells (Figure 6-2) (Lu et al., 2005; Ebbole, 2007). These allow the harvesting of host nutrients. Interestingly, although these hyphae are similarly thought to cause invagination of the host membranes they do not approach the complexity in structure of the haustoria of *Bgh*. Talbot et al., (1993) notes that by 96 h.p.i. the first symptoms are visible as small ellipsoid lesions, resulting from host chlorosis and necrosis (Valent and Chumley, 1991). The hyphae will at some stage breech the leaf surface to permit the formation of conidia (by holoblastic conidiogenesis) and the continuation of the lifecycle (Xue et al., 2004). Spores are transmitted to nearby plants by wind and dew drop (Talbot, 2003).

During the process of forming a “hooked” germ tube signal recognition of the leaf surface, permitting appressorial development, is thought to occur (Gilbert et al., 1996; Choi et al., 1998). Studies have discerned some of the signals and suggest the emerging germ tube tip acts as the vehicle for their sensing (Chun and Lee, 1999). Briefly, these include the need for attachment to a solid surface (Lee and Dean, 1994; Xiao et al., 1994; Gilbert et al., 1996), the presence of soluble cutin monomers and of lipid monomers (i.e. C16 and C18 compounds), potential components of leaf epicuticular waxes (Gilbert et al., 1996, and studies listed in Talbot, 2003). Additionally, nitrogen deprivation and light availability requirements have also been observed, as has stimulation by nutrients (Jelitto et al., 1994; Xue et al., 2004). Further evidence supporting these requirements include the release of cutinase by *M. oryzae* (Sweigard et al., 1992). Many inductive surfaces are hydrophobic in nature although
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hydrophilic surfaces may likewise induce appressoria (Gilbert et al., 1996; Ebbole, 2007). Furthermore, *M. oryzae* forms appressoria on artificial surfaces suggesting the presence of monomers, although stimulating growth, is not essential for it (Gilbert et al., 1996). This has led some authors, e.g. Gilbert et al. (1996), to suggest control via a convergent, multi-stimuli pathway was involved, which may prove essential to permit such a large host range. Many of these requirements show similarities to those required for the development of *Bgh*. Similarly, as in *Bgh* such signals lead to fluxes in certain signalling cascades e.g., the cyclic AMP and the PMK1 MAPK pathway (refer to Talbot, 2003 and Wilson and Talbot, 2009 for details).

During the past decade and beyond this pathogen has begun to fulfil the role of a model plant pathogen (Khang et al., 2005; Wilson and Talbot, 2009). It is considered one of the best studied of the phytopathogenic fungi and features in many studies of host-pathogen interaction (Lu et al., 2005). Important reasons for this include the ability to grow in axenic culture and also the ability to carry out genetic analysis on itself or its rice host (studies are listed in Talbot, 2003; Mitchell et al., 2003). Furthermore analysis is aided by the ability of the fungus to undergo stages of the infection process up to and including appressorium formation on non-host surfaces (studies listed in Talbot, 2003). Previous studies have been able to transform *M. oryzae* via polyethylene glycol utilising such antibiotic markers including hygromycin B (Talbot and Foster, 2001). Talbot (2003) notes that although not overly efficient (averaging only 40 transformants per microgram of transforming DNA), the procedure is considered “sufficient”.

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6.3: Aims and objectives

As observed by Park et al., (2002) one of the challenges of gene regulation is the attempt to define the regulatory regions of genes and link them to the genes known regulation. In studies performed by Dr. Maike Paramor, the effects of four different germination surfaces (barley, wheat, cellulose and glass) on the gene expression of 2027 unigenes was investigated using cDNA microarray. The results of this test revealed genes with altered expression during germination and furthermore genes exhibiting similar expression patterns across the different surfaces. 12 Bgh genes with alternate expressions were chosen as model examples of the different expression profiles encountered during germination on these surfaces. These genes, some of which formed the basis for clusters containing virulence associated genes, included a Bgh plasma membrane ATPase, an extracellular alkaline protease, a Crh-like protein, a Ran GTPase activating protein 1, a Bgh cap20-like protein, an alpha 1,6-mannosyltransferase and a putative structure specific recognition protein, amongst others.

It is a common strategy that gene regulatory elements are predicted by seeking common sequences in the 5’-regions of genes with clustering evident in their expression profiles (Park et al., 2002). This suggests the genes are co-regulated, making in theory the finding of common regulatory sequences easier. Furthermore, studies by authors such as Spellman et al., (1998) demonstrated that some genes with clustered expression belong to, and contain the TF binding sites for, similar regulatory mechanisms.

CMEG regulatory regions were therefore determined by blasting (both Blastn and Blastx) ESTs corresponding to the CMEG in question against genomic contigs available at that time (2007-2008) during early stages of the Blumeria graminis genome sequencing project (www.blugen.org/). Such searches would reveal actual genomic sequence. Linked to this uniprot searches using these ESTs showed whether any known proteins would be a match. When positive matches occurred, a FGENESH search was performed. By optimising the search for Sclerotinia sclerotiorum, a close phylogenetic relative of B. graminis f. sp. hordei, 5’-regions upstream from the TSS were isolated (up to 2 kb, free from any surrounding neighbouring coding sequences). Furthermore, these sequences could then be utilised for further analysis using a phylogenetic footprinting algorithm, alongside five other closely related fungi. This program determines the sequence regions most conserved and consequently most likely to contain the regulatory elements (and was performed by Dr.
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Yusanne Ma, formerly of the Stumpf group, Imperial College). This algorithm utilised Bayesian hierarchical models to compare homologous sequences from the different species used. The algorithm infers ancestral sequences and evolutionary distances between sequences and by employing Bayesian partition models this algorithm partitions the sequence into segments of differing evolutionary conservation.

Subsequently, CMEG clusters were then analysed to determine their *cis*-regulatory regions. Additionally, clusters were analysed *in silico* to see if these co-regulated genes shared similar regulatory elements in their promoter region. This *in silico* testing, however, failed to yield any meaningful results regarding shared elements although some conservation was observed (Dr. Maike Paramor, pers comm.).

As *in situ* analysis is not yet a possibility in *Bgh* due to inability to stably transform it (as highlighted in the previous chapter), other methods of testing must be employed. *Magnaporthe oryzae* (formerly *grisea*) is an ascomycete fungus that has become favoured as a model plant pathogen. Known for its tractability and ease of handling, combined with its phylogenetic relationship to *Bgh*, *M. oryzae* may be suitable candidate to act as heterologous host for the testing of these CMEG regulatory elements. Accordingly it was the aim of this work to test for the activity of a number of *Bgh* CMEG 5’-regulatory regions when part of a GFP-reporter system transformed into *M. oryzae*. This investigation aimed to analyse both the suitability of *M. oryzae* for further use as a heterologous host organism and to demonstrate potential for structural analysis of selected CMEG regulatory regions.
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6.4: Materials and methods

6.4.1: Vectors

For the transformation of *Magnaporthe oryzae* the vector pMJK27.2 (a gift from Dr. Mick Kershaw [Talbot Lab, University of Exeter]) and derivatives of this plasmid containing *Blumeria* promoter regions were used (Figure 6-3). Variants were constructed primarily by Dr. Calin Andras and Dr. Maike Paramor, with some help of the author. pMJK27.2 contains a *sGFP* (Appendix E) gene with a promoter from the hydrophobin gene *MPG1* and a *TrpC* terminator, as well as a gene for hygromycin-B resistance. *M. oryzae* previously transformed with a GFP-containing construct, pCAMgfp, (a gift of Dr. A. Sesma, John Innes Centre, Norwich) was used as an indicator of GFP behaviour within *M. oryzae* and for optimisation of microscopy (Figure 6-3). pCAMgfp contains an *sGFP* gene with a *Pyrenophora tritici-repentis* *TOXA* gene promoter and a hygromycin-B resistance gene with an *Aspergillus nidulans TRPC* promoter within a pCAMBIA-1300 vector backbone.

6.4.2: pMJK27.2 Gateway vector conversion and promoter/pMJK27.2 vector preparation

This procedure was based around the protocols of the manufacturer for the ‘Gateway® Vector Conversion with One Shot® ccdB Survival Cells’ (Invitrogen™: Catalogue number 11828029) and ‘pCR8®/GW/TOPO®® TA Cloning Kit (Invitrogen™: Catalogue Number K250020) with some modifications. The procedure was as follows: PCR primers (*T3 cloning* [5’-CTTTTGTTCCCTTTAGGAG-3’] and *sGFP cloning* [5’-ATGGTGAGCAAGGCG-3’]) binding the flanking regions immediately adjacent to the *MPG1* promoter in the MPG1-*sGFP* reporter construct, pMJK27.2, were designed to allow amplification of the whole vector excluding the *MPG1* promoter. Phusion® high fidelity polymerase (Finnzymes) was used to amplify the approximately 6.5 kb vector fragment. Gateway Cassette A (Invitrogen™: Catalogue number 11828029, Appendix E) was ligated according to the protocol of the manufacturer with the promoter-less pMJK27.2 linear fragment.
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**Figure 6-3**: A schematic diagram of pMJK27.2 and pCAMgfp used during *M. oryzae* transformation attempts.

**A) pMJK27.2.** Restriction digestion sites are shown. This plasmid has a molecular weight of 7.82 Kb. A chloroamphenicol resistance gene is present within the backbone. The MPG1 promoter was originally cloned via restriction digestion using *Bst*II and *Nco*I into the plasmid pAN52.1. The promoter/gfp/terminator cassette was then cloned into the multiple cloning site of pBCSK+ (Stratagene®) via a *Bst*II/*Hind*III digestion and relegation. The *Bst*II site lies within the left extremity of the multiple cloning site (demarcated via blue lines). The *Hind*III site lies within the right extremity of the MCS. (Dr. Mick Kershaw, pers. comm.). The pMJK27.2 derivatives have promoters from the *Blumeria graminis* gene replacing the MPG1 gene promoter. These were inserted utilising Invitrogen Gateway® Vector Conversion system protocols. **B) pCAMgfp.** A ~3 Kb *Xho*I-*Eco*RI restriction fragment containing a modified hygromycin resistance gene under the control of the *Aspergillus nidulans* TrpC promoter together with the sGFP gene under the control of the *Pyrenophora tritici-repentis* ToxA gene promoter was isolated from pCT74. This restriction fragment was ligated into the *Xho*I/*Eco*RI-digested pCAMBIA-1300 to replace a pCAMBIA-1300 cassette containing the hygromycin gene under the control of the cauliflower mosaic virus 35S promoter. The resulting vector was introduced into *Agrobacterium tumefaciens* strain AGL-1 and transformed into *M. oryzae* (Dr. A. Sesma, pers. Comm.)

Promoter sequences were amplified by Phusion polymerase PCR (the reverse cloning primer for all promoters was designed to include stop codons in all three frames), and blunt ends were terminated with the addition of adenine by subsequent incubation with generic Taq polymerase for 10 min at 72 °C. These sequences were cloned into pCR8®/GW/TOPO® vector by TA Topo cloning according to the protocol of the manufacturer (Invitrogen™, Invitrogen™, One Shot® ccdB Survival Competent Cells).
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Catalogue Number K250020) but with the reaction volume reduced to 1/3 - 1/2, using reduced reagent volumes accordingly. The volume of One Shot Top 10 competent cells was also reduced to 1/2. Following transformation 50 µl of cells were plated out and the remaining volume was kept for a second plating (using more or less cells) if necessary. Typically 8 colonies per construct were suspended each in 10 µl of ddH₂O of which 1 µl was used in a 20 µl volume reaction for a PCR insert orientation assay. The M13-Forward primer was used in conjunction with the reverse cloning primer for each promoter insert (Appendix E, 9.9) and positive colonies for the expected size fragment were DNA mini-prepped (the remaining 9 µl of colony suspension were inoculated in 2 ml overnight cultures of which 1.5 ml were used for DNA minipreps using QIAprep® Spin miniprep columns (following the protocols of the manufacturer, Qiagen). DNA from selected Topo constructs was used in an LR reaction with the D10 vector using the Clonase II enzyme according to the protocol of the manufacturer (Invitrogen™, Catalogue number 11828029) but with reaction volumes (and reagents) reduced to half. The presence of promoter inserts in D10 were initially assessed by PCR using M13-Reverse primer (sequence 5’-CAGGAAACAGCTATGACC-3’) and the reverse cloning primer for each promoter (4 colonies each). Finally one of the positives was sequenced using the M13-Reverse primer.
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6.4.3: *B. graminis* f. sp. *hordei* isolate DH14 promoter regions used during transformation

5’-upstream regions presumed to contain promoters from the following genes (*Appendix E, 9.9*) were used during transformation attempts of *Magnaporthe oryzae* (Table 8):

<table>
<thead>
<tr>
<th>EST Library Identifier</th>
<th>CMEG Cluster Leader</th>
<th>Identity/Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>D00146</td>
<td>Cap20</td>
<td>Retinal Short Chain Dehydrogenase Reductase</td>
</tr>
<tr>
<td>C00750</td>
<td>Cap20</td>
<td>Cap20</td>
</tr>
<tr>
<td>C00222</td>
<td>C00206(1)</td>
<td>Unknown</td>
</tr>
<tr>
<td>D01230</td>
<td>C00206(1)</td>
<td>ATP- synthase Protein 9, Mitochondrial Precursor</td>
</tr>
<tr>
<td>C00082</td>
<td>C00206(1)</td>
<td>Gamma subunit of the F1 sector of mitochondrial F1F10 ATP synthase</td>
</tr>
<tr>
<td>D00095</td>
<td>C00206(1)</td>
<td>60S ribosomal subunit nuclear export factor</td>
</tr>
<tr>
<td>D00651</td>
<td>C00206(1)</td>
<td>Malate dehydrogenase</td>
</tr>
<tr>
<td>C00741</td>
<td>Cap20</td>
<td>Signal Recognition Particle 54 kDa protein homolog</td>
</tr>
<tr>
<td>BG22203</td>
<td>Haustoria specific</td>
<td>Glutamate decarboxylase</td>
</tr>
<tr>
<td>C01420</td>
<td>C01417*</td>
<td>Aconitase (carbohydrate metabolism)</td>
</tr>
<tr>
<td>C00879</td>
<td>C00206(*)</td>
<td>H4 Histone</td>
</tr>
<tr>
<td>C01157 (*)</td>
<td>C01417*</td>
<td>Protein Disulfide Isomerase Precursor</td>
</tr>
<tr>
<td>D00154</td>
<td>C01417*</td>
<td>Plasma membrane H+-ATPase gene</td>
</tr>
<tr>
<td>C01518</td>
<td>C01417*</td>
<td>Unknown</td>
</tr>
<tr>
<td>D00014</td>
<td>D00933*</td>
<td>MepB (metalloproteinase)</td>
</tr>
<tr>
<td>D01317</td>
<td>D00933*</td>
<td>Alternative oxidase gene</td>
</tr>
<tr>
<td>C00209</td>
<td>C00606</td>
<td>Mannosyl-oligosaccharide alpha-1 (mannosidase precursor; carbohydrate metabolism)</td>
</tr>
<tr>
<td>C00606 (*)</td>
<td>Cluster Leader</td>
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</tr>
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<td>C00010</td>
<td>C01417(1)</td>
<td>Fructose bisphosphate aldolase</td>
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<tr>
<td>D00573</td>
<td>-</td>
<td>Small GTPase</td>
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</tr>
<tr>
<td>C00056</td>
<td>C06011</td>
<td>Unknown</td>
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</table>

Table 8: A list of *Blumeria graminis* f. sp. *hordei* isolate DH14 CMEG Promoters fused to GFP and used during the transformation of *Magnaporthe oryzae*.

**EST Library identifier** indicates the name of the EST used during the original microarray analysis. **CMEG Cluster Leader** indicates the EST providing the most representative expression profile. Where ESTs provided the most representative profile of their group they are labelled ‘Cluster Leader’. (*) indicate genes tested for expression in Chapter 4 (listed in Table 3). (1) indicates ESTs of the same cluster as C01244 and C00563 (Table 3). (2) indicates ESTs of the same cluster as PS11B04 and C01157 (Table 3). (3) indicates ESTs of the same cluster as D00471 (Table 3). Sequence data and expression profiles are shown in *Appendix E*.

6.4.4: Midiprep plasmid purification

This followed a modified version of the miniprep plasmid purification protocol of Sambrook et al., (1989). It was as follows: 50 ml overnight LB media culture (in 50 ml falcon tube) was cooled on ice before pelleting at 3220 x g, 15 min at 4 °C. Pellet was then re-suspended in solution I (*Appendix E*) by vortexing. 2 ml of solution II (*Appendix E*) was then added and mixed by inversion of the tube 4 to 5 times (until solution was clear, for not longer than 5 minutes) at room temperature. 1.5 ml of ice cold solution III (*Appendix E*) was added and
mixed gently, but thoroughly, by inversion 8 to 10 times. The solution was incubated on ice for 5 minutes. The mixture was then centrifuged for 10 min at 3220 x g at 4 °C and the supernatant was transferred to a new tube (by sieving if the pellet was loose. An equal volume of cold iso-propanol was added, followed by inversion (5 to 6 times) with and incubation on ice for 5 min. Following this, the extraction mixture was centrifuged at 3220 x g for 25 min at 4 °C. The supernatant was then discarded. The pellet was washed with 70 % ethanol, and after gentle shaking to dislodge the pellet, the mix was centrifuge at 3220 x g for 5 min at 4 °C. The supernatant was discarded and all liquid aspirated. The pellet was then re-suspended in 100 µl TE buffer or H₂O.

6.4.5: Precipitation of DNA
If necessary DNA (in TE buffer or ddH₂O) was precipitated by adding Sodium Acetate (pH 5.2) to a final concentration of 0.3 M and 2.5 volumes of cold iso-propanol. The solution was incubated for a minimum of 15 minutes at -20 °C and then centrifuged at 4 °C for 10 min at 18,000 x g. After the supernatant was discarded by aspiration, the pellet was washed with 300 µl of 70 % ethanol and then centrifuged at 18,000 x g for 10 min. The ethanol was removed by aspiration and the DNA was re-suspended in the required of volume of ddH₂O.

6.4.6: Detection of successful insertion into plasmids via microprep and restriction digestion
To show insert presence in pMJK27.2 potentially transformed bacterial colonies were transferred by pipette tip to 20 µl ‘1X Cracking Solution’. This solution was subsequently placed on ice. 3 µl ‘Loading buffer’ was then added. Solution was then heated to 70 °C for 5 min 25 on PTC-200™ Peltier thermocycler and was then centrifuged at 3000 rpm (2415 x g) for 5 min. 20 µl of upper layer of microprep solution was then transferred to 0.8 % agarose gel for visualisation. If successful integration was indicated miniprep preparations were made from the potentially successful transformations to allow restriction digestion utilising BsgRI (New England Biolabs®) for pMJK27.2 destination vectors with conversion cassette. Products were then visualised on an agarose gel and recorded as required.
6.4.7: Sequencing
Performed as noted in section 5.3.4. Primers utilised include M13-Forward (5’-TAAAACGACGGCCAGT-3’) and sGFP reverse (5’-AAGATGGTGCGCTCCTGGAC-3’).

6.4.8: Software and internet based resources
Software used was as noted in section 5.3.5. Additionally, sequences were formatted using the ‘Read Sequence Converter’ (developed by Dr. D. Gilbert, University of Indiana; available at http://www-bimas.cit.nih.gov/molbio/readseq/ as of time of writing). The Phytopathogenic Fungi and Oomycete EST Database by COGEME (Consortium for the Functional Genomics of Microbial Eukaryotes) at http://cogeme.ex.ac.uk/ was accessed during 2008-2009 to view microarray data provided by Dr. Maike Paramor (Spanu Laboratory, Imperial College London). Basic Local Alignment Search Tool (BLAST) Resources hosted by the National Centre for Biotechnology Information (NCBI) at http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome were accessed throughout the period of 2006-2009 for sequence identity and homology searches. PCR and RT-qPCR primers were designed using Primer 3 (version 0.4.0; Rotzen and Skaletsky, 2000) as accessed between 2006 and 2009 (.http://frodo.wi.mit.edu/primer3/).

6.4.9: Magnaporthe oryzae transformation

Culture Conditions
Wild-type and transformed M. oryzae isolate Guy11 was grown in 9 cm Petri dishes at 25 °C and 30 % humidity with a 16/8 hour light/dark cycle. Wild-type Guy11 was a gift from Dr. Mick Kershaw (Talbot lab, University of Exeter) although it was originally sampled from French Guyana (Leung, 1981). Also cultured was a Guy11 strain previously transformed with a GFP expressing plasmid, pCAMgfp. Complete media agar was used (Appendix E; Soanes et al., 2002). For long-term storage and use, filter paper stocks were created by growing M. oryzae isolates on 0.5 cm² filter paper sections (Whatman®) and freeze-drying them before storage at -20 °C. Conidia were harvested from fungal plate cultures grown on CM at 25 °C and 30 % humidity for 12 to 14 days by rubbing colony surface in the presence of distilled H₂O and filtering through sterile Miracloth (Calbiochem®). For transformation, originally, liquid cultures were created by gently rubbing the upper surface of a sporulating 9 cm petri dish of Guy 11 when covered in liquid complete media. This media was then decanted into a
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250 ml conical flask and filled to 150 ml. Subsequently, liquid cultures were created by shredding a 9 cm Petri dish of 2 week old culture growth, and mixing with 150 ml complete media, before decanting into a 250 ml conical flask for incubation. The culture was incubated at incubated 25 °C and 65 % humidity with a 16/8 hour light/dark cycle and agitated at 125rpm. Liquid cultures were grown for 3 to 4 days (rubbing), 48 hours (shredding) until fungal matter was plentiful and then used for transformation.

*Original Transformation Procedure*

A *M. oryzae* culture (in liquid complete media,) that had been grown up for 3 to 4 days was filtered through sterile Miracloth (Calbiochem®). The collected culture was then washed twice with sterile distilled H₂O then with filter-sterilised OM buffer (Appendix E, 9.11). The culture was then placed in a 50 ml polypropylene tube and filter-sterilised OM buffer (containing lysing enzyme from *Trichoderma* species at 10 mg/ml (Sigma-Aldrich®) was added until the culture had achieved a medium viscosity. The culture was first gently shaken at 30 °C at 120 rpm for 15 min, followed by approximately 1.5 hrs at 60 rpm. To collect protoplasts this mixture was then filtered through sterile miracloth. The protoplast volume was overlaid with an equal volume of ST buffer (Appendix E 9.11) and centrifuged for 15 min at 1065 x g using an Eppendorf centrifuge 5810 R (Eppendorf). The ST and OM buffer mixture was removed and replaced with STC buffer (Appendix E 9.11). The pellet was re-suspended and the volume was centrifuged again for 15 min at 1065 x g (and 4 °C). The pellet was then re-suspended in 1 ml of STC buffer (concentration 1.0 x 10⁷) and the protoplasts assessed using a haemocytometer. Where possible, aliquots of 180 μl of protoplasts were removed and incubated with 10μg of plasmid for 5 to 10 min. PTC buffer (Appendix E 9.11) was then added in aliquots: 250 μl + 250 μl +500 μl and left for 15 min without agitation. Following this aliquots were centrifuged at 3000 rpm for 10 min, and PTC buffer was extracted. The pellet was gently re-suspended in YGS medium and agitated slowly at 20 rpm overnight. The following day the aliquots were plated in a mixture of 1X minimal media (final concentration, also containing 1.2 M sorbitol final concentration and vitamins, (Appendix E 9.11) and 0.6 % low boiling point agarose (final concentration). The following day the culture was then overlaid using the same mixture of minimal media, low boiling point agarose and hygromycin B (Calbiochem®) (firstly at overlay concentration 200 μg/ml, then 300 μg/ml, then 600 μg/ml and finally at plate concentration 600 μg/ml [overlay concentration 1200 μg/ml]). Plates were then incubated at 25 °C and 60 % humidity with a 16/8 hour light/dark cycle for two weeks. If possible emergent colonies were excised and
then placed on hygromycin selective media in a 25-well plate (10 x 10 cm, with 2 x 2 cm wells).

**Modified Transformation Procedure**

Same as above except washing of mycelia with OM buffer before digest and ST overlay step, incubation with YGS media and use of minimal media with sorbitol were discontinued. Additionally the enzymes utilised were changed to Glucanex® 200G (Novozyme®). Additionally, all centrifugation was carried out at 1000 x g. Protoplasts were washed from digested mycelia using STC buffer, into a 50 ml polypropylene tube. Tube (containing protoplasts and OM buffer mix) was filled with STC buffer then centrifuged at 4 °C for 15 minutes. Protoplasts were washed three times with STC, and then re-suspended in 1 ml STC at 1.0 x 10⁷ protoplasts/ml. 150 µl aliquots were then incubated at room temperature with 4 to 8 µg DNA for 20 minutes, followed with 1 ml of 60 % PTC for 15 min. Each aliquot was gently mixed by inversion. Each aliquot was then added to 150 ml molten (45 °C) agar (Complete medium/0.8 M sucrose/1.5 % agarose (low boiling point agarose, Sigma-Aldrich®). This was dispensed between 5, 9 cm Petri dishes. After incubation at 25 °C for 16 hours plus each plate was overlaid with 1 volume of CM agar containing at 2X hygromycin B to permit a final concentration of 200 µg/ml.

6.4.10: Single spore isolation of transformants

The transformation plates were incubated for up to 2 weeks at 25°C. Plates were monitored and developing colonies that breeched the selective hygromycin covering were selected for further work. Emergents were grown on complete media agar containing hygromycin (200 µg/ml) in a 9 cm for approximately 1.5 weeks. Following the addition of 150 µl sterile ddH₂O to the colony surface after this time a 1 cm² approx section of the surface was rubbed with a sterile L-shaped spreader (VWR®) and diluted 1 in 100 times. 100 µl of the undiluted spore-containing liquid and 100 µl of the dilution were plated separately on a bi-compartmented 9 cm petri (VWR) and incubated for 2 to 3 days at 25 °C. After this time, colonies developing from a single spore were selected and placed in a separate 25-well Petri-dish (VWR) containing complete media with selective hygromycin B (200 µg/ml). Colonies were grown for 1 week before preliminary screening on a Leica MZ16F with Fluo III/edp100 UV attachment epifluorescence dissection microscope (**Figure 6-4**).
After single spore isolation conidial solutions were germinated on onion epidermis and then barley epidermis to study GFP expression. Following this, successful transformants were germinated on glass and cellulose to study GFP expression and underwent RT-qPCR analysis.

6.4.11: Preliminary screening of transformants (Gelbond)

Early screening attempts utilised the hydrophobic side of Gelbond (Cambrex/Lonza) as a surface to study early-germination stage promoter activity of transformants. Approximately, two weeks after sub-culturing the sporulating colony surface was gently rubbed (after flooding with a solution of 1/1000 dilution of 1 mg/ml 1, 16-hexadecanediol [Sigma-Aldrich®] in 100 % ethanol) to release conidia (Thines et al. 1997). The solution was pipetted onto the hydrophobic side of Gelbond (Cambrex/Lonza). After incubation overnight the conidia were inspected using epifluorescence microscopy. All appressoria were identified under normal light then interrogated via blue-light.

6.4.12: Screening (onion)

Later screening attempts utilised the inner epidermis of the 2nd outer layer of onion (Xu et al., 1997). 1 cm² sections of ddH₂O-washed epidermis were placed on 1.5 % TWA.
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Approximately, 12 to 14 days after sub-culturing the plates the sporulating surface was gently rubbed (after flooding with 150 µl ddH₂O) to release conidia. The solution was pipetting and then placed onto the hydrophobic side of the onion. After incubation for 6 to 8 hrs (or at a time most consistent with the *B. graminis* f. sp. *hordei* promoter’s activity) the conidia were inspected using epifluorescence microscopy. All appressoria were identified under normal light then interrogated via blue-light.

6.4.13: Screening (barley)

Primary leaves from 1 to 1.5 week old barley was used to test the promoter activity of transformed *M. oryzae* isolates. Abaxial epidermal sections were prepared as in section 2. Approximately 30 µl of conidial solution (1x 10⁶/ml) was pipette into the centre of the square for viewing after the required incubation time.

6.4.14: Screening (cellulose)

Cuprophan sheet (Medicell International Ltd.) were boiled three times for 10 minutes each followed by autoclaving at 120 °C for 15 minutes. For viewing of transformed *M. oryzae* activity 1 cm² sections were placed on 1.5 % TWA. 100 µl of conidial solution (1 X10⁷/ml) was pipetted on to the section for viewing after the required incubation time.

6.4.15: Screening (glass slide)

Super premium glass microscope slides (VWR®) were used without pre-treatment for germination tests and viewing by microscopy after the required incubation time. Spore concentrations of 1x10⁵/ml were used as an inoculation load.

6.4.16: Fungal DNA extraction

This followed a protocol of Edwards et al., (1991) with modification. Briefly: approximately 20 to 100 mg of fungal material (excised from a colony grown on CM media on a 9 cm plate) was ground with micro-pestles (VWR®) in a 1.5 ml Eppendorf tube in the presence of 400 µl extraction buffer (200 mM Tris-HCL, pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5 % SDS) and sterile sand. The samples were then centrifuged at room temperature at 18,000 x g and the supernatant was transferred to a new 1.5 ml Eppendorf tube. 1 volume of cold isopropanol was added, mixed by inversion, and the solution was incubated overnight at -20 °C. The samples were then centrifuged at 18,000 x g at room temperature followed by aspiration of the supernatant. The pellet was air dried and then re-suspended in 100 µl of TE buffer. 0.5 to 1 µl of the extract was then used for PCR reactions.
6.4.17: PCR screen of transformants

For the identification of successful fungal DNA extractions a region of the fungal ribosomal DNA was targeted for amplification by PCR. All PCRs were performed using a PTC-200™ Peltier thermocycler (MJ Research Inc.). All oligonucleotide primers were synthesised by Sigma®-Genosys Ltd. The forward primer ITS1 (5’-TCCGTAGGTGAACCTGCGG-3’) and the reverse primer ITS 4 (5’-TCCTCCGCTTATTGATATGC-3’) (Figure 6-5) amplified the internal transcribed spacer 1 region (ITS1), 5.8s ribosomal RNA gene and internal transcribed spacer 2 region (ITS 2), leading to a 600 bp amplicon (White et al., 1990). PCR program used was as follows: 1) 95 °C for 2 min; 2) 95 °C for 45 s; 3) 55.5 °C for 45 s; 4) 72 °C for 40 s; 5) 30 repetitions of steps 2 to 4; 6) 72 °C for 10 min; 7) 4 °C until analysis.

To determine successful transformation with pMJK27.2 and derivative plasmids into M. oryzae PCR amplification of the GFP gene within the plasmid was performed. The primers used include sGFP-1 (5’-CCT GAA GTT CAT CTG CAC CA-3’) and sGFP-2 (5’-TGC TCA GGT AGT GGT TGT CG-3’). The PCR program used was as follows: 1) 95 °C for 2 min; 2) 95 °C for 45 s; 3) 60 °C for 45 s; 4) 72 °C for 30 s; 5) 30 repetitions of steps 2 to 4; 6) 72 °C for 1 min; 7) 4 °C until analysis.

6.4.18: Fluorescence picture acquisition and wavelength emission scanning

Pictures on germination surfaces at 0, 4, 8 and 16 h.p.i were taken using a Leica DMIRE2 Confocal microscope. Pictures were analysed using ‘Leica confocal software’. Version 2.61 build 1537 (Leica Microsystems Heidelberg GmbH © 1997-2007). Laser excitation at 488 nm combined with scan and frame averaging was used for GFP picture acquisition. To detect the exact emission wavelength, laser excitation at 476 nm combined with 15 nm emission detection windows was used to scan wavelengths between 500 and 600 nm.
6.4.19: Infection trials
To test infection procedures and to test the pathogenicity of transformed *M. oryzae* isolates 1 week old barley (*Hordeum vulgare* cv. Golden Promise) plants were used in simple spray and microscopy tests. 2 ml solutions of Miracloth-filtered conidia (1 X $10^6$/ml) from selected transformants were sprayed onto 1 pot each of approximately 40 seedlings (at the primary leaf stage of growth) and incubated at 25 °C at 30 % humidity in a 16/8 hr light/dark period for 1 week at ≥ 95 % humidity in isolation pot covers in perspex isolation tanks. As a positive control a solution of Guy11 wild-type conidia was similarly inoculated. As a negative control 1 pot of seedlings were sprayed at the end of the experiment with pure ddH$_2$O. After 72 hrs and 1 week leaves were inspected and random lesion samples (from a selection of 10 randomly selected lesions) were taken for microscopy staining and photography at 72 hrs and 1 week post-inoculation. This experiment was performed 3 times.

6.4.20: Germination and life-stage frequency
100 *M. oryzae* isolate spores (divided between 3 samples) on all surfaces involved in early germination stage studies were assessed for the stage of life-stage differentiation at 4, 8 and 16 h.p.i. Percentages for life-stages were calculated accordingly (no. of spores at developmental stage/observed spores x 100).

6.4.21: Spore and RNA collection
Spores of *M. oryzae* isolates germinating on barley, epidermis, cellulose and glass were collected at 0, 4, 8 and 16hr post-inoculation for RNA extraction. 3 independent replicates were collected at each time point for all isolates. Filtered-conidial solutions (1x10$^6$ spores/ml-2 ml per barley pot; approx 2.5 x 10$^4$ were sprayed on to onto 3 trays of cellulose sheets [2 ml per tray]; 2.5 x 10$^4$ on to the glass slides. After incubation as required the surfaces were dipped into 5 % (w/v) cellulose acetate in acetone. In the case of barley and glass, after drying the cellulose acetate was stripped off and stored at -80 °C. Cellulose sheets were snap-frozen in liquid nitrogen before storage at -80 °C.

6.4.22: Method for RNA extraction
As described in section 2.13.

6.4.23: Agencourt bead cleaning
As described in section 2.12.
6.4.24: RT-qPCR primer design

Primers for analysis were designed using Primer3 to have an optimum annealing temperature of 65 °C and produce a product size of 150 to 250 bp. GFP primers were (5’-CGGCAAGCTGACCGTGAAGT-3’) and (5’-AAGATGGTGCCTCCTCGGAC-3’). Control primers were designed to amplify the *M. oryzae* Guy11 version of the *B. graminis* f. sp. *hordei* DH14 Oxidoreductase gene used as a control for the original confirmatory RT-qPCR microarray experiment. Actin Control primers were (5’-CCGTCTTCCATCCATTGTC-3’) and (5’-CAACAGAACCGGCT-3’). Since no attempts were to be made to compare the Relative Expression Index (REI, i.e. the ratio of test gene and reference gene expression) of one test gene with that of another, primer binding efficiencies were not determined. Instead primer efficiencies of both the test and reference genes were assumed to be 1, i.e. that binding was 100% efficient. Primers for both test and reference genes were supplied in excess and experiments were conducted such that the same primer ‘master mix’ was used in all reactions for all surfaces tested. Similarly all reactions for a test gene were conducted within the same RT-qPCR run. As a consequence any inefficiency in primer binding for either the test gene, or the control/reference gene, would remain constant across the surfaces tested. In turn this would mean any alterations in expression of the test gene (due to the different surfaces tested) would still be detected and any conclusions based on these alterations would be valid.

6.4.25: RT-qPCR

qPCR analysis followed the protocols of manufacturer (Invitrogen™) the Platinum® Quantitative PCR SuperMix-UDG with ROX dye. All reactions were performed upon an ABI systems 7500 real time PCR system. 20 μl reactions were carried out. The mix was as follows: 10 μl Platinum® Quantitative PCR SuperMix-UDG with ROX, Forward Primer (10 μM) 0.4 μl, Reverse Primer (10 μM) 0.4 μl, ROX fluorogenic probe (0.04 μl) and Sterile HPLC grade water (BDH) to total volume inclusive of 4 μl cDNA template. All analyses were carried out using the Applied Bio-systems Fast System SDS software (Version 1.4) and Microsoft Excel®. All primers were manufactured by Sigma®-Genosys Ltd. The PCR amplification conditions were: 20 s at 95 °C, then 40 repetitions of 3 s at 95 °C and 30 s at 60 °C. This was followed by a dissociation cycle: 95 °C for 15 s, 60 °C for 20s, 95 °C for 1.5 s and 60 °C for 15 sec. Following amplification threshold detection parameters (cycle threshold limit) were adjusted manually to optimise results. Data was logged (base 2) and
Quantities calculated by $2^{-(Ct-20)}$. Ratios were calculated by Quantity (Target)/Quantity (Reference). Statistical analysis was performed utilising 1-Factor ANOVA. Homogeneity of variances was assessed utilising the ‘Levene Statistic’ and where homogeneity was not assured a Games-Howell post-hoc analysis was used. Where homogeneity was assured a post-hoc Tukey Test was employed. As a further check a Welch Test was performed to support the conclusions of the Games-Howell test. All analysis was performed utilising ‘PASW statistics 18, release 18.0.0 (Jul 30, 2009) SPSS inc.’
6.5: Results

6.5.1: Magnaporthe oryzae development on barley

To observe the developmental progression of M. oryzae on barley throughout the asexual developmental cycle, barley leaves were infected with spores from M. oryzae strain Guy 11, an isolate known to be pathogenic on both rice and barley. At time points relevant to Bgh development (0, 4, 8, 16 h.p.i. and 7 d.p.i.) leaves were stained with trypan blue to allow observation of the equivalent stage of M. oryzae development. As with Bgh the infection starts with a conidium landing on a surface (Figure 6-6A). Visible by 4 h.p.i., a short germ tube emerges (Figure 6-6B). This germ tube elongates, hooks and swells to form a prominent appressorium that supports host invasion (often developing by 4 h.p.i., with melanisation readily visible by 8 h.p.i (Figure 6-6: C, D, E). By 16 h.p.i., the formation of an invasive

Figure 6-6: Developmental cycle of Magnaporthe oryzae strain Guy11 on barley.

A) Non-germinated conidium. B) Germ tube emergence seen at 4 h.p.i. C) Germ tube hooking and swelling (4 h.p.i approx). D-G) Development of appressorium proper (marked by arrow in D, 4-8 h.p.i.) followed by penetration of epidermal cell and formation of an invasive hypha (marked by arrow in F and G, 16 h.p.i. and longer). H) Creation of epiphytic mycelia formation and conidiophores, by 7 days post-inoculation. The release of dew/air-borne spores follows the formation of conidiophores. I) Mature Blast legions visible 7 days post-infection of barley xv. ‘Golden Promise’. Stained using Trypan blue/lactophenol and cleared with chloral hydrate as described in section 2.3.1 Scale bar (images A-G) = 10 µm approx. Scale Bar (image H) = 150 µm
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hypha was evident (Figure 6-6: F, G). Although in appearance a less complex structure than the dedicated haustorium of *Bgh* such hyphae sustain the formation of epiphytic mycelia growth across the leaf surface. From this mycelia airborne/dew-borne spores develop on conidiophores (Figure 6-6H). Approximately 1 week after inoculation the symptomatic grey necrotic lesions (Figure 6-6I) associated with *M. oryzae* infection are easily visible to the eye. It is within these lesions that the conidiophores are found. In conclusion, the development of these features correlate with the similar structures of *Blumeria graminis* f. sp. *hordei* at these time points.

6.5.2: Transformation and screening of *Magnaporthe oryzae* with *Bgh* CMEG Promoter/GFP plasmid constructs

In order to test the hypothesis that promoters of *Bgh* genes (found to be responsive to different surface stimuli) may be similarly regulated. The 5’-regulatory regions from 22 different CMEG genes (from 9 separate clusters) (listed in Appendix 9.9) were cloned into the plasmid backbone of pMJK27.2 by Dr. Maike Paramor and Dr. Calin Andras. This plasmid features a sGFP gene driven by the endogenous *M. oryzae* MPG1 hydrophobin gene promoter. After confirmation of the CMEG regulatory regions successful replacement of the MPG1 Promoter, these regulatory region/reporter constructs were transformed into *M. oryzae*. At times during this process (transformation, screening and harvesting) the author received help from Natasha Cain and Nurul Ismail.

Early transformation attempts followed a previously determined protocol (section 6.4.9) and featured selection pressures of 100 μg/ml to 600 μg/ml hygromycin B. Later attempts follow a modified protocol recommended by Dr. Mick Kershaw (University of Exeter) (Section 6.4.9). For both protocols the creation and isolation of protoplasts, the regeneration of *M. oryzae* colonies and the germination of conidia from these colonies was feasible (Figure 6-7).

Although yielding colonies that grew well on selective media, attempts based on the original protocol did not lead to successful transformation, as indicated by GFP expression. Experiments featuring non-transformed protoplasts incubated on 200 μg/ml selective media overlays (100 μg/ml final concentration) revealed a high background of non-transformed protoplasts surviving the treatment regime. Attempts were first modified to increase the selection to 150 μg/ml and then 300 μg/ml. Further modification took place in the use of multiple plates rather than a sole plate for the regeneration of protoplasts. This allowed for a
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reduction in hygromycin B selection pressure whilst also reducing protoplast background. Similarly, a change to the use of 60 % PEG solution was initiated.

![Image](image.png)

**Figure 6-7: Magnaporthe oryzae transformation and screening.**

A) Regenerating mycelia. After shredding a 1.5 week old Guy 11 colony, *M. oryzae* colonies were left to regenerate for 3 days in liquid complete media before the creation of protoplasts (B). C and D) After transformation protoplasts were mixed with agar and plated in hygromycin-laced selective complete media. Colonies that were transformed grew faster than surrounding colonies (arrow) and breeched the hygromycin selective layer first. These colonies were selected for further analysis (E). **Scale bar B** = 8 µm approx. C and D) 1.24 cm approx E) 2 cm

Although these steps led to a reduction in background the majority of colonies sub-cultured often failed to show the phenotype of development consistent with wild-type Guy 11 or Guy 11 transformed with pCAMgfp (a positive control) on either selective or non-selective media. Selected colonies, often exhibited uneven growth and demonstrated a sectored appearance.
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Combined with this, spores that had germinated failed to fluoresce when checked with epifluorescence microscopy.

Accordingly as noted in **section 6.4.9**, the procedure was altered by discontinuing the overnight incubation in YGS and the use of sorbitol-containing minimal media for regeneration. Also Glucanex enzyme was used instead of lysing enzymes from *Trichoderma* species. This change lead to the successful transformation (as indicated by even growth on hygromycin of colonies) of *M. oryzae* with the *Bgh* regulatory region/GFP reporter constructs. In total 504 colonies, representative of transformations of 22 different promoter/reporter constructs, grew on media containing concentrations 200 to 600 μg/ml hygromycin B and with similar appearances to that of the wild-type Guy 11 isolate (**Figure 6-8**). These colonies were then screened for fluorescence according to protocols in **section 6.4**.

![Figure 6-8: Growth of *M. oryzae* wild-type strain (Guy11) and transgenic *M. oryzae* strains growing on complete medium (CM) and complete medium containing hygromycin B. (source: N. Cain)](source)

Transgenic strains displayed in this image contained either the pMJK27.2 plasmid or a plasmid containing the 5’-regulatory region of EST C00879. Plates were made containing CM only or CM + 150 μg/ml Hygromycin B. The colonies were allowed to grow for 7 days before inspection. The effect of Hygromycin B on the untransformed Guy11 is clearly visible as a reduction in growth and a stunted sectored appearance.

As shown in **Figure 6-7 (C, D)**, during transformation certain regenerating colonies began to grow at faster rates than others, with a lesser number appearing (within the 2-3 weeks after transformation) to continue growth through the selective overlay. These emergent colonies were selected for further analysis. Early screening consisted of selected colonies being grown within multi-welled petri dishes (**Figure 6-7E**) and sub-cultured multiple times on selective media (at 200 μg/ml Hygromycin B) before undergoing germination analysis and epifluorescence microscopy on the hydrophobic surface of gelbond, a substrate used in previous *M. oryzae* germination studies (Thines et al., 1997) (**Figure 6-9**).
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However, due to the colony size being limited to 4 cm\(^2\) only small numbers of conidia were produced. This made visual screening time consuming. Subsequently, further sub-culturing took place with individual colonies grown upon 9 cm Petri dishes. Additionally, the gelbond surface used to stimulate germination exhibited a level of background fluorescence that made the screening of suspected isolates difficult. As a result colonies were then analysed by direct inspection of colony mycelia whilst still on the plate, and via the germinating of spores of onion epidermal peels, using epi-fluorescence microscopy (Figure 6-9; Figure 6-10).

![Figure 6-9: *Magnaporthe oryzae* germination screening.](image)

- **A)** Germination with appressorial formation (8 h.p.i.) of spores of an *M. oryzae* strain transformed with pMJK27.2 on gelbond.
- **B)** Fluorescence of the same image, via blue light illumination as visualised though a FITC filter (BP450-490, FT510)
- **C and D)** The same *M. oryzae* pMJK27.2 transformant during germination and appressorium formation on onion epidermis. **Scale Bar = 20 μm**

During the observational screen an isolate of Guy 11 previously transformed with a plasmid containing GFP, pCAMgfp was used as a positive control to assay for fluorescence (section 3.3) (Figure 6-10). During transformation itself the vector pMJK27.2 functioned as a transformational positive control. Following its successful incorporation into *M. oryzae*, because of the brightness of fluorescence exhibited by the germinating spores, the transformants then became a positive control for all observational studies.
Although not definitive for the presence of GFP, during observation of *M. oryzae* germination and colony formation, screening by epifluorescence microscopy employing filters allowed a rapid visual determination of autofluorescence. As shown in Figure 6-11 when viewed through filter set (BP450-490, FT510) **Figure 6-11:** both fluorescence (GFP or autofluorescence) was easily visible. Importantly under filter set (BP565/30, FT585, BP620/60) autofluorescence was still easily visible, whilst that associated with GFP was not visible **Figure 6-11** (compare figure D and G). One drawback to using this filter as a sole indicator of GFP presence was that low level autofluorescence was harder to pick up visually.

In cases where such a situation arose filter set (BP485/20, FT510, LP515) was used as a further level of assessment. When viewed through this filter GFP fluorescence appeared greener in hue than autofluorescence which appeared yellow **Figure 6-11, C**. This test, combined with the use of hygromycin selective media, allowed a sorting of colonies for further testing which included confocal microscopy with wavelength analysis to conclusively prove the presence of GFP at set time points during development.

Combined with this use of visual screening, PCR analysis was used to determine the presence of the GFP gene in transformants that, although successfully growing on hygromycin media, were not observed to fluoresce. A simple method of DNA extraction, first developed by Edwards et al., for use on plants, was adapted to permit large scale screening of colonies (333 of the total). ITS region specific primers for PCR were used to indicate successful DNA extraction by the amplification of a product approximately 600 bp in size. GFP specific primers were then used to indicate which of these colonies
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contained the GFP gene by permitting amplification of a PCR product approximately 500 bp in size. **Figure 6-12** illustrates an example of this.

![Figure 6-12](image)

**Figure 6-11**: Blue-light epifluorescence images of *Magnaporthe oryzae* mycelia grown on complete media viewed through discriminatory filters.

A) White light image of a mycelia crush of a potential *M. oryzae* transformant for EST D00154. B-D) Mycelia viewed via epifluorescence through filter set B) BP450-490, FT510; C) BP485/20, FT510, LP515; D) BP565/30, FT585, BP620/60. The presence of fluorescence when viewed through all filter sets indicates the presence of autofluorescence. E) Non-germinated spore of *M. oryzae* transformed with pMJK27.2. F) Spores exhibiting GFP fluorescence under blue-light. G) Spores as viewed through filter set BP565/30, FT585, BP620/60. Oval indicates position of spores. **Scale Bar** = A-D 40 μm approx. F-G) 25 μm approx.

To summarise these tests, it was revealed a successful GFP transformation rate of approximately 17.9 % which suggested significant numbers of the hygromycin-resistant colonies retained solely the hygromycin resistance gene (although this was not tested by specific PCR examination). Nevertheless transformants were confirmed by epifluorescence microscopy and/or PCR analysis to have successfully incorporated certain *Bgh* promoter/GFP constructs. Several transformants were observed for the promoter of EST C00750 (encoding a protein with similarities to CAP20) but did not prove stable and so could not be analysed.
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Additionally one unstable transformant was observed, and confirmed by PCR, for the promoter of EST C01157 (encoding a protein disulphide isomerise precursor involved in electron transport). Conversely 3 stable transformants for the *Bgh* promoters for EST C00879 (believed to drive expression of a mildew H4 histone gene), (referred to as C00879 ‘A’, ‘B’, ‘C’) and 1 transformant for the promoter of EST C01420

Additionally, to test the hypothesis that regions of the promoters most critical for their functions would show higher evolutionary conservancy than non-critical regions, a shortened version of the regulatory region of EST C00879 (from this point onwards referred to as C00879[s]) containing the most evolutionary conserved section of the putative promoter region was inserted in replacement of the MPG1 promoter and was also transformed into *M. oryzae* (sequence available in Appendix E 9.12). 3 independent, stable transformants were successfully generated (from now on labelled as ‘A’, ‘B’, ‘C’). All of these transformants are in addition to 3 independent transformants of the vector pMJK27.2, used as a positive control.

Figure 6-12: Gel electrophoresis of results of PCR–based transformant screening.

A) Results of PCR amplification using ITS region specific primers. Bands indicate successful extraction of fungal DNA from colonies. Lanes 1-12 are separate DNA extractions from *M. oryzae* transformant colonies, m = Generuler™ 1 Kb DNA plus ladder (Fermentas). 1.5 % agarose gel. B) Results of PCR using GFP specific primers with DNA extracted from transformant colonies as a template. Lanes 1-3, 5 and 7 indicate successful amplifications. These samples include DNA extracted from colonies containing the promoter construct for EST C00879 (lane 2). M = Generuler™ 100 bp DNA ladder (Fermentas). 1.5 % agarose gel. Numbers alongside ladder indicate base pair number.
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for transformation optimisation. Due to their fluorescence it is these transformants of the full-length and shortened selected regulatory regions of C00879 and the proposed regulatory region of EST C01420 which were taken for further analysis. These results are summarised in Table 9.

<table>
<thead>
<tr>
<th>EST Library Identifier</th>
<th>CMEG Cluster Leader</th>
<th>Gene Identity/Function</th>
<th>Stable Transformants</th>
<th>GFP confirmed</th>
</tr>
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<tbody>
<tr>
<td>D00146</td>
<td>Cap20</td>
<td>Retinal Short Chain Dehydrogenase Reductase</td>
<td>29 (6)</td>
<td>2</td>
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<tr>
<td>C00750</td>
<td>Cap20</td>
<td>Cap20</td>
<td>56 (17)</td>
<td>1</td>
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<tr>
<td>C00741</td>
<td>Cap20</td>
<td>Signal Recognition Particle 54 kDa protein homolog</td>
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<td>C00222</td>
<td>C00206</td>
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<td>ATP- synthase Protein 9, Mitochondrial Precursor</td>
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<tr>
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<tr>
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</tr>
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<td>C00206</td>
<td>Histone H4</td>
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<td>C00206</td>
<td>Histone H4</td>
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</tr>
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<td>C06011</td>
<td>Unknown</td>
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Table 9: Summarisation of results of both visual and PCR screening of *M. oryzae* transformants containing a CMEG 5’-regulatory region/GFP fusion.

**EST Library identifier** indicates the name of the EST used during the original microarray analysis. **CMEG Cluster Leader** indicates the EST providing the most representative expression profile. **Stable Transformants** indicates the numbers of colonies stably growing on hygromycin selective media. Number in brackets indicates number of independent transformations from which these colonies originate. **GFP confirmed** indicates the number of colonies confirmed by PCR to contain the GFP gene.
6.5.3: Pathogenicity assay on barley

All stable transformants for the regulatory region of EST C00879 (full-length and shortened), and the regulatory region of EST C01420, alongside 1 isolate of Guy 11 transformed with pmjk27.2 and one wild type Guy 11 isolate as controls were tested for their ability to complete their life cycle on barley. Although virulence differences between isolates were expected due to the random nature, and multiple events, of construct insertion this basic test would give an indication of any critical disruption or enhancement to disease pathways of the disease cycle caused by the transformation process.

Although the close proximity of *M. oryzae* spores to one another can lead to mutual inhibition of germination, signals present on the leaf surface may relieve this inhibition. Therefore simple inoculation tests were used to ascertain the correct concentration of conidia to be sprayed onto the barley leaves. Consequently, \(1 \times 10^6\) conidia/ml spore solutions (in the case of EST C00879 promoter isolate ‘A’ solutions of \(1 \times 10^4\) conidia/ml were used due to its reduced sporulation rate compared to both wild type and other C00879 promoter transformation isolates) were sprayed onto 7-day-old barley and 5-7 days after infection plants were inspected for their ability to form lesions. In a simple assay 30 lesions per isolate were inspected and compared to those caused by the wild-type Guy 11 infection. Indicative results are shown in **Figure 6-13** and **Figure 6-14**. Non-infected barley leaves remained healthy, with, in some cases, a small degree of dark speckling. This was later attributed to prolonged contact with soil after germination and was similarly also present in infected leaves. As shown in **Figure 6-14** all isolates were able to infect barley and formed the necrotrophic lesions associated with Rice Blast infection of barley. Lesions formed by isolates C00879 ‘B’, C00879 ‘C’, C00879[s] ‘A’, C00879[s] ‘B’, C00879[s] ‘C’, the Guy 11 wild-type and pmjk27.2 containing Guy 11 isolate were of a similar type. To generalise although the lesions themselves appeared a dark grey (showing a degree of translusance as tissue death spreads) it was surrounded by a slightly darker green margin. The leaf epidermis itself showed a slight chlorosis spreading from the lesion into the surrounding tissue. This chlorosis was most apparent around larger lesion types. Such lesions (approximately 5 mm in length) appeared equivalent to lesion categories Type 4/5 (as defined on rice by Valent and Chumley, 1991). However, instead of a lighter tan centre to the eyespots present during that study, gray mycelia was present in these lesions. As noted by Valent and Chumley (1991) this mass was associated with conidiation. Trypan blue staining of these infected leaves indicated that all isolates were capable of completing their lifecycle (defined as the formation
CMEG 5’-Regulatory Region driven GFP expression in *M. oryzae*

of mature conidiophores and spores) and were therefore pathogenic. It should be noted however that although capable of forming mature lesions isolate C00879 promoter isolate “A” (previously noted for its reduced sporulation) and the isolate C01420 formed smaller lesions (approximately, on average, 2 mm in size). These were equivalent in appearance to the type 2 lesions noted by Valent and Chumley (1991). Although still considered pathogenic due to an ability to sporulate, this reduced lesion size may suggest a reduction in virulence of these two isolates, possibly due to a reduced rate of development on the leaf. Inspection of early stage pre- and post-penetration stage infection events suggested that the attenuation was not overtly present at these points and so may feature solely in later stages of development.

![Figure 6-13: Transformed *Magnaporthe oryzae* strain pathogenicity assay.](image)

Transformed strains of *Magnaporthe oryzae* strain Guy 11 showing infection on barley leaves, 7 d.p.i. approx. The upper set of images indicates visible eyespot lesion symptoms whilst the lower set indicates lesion symptoms visible after staining with trypan blue. **A**) Non-infected barley leaf. The slight speckling associated with prolonged soil contact is apparent on the lower leaf. **B** and **C**) C00879 isolates ‘B’ and ‘C’ respectively. The lesion appears similar to ‘Type 4/5’ lesions as categorised by Valent and Chumley, 1991. **D**) C00879 isolate ‘A’. The lesion appearance suggests a similarity to a type 2 in size. **E-G**) C00879 isolates ‘A’, ‘B’, ‘C’
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showing lesions symptoms equivalent to eyespot Type 4/5. **H)** C01420 isolate ‘A’ showing similarity to isolate Type 2. **I)** Wild-type Guy11 infected leaf. Inset pictures show lesions at closer magnification.

Figure 6-14: Blast lesions on the barley leaf caused by transformed M. oryzae strains.

**A-C)** lesions caused by C00879 isolate ‘A’-‘C’. **D-F)** C00879[s] isolate ‘A-C’ lesion. **G)** C01420 isolate ‘A’ lesion. **H)** infected with Wild-type Guy11 lesion. **I)** Uninfected barley leaf. Inset pictures show the necrotic lesions, or leaf at a lower magnification. Lesions were stained after 10 d.p.i. with lactophenol/trypan blue and then cleared in chloral hydrate.

In conclusion although some transformants exhibited lower virulence than others all isolates tested retained pathogenicity. As such all were eligible for further testing.

6.5.4: Application of transformants to target surfaces

Before determining GFP expression after application to different surfaces of the various CMEG promoter/GFP, it was necessary to assess the correct spore concentration and delivery method for the inoculum. An inoculation test with a range of spore concentrations on the selected surfaces (barley leaf epidermis, glass slides and cellulose sheets) indicated spore concentrations that permitted both maximal germinating development (section 6.4.20) on that specific surface as well as maximised the ability to harvest RNA for quantitative real-time
PCR analysis. It was observed that on barley spore suspensions of $1 \times 10^6$/ml allowed full life-cycle development without any significant self-inhibition between spores. Similarly $1 \times 10^4$ spores/ml permitted the development of appressoria on cellulose, with higher concentrations reducing the likelihood of this stage of development. On glass the maximum level of development attained was the formation of germ tubes (at $1 \times 10^5$ spores/ml or less). Therefore these concentrations were selected to offer similar development to that of $Bgh$ development also seen on these surfaces.

In regards to the application of the spores to the surfaces, spraying was believed to be the most efficient application method as it would permit an even coverage of the test surface. Even so perceived disadvantages regarding this method included the damage incurred by the spores due to shearing forces present on passage through the sprayer nozzle as well as during surface impact. As shown in Figure 6-15 tests where spores were sprayed from a 5 cm distance onto glass slides revealed that spore integrity would remain unaffected by the spraying process. Another consideration was that the evaporation of the spore solution and a reduction in humidity to levels detrimental to germination and appressorial formation could also occur. As such glass slides and cellulose sheets were mounted on 1.5% TWA held in closed top trays and had a minimum of 2 ml of spore solution applied per tray. Barley plants were sealed in using isolation top covers allowing the maintenance of humidity levels at over 90% at 25°C. 5 ml of spore solution was applied per pot, at a distance of 5 cm from the barley seedlings. This provided enough moisture for development without the risk of droplet run-off from the barley seedlings. These measures permitted $M. oryzae$ development on these target surfaces during the required time periods.
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Figure 6-15: Spray inoculation test of *M. oryzae* on glass and barley.

A and B) Spores from *M. oryzae* transformed with promoter C00879 (isolate ‘A’) sprayed on to glass. White light view and epifluorescence showing spore integrity has been maintained. Central picture) 7-day-old barley plant sprayed with spore inoculum. Scale bar = 10 µm.

6.5.5: *M. oryzae* transformants developing on target surfaces

As a prelude to quantitative real-time PCR (aimed at determining the abundance of GFP mRNA as driven by these CMEG promoters on barley and other surfaces), *M. oryzae* transformants containing the C01420 promoter/GFP construct, the C00879 full length promoter/GFP construct and the shortened C00879 promoter/GFP construct were viewed at various stages of their lifecycle on barley, cellulose and glass surfaces to determine if GFP could be visualised. Alongside investigation of promoter-driven GFP behaviour at times (0, 4, 8 and 16 h.p.i.) relevant to differential activity of CMEGs on substrate surfaces of different inductive properties, transformants were inspected for the presence of GFP during colony growth. Additionally observation occurred at 31 h.p.i. when relatively well developed invasive growth should be present when infecting the barley leaf. Mycelial preparations of transgenic colonies were also viewed. At the time points viewed emissions were analysed to determine the peak wavelength emitted. Development stages as shown by *Magnaporthe oryzae* wild-type strain Guy11 and transformants are summarised in Table 10.
CMEG 5’-Regulatory Region driven GFP expression in M. oryzae

<table>
<thead>
<tr>
<th>Surface</th>
<th>Germ tube</th>
<th>Developing appressorium</th>
<th>Appressorium and invasive hypha</th>
<th>More than one germ tube</th>
<th>More than one germ tube with branching</th>
<th>Multiple germ tubes, branching and appressorium formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cellulose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glass</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 10: Developmental stages as seen during M. oryzae wild-type and transformant strain growth on barley, cellulose and glass.

+ = stage observed  ++ = very common - = stage absent

Activity of promoters of EST C01420 and EST C00879 during colony growth

As can be seen in Figure 6-16 and Figure 6-17 GFP is present in hyphae, developing conidiophores and also mature spores in colonies of M. oryzae transformants of C00879 and C01420 promoter/GFP constructs. The presence of the GFP in the mature spores was seen for all transformants, and importantly, in all the cellular compartments when spores were added to all the test substrates. As such these images indicate CMEG promoter activity in M. oryzae at 0 h.p.i. It should be noted that all independent C00879 transformants showed similar properties and as such only one set of images (collated from images collected from all isolates) has been presented as an example of behaviour.

On comparison with the fluorescence exhibited by similar structures of the wild-type Guy11 (shown in the Appendix E 9.13), which appear quite dark in comparison (with fluorescence often being limited to cell walls) the brighter fluorescence in the transformant is easily apparent. M. oryzae transformed with pMJK27.2 acting as a transformational positive control is also shown (Appendix E 9.14). To ensure that fluorescence was primarily due to the presence of GFP wavelength emission scanning was performed on randomly selected regions of interest. Exciting the tissue at 476 nm allowed analysis of all fluorescence wavelengths emitted between 500 nm to 600 nm. Wavelength spectra graphs displayed in all figures (Figure 6-16 to Figure 6-36) are expressed as a function of relative fluorescence units according to the wavelength (measured from 500 nm to 600 nm). The region of interest for which they relate are labelled on the adjoining fluorescence image (and are identifiable by their colour). As is shown in Figure 6-16 C, F, I and Figure 6-17 C, F, I the emission
maxima appears in the region of 507-510 nm, associated with the maximal fluorescence of GFP (Czymmek et al., 2002). Autofluorescence, as seen in appendix Figure 9.1D-F (Appendix E, 9.13), provided returns with multiple peaks in the region of 520 nm or higher. As a consequence of these results it may be assumed that the promoters of EST C01420 and C00879 (driving expression of an aconitase and H4 histone in Bgh) are active during this time period and in the cell types observed.

Figure 6-16: Mycelial preparation of M. oryzae transformed with C01420/GFP growing on hygromycin B selective complete medium.

A) Hypha growing through agar. B) Confocal fluorescence image of hypha. Green notation equates to the ‘Region of Interest’ selected for wavelength analysis. C) Emission spectrum showing peak approximately 510 nm, which accords to the region associated with GFP fluorescence. D) Putative developing conidiophore. E) Confocal fluorescence image of developing conidiophore. Dark section indicates a lack of GFP, with selected of region of interest in close proximity to this. F) Emission Spectrum indicating a weak peak near 510 nm. Other peaks are associated with autofluorescence G) Mature M. oryzae spore. H) Confocal fluorescence image of mature M. oryzae spore. This equates to the baseline activity of the C01420 promoter at 0 Hours on all surfaces. I) Emission spectra, with emission maxima at 507-510 nm, suggesting that the fluorescence in all compartments of the conidium is due to the presence of GFP. Scale Bar = 10 µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
CMEG 5’-Regulatory Region driven GFP expression in *M. oryzae*

Figure 6-17: Mycelial preparation of *M. oryzae* transformed with C00879/GFP growing on hygromycin B selective complete medium.


**Activity of promoters of EST C00879 and EST C01420 during growth on barley leaf epidermis**

As a direct test of the feasibility of using *M. oryzae* as a heterologous test organism for future analysis of CMEG promoters, observations were made to detect the presence of GFP during...
CMEG 5’-Regulatory Region driven GFP expression in *M. oryzae*

*M. oryzae* development on barley. As previous research had monitored the activity of the genes from which these promoter derive in *Bgh* at 0, 4, 8 and 16 h.p.i. on the different test substrates, the same time points were chosen during *M. oryzae* development on barley (section 6.5.1: *Magnaporthe oryzae* development on barley). Additionally viewing was also conducted at 31 h.p.i. According to these studies at 4 h.p.i. *M. oryzae* germ tube formation is present, although often by this time appressorial development would also be apparent. By 8 h.p.i. mature appressorium formation by *M. oryzae* is evident, whilst by 16 h.p.i. and 31 h.p.i. penetration of the leaf surface and the development of invasive hyphae is visible. All isolates tested during this study followed such patterns of development (although they did show variation in the frequency of developing such structures).

At 4 h.p.i. in germlings of both C01420 and C00879 transformants, GFP fluorescence was present at the various time-points, and confirmed by wavelength analysis, within the germ tube cytoplasm as this structure developed (Figure 6-18 A, B, C and Figure 6-20 A, B, C). Appressoria had also begun to develop, and GFP built up within these structures suggesting expression occurred in this cell type (Error! Reference source not found. Figure 6-18 D, E, F, and 6-20 D, E, F). In comparison the *M. oryzae* wild-type strain appressoria showed only low levels of autofluorescence (Appendix E 9.13, Figure 9.1 A-C).

By 8 h.p.i., the majority of spores in the isolates tested on barley leaves were forming appressoria (e.g. an average of 93.8 % ± 2.93 % s.d. in the case of C00879 ‘A’). Figure 6-18 G, H, I, J, K and Figure 6-20 G, H, I). In all transformants these structures appeared to contain the greatest concentration of GFP, and as a result glowed the brightest of all the structures observed over the time points. This may have been the consequence of direct GFP production within the appressorium, or due to an influx of the molecule during sub-cellular movement of internal stores of the spore, or both.

Interestingly the lysis and emptying of different cellular compartments became apparent during *M. oryzae* development. Although more work would be needed to clarify this point it appeared that the cell closest to a functioning appressorium began to empty first (as seen by a reduction in GFP, potentially, as the lytic vacuole increased). Then as time progressed, and as components from the two other cells within the spore were mobilised, the spore closest to the appressorium appeared to “re-fill”, leaving the cell furthest away as the most empty. By 16 h.p.i. the spores had begun to invade the host cell, and invasive hyphae were visible within
CMEG 5’-Regulatory Region driven GFP expression in *M. oryzae*

the epidermis (in the case of C00879 ‘C’ 35 % ± 3.49 % s.d. of spores had begun (Figure 6-19 L, M, N, O, P and Figure 6-20 J, K, L, M, N, O). The invasive hyphae of all transformants showed some fluorescence, although in cases this could be weak- potentially either as a result of “dilution” of the GFP molecule in a larger cytoplasmic volume or because of reduced promoter activity (Figure 6-19, P). By 31 h.p.i it was apparent that promoter C01420 in the sole isolate studied did not appear to be active as no GFP was present (Figure 6-19 T, U, V). In comparison, the well developed hyphal structures of C00879 promoter/GFP construct transformants showed some fluorescence- although as seen in Figure 6-21 S, T, U, this was often limited to cytoplasmic regions outside the vacuole.

To summarise these results it appeared that GFP expression as driven by the selected promoter region of EST C00879 was present in developing structures formed at all time points of observation on barley. If GFP decreased in a particular cell type this could be explained by the auto-phagocytotic cell death combined with the *M. oryzae* lifecycle (Veneault-Fourrey et al., 2006). GFP expression as driven by the 5’-regulatory region of C01420 was present in all developing structures bar invasive hyphae at 31 h.p.i.

As support for conclusions drawn from the pathogenicity assay of section 6.5.3 the early development over the time period studied of both C00879 isolate ‘A’ and the C01420 isolate did not appear to differ from that of isolates C00879 ‘B’ and ‘C’ which produced barley equivalent Type 4/5 lesions. As such these isolates were included in qRT-PCR studies detailed below.
Figure 6-18: Development of *M. oryzae* transformed with C01420/GFP developing on barley leaf epidermis at 4 and 8 h.p.i.

A) Spore with developing germ tube on barley at 4 h.p.i. B) Confocal fluorescence image of germling, with 3 regions of interest highlighted C) Emission spectra with peaks clustering at 510 nm. D) Spore with a developing appressorium at 4 h.p.i. E) Fluorescence image F) Emission spectra suggesting the presence of GFP in cellular compartments. G) Spore development with melanised appressorium present at 8 h.p.i. on barley. Dashed inset pictures indicate regions on different focal plains) H) Confocal fluorescence image of the developing *M. oryzae* spore at different focal planes I) Emission spectra with maxima at 508 nm approx. Scale Bar = A-E) 8 µm, G-H) 5 µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 6-19: Development of \textit{M. oryzae} transformed with C01420/GFP developing on barley leaf epidermis at 16 and 31 h.p.i.

L) Developing spore at 16 h.p.i. (dashed inset pictures indicate regions on different focal plains). M/O) Confocal fluorescence image of hyphae with regions of interest labelled. N/P) Emission spectra showing peak approximately 510 nm, although peak present in P is weak. Q/T) Germling at 31 h.p.i. (inset shows lower magnification image) R/U) Confocal fluorescence image of the germling and internal hyphae. No GFP appears present. S/V) Emission Spectra with multiple weak peaks suggest no GFP is present. Scale Bar = 5 µm.

Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 6-20: Development of *M. oryzae* isolates transformed with C00879/GFP developing on barley leaf epidermis at 4-16 h.p.i.

A) Spore with developing germ tube on barley at 4 h.p.i. The germ tube appears to not contact the barley leaf surface as its tip is just out of the plane of focus. B) Confocal fluorescence image of germling, with 4 regions of interest highlighted C) Emission spectra with peaks clustering at 510 nm suggesting the presence of GFP in all compartments -adjoining conidial cell. D) Spore with developing appressorium at 4 h.p.i. E) Fluorescence image showing a reduction of GFP in the appressorium-adjoining conidial cell. F) Emission spectra suggesting the presence of GFP in all cellular compartments, although the peak indicated by the orange spectra (analysing GFP content in the cell adjoining the appressorium) suggests GFP presence is weak. (continued overleaf) Scale Bar = A-B) 8 µm, D-E) 5 µm approx
CMEG 5’-Regulatory Region driven GFP expression in *M. oryzae*

**Figure 6-15 contd.** G) Spore development with melanised appressorium present at 8 h.p.i. on barley. H) Confocal fluorescence image of the developing *M. oryzae* spore, with highly fluorescent appressorium, and conidial body cells exhibiting reduced fluorescence. I) Emission spectra with strong maxima at 508 nm approx. J/M) Developing germling at 16 h.p.i. dashed boxes indicate structures at different focal plains. K/N) Confocal fluorescence image of the developing structures, showing highly fluorescent appressoria and invasive hyphae. Regions of interest are labelled. L/O) Emission Spectra showing emission wavelength associated with their equivalent region of interest. Although the majority of structures analysed suggest the strong presence of GFP, the germ tube and spore as shown in image N (ROI is labelled in purple and green respectively) suggests fluorescence is weakening in these structures. **Scale Bar = A-E** 8 µm, **G-H** 5 µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.

**Figure 6-21:** *M. oryzae* isolates transformed with C00879/GFP developing on barley leaf epidermis at 31 h.p.i.

A/D) C00879 isolate ‘C’ germling development at 31 h.p.i. on barley., showing the invasive hyphae present. Inset pictures show the structures at a lower magnification. B/E) Confocal fluorescence image showing GFP appears weak in above surface structures, whilst in invasive hyphae it appears excluded from the vacuole. C/F) Emission spectra. **Scale Bar =** 10 µm approx. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.

**Activity of promoters of EST C00879 and EST C01420 during growth on cellulose**

As with *Bgh*, germination on cellulose does not permit the full life-cycle of *M. oryzae* to occur. Although inspection of the underlying TWA (used to maintain hydration of cellulose) was conducted, no invasive hyphae were observed. As with *Bgh*, germination and development of *M. oryzae* appears delayed as the highest level of development attainable on this surface, the formation of appressoria, is reached by fewer spores and often at slightly later times (for example in the case of the single isolate C01420 where by 31 hrs 62 % ± 17 % s.d. formed appressoria). As such the author concludes the cellulose used in this study only
CMEG 5’-Regulatory Region driven GFP expression in *M. oryzae*

permitted the formation of appressoria (and if penetration did occur) the lack of development inducing signals caused development to cease before invasive hyphae were obvious.

As with the behaviour of the C00879 and C01420 promoters in *M. oryzae* on barley, GFP appears to be present in all life-stages viewed on cellulose. These results are displayed in **Figure 6-22** through **Figure 6-25**. At 4 h.p.i. where germ tubes were present, and in cases where terminal swelling suggested the potential formation of an appressorium, GFP presence was as noted by wavelength analysis (**Figure 6-22 A, B, C** and **Figure 6-24 A, B, C**). Wild-type Guy 11 showed negligible fluorescence at this time point (**Appendix E, 9.13, Figure 9.4**). What fluorescence was noticeable, appeared to originate from autofluorescence from cell wall materials. Autofluorescence is more noticeable on cellulose (and glass), as the plant surface exhibits more background autofluorescence. At 8 h.p.i. if appressoria had developed, GFP was similarly present in these structures as in appressoria developing on barley (**Figure 6-22 G, H, I** and **Figure 6-24, D, E, F**). Of particular note, where spores did not develop melanised appressoria (which were often of a darker in appearance than their counterparts on barley itself suggesting heavier melanisation) they developed, by 16 and 31 h.p.i., increasingly long and complex germ tube structures. Such structures also demonstrated GFP presence (**Figure 6-24 G, H, I, J, K, L**).

During development on barley a gradual, sequential “emptying” of the spore cells of GFP was observed. This was similarly seen during *M. oryzae* development on cellulose up to and including appressorial formation, as typified in **Figure 6-23 S, T, U**. Interestingly it appeared during *M. oryzae* development where appressoria were not formed as time progressed the cell nearest the germ tube became depleted (followed by the cell in the middle of the spore). The cell furthest way retained its cellular contents for a protracted period of time. At a certain length of germ tube however even this cell became depleted, after which a resurgence in GFP presence appeared. This is most evident in **Figure 6-25 (M, N, O, P, Q, R, S)** of C00879 isolate activity.

As seen **Figure 6-23** and **Figure 6-25** by 31 h.p.i. such structures were very complex with multiple swollen/thick germ tubes that demonstrated branching. In the more advanced cases these could be interpreted as equivalent to the aberrant classes of *Bgh* spores of multiple and branched character discussed
CMEG 5’-Regulatory Region driven GFP expression in *M. oryzae*

in Chapter 3. Other examples of aberrant structural formation include the formation of sub-apical structures (Figure 6-22 J, K, L). These structures may be a result of appressorial development being aborted.

To summarise, as with development on barley, it appears that all cell types and structures during *M. oryzae* germination on cellulose contain GFP at the time points investigated. This suggests that the 5’-regulatory regions of EST C01420 and EST C00879 are capable of driving expression at these times.
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Figure 6-22: *M. oryzae* transformed with C01420/GFP developing on cellulose from 4, 8, and 16 h.p.i.

A) Spore with germ tube at 4 h.p.i.  B) Confocal fluorescence image with 3 regions of interest highlighted.  C) Emission spectra all showing maxima indicative of GFP presence.  D) Spore with germ tube exhibiting terminal swelling at 4 h.p.i.  E) Confocal fluorescence image with 4 regions of interest highlighted.  F) Emission Spectra with fluorescence maxima at 505-10 nm approximately.  G) *M. oryzae* spore. Germling with heavily melanised appressorium at 8 h.p.i.  H) Confocal fluorescence image. A lack of fluorescence suggests that no GFP may be present in the appressoria. This stands in contrast to many spores visualised. Also present are large, expanding vacuoles in the cytoplasm of the cells of the conidium. GFP appears to be excluded from these.  I) Emission spectra suggesting that at all regions of interest GFP is present: even if in a reduced quantity.  J) Spore at 16 h.p.i. exhibiting sub-apical structure formation.  K) One appressorium appears to be developing successfully, as suggested by the accumulation of GFP. Confocal Fluorescence image. 8 regions of interest are highlighted.  L) Emission Spectra showing maxima at 505 nm for most region of interest selected. Of note is the spectra for the vacuole nearest to the brightly glowing appressorium in image K. Labelled in purple, this spectrum shows only a minimal peak suggesting that GFP is absent from this organelle. **Scale Bar** = 8 µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 6-23: *M. oryzae* transformed with C01420/GFP developing on cellulose at 31 h.p.i.

**M/P** Complex germ tube network formed by spore at 31 h.p.i. Inset picture shows the network at lower magnification. Inset pictures where present indicate fungal structures at lower magnification. **N/Q** Confocal fluorescence image showing several regions of interest selected for emission wavelength analysis. Of note in this image are the presence of vacuoles within the hyphal network (as indicated by the *). **O/R** Emission Spectra suggesting that GFP is present at all of the selected regions of interest. **S** Spore at 31 h.p.i. with mature appressorium. **T** Confocal fluorescence image suggesting that at this time point GFP appears to be mainly localised to the appressorium. Regions of interest have been selected, including for the appressorium, the germ tube and all cellular compartments of the spore. **U** Emission Spectra displaying an emission maxima at approximately 507 nm for the region of interest within the appressorium. Other regions of interest have maxima at greater wavelengths than 507 nm suggesting any fluorescence is not attributable to the presence of GFP. **Scale Bar** = 5 \( \mu \)m. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 6-24: *M. oryzae* isolates transformed with C00879/GFP developing on cellulose at 4-16 h.p.i.

A) C00879 isolate ‘B’ germling development at 4 h.p.i. on cellulose. A large germ tube is evident. B) Confocal fluorescence image suggesting the presence of large vacuoles in two of the conidial cells, and also a weak GFP presence in the body of the germ tube itself. C) Emission spectra suggesting that GFP is present in the germ-tube, and in the cell from which the germ tube originates. D) *M. oryzae* spore originating from C00879 isolate ‘A’ with developing appressorium at 8 h.p.i. E) Confocal fluorescence image indicating the presence of GFP in all germling compartments. F) Emission spectra with maxima at approximately 510 nm suggesting fluorescence is attributable to GFP. G/J) Developing spore from C00879(S) ‘A’ at 16 h.p.i. with multiple germ tubes, some with branching. Inset picture showed fungal structure at lower magnification. H/K) Confocal fluorescence images showing fluorescence in the all 3 cells of the spore body and in the developing hyphae. I/L) Emission Spectra showing maxima indicative of the presence of GFP at the selected regions. Inset pictures where present indicate fungal structures at lower magnification. **Scale Bar = A, B, G, H, J, K) 4 µm D, E) 8 µm**
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**Figure 6-25**: *M. oryzae* isolates transformed with C00879/GFP developing on cellulose at 31 h.p.i.

**M** Spore with heavily branched structure and appressorial formation at 31 h.p.i. (The speckling present is due to blemishes on the microscope lens.). Inset picture shows the structure fluorescing at a lower magnification. **N/P/R** Higher magnification confocal fluorescence images of the structure shown in M, showing fluorescence of fungal structures, and also the presence of vacuoles, for example in image P (*). **O/Q/S** Wavelength emission spectra. Although confirming that fluorescence is due to GFP presence, a spectrum in image Q confirms the lack of fluorescence in the vacuole in image P (marked by an *). **Scale Bar** = M 30 µm N) 10 µm, P and R) 10 µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.

**Activity of promoters of EST C00879 and EST C01420 during growth on glass.**

Unlike on the two other surfaces, *M. oryzae* appressorial formation on glass was never seen. This matches the behaviour of *Bgh* on the glass slides used both in chapter 3 and also in the original microarray studies performed by Dr. Maike Paramor. Instead, in all isolates including the Wild-type isolate Guy 11, long germ tube formation was the usual occurrence (for example, in the case of C00879 isolate “B”, at 8 h.p.i. 75 % ± 6.06 % s.d. formed just one germ tube) (**Figure 6-29**). Development and GFP expression at 4, 8, 16 and 31 h.p.i. for *M. oryzae* isolates transformed with the 5’-regulatory regions for EST C01420 and C00879 are displayed in images **Figure 6-26** through **Figure 6-29**. It is was a common occurrence
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that more than 1 germ tube may also be formed, most often from the two terminal cells as both

![Image of M. oryzae](image1.png)

**Figure 6-26:** Development of *M. oryzae* transformed with C01420/GFP on glass at 4-16 h.p.i.

A) Spore with singular germ tube. B) Confocal fluorescence image of germinating spore with 4 regions of interest highlighted. As the germ tube does not remain in the same plane of focus sections appear to fluoresce less than others, although this is an artefact of viewing. C) Emission spectra of all highlighted regions of interest, all show a maxima in the region of 508 nm approximately. D) Spore with an elongating germ tube at 8 h.p.i. E) Confocal fluorescence image of the spore in E. F) Emission Spectra with maxima suggesting again that fluorescence is due to the presence of GFP. Also maxima are strongest for the germ tube and the cell from which it emerges, suggesting a build-up of GFP in these structures. G) Mature *M. oryzae* spore at 16 h.p.i with two germ tubes both elongating at various focal planes. J) One of the germ tubes of the spore shown in G magnified. H/K) Confocal fluorescence images of spore body and the developing germ tube. I) Emission spectra suggesting all 3 cells of the conidia provide varying levels of fluorescence attributed to GFP L) Emission Spectrum suggests high fluorescence as attributable to GFP with a maxima at 510 nm approx. Scale Bar = A,B, D, E) 8µm G,H, J, K) 5 µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 6-27: Development of *M. oryzae* transformed with C01420/GFP on glass at 31 h.p.i.

**M/P** Spore with long and branched germ tubes growing on glass at 31 h.p.i. **N/Q** Confocal fluorescence image suggesting GFP presence throughout the cellular structures, with some localisation of the GFP to the periphery of the germ tube. **O/R** Emission spectra displaying fluorescence maxima at approximately 510 nm. Inset pictures where present indicate fungal structures at lower magnification. **Scale Bar** = **M, N** 10 µm; **P, Q** = 3 µm approx. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.

are capable of independent germination. Unlike during development on cellulose, structures formed by 31 h.p.i. on glass were less complex and showed less branching – if any was present at all (Figure 6-27). In similarity to *Bgh* the germ tubes were often projecting above the surface of the glass, suggesting a lack of contact for further surface sensing. The slides used in this section were equivalent to those used in **Chapter 3** and not cleaned with chromic acid as in **Chapter 4**. Although this meant *M. oryzae* spores were, in theory, experiencing the same stimuli as *Bgh* on glass in the original study of EST expression activity performed by Dr. Maike Paramor, this also meant that the glass was a very hydrophilic surface. This would explain the apparent contradiction in the lack of appressorial formation on glass in this study compared to other studies (Lin, 2001). As with promoter activity on the other two surfaces, promoters in all independent isolates for C00879 and C01420/ GFP construct transformants appeared to drive, or have led to GFP expression, during all stages of development. Unlike on barley and cellulose where spores may form appressoria by 31 h.p.i., but in similarity to those spores on cellulose that focused on germ tube formation, GFP was still present at this time.
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**Figure 6-28: *M. oryzae* transformed with C00879/GFP on glass at 4-16 h.p.i.**

A) C00879 isolate ‘C’ germling development at 4 h.p.i. on glass with two germ tubes. B) Confocal fluorescence image. C) Emission spectra showing maxima attributable to the presence of GFP. D/G) *M. oryzae* spore originating from C00879 isolate ‘C’ with germ tubes at 8 h.p.i. E/H) Confocal fluorescence image. F/I) Emission spectra suggesting a strong presence of GFP in both germ tube and the cell from which it originates, whilst the two other cells of the conidia show a reduced presence. J/M) Developing spore from C00879(S) ‘A’ at 16 h.p.i. with two germ tubes. K/N) Epifluorescence images of both the spore body and one germ tube. L/O) Emission Spectra with maxima present in the region of 505-510 nm. **Scale Bar** = A, B) 15 µm; D, E, G, H) 5 µm J, K) 5 µm M, N) 3 µm.

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Figure 6-29: *M. oryzae* isolates transformed with C00879/GFP on glass at 31 h.p.i.

P) Germling development at 31 h.p.i. on glass showing two long germ tubes. Q) Confocal fluorescence image showing the presence of GFP in the germ tube and a reduction of GFP in cells within the spore body, potentially due to the enlargement of the lytic vacuole. The image also suggests that one of the terminal cells is empty of all GFP. R) Emission spectra suggesting that the strong fluorescence in the central cell periphery is due to the presence of GFP, both vacuoles appear S) Image of the developing germ tube T) Confocal fluorescence image, T) Emission spectrum showing a maxima in the region of 505nm suggesting that the fluorescence of the germ tube is due to the presence of GFP. Scale Bar = P, Q) 3 µm approx; S, T) 8 µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units

To summarise, *M. oryzae* resembled *Bgh* during germination on differing surfaces by displaying alternate growth morphologies than those seen during development on barley. Additionally the results for the full-length promoters of EST C01420 and EST C00879 visual observation indicated, that the promoters were each capable of driving GFP expression, as determined via the presence of fluorescence (attributed to the presence of GFP, by wavelength emission analysis) for the majority of fungal structures observed at 0, 4, 8, 16 and 31 h.p.i. on barley, cellulose and glass.

6.5.6: Promoter C00879(s) behaviour on barley, cellulose and glass

As an additional question to our main aim of validating *M. oryzae* as a potential host for future analysis of CMEGs promoters, attempts were also made to test truncated versions of the promoters in question. This truncation was based on the theory that necessary sections of the promoter would show greater evolutionary conservation in comparison to less necessary non-important sections. Therefore alongside transformations of full length promoters, 3 independent transformants of the shortened C00879 promoter were generated and visually analysed for the production of GFP. These “shortened” regulatory regions (Appendix C:
9.15) differed from the full length promoter in being 567 bp in length rather than 1035 bp and most likely contained the regions thought to be evolutionary most conserved. If the hypothesis was correct these shorter promoter regions would still be able to drive GFP expression in a similar fashion to the full-length promoters.

![Image of mycelial preparation of M. oryzae transformed with C00879(s) isolate ‘C’.

A) Hyphae growing through complete medium agar with developing spore present. B) Confocal fluorescence image. C) Emission spectra with maxima at 505-510 nm suggesting that fluorescence present in both cells of the spores and in the surrounding hyphal mass is due to the presence of GFP. D) Mature spore released during preparation of mycelia crush, representing the activity of the shortened C00879 promoter at 0 h.p.i. E) Confocal fluorescence image with 3 regions of interest highlighted, one in each of the spore cells. F) Emission spectra showing maxima present in the region of 510 nm approx, suggesting that fluorescence is due to GFP rather than autofluorescence. Scale bar = 8 µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.

The spores generated from these isolates did not show any deviance from the developmental patterns of the full-length promoter/GFP isolates, as discussed in section 6.5.3. Development on barley reached the invasive hyphal stage, development on cellulose led to either the formation of appressoria or the formation of long, intertwined germ tubes and development on glass led to the formation of multiple, long germ tubes. These results are shown in Figure 6-30 through Figure 6-36. Again, as with the images displayed for the 3 independent transformants of the full length regulatory region of EST C00879, because all isolates demonstrated similar fluorescence properties the figures contain ideal pictures from selected isolates. To summarise promoter activity as determined by GFP presence, GFP was observed
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at all times during all developmental permutations. This suggests the shortened version of the *Bgh* Histone H4 promoter is indeed operational and, that to a degree, the theory that important sections of the promoter would show less mutation has proven to be correct.

To fully test this theory a promoter/GFP construct consisting solely of the least conserved sequence of the promoter should be transformed into *M. oryzae*. In this particular study although attempts were made to create and transform *M. oryzae* with such a construct these proved unsuccessful during the time available and so observational studies could not be carried out.
Figure 6-31: *M. oryzae* transformed with C00879(s)/GFP on barley at 4-16 h.p.i.

A) C00879(s) isolate ‘B’ germling with a germ tube at 4 h.p.i. on barley. Continued overleaf.
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B) Confocal fluorescence image with 4 regions of interest highlighted  
C) Emission spectra, which although showing noise seem to indicate that fluorescence is due to the presence of GFP as maxima are present in the region of 507 nm.  
D) C00879(s) ‘B’ germling with appressorium at 4 h.p.i.  
E) Confocal fluorescence illustrating the build-up of GFP in the appressorium of the germling. Regions of interest are highlighted.  
F) Emission Spectra suggesting maxima in the region of 505-510 nm.  
G) *M. oryzae* spore originating from C00879(S) isolate ‘A’ with mature appressorium at 8 h.p.i.  
H) Confocal fluorescence image with strongly fluorescing appressorium, again with regions of interest including all cells of the conidium, germ tube and appressorium highlighted.  
I) Emission spectra, notably confirming stronger fluorescence of wavelengths associated with GFP in the appressoria  
J/M) Developing spore from C00879(S) ‘A’ at 16 h.p.i. showing the invasive hypha penetrating the epidermal cell. Image M is at a deeper focal plane than M.  
K/N) Confocal fluorescence image suggesting weakened fluorescence in the spore, and noticeably the appressorium, but also fluorescence in the invasive hypha.  
L/O) Emission Spectra, which although with maxima in the region of 510 nm, also have competing maxima at alternate wavelengths, suggesting a decrease in the fluorescence of the GFP compared to surrounding structures.  

**Figure 6-32: M. oryzae transformed with C00879(s)/GFP on barley at 31 h.p.i.**

P(S) C00879(s) isolate ‘C’ germling development at 31 h.p.i. on barley with invasive hyphae formation. Picture P suggests spore collapse has occurred.  
Q(T) Confocal fluorescence image suggesting no fluorescence in the spore body itself or germ tube, and a residual fluorescence present in the appressorial structure. In comparison invasive hyphae do show stronger fluorescence. Regions of interest are highlighted.  
R/U) Emission spectra suggesting that fluorescence present in the above-leaf structures is not due to the presence of GFP but due to autofluorescence. Fluorescence present in the invasive hyphae shows a maxima at 507 nm, supporting the presence of GFP. Inset pictures where present indicate fungal structures at lower magnification.  

**Scale Bars** = A, B, D, E) 8 µm approx  
G) H) 5 µm  
J, K, M, N) 6 µm approx.  

Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
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Figure 6-33: *M. oryzae* transformed with C00879(s)/GFP on cellulose at 4-16 h.p.i.

A) C00879(s) isolate ‘A’ germling development at 4 h.p.i. on cellulose. Spore germination from central cell indicates damage to both terminal cells. B) Confocal fluorescence image. C) Emission spectra confirming the presence of GFP in the periphery of the central conidial cell, and in the germ tube. D) C00879(s) ‘B’ germling with melanised appressorium at 8 h.p.i. E) Confocal fluorescence showing the build-up of GFP in the appressorium, and a reduction of fluorescence in cells in the conidium. F) Emission spectra with maxima suggesting that the fluorescence in the appressorium of the germling is due to GFP. G) *M. oryzae* spore originating from C00879(S) isolate ‘C’ with mature appressorium at 16 h.p.i. showing an enlarged and branched germ tube H/J) Confocal fluorescence image of the spore body and the branch junction of the germ tube. Regions of interest are highlighted. I/K) Emission spectra confirming the presence of GFP in all regions tested, although of varying intensities. **Scale Bar** = A, B, D, E) 8 µm G) 15 µm approximately.
Figure 6-34: M. oryzae transformed with C00879(s)/GFP on cellulose at 31 h.p.i.

L/O/R/U) C00879(s) isolate ‘C’ germling development at 31 h.p.i. on cellulose. Spore development includes complex, intertwining germ tubes as well as appressorium formation at this time point. M/P/S/V) Confocal fluorescence images showing fluorescence in all structures, although in the case of spores forming appressoria some conidial cells show a decrease in fluorescence. Regions of interest are highlighted. N/Q/T/W) Emission spectrum of the associated regions of interest with maxima in the region of 505nm- indicative of the presence of GFP. Notably one spectrum (displayed in image W) suggests that one area of fluorescence at wavelengths not associated with GFP. Inset pictures where present indicate fungal structures at lower magnification. Scale Bar = L, M) 20 μm approx O, P, R, S) 8 μm approx U, V) 5 μm approx. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 6-35: *M. oryzae* transformed with C00879(s)/GFP on glass.

A) C00879(s) isolate ‘C’ germling with 1 germ tube at 4 h.p.i. B) Confocal fluorescence image with regions of interest highlighted. C) Emission spectra with maxima at 505-510 nm showing that fluorescence in all compartments is at the wavelength associated with GFP. D/G) C00879(s) ‘A’ germling at 8 h.p.i. with an elongated germ tube E/H) Confocal fluorescence image of the spore body and the germ tube. Due to undulation the germ tube does not remain in the same plane of focus within image E. Continued overleaf.
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**F/I** Emission Spectra showing strong maxima at 507 nm. **J/M** *M. oryzae* spore originating from C00879(S) isolate ‘C’ at 16 h.p.i. **K/N** Confocal fluorescence image of both the spore body and the germ tubes, showing a decrease in fluorescence in conidial cells. **L/O** Emission spectra suggesting that although GFP is present in all structures that in the three cells of the spore it is decreasing. Scale Bar = 5 µm approx. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.

![M. oryzae spore originating from C00879(S) isolate ‘C’ at 16 h.p.i.](image)

**Figure 6-36: M. oryzae transformed with C00879(s)/GFP on glass.**

**P/S/V** Germling originating from transformant C00879(s) “C” at 31 h.p.i. showing a highly branched germ tube structure. Inset picture shows the germling at a lower magnification. **Q/T/W** Confocal fluorescence image showing pockets of reduced fluorescence, possibly due to the expansion of vacuoles within the *M. oryzae* cell cytoplasm. Regions of interest are highlighted. **R/U/X** Emission spectra suggesting all regions of interest show fluorescence associated with the presence of GFP, even if it is at reduced intensity. Inset pictures where present indicate fungal structures at lower magnification. Scale Bar = 5 µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.

6.5.7: **C00879 and C01420 CMEG promoter activity in *M. oryzae***

*(Conducted with the aid of Nurul Ismail, MRes student).*

In order to further ascertain whether the activity of *Bgh* CMEG promoters was similar in *M. oryzae* to activity seen in *Bgh*, RNA was collected from germinating spores of the GFP-expressing transformants at time-points of 0, 4, 8, 6 h.p.i. This was used as a template for
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creating cDNA. Real-time quantitative PCR was used to assess the relative expression of GFP driven by the 5’-regulatory regions of ESTs C00879 and C01420. Target gene expression was normalised using the \textit{M. oryzae} actin gene as an internal control. This gene has been used in other studies for a similar role (Mosquera et al., 2009). The original microarray (in the case of C00879 please refer to \textbf{Figure 6-37}) derived expression data for the activity of these EST promoters in \textit{Bgh} was confirmed by using the gene D00403 (an NADH-ubiquinone oxidoreductase) as a normalisation control as this gene was believed to be expressed relatively uniformly across all the conditions tested. Consequently, the \textit{M. oryzae} gene equivalent was used as a secondary control to re-enforce the assessment of CMEG promoter behaviour. Its use was based on the hypothesis that to truly compare CMEG promoter activity once in the heterologous host, the same controls should be used. However, this was based on the assumption that behaviour of the control gene would remain the same between organisms- an idea that was itself under test during this experiment.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Relative expression of the EST C00879 in \textit{Blumeria graminis} f. sp. \textit{hordei} during development on barley (B), wheat (W), cellulose (C) and glass (G).}
\end{figure}

\begin{flushleft}
Derived from microarray data provided by Dr. Maike Paramor. \textbf{REI} = relative expression index. expression values being displayed as the log$_2$-transformed ratios of sample/universal reference intensities. REI values have three replicate hybridisations averaged, with the standard deviation displayed as a bar. Time points include 0, 4, 8, and 16 h.p.i. Error bars = standard deviation
\end{flushleft}

Interestingly, the majority of transcription patterns analysed for independent isolates using one control gene appeared to match their counterpart analysed using the other control gene (please refer to \textbf{Figure 6-38}, to compare visual trends for example of C00879 isolate ‘A’). Such results suggest both genes were expressed in a similar fashion and suggests the choice
of either gene as a control for expression normalisation would be appropriate – at least on these two surfaces utilised (readers are referred to the discussion of Chapter 4 for other remarks on the behaviour of this gene). However, because of its ubiquitous use in other studies the actin gene was chosen as the primary control gene during this study.

As shown in Figure 6-37 (provided by Dr. Maike Paramor) the expression of EST C00879 in Bgh during early development on barley is up regulated quite strongly at 4 h.p.i. After this time point a decrease, not as sharp as the initial increase is seen, until at 16 h.p.i. the gene expression (encoding a H4 histone) is approximately half that recorded at 4 h.p.i. A similar profile is seen during development on wheat. In comparison to this, expression on glass and cellulose remains relatively consistent, with results indicating (although not statistically significant) that the highest expression is see at 16 h.p.i. at a level approximately 1/3 of that observed at 4 h.p.i during development on barley.

The independent isolates used during this experiment exhibited expression profiles during development on barley as shown in Figure 6-38. All three isolates showed GFP gene expression which differed to that encountered during Bgh development on barley. Where possible statistical analysis utilising 1-factor ANOVA combined with a post-hoc Tukey or Games-Howell test was used to confirm visual trends.

Two out of the three isolates “A” and “C” show very similar visual profiles with relative expression appearing to climb during the period of assessment 0 – 16 h.p.i., In the case of isolate ‘A’, a significant 2-fold increase was seen between 0 h.p.i. and 16 h.p.i. for data normalised against both the actin gene, and the M. oryzae homologue of the Bgh NADH oxidoreductase gene (at α = 0.05, appendix E, 9.14). However, no peak is seen at 4 h.p.i. unlike in the original microarray data. Such significance was the same for the profile generated for C00879 isolate ‘C’ when normalised against the actin gene, appendix E, 9.14). In comparison isolate “B” appears to show expression at 4 h.p.i matching 16 h.p.i at levels 4 times higher than expression at 0 and 8 h.p.i. This decrease at 8 h.p.i. is nearly significant (at α = 0.05, appendix E 9.14).
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Figure 6-38: Relative expression of GFP driven by the promoter of EST C00879 in *M. oryzae* isolates during development on barley.

Based on qPCR data. Time points include 0, 4, 8, and 16 h.p.i. **Left Graph** = Relative expression compared to that of the *M. oryzae* actin gene. **Right Graph** = Relative expression compared to that of the *M. oryzae* equivalent of the *B. graminis* NADH ubiquinone oxireductase gene. If all 3 biological replicates not available then data points are plotted independently. Error Bars= ± standard error.

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During this trial, attempts were made to extract the RNA from on one other surface apart from the host. The method of RNA extraction used during this investigation meant that extraction from spores developing on glass was not possible due to the presence of water (derived from the spore inoculation solution) interacting with the cellulose acetate on the slides. Therefore cellulose was chosen as the second surface to investigate. As described earlier, activity during *Bgh* development on cellulose showed relatively low expression during the time period assessed with results suggesting an expression climbing from 0 to 4 h.p.i. and reaching a maximum at 16 h.p.i.

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**Figure 6-39:** Relative expression of GFP driven by the promoter of EST C00879 in *M. oryzae* isolates during development on cellulose.

REI = relative expression compared to that of the *M. oryzae* actin gene. Based on qPCR data. Time points include 0, 4, 8 and 16 h.p.i. If all 3 biological replicates not available then data points are plotted independently. Error Bars = ± standard error.
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As shown in Figure 6-39 of the 3 independent isolates tested two (C00879 ‘A’ and ‘B’) provided profiles that suggest no significant increase in expression over the time period shown. C00879 Isolate “A” results seem to suggest a drop in expression followed by an increase in levels at 16 h.p.i. to match those experienced in the freshly inoculated conidia at 0 h.p.i. Isolate “B” results indicate a potential rise in expression at 4 h.p.i followed by a drop thereafter. Isolate “C” shows a drop at 4 h.p.i followed at 8 h.p.i that remains constant at 16 h.p.i. The third independent transformant (C00879 isolate ‘C’) did show a significant increase in expression when data collected at 16 h.p.i. was compared to both 0 h.p.i. and 4 h.p.i. The data points provided suggest that expression at 8 h.p.i. would be similar to that collected at 16 h.p.i. However, there was a significant drop in expression between 0 and 4 h.p.i (significant at α = 0.05, appendix E, 9.14). Consequently, this is a deviation from the expression profile exhibited in Figure 6-37. As a result of these observations attempts to collect RNA from *M. oryzae* transformants containing the C00879[s]/GFP fusion were terminated as being impractical within the time frame of the experiment.

As shown in Figure 6-40 (provided by Dr. Maike Paramor) the expression of EST C01420 in *Blumeria graminis* f. sp. *hordei* during development on barley (B), wheat (W), cellulose (C) and glass (G).

![Figure 6-40](image)

Derived from microarray data provided by Dr. Maike Paramor. REI= relative expression index. expression values being displayed as the log₂-transformed ratios of sample/universal reference intensities.REI values have three replicate hybridisations averaged, with the standard deviation displayed as a bar. Time points include 0, 4, 8, and 16 h.p.i.
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h.p.i. Again, although not statistically significant, the expression profile suggests a drop in expression at 16 h.p.i. In comparison to this, behaviour on wheat clearly shows that gene expression is at its highest at 8 h.p.i, followed by a decrease at 16 h.p.i. Expression on glass and cellulose remains at a low level consistent with expression seen at 0 h.p.i.

The single isolate that contained the regulatory region for CMEG C01420 was tested for its expression of GFP during its development on barley. As shown by the profiles in Figure 6-41 illustrating GFP EST C01420 driven-expression (when compared relative to the activity of both control genes), although not significantly different from later time points, visual trends suggest a peak at 4 h.p.i. before dropping at 8 h.p.i. ([appendix E, 9.14](#)). If this were the case then it suggests GFP expression as driven by this promoter region in *M. oryzae* does not match the expression behaviour of the aconitase gene in *Bgh*.

![Figure 6-41: Relative expression of GFP driven by the promoter of EST C01420 in *M. oryzae* isolates during development on barley.](#)

REI = relative expression compared to that of the *M. oryzae* actin gene. Based on qPCR data. Time points include 0, 4, 8 and 16 h.p.i. If all 3 biological replicates not available then data points are plotted independently. Error Bars = ± standard error.

In conclusion these results displayed in this chapter suggest firstly, that the majority of CMEG regulatory regions transformed into *M. oryzae* in this study are not capable of driving the expression of GFP in that heterologous host. Secondly, although the two promoters regulate GFP expression in *M. oryzae*, containing the full-length selected 5’-regulatory
regions of EST C00879 and EST C01420 do not drive expression as experienced by the gene in *Bgh*. Thirdly, truncation of the 5’-regulatory region of EST C00879 (so that it contains a region showing higher conservation than surrounding sequences) still permitted the expression of GFP whilst in *M. oryzae*. 

CMEG 5’-Regulatory Region driven GFP expression in *M. oryzae*
6.6: Discussion

Allocco et al., (2004) stated that it is ‘axiomatic in functional genomics that genes with similar mRNA expression profiles are likely to be regulated via the same mechanisms’. Studies such as Thorsen et al., (2007) have identified genes with similar transcriptional profiles and followed up with consecutive testing for cis-regulatory regions. It is a similar hypothesis that underpins attempts to use the results of earlier microarray studies to understand gene regulation of the CMEGs that demonstrate expression clustering. It would be useful to understand the promoter construction of set genes so that the regulatory networks of Bgh can be understood. Furthermore it will allow a better understanding of which stimuli or host signals spur the development of this very specific fungus. By fully understanding these control pathways, and seeing where they interlink with others, it will become easier to both create better ways of combating it in the field, but also understanding not just other B. graminis formae speciales but other powdery mildews.

One of the hardest tasks in the annotation of whole genomes remains the accurate identification and delineation of promoters (Abeel et al., 2008). Due to the diversity of eukaryotic promoters and the fact that promoter prediction software is often species specific, experimental data (in the form of deletion and mutagenesis studies) must be collected to “train” such programs (Wasserman and Sandelin, 2004; Munch and Krogh, 2006). Once enough data has been collected it would be possible to apply the models to screening the recently annotated Bgh genome. By doing so, apart from potentially showing the genes involved in similar regulatory networks it may also help identify genes of unknown function which genome annotation programs have failed to identify (Abeel et al., 2008). Therefore, as functional regulatory regions tend to be proximal to the initiation site of transcription, regions approximately 2 kb in size (minus coding sequence) were amplified from the upstream region of selected CMEGs (Wasserman and Sandelin, 2004). Removal of coding sequence is important, especially when looking for conserved regulatory sequences as coding regions naturally tend to be highly conserved. This will affect motif detection programs (Chowdhary et al., 2006). It was believed therefore that these selected regions would contain the core promoter and possibly proximal elements. As noted previously attempts at finding motifs (especially for transcription factors) were unsuccessful, possibly because such TFBS are short and variable making detection difficult (Bucher, 1999).
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Since transformation of *Blumeria graminis* is not an option at this time-point another approach needed to be taken to try to dissect the method of regulation of these CMEGs. Many regulatory modules (e.g. cis-regulatory elements and TFs) are conserved across the *Ascomycota* (Wohlbach et al., 2009). Therefore the option chosen in this study was that of testing these proposed promoter regions in *M. oryzae* a related, easier-to-handle fungus whose genetic manipulation has become routine (Wilson and Talbot, 2009). Unlike other techniques also used for experimentally determining important regulatory regions, such as the yeast 1-hybrid system, this approach would (if successful) permit the rapid analysis of several promoters from the same CMEG cluster. Therefore the aim of this experiment was to determine if CMEG promoters would drive the expression of GFP whilst present in *M. oryzae*. This would then demonstrate its suitability as a heterologous host to trial deletion analysis or mutagenesis on these regulatory regions.

The hemibiotroph *M. oryzae* shares many important similarities with *Bgh*. These include the formation of appressoria and intracellular tissue invasion (Caracuel-Rios and Talbot, 2007; Wilson and Talbot, 2009). This initial host-internal growth (approx 72 h.p.i) is biotrophic in nature, with the invasive hyphae causing an invagination of the host cell membranes, in a similar fashion to *Bgh* haustorial growth (Wilson and Talbot, 2009). *M. oryzae* also has the ability to infect barley, as well as utilising similar germination stimuli as *Bgh* (for example hydrophobicity and cutin monomers). Additionally during this thesis, *M. oryzae* demonstrated morphological similarities to *Bgh* during development on surfaces other than barley. Consequently, it was thought to be suitable as such a host may contain similar signal transduction pathways. Additionally, GFP was first expressed in *Magnaporthe* in 2002 by Czymmek et al. In that study transformation with GFP resulted in high expression, with fluorescence being excluded from large organelles such as vacuoles and mitochondria, although it did accumulate in inter-phase nuclei. According to the authors no discernible difference in appressorium formation or function was observed in transformants expressing the fluorescent protein compared to the wild-type (Czymmek et al., 2002). Therefore this robustness suited it to these expression studies.

As CMEG gene expression was originally assessed at 0, 4, 8, 16 h.p.i. efforts were made to discern the morphological development of *M. oryzae* at those time points. As demonstrated, the lifecycle of *M. oryzae* on barley shows some morphological and temporal similarities to that of *Bgh* (Figure 6-6). By 4 h.p.i. the sole germ tube has emerged. In many cases during
CMEG 5’-Regulatory Region driven GFP expression in *M. oryzae*

development on barley germ tube hooking was present suggesting maturation of the germ tube was already underway (i.e. its development from germ tube to appressorial germ tube). The germ tube of *Magnaporthe* appears to combine some of the functions of both *Bgh* germ tubes, both sensing stimuli present on the substrate (like the PGT of *Bgh*) and also permitting the invasion of the host (like the appressorial germ tube of *Bgh*). By 8 h.p.i., as in *Bgh*, maturation of the appressorium is occurring. At the final time-point assessed by RT-qPCR analysis (16 h.p.i.) invasion of the barley host has begun, just as with powdery mildew. Interestingly the timing of this penetration of barley by *M. oryzae* appears to occur at a faster rate than during development on rice (the primary host of the pathogen). In section 6.2 it was noted that during development on rice between 4 to 8 h.p.i. melanisation of the appressorium begins (as does an increase in turgor generation). Following this at 24 h.p.i. an appressorial pore ring will form from which the penetration peg emerges between 24 to 31 h.p.i (Howard et al., 1991; Lu et al., 2005). However as the results clearly show penetration of barley has may already have begun by 16 h.p.i. Reasons for this faster penetration may lie with the barley epidermal surface either offering less resistance to the penetration peg (and as a consequence yielding after less turgor pressure has built-up) or because it is more susceptible to attack by the enzymes released by the developing *M. oryzae* spore.

During this study as with the alternate surfaces used in the original microarray study of *Bgh*, *Magnaporthe* also displayed alternate morphologies when germinating on surfaces other than the host. Spores were delivered in liquid and earlier studies (e.g. Hegde and Kolattukudy, 1997) had noted a spore-concentration based inhibition mechanism. As a result spore levels on the different surfaces were tailored such that any morphologies present were dependent on surface-based stimuli rather than inhibition by spore concentration. On glass, again a sign of how hydrophilic the slides were that were employed for the original study conducted by Dr. Maike Paramor, germ tube rather than appressorium formation was the usual end state of germination (for example Figure 6-26). On cellulose formation of appressoria was also seen (for example Figure 6-22), although at a reduced rate compared to that seen on the host. This alternate germination behaviour further re-enforced the selection of this fungus as a heterologous host.

During this study of the 22 different CMEG regulatory regions that were successfully transformed, 2 showed activity when present in *M. oryzae*. In the case of the regulatory region of EST C00879 3 independent transformants of the “full length” region demonstrated
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GFP expression whilst in M. oryzae. Three independent transformants of “shortened” regulatory regions (containing a section believed to show higher conservation than the remainder of the original sequence section) also showed GFP expression. This would suggest that the region selected did indeed contain the core promoter (and most likely proximal sequences) required to drive expression and that in the case of this gene M. oryzae may indeed be an acceptable host for further promoter structural analysis.

In all cases these EST C00879 regulatory-region containing M. oryzae transformants demonstrated visible GFP presence in each of the conidial cells, the developing germ tube, the appressorium and shortly after infection of barley. Furthermore, it appeared that although different germination states could be seen when on surfaces other than the host, for example (if appressorium were not formed) the large hyphal networks present on cellulose (e.g. Figure 6-25), that GFP expression was still present. Such behaviour suggests that expression could have been driven by a signal transduction pathway active early on in the development or more probably that expression was not being regulated in a surface dependent fashion in this host, but would occur at whichever developmental stage the spores in question reached. This seems to be supported by the fact that the hyphae of a transformed colony also demonstrated fluorescence.

As an indicator of the behaviour of these promoters in Magnaporthe, and to permit an extra comparison to behaviour in Bgh, expression of regulatory region-driven GFP was assessed by RT-qPCR. RNA was extracted from germlings developing on barley surfaces at 0, 4, 8, 16 h.p.i. In an attempt to see alternate expression on a surface other than the host only cellulose was tested. Limitations of the RNA collection method meant that RNA could not be collected from glass slides, therefore collection from cellulose was chosen instead. In the case of expression during development on barley the isolates did not match expression seen in Blumeria during germination on that surface. This suggested expression as commanded by the regulatory region selected was regulated in a different fashion when present in Magnaporthe. In the case of expression on cellulose, visual trends did not match the profile collected during the original microarray experiment, again suggesting there may have been some difference in regulation. In the case of one isolate (Figure 6-38) a significant increase was seen by 16 h.p.i. (and possibly by 8 h.p.i), however a decrease seen from 0 to 4 h.p.i again suggests expression did not match of the H4 gene during Bgh development on cellulose.
The gene C00879 encodes a histone H4 involved in histone formation (Luger et al., 1997) whose expression, at least during infection on barley, appears to increase at 4 h.p.i. before decreasing thereafter. It is at this time-point the formation of the second germ tube, destined to become the AGT, takes place. Although nuclear structure of Bgh, to the knowledge of this author, has not been confirmed at this time it is thought the second, septate, germ tube contains its own nucleus. By 16 h.p.i., at least on barley mRNA abundance has decreased. This may be due to the fact that either histones are no longer needed as nuclear formation has reduced, or that the sampling method failed to accurately sample RNA present in the haustorium. As a gene with an essential role in the cell its control may not need to be accurately tuned to the behaviour of external stimuli, instead being determined by the cell's own requirements. As such the length of the functional promoter may be shorter than other regulatory regions as complex signal integration (with multiple regulatory gene binding sites) may not be required.

The fact that other regulatory regions from the same cluster were chosen (for example EST D00651 and EST D00095), and although successful transformants were detected by GFP marker specific PCR, failed to glow may suggest that this cluster (although co-expressed) is actually regulated by different mechanisms. Authors such as Park et al., (2002) have noted that different transcriptional mechanisms may have the same effect on expression. If this is the case then understanding the regulatory networks of Bgh may be more complicated than expected. Why is there this apparent diversity of promoter elements in Bgh? It may be because the complex lifecycle requires very careful regulation to ensure that biotrophy is successfully initiated and then maintained for as long as possible. The ability to respond to a host that is itself responding both to the presence of fungus, to other pathogens, and also the environment would require complex regulation. The fungus must be able to “tweak” responses spatially and temporally where and when necessary without having to worry about initiating widespread gene activation. The presence of such complex pathways incurs the question of just how did they evolve and how long did the fungus need to be in contact in the host before it assumed such intimate levels of contact? Wohlbach et al (2009) suggests regulatory divergence occurs at an elevated rate compared to the divergence of coding sequences. Therefore the genomic repetition present in Bgh may have permitted the fungus the option of developing such complex regulation of its genes: all the better to ensure compatibility with the host.
In the second case of a GFP-expressing transformant, the 5’-regulatory region of EST C01420, GFP appeared to be expressed at all time-points assessed except at 31 h.p.i. on barley where GFP expression appeared minimal. However, only 1 transformant (from a total of 24 hygromycin resistant colonies analysed, originating from 7 separate transformant isolations) demonstrated the capability to drive GFP expression when in the heterologous host. It is therefore possible that this successful transformant was active due to the effect of a nearby *Magnaporthe* enhancer element or promoter having an effect. If this was the case then it is more than likely that the regulatory region selected is not complete and normal expression in Bgh requires elements, in this test left unselected, to propel transcription. As this is the case it suggests that *M. oryzae* is not an optimal test environment for this promoter.

In the original microarray data expression for EST C01420 during development on barley, mRNA abundance increased rapidly from 0 to 4 h.p.i, before reaching a maximum at 8 h.p.i and then dropping again at 16 h.p.i. The gene of this promoter encodes an aconitase. Present in both the mitochondria and cytoplasm, this enzyme catalyzes the interconversion of citrate and isocitrate (Tong and Rouault, 2007). Catalysing part of the tricarboxylic acid cycle, at least in its mitochondrial form, this enzyme therefore has an important role in energy generation. As a consequence it would be expected that its expression would increase to match cellular requirements during the production and maturation of the appressorium which happens at 4 to 8 h.p.i. When present in the cytoplasm aconitase has roles in iron metabolism and the flow of citrate (Tong and Rouault, 2007). Regrettably, RT-qPCR analysis did not confirm this expression during *M. oryzae* development on barley (Figure 6-41), with visual trends suggesting an increase a maximum abundance at 4 h.p.i. with a reduction in expression at later time points. This would confirm the belief that the GFP expression was being modulated by a nearby *M. oryzae* element.

Both the regulatory regions demonstrating the ability to drive GFP expression originated from different CMEG clusters. Therefore the likelihood that these genes would have the same regulatory mechanisms is reduced. This means whatever *cis*-element information is found by analysing one may not be useful in understanding the other. Therefore at the present time any contribution to understanding Bgh regulatory mechanisms remains fragmentary at best.
Although some positive results were achieved during this study there were several drawbacks to the approach used. The biggest revolves around removing the promoters from their native environment. Although unavoidable if widespread screening of activity was to be attempted, the potential exists that the selected regions were separated from any influencing enhancer and silencer elements. This would consequently alter their behaviour outside the donor and furthermore not allow full investigation of CMEG regulation. Additionally, specific copy number and positioning of genes are required for the optimal production of a gene product as well as to allow co-ordination of expression of genes involved in related metabolic pathways (Saez-Vasquez and Gadal, 2010). Therefore any information gathered about the gene regulation would also be tempered by this dissimilarity.

Since the majority of promoter regions tested failed to permit GFP expression when transformed in *M. oryzae* it means that for those promoter regions further work utilising this host is not possible. This lack of activity may be due to several reasons. Firstly, the regions used did not include all of the required promoter sequence. Although the core promoter (and proximal elements) may have been selected a lack of enhancer sequences could mean that even though the relevant transcription factors were present in *Magnaporthe* expression of the GFP was not possible. This may suggest that local DNA sequence in the case of the majority of these genes was not sufficient to drive expression and that even in the host long range interactions were required.

Secondly, it is possible that all the relevant core and proximal promoter sequences were selected. However, *M. oryzae* may not contain the relevant transcription factors to bind to the TFBSs present in those regions, meaning expression was not possible. Alternatively, many promoters contain multiple binding sites for the same TFs (Paixao and Azevedo, 2009). Therefore if enough TFs were not present at the time (due to other cellular requirements) then true promoter activity may not be seen (Pedersen et al., 1999). This is assuming that TFs work or bind alone. Often they bind in combinations, and non-independently, at CRMs (Wasserman and Sandelin, 2004). Therefore even if the majority of TFs are present the lack of one may cause others to fail or lead to greatly reduced expression. To add further complexity to the matter transcription factors are themselves transcriptionally regulated, meaning that by assuming their presence in *M. oryzae* we are also implicitly assuming they themselves are regulated in a similar fashion to those in *B. graminis.*
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Thirdly, being a hemi-biotrophic pathogen with a considerable host range, *M. oryzae* probably does not require all of the signals which *Bgh* does to complete its invasion of the host. As a result less complex signal transduction pathways may exist, or in some cases not at all. Therefore multiple assumptions had to be proved correct in order for this testing scheme to work – and in actuality they were not. This would mean again that GFP expression would be hard to detect. As it was not practicable to perform RT-qPCR on all transformants only those that showed the strongest (or in this case visible) expression were selected for.

There were also drawbacks to the choice of visual marker. Although the exact half-life of the sGFP molecule was unknown it was originally selected as a requirement for easily visible GFP during the screening process. This made the screening process easier as the prospect of protein degradation was reduced and human errors, such as missing GFP expression, could be minimised. However, in attempts to spot the temporal and cell-type localisation of the GFP problems could be encountered. Combined with a lack of sub-cellular targeting, the assumed long-life of the GFP molecule means residual GFP may be present over extended periods these times, so an assumption that the promoter is solely active at this time is not altogether applicable. Cytosolic movements between cells of the developing *M. oryzae* germling could have led to a “blurring” of the lines regarding actual site of production with GFP flowing, for example between the germ tube and developing appressorium.

Although attempts were taken to understand expression by using RT-qPCR to detect relative levels of GFP mRNA, a drawback was encountered in the use of vectors which integrated randomly, and with an unknown copy number, rather than at a set region. This meant that intrinsic *Magnaporthe* elements could be affecting exact regulation, for example by placing the promoters near enhancer elements or leading to integration of upstream of native promoters. These factors could have resulted in the alternate expression profiles seen for the independent transformants of the regulatory region of EST C00879. Since expression was variable the decision was made not to test the mRNA abundance expression of the “conserved” C00879 promoter regions. Instead presence of visible GFP was the sole indicator of activity for that promoter section.

Since only a few positive results were gathered in this study a change of approach may be necessary for future work. In the short term, the regulatory region of EST C00879 could still be analysed in *M. oryzae*. These tests should focus on using a vector that targets to a
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...genetically neutral site (i.e. one where previous transgene incorporations have shown no apparent effects due to surrounding *M. oryzae* sequences). Also, *Agrobacterium*-mediated transformation, already in use with *M. oryzae* and with its high rates of transformation and lower levels of multi-gene insertion, should be considered (Jeon et al., 2007). With such a system it would then be possible to accurately compare expression of the regulatory region at the length originally chosen for this investigation and the conserved region. In this study attempts were made to create transformants with solely the upstream sequences that showed the least conservation, as a form of negative control. However these efforts proved unsuccessful. Future work should re-attempt the creation of such promoter/GFP fusions. This promoter, if not providing the binding sites for any transcriptional machinery would fail to drive GFP expression. This would then allow full confirmation of the role of the conserved regions of the C00879 promoter.

Building on the results presented herein, the regulatory regions (for EST C00879 and EST C01420) could also be utilised in a yeast 1-hybrid system. This could identify the *Bgh* transcription factors that bind to them. This would be possible by placing this promoter region in front of a screenable marker such as GFP, the GUS gene (encoding β-glucuronidase an enzyme from *Escherichia coli* that hydrolyses β-glucuronides, many of which on cleaving produce coloured products) and then by inserting a library of *Bgh* cDNAs (linked to constitutive promoters) already used for the microarray work into yeast (Blanco et al., 1982; Hirt, 1991). It would then be possible to test for expression of binding products. Transcription factors seen to bind could then be used themselves as a source for further work to see what other regulatory regions they bind to. As noted by Reid et al., (2009) to start an analysis of transcriptional regulation we need to consider the single TF level first. Example techniques include that of Mukherjee et al., (2004) and Bonham et al., (2009) where the use of DNA-based protein binding microarrays can rapidly identify transcription factor binding sites. Furthermore characteristics of this transcription factor may also be known in other species so data about behaviour could be gathered in this fashion. TFs have distinct preferences towards specific target sequences. By finding known binding sites it is then possible to model the target sequence properties. This may then lead to the creation of more accurate models for promoter prediction and allow some level of prediction throughout the genome (Wasserman et al., 2004). It may also be possible that in silico methods have developed since the time of original testing (2007-2008) to the degree that elements within the promoters may now be identifiable based on information gathered from other filamentous fungi. This is worth
investigating alongside further experimental work. If experimental work is to be continued then maybe the entire region between ORFs (including the 5’-UTR) could be used in future to ensure no regulatory region is missed.

In conclusion, two CMEG promoter regions showed activity whilst present in *Magnaporthe oryzae*. However, another 20 CMEG/construct were tested and failed to show any activity. Although the methodology used during this study did have disadvantages (including unknown copy number and lack of targeting) this result suggests that the usage of *M. oryzae* is not feasible when it comes to widespread testing the of *Bgh* CMEGs regulatory regions. This is not necessarily surprising when it is very possible that due to its difference in lifestyle compared to *Bgh* (a hemi-biotroph vs. a true biotroph), *Bgh* may need more complex control pathways to maintain its highly tuned relationship to that of the host. The ability of *M. oryzae* to grow on multiple hosts mean it has not evolved to be solely in concert with one and therefore has less of a need of such complexity.

Even if the *Bgh* genomic sequence is successfully analysed for an initial set of TFs the higher order of transcriptional regulation, transcriptional cascades, can still remain something of a mystery (Eckert and Muhlschlegel, 2009). Also the regulation of any gene may occur at any point during the progress of transcripts into functional (e.g. splicing or protein modification) (Wasserman and Sandelin, 2004). Therefore discerning the *cis*-elements in front of genes is merely the first step in trying to understand this complex organism.
Chapter 7: Discussion and concluding remarks

In the introduction to this thesis the author referenced J. A. Lucas who (in his presidential address of 2003 for the British Society of Plant Pathology) observed that to successfully infect a host a pathogen must utilise both host and environmental cues to marshal its developmental processes in such a manner that it can successfully infect the host. It is the sensing of the host by barley powdery mildew, and the effect such sensing has on its gene expression that has been studied in this thesis.

Progress of Bgh research during the course of this investigation.

Schneider and Collmer (2010) identified 4 eras of molecular plant pathology research. The first they define as that of ‘disease physiology’ (lasting from the 1900’s to the 1980’s). This era featured observational studies (for example that of Russell et al., 1975) and was based on using “cell-free extracts” showing biological activity. As the authors observe during this period the molecular basis for biotrophy remained unknown (Schneider and Collmer, 2010). The next era was that of ‘molecular genetics’ (1980’s to 2000) which was focused on small numbers of genes (Schneider and Collmer, 2010). Following this research moved into the ‘genomics era’, beginning with the sequencing of Xylella fastidiosa by Simpson et al., (2000) (although other authors suggest it truly began with the sequencing of Saccharomyces cerevisiae by Goffeau et al., 1996)(Nakayashiki and Nguyen, 2009; Schneider and Collmer, 2010). Genomic era investigations centred around the determination and use of an organism’s genome sequence to identify both the genes themselves but also non-coding yet functionally important regions of the genome (Archer and Dyer, 2004 and references there-in). Finally, the authors go on to note a new era of research, that of ‘systems biology’, where investigators try to integrate knowledge of all parts of a pathosystem gathered by transcriptomic, proteomic and metabolomic studies into a viable model (Ideker et al, 2001, Collmer and Schneider, 2010).

Until recently the investigatory approach to Bgh has been reminiscent of strategies found within the molecular genetics era. The technical limitations of working with a biotroph have, essentially, forced investigators to approach its study in a piece-meal fashion based on ideas
garnered from other pathogens. Examples of such approaches include the investigations of Zhang and Gurr (2001) who attempted to investigate the role of MAPK in *Bgh* development based on the presence of homologs in *M. oryzae* or Bindslev et al., (2001) who uses *M. oryzae* as a heterologous host for the testing cAMP subunits. This is not to say that techniques associated with the genomics era (such as transcriptomics) have not been utilised or that advances have not been made using these techniques. Focusing on areas pertinent to the questions posed within this thesis when this investigation began (2006-2007) the role of hydrophobicity in development was proposed given its ability to induce varying amounts of ECM. In 2008 Zabka et al., further re-iterated the role of surface hydrophobicity as a prime factor in developmental inductivity by demonstrating that highly hydrophobic enhanced formation of later developmental stages (e.g. appressorial formation) of *Bgh*. With regards to compounds capable of inducing *Bgh* development the inductive capability of cutin monomers and aldehydes had already been cited in 1996 and 2002 by Francis et al., (1996) and Tsuba et al., (2002) respectively. A further advance in this area has recently been published by Hansjakob et al., 2010 again utilising observational studies. In this study multiple even chain length aldehydes were tested to determine their affect on germination and differentiation. Again 1-hexacosanal was shown to be the primary inducer of differentiation. These authors also noted other aldehydes such as the C$_{28}$-aldehyde octacosanal, and even interestingly n-hexacosanol, were effective inducers in both a dose- and chain length-dependent manner under the experimental conditions employed.

With regards to gene transcription levels during *Bgh* development no studies have been published since 2005 which includes the first use of Serial Analysis of Gene Expression (SAGE) in a fungal plant pathogen (Thomas et al., 2002) and the microarray-based work of Both et al., 2005 (A, B); upon which sections of this thesis is based. To the author’s knowledge the studies featured within this thesis are the first attempt to take the next step and link the activity of inductive compounds/conditions directly to gene expression. Similarly, no further feasibility studies of *Bgh* transformation have been published (although as mentioned at the end of Chapter 5 they are still being attempted by other research groups). Instead attention of the *Bgh* research community appears to be moving towards the use of proteomics with studies released in 2008 and 2009 looking at the proteome found within haustoria, conidiophores and spores (Bindschedler et al., 2009; Godfrey et al., 2009; Noir et al., 2009). In fact the study of *Bgh* conidiophores using 2D-gel electrophoresis by Noir et al., (2008) marked the first proteomic study of a fungal phytopathogen (Tan et al., 2009). Other efforts,
focusing just on investigations of post-penetration development, have begun to utilise ‘Host Induced Gene Silencing’ where dsRNA is transferred from the host into Bgh, via the haustoria, permitting the silencing of genes required for virulence (Nowara et al., 2010).

At the beginning of this study (2006-2007) the complete sequence of only one plant pathogenic fungus (M. oryzae) was available (although others were in the process of being sequenced) (Dean et al., 2005, Xu et al., 2006). However by 2009, the complete sequences of 12 fungal plant pathogens had been released (including two biotrophs: Puccinia graminis and Ustilago maydis, Bhaduaria et al., 2009). In 2010, the sequence of Bgh was published, marking the most significant advance seen in the field during the course of this thesis (Spanu et al., 2010). With this achievement, it could be argued that research into Bgh has only now moved definitively into the genomics era. Schneider and Collmer (2010), as well as other authors, refer to organisms under investigation (and without sequence information) as “black boxes” (Schneider and Collmer, 2010; Xu et al., 2006). This advance provides a platform for comparative genomic studies that may explain the obligate nature of Bgh infection (Xu et al., 2006).

It has been the intention of the author to contribute to this general progress in Bgh research by highlighting the strong reliance that barley powdery mildew places on its host and how development of the fungus relies strongly on select cues present at different stages of development. The thesis began with an example of the differential growth behaviour on different surfaces (Chapter 3). It followed with attempts to elucidate the signals that spur development in the germinating pathogen and also to see how exactly the gene expression of the pathogen responds to those signals (Chapter 4). In the second half of this thesis investigations focused initially on the use of Agrobacterium tumefaciens as a method of transforming Bgh (Chapter 5). The inability to transform this fungus and allow genetic manipulation has proven a hindrance to analysis of the lifecycle of this pathogen. As this was not overcome, the latter part of the thesis (Chapter 6) documents attempts to use a model fungal pathogen, Magnaporthe oryzae, as a heterologous host for deciphering the regulatory systems of genes known to both show co-expression and also responsiveness to the substrate the pathogen was developing on.

To summarise the results observed, it is clear that Bgh responds to underlying substrata with alternate genetic expression resulting in varied morphological development. In Chapter 3
this was demonstrated most clearly when germling development on the host was compared with that on glass. On the artificial surface the majority of spores at all time points tested were limited to the earliest pre-penetration developmental stages and often produced just one germ tube. In comparison on the host all stages of development were observed including post-penetration events such as haustorial formation. From these results we may conclude that to ensure correct functional development, energy and resources must be employed at the right stages of infection otherwise no infection is achieved at all. Additionally, the fact that differential behaviour was seen on different surfaces, which offer significantly different cues (for example, glass slides lacking the cutin monomers and cuticular waxes of the host), suggests that a Bgh germling is indeed sensing the substrate upon which it lands and not just responding to a “touch” stimulus. Such a conclusion is not novel, since other authors have made similar observations but the study in Chapter 3 adds further support for such a hypothesis. Therefore, during development the signals from the host are taken up and stimulate advanced stages of development. These signals can range from cutin monomers to aldehydes to more prosaic characteristics including hydrophobicity. Even though attempts were made in Chapter 4 to trial a number of these signals to see their effect on development and expression, morphological effects were often easier to discern than changes in gene expression. In support of studies by Tsuba et al., (2002) (and more recently Hansjakob et al., 2010), the aldehyde 1-hexacosanal was shown to induce Bgh appressorial formation. Its addition to glass slides led to a significant increase in appressorial formation, which outweighed all other artificial surfaces/coatings tested. This highlights the importance of this aldehyde over other components in induction, and supports both Tsuba et al., (2002) and now Hansjakob et al., (2010) in their conclusion that this component of the wax was the most inductive of all. In addition for the first time (to the author’s knowledge), the barley cutin monomer, 16-hydroxyhexadecanoic acid, was shown to also have inductive potential (although potentially less than 1-hexadecanol). Of the genes tested, although hindered to a degree by a lack of resolution, it appeared that many genes did not just respond to one sole stimulus but rather to a combination of stimuli. Further analysis suggested that some of these genes had no known function (‘Orphans’) and furthermore had no identifiable homologs in other species. Others encoded proteins with identifiable functions. These included glycosyltransferases, potential glucan-1,3-glucanases, and MAPK interacting proteins. Additionally, these proteins had homologs within varying numbers of ascomycete species, including organisms that were necrotrophs or hemibiotrophs. Both the unidentified and identifiable genes are candidates for future investigations. Those that have homologs in
organisms that are transformable are candidates for complementation experiments. Those that are unidentifiable become points of interest in attempts to define genes that are \textit{Bgh} specific genes.

In the second half of the thesis, work with an \textit{Agrobacterium}-based transformation system (Chapter 5) was not conclusively successful with just one potential transformant. Nevertheless it did provide both the rationale and the impetus to trial a heterologous host whilst also forming the basis for experiments in other research groups. Attempts to utilise \textit{M. oryzae} as a host to examine the proximal promoter regions from CMEG genes (‘Co-ordinate Mis-expressed Genes’) (Chapter 6) highlighted two promoters in particular that were active in this heterologous host. This result offers the potential, in the form of deletion or mutagenesis, for discovering the regulatory elements contained within these sequences. Notably these results argue that for the greater majority of sequences tested (at least in the context of \textit{Bgh} promoters), the use of \textit{M. oryzae} as a heterologous host is not a viable strategy. As this was the case it was not possible to attempt the analysis of promoters for the genes studied in Chapter 4. Of those that were tested (readers are referred to table 8 for details), no fluorescence was detected; and as such further analysis could not be conducted. Furthermore, for at least some of the regions tested, results (a lack of fluorescence but PCR analysis confirming proving that the transformation was successful) suggest that even though some of these genes may show co-expression with one another, their regulation is not necessarily achieved via the same mechanism. This is not surprising and has been encountered with other data sets (Clements et al., 2007). However, these results reinforce the notion that \textit{Bgh} relies on complex regulatory control networks that synthesise inputs in the form of multiple host-originating signals to ensure dynamic integration with the host and successful development. This seems necessary as nutrients of the spores are limited and mis-regulated development could lead to impaired function and a lack of infection.

How and why could such complexity of regulation have arisen? During the sequencing of the genome of \textit{Bgh} a large proliferation of transposable elements (TEs) (accounting for 63 \% of the genome size) was observed (Spanu et al., 2010). These TEs include Non-LTR Retrotransposons, which encode activities permitting their retrotransposition (Kazazian, 2004). In comparison only 9.7\% of the \textit{M. oryzae} genome comprises repetitive DNA (formed of retroelements and DNA transposons) (Dean et al., 2005, Xu et al., 2006). Their presence, linked to the apparent lack of the Repeat-Induced Point Mutations Pathway, may have
allowed increased genetic variation of the pathogen without the need of sexual recombination (Spanu et al., 2010). Although advantageous when thought of in terms of the plant-pathogen “arms race” (specifically the interactions of pathogen released effectors, pathogen associated pattern triggered immunity and effector triggered immunity), TE insertions into both genes and their regulatory elements may also have had detrimental effects by disrupting function and control (Biemont and Vieira, 2006). Also observed during the sequencing of Bgh was the relatively small numbers of genes involved in common biosynthetic pathways – except genes essential for biotrophy (Spanu et al., 2010). This may be indicative of the cumulative damage caused by TEs during co-evolution with the host. Given the potential for TEs to cause damage over time, the fungus may have begun to lose the ability to regulate some of its own genes. Simultaneously, in a form of simplification of control, Bgh evolved to place part of the “responsibility” for regulation on the host. By depending on signals normally generated at specific points in infection to regulate development the fungus was reducing the potential for mis-regulation by a TE insertion in a gene upstream of a critical process. Alternatively to this the very presence of the TEs, with insertions leading to chromosomal re-arrangements and movement of genetic elements, may have permitted the diversity of cis-elements required to begin developing these complex networks. This could be due to some form of redundancy in copy numbers, now lost, which would have permitted regulatory sequence divergence (Wohlbach et al., 2009). Such divergence could be due to mutations in the cis-elements (or by changing the gene product itself thereby altering feedback) (Ronald et al., 2005). Alternatively because of replication, altered genes and their products then go onto affect other genes in trans (Wohlbach et al., 2009).

Chapter 3 (Characterising Blumeria graminis f. sp. hordei Germination Behaviour) and Chapter 4 (Germination on Modified Surfaces): critique and future work.

In the first half of the thesis experiments relied heavily on microscopy analysis to obtain evidence of Bgh development. Bgh only attacks epidermal cells and the majority of its structures (except the haustoria) grow epiphytically in a synchronous fashion. As such microscopy coupled with fungal staining offers both a convenient and reliable method of observing early development (Panstruga, 2004). Based on such techniques visual counting and classification provide a relatively reliable system of categorising spore development not just on the host but on a range of surfaces. It allows simple conclusions to be deduced
regarding inductivity and has been used in other studies (such as Tsuba et al., 2002) as the basic method of information gathering from which future investigations are often initiated.

However, microscopy and counting do have limitations and shortfalls. Although one of the few techniques that can be applied with ease to the study of Bgh, it provides only the most basic of information and does not reveal what is actually going on within the fungus itself. It may indicate, for example, that a certain wax component has an inducing effect, but not the mechanism or indeed how this effect is manifested. Furthermore, the Bgh developmental cycle is a continuous process and the developmental stage classifications used are a crude attempt to profile spore development. As such, if these categories are not specifically defined uncertainty can arise when describing an observation or in terms of the ability to compare different studies. As an example the classification used during the ‘stimuli screen’ (section 4.3.1) in Chapter 4 utilised three categories which offered the potential for confusion. The system tried to differentiate between a developing secondary germ tube, an elongating secondary germ tube with appressorial characteristics, and an appressorium proper. In the case of this germ tube such classifications are highly subjective and evidence will be biased dependent on the observer involved; potentially resulting in inaccurate conclusions. With such facts in mind it is no surprise that other authors (dependent on the aim of the experiment for example Tsuba et al., 2002) all utilise or emphasise different styles of classification. Consequently, it can be difficult to integrate the information gathered in different studies into a global picture for Bgh – a strategic aim of Bgh studies.

Other limitations existed with the exposure of stimuli to spores. Although providing an example of differing hydrophobicity, the polyacrylamide gel backing (gelbond) used in this study failed to spur development to the same degree as seen with other studies that used surfaces of increased hydrophobicity (but different formulation) e.g. Zabka et al, (2008). This was believed to be due to an inhibitor of development on the gelbond. Consequently, the author believes this surface does not accurately portray the effects of hydrophobicity on gene expression selected in this study. In the case of the cuticular products application was dependent on manual spreading which resulted in an uneven presentation of the chemical to the fungus. To reduce variability in application (which contributed to variability in growth and gene expression) future studies need to meet the application standards of other investigations which used a spray based system followed by slow evaporation. This would
allow better comparison of any results obtained with these studies with the research found of other groups – a necessary feature of the ‘systems biology approach’.

In this thesis observation time points to assess the effect of stimuli on Bgh development were chosen at 4 and 16 h.p.i. (Figures 4.8 and 4.9). The 4 hour time point was chosen to match the microarray time point upon which the premise for the RT-qPCR assays were based. However, if we want to judge the effect of monomers and aldehydes on emergence of the primary germ tube, earlier observation time points may be more beneficial for example 30 minutes to 1 hour post inoculation (the time frame within which the PGT is known to emerge). Potentially by 4 h.p.i. differences in the rate of PGT emergence are camouflaged as only the final proportion of the germ tube is considered. In this study compounds that induced the greatest proportion of PGT, SGT and APP structures were assessed in the hope that more noticeable changes in gene expression may be observed. In an alternative experiment it may have been interesting to choose inductive substrates that cause similar levels of structural development and analyse the speed at which this takes place. This may indicate the importance the developing Bgh spore places on specific stimuli.

With regards to future work there are a number of observational experiments that would build on the results detailed in Chapters 3 and 4. Although a variety of stimuli were tested for their effect on gene expression (including an extract from the barley cuticle), none came close to the relative speed of induction, or the advanced nature, as seen during development on the host (and to a lesser degree on wheat). Therefore future work should aim to expand the range and combination of stimuli presented. Aims should include trying to see how each compound is linked to Bgh inductivity, how they relate to hydrophobicity (as an inductive feature of the cuticle) and if there is some form of redundancy between a stimuli’s chemical or hydrophobic properties.

The first step in such an investigation would use a combination of monomers studied by Francis et al., (1996) (cis-9,10-epoxy-18-hydroxy-stearic acid and 8,16-dihydroxy-palmitic acid), 16-hydroxyhexadecanoic acid and 1-hexacosanol (the cutin monomer and aldehyde tested with in Chapter 4) on chromic acid cleaned glass slides. Levels of appressorial development could then be monitored to see if they matched that of the host. If it did then it would suggest that all of the factors needed to cause successful pre-penetration induction would be known. If not further compounds would need isolating from the barley leaf. This
hunt would aid in the discovery of their receptors and delineating the signalling pathways downstream of them.

If the full range of stimuli (inducing host-like levels of development) were added to the glass slides in a similar fashion as to that described in this thesis (where levels of hydrophobicity remained unchanged) then it could suggest that biological/chemical structures of the stimuli were the key to germination rather than just their hydrophobic properties. The results of Zabka et al., (2008) which demonstrated that increased hydrophobicity led to increased appressorial formation would suggest this may not necessarily the case. If host like-levels of appressorial formation could not be induced then applying the stimuli combination in a fashion that allows widespread crystal formation (resulting in increased hydrophobicity) could be attempted. If host like-levels were induced it would also suggest all of the major inductive compounds of the barley leaf surface had been isolated and furthermore that induction was based on both the chemical and hydrophobic properties of the compounds involved.

Combined with these studies, analysis of surfaces with highly increased hydrophobicity (i.e. those of contact angles close to those generated by the barley cuticle, as suggested by Zabka et al., (2008) should be made. If induction levels matched that of the host, combined with the results of Chapter 4, then it would be confirmed that Bgh responds to both hydrophobicity and chemical properties of stimuli but that hydrophobicity was the more important factor. Results presented in Chapter 3 (where wheat leaf surfaces presented hydrophobicity of similar levels to the barley leaf) may already hint at the results to be expected. As an example, conidial germination at all the time points tested, was less than that seen on barley, suggesting that hydrophobicity by itself will not lead to host-like levels of developmental induction and so a combination of stimuli is required.

Based on the inability of Bgh to survive on any host except barley the initial reaction would be to suggest that the lifecycle of the spore and its requirement for a specific host would suggest stimuli redundancy was not the case. However bearing in mind the development on wheat (and the potential role of host defence reactions) then such redundancy becomes a more realistic possibility. If this redundancy does indeed exist, then the obligate nature of this fungus on the host may not be based on the inability to “sense” (inductive) compounds but the inability to parasitise any plant but barley.
Identification of all leaf surface compounds would aid in the search of their *Bgh* receptors and comparisons to the behaviour of other *forma speciales*. Proteins predicted to be membrane-bound and that harbour typical binding sites (based on the translated gene sequence from the recently annotated genome) could be over-expressed in bacteria and structurally analysed. Binding site features to consider include those with size-selective hydrophobic cavities (Hansjakob et al., 2010). Those with active sites that have properties that suggest potential binding with for example, 16-hydroxyhexadecanoic acid, could be then be listed and featured in (if transformation allows) knockout studies or complementation studies in other pythopathogens such as *M. oryzae* which shows increased germination on the presence of cutin monomers.

Future experiments could also look at the timed application of stimuli. The application of the monomer and aldehyde stimuli used in this investigation meant that the fungus was continuously exposed. Compounds may also affect more than one developmental process (for example the role of 16-hydroxyhexadecanoic and 1-hexacosanal in inducing both the PGT and the SGT) so it is impossible to state at exactly which point the compound is sensed and how often. In order to determine exactly when the compound is sensed it would be necessary to expose the monomer to the fungus for a set time, and then to remove the stimulus. In one scenario slides could be partially covered with a stimulus and spores that have reached a certain level of development (for example PGT) could then be rolled via micromanipulation (in a similar process to Wright et al., 2002) onto another area of the slide that (for example) had been treated. Advances in development could be then be monitored by microscopy and potentially allowing differential reliance for stimuli to be discerned for each germ tubes.

As noted previously to the author’s knowledge the RT-qPCR studies featured within this thesis are the first that try to link *Bgh* development directly to gene expression. There were several drawbacks with the RT-qPCR technique apart from the technical problems already discussed in Chapter 4. During the writing of this thesis it was noted that several improvements to this process have subsequently been made. A prime example being the reliance on just one reference gene (here NADH ubiquinone oxioreductase), which was used as a “marker” to gauge relative differences in expression levels of the genes tested. Although other reference genes were examined the NADH ubiquinone oxioreductase was chosen as the alternatives demonstrated uneven expression (after inspection of microarray data). Such a
dependence on one reference gene is no longer accepted practice with authors such as Derveaux et al., (2010) advising the use of between 3 to 5 reference genes. As such the data cannot be accurately compared to new data generated in future studies. Conceptually this is problematic given that work will focus on systems biology approaches.

Another drawback (although one that could be remedied in future experiments) featured the extraction of RNA from a population of spores, not only those which showed the most advanced state of development on the surfaces under investigation. Spores vary in fitness and so hoping all would respond uniformly with sufficient speed to provide a significant change in expression is not feasible. The spores would (at 4 h.p.i.) have been at slightly different stages of development. Any induction by a compound would therefore be complicated by the fact that gene expression in each of the spores is not uniform. In future experiments the use of laser capture micro-dissection may be useful in harvesting RNA solely from the proportion of spores demonstrating the most representative level of development for that surface. Although this technique is not new (having originated with Emmett-Buck et al., 1996) and it would be laborious to apply, it could be coupled to more modern technologies such as RNA pre-amplification to increase RNA levels to a more experimentally useable level (Vermeulen et al., 2009). RT-qPCR could then be performed with samples that were more distinct in their mRNA abundances.

An alternative to the RT-qPCR experiments would involve the use of microarrays comprising the 2072 unigenes (as used previously by Dr. Paramor), to detect changes in gene expression in response to the inductive compounds. An example of the use of this technique is shown in the study by Oh et al., (2008), where they monitor M. oryzae germination on a hydrophobic surface following stimulation by cAMP. As a proven technique, microarray is an obvious choice for assaying gene expression levels between spore populations, allowing the tracking of thousands of genes over multiple conditions (Lorenz et al., 2002). Any changes in gene expression would have been referenced against a universal standard negating the problems associated with the reliance on one reference gene with potentially non-uniform expression. One obvious advantage with this technique is the ability to monitor gene expression in response to different stimuli, which can inform about the function of specific genes (Lorenz et al., 2002). However, such an experiment was not undertaken, given the time invested in attempts to use M. oryzae as a heterologous host. As such the decision was made to measure a select number of genes via RT-qPCR and then to compare their profile back to the data
gathered by microarray. However now that the full genomic sequence of \( Bgh \) is known, it should be possible to construct microarrays based on the full gene complement of the organism rather than just an EST library (as utilised during the earlier studies). This would ensure the expression of all annotated genes was monitored not just those that were present in the EST libraries on which the studies of Both et al., 2005 (A,B) were based. If such arrays were employed to test the effects of 16-hydroxyhexadecanoic acid and 1-hexacosanal on early gene expression, then a more complete picture of gene activation in response to specific compounds could be produced. By noting the genes which were activated, it may be possible (with some form of comparative analysis with other pathogens and data mining of genomes) to discover not only the signalling pathways involved, but also some of the transcriptional networks that they utilise. In a complementary approach, data mining of the \( Bgh \) genome with the aim of identifying genes thought to encode surface based receptors.

Alternatively, Serial Analysis of Gene Expression (SAGE) (as performed in the study by Thomas et al., 2002 mentioned earlier) could be used, as it has a number of advantages over microarrays. It does not need sequence information and has been shown to identify small Open Reading Frames (ORFs) missed during genome annotation (Lorenz et al., 2002). This technique scores mRNA levels dependent on the abundance of sequences constructed from short cDNA segments that were originally converted from cellular mRNA (Lorenz et al., 2002, and studies therein). Irie et al (2003) utilised SAGE to study gene expression during appressorium formation in \( M. \) oryzae conidia in the presence of cAMP. A previous drawback to the use of SAGE technologies was the cost. However, with the advent of next generation sequencing this will decrease (Tan et al., 2009). Additionally, SAGE requires large amounts of RNA, however refinements have been made, such as microSAGE (Datson et al., 1999), which require up to 5000-fold less RNA than normal SAGE procedures (Bhadauria et al., 2007A). These technologies could be applied to others such as laser micro-dissection to allow for a more cell-type specific analysis.

**Chapter 5 (Toward the Agrobacterium mediated transformation of Blumeria graminis f. sp. hordei): critique and future work.**

Technical drawbacks to the experimental approach used in this chapter have already been covered (See the discussion section 5.5 in Chapter 5). These included, for example, use of a liquid based inoculation strategy or the lack of continuous selection. Another drawback lay
not so much with technique but with approach. Due to the significant potential for failure (as evidenced by the continuing attempts over the past 15 years to create a reproducible method of transformation; see Christiansen et al., 1995; Chaure et al., 2000; Matsuda et al., 2000) the study was limited with attention focused on the use of M. oryzae as a heterologous host. This fact, coupled with the inexperience of the author, meant that no robust downstream screening procedure was in place to analyse transformants should they have arisen. As such, when a transformant was observed (as evidenced by its ability to grow on triadimenol selective plants and the presence of fluorescence), the sample was lost before conclusive PCR analysis to determine the presence of GFP could be performed. This, however, did prompt the implementation of a robust screening system for experiments in Chapter 6. With hindsight, rather than attempting to harness M. oryzae as a heterologous host, more strains of Agrobacterium (including those of increased virulence), different dilution concentrations of bacterium, as well as varying ages of Bgh colonies could have been tested. However, it is difficult to suggest alternative ideas for the transformation system itself when it is clear that common alternatives (such as protoplast-based transformation approaches) are either inappropriate, due to the constraints of the Bgh lifecycle, or have previously been tried and proved unsuccessful (for example biolistic-mediated transformation). One alternative that has yet to be tried includes the use of microinjection of plasmid vectors (with increased flanking regions to enhance homologous recombination) into the developing conidiophores or spores that have landed on the leaf surface and begun germination (Prof. Kim Hammond-Kosack, personal communication). This may be a more reliable method of transformation in comparison to the literally “hit or miss” approach of biolistics, however it will lack the accompanying proteins utilised as part of the Agrobacterium T-DNA system that, when successful, ensure higher transformation rates. Future work should also continue to focus on trying to transform Bgh, if possible using Agrobacterium-mediated transformation since there were indications presented in this thesis that, as a technique, it may be successful.

Now the genome sequence is available, a successful transformation system would be more advantageous than ever before. Although genomic techniques are powerful, without a genetic system which allows gene function validation their value is reduced (Yoder and Turgeon, 2001). Therefore the ability to transform this pathogen would allow manipulation of genes, the conformation of identity, discernment of role, and the piecing together of the intertwining of pathways and interactions that lead to pathogenicity. For such functional analysis of annotated genes, homologous recombination (mediated by DNA transformation) is necessary
to allow a better understanding of gene function via target gene disruption/knock-out or manipulation (Kuck and Hoff, 2010). Therefore the inability to transform is an obstacle that needs overcoming. According to Bhaudauria et al., (2009) several disruption approaches exist. They list disruption of the gene, alteration in expression levels and gene replacement. Gene disruption has been used in *M. oryzae* to discern the role of *MPG1* in germination, the promoter of which was used as part of the transformational control vector pMJK27.2 in Chapter 6 (Talbot et al., 1993). A systematic approach to mutagenesis includes creating a library of strains where every non-essential gene is mutated (Lorenz et al., 2002). If experiments with *Agrobacterium* do prove fruitful, then a large scale insertional mutagenesis screen, similar to that carried out in *M. oryzae* by Jeon et al., (2007), could be performed. Furthermore by having the complete genome sequence it will be easier to identify any tagged locus (Lorenz et al., 2002).

However, even if transformation is achieved as noted by Talbot and Hamer (2000) with an obligate pathogen, trying to target genes for disruption may not be a viable strategy. Although some *Blumeria* genes may not be essential, if the disrupted genes are essential for life (especially if they are single copy) then the fungus may simply fail to grow on the host. Unlike non-obligate pathogens where additives in the media can compensate for example, for an inability to metabolise a compound, it would not be possible to provide such supplements to a pathogen that only grows on the host. Instead gene disruption in this organism could be based on a temporary gene silencing mechanism, manipulated by a switch promoter system (Zhang and Gurr, 2001). RNAi (RNA interference, a technique utilising double stranded RNA triggered degradation of homologous mRNA), for example, has been used to successfully down regulate genes in other filamentous fungi e.g. *Cryptococcus neoformans* (Liu et al., 2002). RNAi has several advantages over actual gene disruption in this regard (Nakayashiki and Nguyen et al, 2009; Bhaudauria et al., 2009; Kuck and Hoff 2010). It may only cause a partial reduction in gene transcript rather than a complete loss allowing for some growth and may knock-down the expression of multiple genes so long as they are of sufficient sequence similarities (Nakayashiki and Nguyen, 2009). This can reduce any compensation by other genes, which may be mitigating any resultant phenotype. There are disadvantages with RNAi including “off target gene effects” where genes (of high sequence similarity to the intended target) are also silenced. This can complicate interpretation of phenotypes (Bhaudauria et al, 2009; Nakayashiki and Nguyen, 2009). However, it is possible to screen for such potential effects if a genome sequence is available, as is the case with *Bgh*
(Weld et al., 2006, Bhaudauria et al, 2009). RNAi has also been used in *M. oryzae* to study calcium signalling proteins during infection related development (Nguyen et al., 2008). Ideally, with a functional transformation system, one could insert plasmid constructs that express homologous sequences, hairpin RNA or intron containing hairpin RNA, the latter two of which Nakayashiki and Nguyen (2009) consider more reliable for inducing RNAi based silencing in fungi. With an inducible promoter of some kind (for example linked to plant stimuli sensing pathways) silencing at a certain point in development could be achieved. This promoter would need to be tightly controlled otherwise there would be difficulty in determining the difference between RNAi induced and uninduced phenotypes (Kuck and Hoff, 2010), unless a system such as that used by Nguyen et al., (2008) in *M. oryzae* is obtained. In this system (to aid high throughput analysis), an eGFP gene was incorporated into the vector used, and was transcribed as a chimera with the gene of interest. As a consequence, spores that were undergoing silencing also fluoresced. In the case of *Bgh* such a system would prove useful in determining between spores that were actually affected by silencing, and the sizeable proportion (especially on glass) that were merely less fit and failing to germinate for other reasons.

One significant obstacle to the use of RNAi is the knowledge that in some fungal species, proteins that comprise the RNA silencing pathways appear to be missing (as in *Ustilago Maydis*; Nakayashiki and Nguyen, 2009). The fact that high numbers of transposons are active in *Bgh* would initially suggest at least some of the RNAi machinery may not be functional - as in some fungi it is known to suppress transposons (for example LTR-retrotransposons in *M. oryzae*; Murata et al., 2007). Fortuitously, studies utilising barley (‘Host induced Gene Silencing’) as an expressor for dsRNA or siRNA which can be transferred into *Bgh* have demonstrated that RNAi is indeed a valid strategy to employ in *Bgh*. However this system cannot be used to silence genes before penetration of the host (and formation of a functioning haustoria proper has occurred at approximately 16 h.p.i.) and so is not really suitable with regards to the questions addressed within this thesis. Therefore transformation of *Bgh* would still probably form the cornerstone of any indepth attempt to analyse gene behaviour pre-penetration.

With a successful transformation system it could be envisaged that goals for future work would include the knocking down of genes with differential expression at 4 h.p.i. (i.e. those identified in Chapter 4) to see exactly what kind of effect that would have on development.
Roles in glycosylation, penetration and cell wall remodelling could be confirmed as could the affect of a protein on MAPK signal pathways. More importantly genes of unknown function could be analysed allowing potentially novel infection factors to be revealed. Since many of these genes are present as single copies the problems associated with redundancy muting phenotypes would be reduced. Observational studies of development (as well transcriptomics) on the host would be used to determine at what stage of pre-penetration the phenotype exhibited itself. It would also be interesting to determine the contribution and importance of aldehydes or cutin monomers in silencing part of the signal apparatus or receptors that transduce changes in gene expression. In this case the phenotype may, for example, exhibit itself as an inability to progress past the formation of primary or secondary germ tubes.

Other uses of transformation include the \textit{in-situ} localisation of product, assessment of translation rates and the use of fluorophore-tagged proteins to understand interactions between specific proteins (for example receptors and signal pathway components) during \textit{Bgh} germination. When a donor and acceptor fluorophore on different proteins reach a certain proximity from one another non-radiative energy transfer between them can be monitored via ‘Fluorescence Resonance Energy Transfer’ (FRET) analysis indicating the time and location of successful interaction (Panstruga, 2004). Transformation would permit endogenous fluorescent tagging and allow the study of gene position in living cells. This would permit studies of the dynamic genome organisation and the plotting of nuclear sub-domains which could explain gene activity (Saez-Vasquez and Gadal, 2010). The role (temporal and spatial) of secondary messengers (such as calcium) could also be studied via the use of ‘cameleon’ proteins which have a calmodulin binding domain between two fluorophores, as have been used in other studies (Panstruga, 2004). Most importantly we could also track effectors leaving the host cell. This could make R-genes easier to study as “gene-for-gene” interactions mediating barley defence could be visualised. By producing transformants of the powdery mildew carrying a putative avirulence gene (for example thought to transfer to the host via the haustorium) we may then be able to observe its appearance in the host by using GFP-fusion tags. This may tell us its roles in infection. Furthermore it may even allow the matching of \textit{avr} genes to known resistance genes in the host (Talbot and Hamer, 2000). A press release at the 10\textsuperscript{th} International Barley Genetics Symposium (IBSC), 2008) suggested genomic data is soon to become available for barley. Furthermore transformation protocols already exist for this model plant, including for ‘Golden Promise’ the cultivar used during
this work (Hensel et al., 2008). This will mean it will be easier to analyse both sides of this host-pathogen interaction. By doing so you would complete both sides of the picture in regards to host/pathogen defence.

Until transformation is achieved many of these plans will remain unfulfilled. In the meantime if direct transformation cannot be achieved then the use of synthetic siRNA may be considered. Still an emerging technology, synthetic siRNA has been used in studies of *Aspergillus nidulans* to silence an ornithine decarboxylase gene during germination (Khatri et al., 2007). Of course since the addition of siRNA to the culture medium (as performed by Khatri et al., 2007) is not an option for *Bgh*, alternative methods must be considered. The coating of germination surfaces followed by the application of spores is one option, although the presence of fluid may impede germination (one of the reasons for failure mentioned in Chapter 5). In the case of *Bgh* synthesis siRNA could be injected into the spore to inhibit gene function during development, dependent on the survival of the cell after the wall is breeched.

*Chapter 6 (CMEG 5'-regulatory region driven GFP expression in Magnaporthe oryzae): critique and further work.*

As early as 1999, questions were being asked regarding the correlation between protein levels and mRNA (Lorenz et al., 2002 and studies therein). Therefore the testing of *Bgh* promoters in *M. oryzae* to understand transcriptional regulation already has its limitations in trying to decipher mechanisms of *Bgh* development, even before shortfalls in using a heterologous host (such as alternate TF networks) are considered. It is known that post-translational regulation (turnover and modification such as glycosylation) leads to effects on both protein activity, abundance as well as temporal and spatial location (Lorenz 2002, Kim et al., 2007, Bhadauria et al., 2007). Therefore even though promoter analysis (and RT-qPCR analyses) may begin to unravel some of the methods of gene regulation, conclusions must necessarily be tempered with caution. Many such modifications are reversible and have roles in regulation not readily detectable from either genomic sequence data or mRNA expression data (Bhadauria et al., 2007). Furthermore proteins often act as complexes. Therefore just because one gene may be transcribed in *M. oryzae* in a similar fashion to *Bgh*, this may not be true of their action as proteins (whose activity may depend on another gene that is not similarly transcribed at that time).
As for future work regarding promoter regions efforts should be made to build on the results presented in this thesis. Further structural analysis of the regulatory region of EST C00879 could be performed in *M. oryzae*, and combined with work involving the yeast 1-hybrid system, the identity of *Bgh* transcription factors that bind to the region (and the motifs they recognise) could be elucidated. Other regulatory regions from the same expression cluster as EST C00879 could be tested in *M. oryzae*. Although the few tested alongside EST C00879 did not show fluorescence there is no guarantee that others may not. Furthermore, other conserved genes, for example encoding other histones, could also be tested to establish whether they are active in *M. oryzae*. On discovering the TF that binds EST C00879, it would be possible to employ the strategy (i.e. microarrays) used by Odenbach et al., (2007) to identify genes that are regulated by a specific TF (in this case Con7p). Microarrays can then be performed to determine which other genes, and most importantly which CMEG genes are regulated by these TFs.

One drawback not previously mentioned includes the low number of regulatory region types actually tested in *M. oryzae*, primarily due to logistical reasons. Although suggesting that *M. oryzae* is not suitable for use as a heterologous host for analysis of *Bgh* regulatory regions, the transformation and screening process (acting as a logistical bottleneck) meant only 22 CMEG promoters were successfully screened for activity. Therefore if further regulatory regions were screened a different conclusion may have been formed. Whether this would have been the case is debatable. However even if this had occurred, limited options due to the nature of *Bgh* biotrophy meant the use of heterologous hosts may be unavoidable. Therefore it may be better to use such hosts to study the protein interactions directly, rather than trying to understand regulation with all of the assumptions such a study would require. If future heterologous host usage is to be considered it may be best to use *Saccharomyces cerevisiae*. Although not a close relative of *Bgh* as it has a well-annotated genome, well-studied physiology, and a number of mutant strains are available for functional complementation analysis (Bachi et al., 2008). Also as noted by Bachi et al., (2008) multiple genomic, transcriptomic and proteomic studies have been carried out on this yeast so such studies may go some way to nullifying uncertainties. Yeast two hybrid analysis (to study protein-protein interactions) could also be used to understand signal pathways downstream of stimuli receptors. In such analysis one protein of interest is bound to a DNA binding domain and another protein of interest fused to a transcription activating domain, which when an
interaction occurs, activation of a transcriptional reporter occurs (Bhaudauria et al., 2007). After data mining the Bgh genome, genes identified as receptors could be analysed in the yeast two hybrid system and protein-protein interactions scored.

Future work should investigate the signal transduction pathways in Bgh, knowledge of which remains very fragmented. This would start by cataloguing all the genes in Bgh with homologues in other organisms known to be involved in signalling. Future work must try to link surface signals to these transduction pathways (by locating receptors for known stimuli) and to try to fully understand what roles they play in the regulation of gene expression. Concerted efforts should be made to data-mine this information to spot components (for example G-protein coupled receptors) of, and piece together, these signal regulatory pathways. By knowing full signal chains rather than fragmented information it would be easier to discern the role of these chains in Bgh by comparing it to other fungi, but may also allow better methods of analysing these pathways. Another, more short-term benefit, would include a more focused investigation during future expression studies where the fungus is exposed to other signals. Also now we have more information regarding the Bgh genome maybe one of the next steps to aid elucidation of the function of genes is to start mapping where they exist in the genome compared to similarly regulated genes. This could lead to the identification of clusters of genes and give added clues to determining nearby regulatory elements.

Concluding remarks.

More fundamental questions also exist with regards to barley powdery mildew. When did Bgh become solely specific on barley? Is it assumed that Blumeria has co-evolved with the cereals since their domestication in the fertile crescent? How would we find this out? Ancestral populations may contain non-essential genes showing greater genetic diversity. This has been done before in the case of Rhynchosporium secalis and suggested this pathogen (once thought to originate in the middle east) has Scandinavia as its source of origin (Zaffarano et al., 2009). By pinpointing where these populations lie, we can track the evolution of the organism and help answer why exactly some pathogens become obligate. In addition to this the sequencing of the wheat powdery mildew (B. graminis f. sp. tritici) is underway, with hopes that it will be completed by April 2011 (Shimizu and Keller, January 2010). When this occurs an ideal opportunity will arise to compare the two genomes, identify
“co-owned” genes (and to compare expression) and also to try to figure out what exactly leads to their host specificity. It would be interesting to see how if this difference is manifested in differing surface receptors.

To finish, at the time of writing evidence is building for potential future climate fluctuations, with concern being focused on anthropogenic climate change due to build-up of greenhouse gas emissions (Tuttolomondo et al., 2009; Legreve and Duveiller). With changes in climate, disease epidemics and their spread can become harder to predict. This change (with alterations in precipitation and temperatures) will affect crop yields, even though modern agriculture uses high levels of pesticides and fertilisers, as cereal production often uses high acreage and use in areas of often poor irrigation (Tuttolomondo et al., 2009). In past times of climatic turbulence or anthropogenic related instability (i.e. farming induced salinity), for example during the Mesopotamian era, with its greater robustness barley increased in cultivation whilst other crops were hard to farm (Murphy, 2007). With the withdrawal of certain fungicides and the potential for new virulence alleles that could overcome host resistance it is becoming ever more important that attempts are continued to understand one of more destructive pathogens of barley – powdery mildew (Bindschedler et al., 2009).
7.1: Acknowledgements

“The drama’s done. Why then here does any one step forth? — Because one did survive the wreck.”
- ‘Moby Dick, or the Whale’.

I would like to thank a number of people. Firstly my supervisor: Pietro Spanu. I told him before I started that I wasn’t the best thing since sliced bread but that I worked hard. His reply was that sliced bread wasn’t that good an invention. So there we go. That kind of set the tone of all that followed.

Secondly, I should mention my other three “lab-mentors” during these times: Maike Paramor, Mike Ray, and my good friend Calin Andras. All got me out of the different scrapes and problems all PhD students face. Maike was a tower of strength during the writing of this thesis and I can only offer my humble thanks in regards to that. And maybe something from Harrods.

Mick Crawley and Ryo Murakami also deserve my thanks. The former for his patience when it came to explaining statistics (and his lack of imagination when it came to finding places to hide in Silwood whilst I was about) and the latter for pointing me to that damned infuriating device of a goniometer.

My friends from the old Cambridge bunch to those in this department, too numerous to talk about individually, also share my thanks and maybe a pint if they ask nicely. Then again they owe me pints for all the Saturday mornings that I did their experiments for them.

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Finally, I would be remiss if I did not highlight my gratitude to Rachel.

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Chapter 9: Appendixes

9.1: Appendix A: Chapter 2 Material and Methods (General)

9.1.1: Tap water agar (TWA)
15 g agar (Agar Technical (Agar No. 3), Oxoid Ltd) in 1 Litre ddH₂O

9.1.2: Escherichia coli growth media and solutions
pH was altered as required using 1 M KOH and 2 M NaOH. All media was autoclaved for 20 minutes at 121 °C and allowed to cool to approximately 50 °C before any addition of selective antibiotics (Ampicillin: stock 100 mg/ml, selective levels: 500 µg/ml; Kanamycin: stock 100 mg/ml, selective levels: 500 µg/ml; chloramphenicol at 100 µg/ml were used as required by plasmids.

Lysogeny-Broth (LB) media 1 litre contains: 10 g Tryptone (Oxoid Ltd.), 5 g Yeast extract (Oxoid Ltd.) and 5 g NaCl (BDH) for agar plates add 15 g/litre agarose (Agar Technical, Number: 3, Oxoid Ltd.) Sterile water is used to bring volume to 1 litre. pH was adjusted to 7.5.

SOB media 0.5 % Yeast Extract. 2 % Tryptone (Oxoid Ltd), 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄

SOC media Glucose (20 mM), KCl (2.5 mM), MgCl₂ (10 mM), MgSO₄ (10 mM), NaCl (10 mM), Tryptone (20 g/L) (Oxoid Ltd.), Yeast Extract (5 g/L). Required pH = 7.0 (Sambrook et al., 1989)

TB solution 10 mM Heps (or Pipes), 15 mM CaCl₂, 250 mM KCl. Adjust pH to 6.7 with KOH. Then dissolve MnCl₂ to have final concentration of 55 mM. Sterilize solution by filtration through 45 µm filter and store at +4 °C.
**GTC** 4M Guanidine Thiocyanate (Fluka Biochemika); 25 mM Na citrate pH 7.0 (BDH AnalR®); 0.5 % Sacrosyl (N-Lauriyl sarcosine, Sigma-Aldrich®)

**GTC-phenol-β-mercaptoethanol** 1:1 volume GTC: unbuffered, equilibrated phenol (pH 4.5); 7 µl/ml β-Mercaptoethanol (2-mercaptoethanol, Sigma)

**DEPC water** 1 Litre DEPC ddH₂0 was produced by the addition of 1000 µl Diethyl Pyrocarbonate (Sigma-Aldrich®) to 1 Litre of ddH₂0. Solution was left for 16 hours stirring/aeration before autoclaving for 15 min at 120 °C.
9.2: Appendix B: Chapter 3 (Characterising Blumeria graminis f. sp. hordei Germination)

9.2.1: Statistical analysis of Bgh germination on barley

(1) Germination development on barley, glass, cellulose, wheat at 4, 8 and 16 h.p.i.

3-Factor Anova Analysis:

Error: Pot
   Df Sum Sq Mean Sq
   Time 1 0.31795 0.31795

Error: Pot:Slide
   Df Sum Sq Mean Sq F value Pr(>F)
   Residuals 7 0.97209 0.13887

Error: Within
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   Surftype 3 32.3 10.8 32.3796 < 2.2e-16 ***
   Time 7 0.7 0.1 0.3102 0.949655
   GermState 7 8000.8 1143.0 3433.9061 < 2.2e-16 ***
   Surftype:Time 24 15.6 0.7 1.9581 0.003638 **
   Surftype:GermState 21 871.6 41.5 124.6966 < 2.2e-16 ***
   Time:GermState 56 194.2 3.5 10.4173 < 2.2e-16 ***
   Surftype:Time:GermState 168 236.1 1.4 4.2215 < 2.2e-16 ***
   Residuals 2009 668.7 0.3

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(2) Comparison of PGT vs SGT on barley at 4 h.p.i.

Error: Pot
   Df Sum Sq Mean Sq F value Pr(>F)
   Residuals 2 0.78884 0.39442

Error: Pot:Slide
   Df Sum Sq Mean Sq F value Pr(>F)
   Residuals 21 14.637 0.697

Error: Within
   Df Sum Sq Mean Sq F value Pr(>F)
   GermState 1 26.461 26.461 36.298 3.823e-06 ***
   Residuals 23 16.767 0.729

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(3) Comparison of SGT vs AGT on barley at 4 h.p.i.

Error: Pot
Df  Sum Sq Mean Sq  F value  Pr(>F)
Residuals  2 2.1438  1.0719

Error: Pot:Slide
Df  Sum Sq Mean Sq  F value  Pr(>F)
Residuals 21 31.028  1.516

Error: Within
Df  Sum Sq Mean Sq  F value  Pr(>F)
GermState  1  5.7492  5.7492  8.5575  0.00761 **
Residuals 23 15.4522  0.6718

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(4) Comparison of SGT vs PGT on barley at 8 h.p.i.

Error: Pot
Df  Sum Sq Mean Sq  F value  Pr(>F)
Residuals  1 0.02821 0.02821

Error: Pot:Slide
Df  Sum Sq Mean Sq  F value  Pr(>F)
Residuals  1 0.11069 0.11069

Error: Within
Df  Sum Sq Mean Sq  F value  Pr(>F)
GermState  1  4.3674  4.3674  8.3483  0.005971 **
Residuals 44 23.0184  0.5231

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(5) Comparison of Pgt vs A on barley at 8 h.p.i.

Error: Pot
Df  Sum Sq Mean Sq  F value  Pr(>F)
Residuals  2 2.4463  1.2232

Error: Pot:Slide
Df  Sum Sq Mean Sq  F value  Pr(>F)
Residuals 21 15.8702  0.7557

Error: Within
Df  Sum Sq Mean Sq  F value  Pr(>F)
GermState  1 131.078 131.078  99.46  8.044e-10 ***
Residuals 23  30.311  1.318

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(6) Comparison of SGT vs A on barley at 8 h.p.i.

Error: Pot
  Df  Sum Sq Mean Sq  F value   Pr(>F)
Residuals  2 0.28002 0.14001

Error: Pot:Slide
  Df  Sum Sq Mean Sq  F value   Pr(>F)
Residuals 21 23.815 1.134

Error: Within
  Df  Sum Sq Mean Sq  F value   Pr(>F)
GermState  1  87.593 87.593  86.29 3.015e-09 ***
Residuals 23 23.347 1.015

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(7) Comparison of Agt development at 8 and 16 h.p.i. on barley.

Error: Pot
  Df  Sum Sq Mean Sq  F value   Pr(>F)
Time       1  5.0813  5.0813  7.2347 0.05467 .
Residuals  4 2.8094  0.7024

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: Pot:Slide
  Df  Sum Sq Mean Sq  F value   Pr(>F)
Residuals 42 51.153  1.218

(8) Comparison of Ng development on wheat compared to barley at 16 h.p.i.

Error: Pot
  Df  Sum Sq Mean Sq  F value   Pr(>F)
Residuals  2 1.66974 0.83487

Error: Pot:Slide
  Df  Sum Sq Mean Sq  F value   Pr(>F)
Residuals 21 14.6906 0.6996

Error: Within
  Df  Sum Sq Mean Sq  F value   Pr(>F)
Surftype   1  6.0899  6.0899  17.011 0.0004129 ***
Residuals 23 8.2341  0.3580

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(9) **Comparison of Agt development on wheat compared to barley at 16 h.p.i.**

Error: Pot
   Df Sum Sq Mean Sq F value  Pr(>F)
Residuals  2  2.7795  1.3897

Error: Pot:Slide
   Df Sum Sq Mean Sq F value  Pr(>F)
Residuals 21  34.87  1.66

Error: Within
   Df Sum Sq Mean Sq F value  Pr(>F)
Surftype  1  0.0107  0.0107 0.012 0.9137
Residuals 23 20.5663  0.8942

(10) **Comparison of H development on wheat compared to barley at 16 h.p.i.**

Error: Pot
   Df Sum Sq Mean Sq F value  Pr(>F)
Residuals  2  0.7128  0.3564

Error: Pot:Slide
   Df Sum Sq Mean Sq F value  Pr(>F)
Residuals 21  13.71  0.6529

Error: Within
   Df Sum Sq Mean Sq F value  Pr(>F)
Surftype  1  63.96  63.96 102.07 6.301e-10 ***
Residuals 23 14.41  0.627

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

9.2.2: **Statistical comparison of Bgh germination on barley, and glass at 4, 8 and 16 hours after inoculation, and statistical analysis of Bgh germination on glass**

(1) **Comparison of Ng on glass compared to barley at 4 h.p.i.**

Error: Pot
   Df Sum Sq Mean Sq F value  Pr(>F)
Residuals 2  0.1518  0.0759

Error: Pot:Slide
   Df Sum Sq Mean Sq F value  Pr(>F)
Residuals 21 10.27  0.4892

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(2) **Comparison of PGT on glass compared to barley at 8 h.p.i.**

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Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(3) **Comparison of PGT on glass compared to barley at 16 h.p.i.**

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(4) **Comparison of PGT vs SGT on Glass at 16 h.p.i.**

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Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

336
(5) Comparison of Ng on glass at 8 h.p.i. and 16 h.p.i.

Error: Pot
  Df  Sum Sq Mean Sq F value  Pr(>F)
Time       1 2.18759 2.18759   9.564 0.03648 *
Residuals  4 0.91493 0.22873
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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: Pot:Slide
  Df  Sum Sq Mean Sq F value  Pr(>F)
Residuals 42 3.6365  0.0866

(6) Comparison of PGT on glass at 8 h.p.i. and 16 h.p.i.

Error: Pot
  Df  Sum Sq Mean Sq F value  Pr(>F)
Time       1 15.3467 15.3467  24.536 0.007743 **
Residuals  4  2.5019  0.6255
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: Pot:Slide
  Df  Sum Sq Mean Sq F value  Pr(>F)
Residuals 42 20.2253  0.4816

(7) Comparison of SGT on glass at 8 h.p.i. and 16 h.p.i.

Error: Pot
  Df  Sum Sq Mean Sq F value  Pr(>F)
Time       1  7.0994  7.0994   8.5099 0.04337 *
Residuals  4  3.3370  0.8342
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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: Pot:Slide
  Df  Sum Sq Mean Sq F value  Pr(>F)
Residuals 42 19.6893  0.4688

9.2.3: Statistical comparison of Bgh germination on cellulose, glass and wheat at 4, 8 and 16 hours after inoculation, and statistical analysis of Bgh germination on cellulose

(1) Comparison of Ng on cellulose compared to glass at 4 h.p.i.

Error: Pot
  Df  Sum Sq Mean Sq F value  Pr(>F)
Residuals  2  0.52099  0.26049

Error: Pot:Slide
   Df  Sum Sq  Mean Sq   F value  Pr(>F)
Residuals 21  4.9773  0.2370

Error: Within
   Df  Sum Sq  Mean Sq   F value  Pr(>F)
Surftype  1  0.5236  0.5236  1.9081  0.1804
Residuals 23  6.3116  0.2744

(2) Comparison of Ng on cellulose compared to glass at 8 h.p.i.

Error: Pot
   Df  Sum Sq  Mean Sq   F value  Pr(>F)
Residuals  2  0.024451 0.012226

Error: Pot:Slide
   Df  Sum Sq  Mean Sq   F value  Pr(>F)
Residuals 21  4.8298  0.2300

Error: Within
   Df  Sum Sq  Mean Sq   F value  Pr(>F)
Surftype  1  0.8479  0.8479  3.841  0.06224 .
Residuals 23  5.0770  0.2207

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(3) Comparison of Ng on cellulose compared to glass at 16 h.p.i.

Error: Pot
   Df  Sum Sq  Mean Sq   F value  Pr(>F)
Residuals  2  0.31012  0.15506

Error: Pot:Slide
   Df  Sum Sq  Mean Sq   F value  Pr(>F)
Residuals 21  2.7364  0.1303

Error: Within
   Df  Sum Sq  Mean Sq   F value  Pr(>F)
Surftype  1  7.1892  7.1892  45.077  7.547e-07 ***
Residuals 23  3.6682  0.1595

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(4) Comparison of PGT on cellulose compared to glass at 4 h.p.i.

Error: Pot
   Df  Sum Sq  Mean Sq   F value  Pr(>F)
Residuals  2  4.1648  2.0824

Error: Pot:Slide
(5) Comparison of PGT on cellulose compared to glass at 8 h.p.i.

Error: Pot
  Df  Sum Sq Mean Sq F value  Pr(>F)
  Residuals  2  0.59672 0.29836

Error: Pot:Slide
  Df  Sum Sq Mean Sq F value  Pr(>F)
  Residuals 21 16.6978  0.7951

Error: Within
  Df  Sum Sq Mean Sq F value  Pr(>F)
  Surftype  1  0.6166  0.6166  1.1876 0.2871
  Residuals 23 11.9417  0.5192

(6) Comparison of PGT on cellulose compared to glass at 16 h.p.i.

Error: Pot
  Df  Sum Sq Mean Sq F value  Pr(>F)
  Residuals  2  1.33556 0.66778

Error: Pot:Slide
  Df  Sum Sq Mean Sq F value  Pr(>F)
  Residuals 21 10.5120  0.5006

Error: Within
  Df  Sum Sq Mean Sq F value  Pr(>F)
  Surftype  1 28.822  28.822  48.201 4.461e-07 ***
  Residuals 23 15.140  0.658

(7) Comparison of PGT on cellulose and wheat at 4 h.p.i.

Error: Pot
  Df  Sum Sq Mean Sq F value  Pr(>F)
  Residuals  2  0.95237 0.47618

Error: Pot:Slide
  Df  Sum Sq Mean Sq F value  Pr(>F)
  Residuals 21 5.3834  0.2564

Error: Within
  Df  Sum Sq Mean Sq F value  Pr(>F)
  Surftype  1 31.728  31.728  48.201 4.461e-07 ***
  Residuals 23 15.140  0.658
(8) Comparison of PGT on cellulose and wheat at 8 h.p.i.

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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(9) Comparison of PGT on cellulose and wheat at 16 h.p.i.

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## 9.3: Appendix C: Chapter 4 (Germination on Modified Surfaces)

### 9.3.1: Stimuli screen

#### (1) Germination Development on Treated Glass Slides at 24 h.p.i.

**2-way Anova Analysis:**

**a. (Additives dissolved in di-ethyl ether)**

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**b. (Additives dissolved in dichloromethane)**

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**Signif. codes: 0 ‘****’ 0.001 ‘***’ 0.01 ‘**’ 0.05 ‘.’ 0.1 ‘ ’ 1**

#### (2) Comparison of non-germinated spores on slides treated with di-ethyl ether and dodecanol

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(3) Comparison of non-germinated spores on slides treated with dichloromethane and hexacosanoic acid

(4) Comparison of primary germ tubes on slides treated with 1-hexacosanol and hexacosanoic acid

(5) Comparison of primary germ tubes on slides treated with barley cuticular extract and di-ethyl ether
(6) Comparison of secondary germ tubes on slides treated with barley cuticular extract and di-ethyl ether

(7) Comparison of Egst/App formation on slides treated with di-ethyl ether and barley cuticular extract

(8) Comparison of App formation on slides treated with di-ethyl ether and barley cuticular extract
Residuals  1 0.60892 0.60892

Error: Well:Slide
  Df  Sum Sq  Mean Sq  F value  Pr(>F)
Residuals  1 0.23492 0.23492

Error: Within
  Df  Sum Sq  Mean Sq  F value    Pr(>F)
Surftype   1  3.7488  3.7488  14.148 0.0004957 ***
Residuals 44 11.6586  0.2650
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> (9) Comparison of Egst/App formation on slides treated with di-ethyl ether and 16-hydroxyhexadecanoic acid

Error: Well
  Df  Sum Sq  Mean Sq  F value  Pr(>F)
Residuals  1 0.20241 0.20241

Error: Well:Slide
  Df  Sum Sq  Mean Sq  F value    Pr(>F)
Residuals  1 1.6393  1.6393

Error: Within
  Df  Sum Sq  Mean Sq  F value    Pr(>F)
Surftype   1 17.314  17.3137  36.991 2.557e-07 ***
Residuals 44 20.594  0.4681
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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> (10) Comparison of App formation on slides treated with di-ethyl ether and 16-hydroxyhexadecanoic acid

> model<-aov(sqrt(Count)~Surftype+Error(Well/Slide),subset=(GermState=="5"),data=d2)
> summary(model)

Error: Well
  Df  Sum Sq  Mean Sq  F value  Pr(>F)
Residuals  1 0.63227 0.63227

Error: Well:Slide
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Residuals  1 0.15629 0.15629

Error: Within
  Df  Sum Sq  Mean Sq  F value    Pr(>F)
Surftype   1 27.985  27.9846  57.871 1.488e-09 ***
Residuals 44 21.277  0.4836
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> 

344
(11) Comparison of Egst/App formation on slides treated with 1-hexacosanal and dichloromethane

Error: Well
   Df  Sum Sq  Mean Sq   F value    Pr(>F)
Residuals  1 0.011318 0.011318

Error: Well:Slide
   Df  Sum Sq  Mean Sq    F value   Pr(>F)
Residuals  1 0.44737 0.44737

Error: Within
   Df  Sum Sq  Mean Sq   F value   Pr(>F)
Surftype  1 12.351 12.3505 21.466 3.205e-05 ***
Residuals 44 25.315  0.5753

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(12) Comparison of Egst/App formation on slides treated with 1-hexacosanal and dichloromethane

> model2<-aov(sqrt(Count)~Surftype+Error(Well/Slide),subset=(GermState=="5"),data=d2)
> summary(model2)

Error: Well
   Df  Sum Sq  Mean Sq   F value   Pr(>F)
Residuals  1 0.035151 0.035151

Error: Well:Slide
   Df  Sum Sq  Mean Sq    F value   Pr(>F)
Residuals  1 0.39969 0.39969

Error: Within
   Df  Sum Sq  Mean Sq   F value   Pr(>F)
Surftype  1 23.0611 23.0611 120.18 3.638e-14 ***
Residuals 44  8.4429  0.1919

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

9.3.2: Overall comparison of germination development upon non-chromatic acid cleaned glass and treatments

Error: Rep
   Df  Sum Sq  Mean Sq    F value   Pr(>F)
Residuals  3 5.3428  1.7809

Error: Rep:Slide
   Df  Sum Sq  Mean Sq    F value   Pr(>F)
Residuals 36 8.9607  0.2489
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Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(1) Comparison of SGT between ether and glass

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Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

9.3.3: Overall comparison of germination development upon chromic acid cleaned glass and treatments

Error: Tray

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Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(1) Comparison of PGT between 16-hydroxyhexadecanoic acid treated glass and cleaned glass

Error: Tray
Df Sum Sq Mean Sq F value Pr(>F)
Residuals 8  9.7940  1.2242

Error: Tray:Rep
Df Sum Sq Mean Sq F value Pr(>F)
Residuals 18  7.3782  0.4099

Error: Within
Df Sum Sq Mean Sq F value Pr(>F)
Status     1   0.694   0.694  0.5441 0.4674
Residuals 26  33.147   1.275

(2) Comparison of appressorium formation on clean glass and 16-hydroxyhexadecanoic acid treated glass

Error: Tray
Df Sum Sq Mean Sq F value Pr(>F)
Residuals 8  6.7028  0.8379

Error: Tray:Rep
Df Sum Sq Mean Sq F value Pr(>F)
Residuals 18  9.1639  0.5091

Error: Within
Df Sum Sq Mean Sq F value Pr(>F)
Status     1  43.339  43.339  46.113 3.308e-07 ***
Residuals 26  24.436   0.940
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(3) Comparison of appressorium on 16-hydroxyhexadecanoic acid treated glass and ether treated glass

Error: Tray
Df Sum Sq Mean Sq F value Pr(>F)
Residuals 8  11.2028  1.4004

Error: Tray:Rep
Df Sum Sq Mean Sq F value Pr(>F)
Residuals 18  15.9337  0.8852

Error: Within
Df Sum Sq Mean Sq F value Pr(>F)
Status     1  44.979  44.979  54.885 7.24e-08 ***
Residuals 26  21.308   0.820
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

>
9.3.4: Overall Comparison of non-Germination development on host, non-host and artificial surfaces at 4 h.p.i.

(1) Comparison of germination on barley and aldehyde treated glass

Barley versus aldehyde ng
summary(model)

Error: Batch
  Df  Sum Sq Mean Sq F value Pr(>F)
Residuals  2 0.5495 0.2747

Error: Batch:Tray
  Df  Sum Sq Mean Sq F value Pr(>F)
Residuals  6 1.0952 0.1825

Error: Batch:Tray:Rep
  Df  Sum Sq Mean Sq F value Pr(>F)
Residuals 18 1.8435 0.1024

Error: Within

  Df  Sum Sq Mean Sq  F value  Pr(>F)
Status        8  29.7 3.7  15.861  < 2.2e-16 ***
GermState     7 4171.8 596.0 2550.116  < 2.2e-16 ***
Status:GermState 56 379.9  6.8  29.025  < 2.2e-16 ***
Residuals 1846 431.4 0.2

---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> (2) Comparison of non-germination on barley and wheat at 4 h.p.i.

> summary(model)
(3) Comparison of non-germination on barley and wheat at 4 h.p.i.

> summary(model)

Error: Batch
  Df  Sum Sq  Mean Sq   F value     Pr(>F)
Residuals  1 0.0028239 0.0028239

Error: Batch:Tray
  Df  Sum Sq  Mean Sq   F value     Pr(>F)
Residuals  1 3.8891  3.8891

Error: Batch:Tray:Rep
  Df  Sum Sq  Mean Sq   F value     Pr(>F)
Residuals  1 0.8357  0.8357

Error: Within
  Df  Sum Sq  Mean Sq   F value     Pr(>F)
Status  1  1.6462  1.6462  2.6798  0.1080
Residuals 49 30.1005  0.6143

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(4) Comparison of non-germination on aldehyde and cuticular extract at 4 h.p.i.

> summary(model)

Error: Batch
  Df  Sum Sq  Mean Sq   F value     Pr(>F)
Residuals  1 1.1242  1.1242

Error: Batch:Tray

(5) **Comparison of PGT formation at 4 h.p.i. between hexacosanal treated slides and chromic acid cleaned slides**

(6) **Comparison of PGT formation at 4 h.p.i. between the Cuticle Extract treated slides and chromic acid cleaned slides**

> summary(model)
(7) Comparison of PGT formation at 4 h.p.i. between 16-
Hydroxyhexadecanol treated slides and chromic acid cleaned slides

(8) Comparison of PGT formation at 4 h.p.i. on hydrophilic gelbond
compared to hydrophobic gelbond
(9) **Comparison of SGT formation at 4 h.p.i. on hexacosanal treated slides and wheat**

> summary(model)

Error: Batch
   Df Sum Sq Mean Sq F value Pr(>F)
Residuals  1 0.52281 0.52281

Error: Batch:Tray
   Df Sum Sq Mean Sq F value Pr(>F)
Residuals  1 0.94129 0.94129

Error: Batch:Tray:Rep
   Df Sum Sq Mean Sq F value Pr(>F)
Residuals  1 3.0249  3.0249

Error: Within
   Df Sum Sq Mean Sq F value Pr(>F)
Status  1 0.379  0.379  0.5308 0.4697
Residuals 49 35.022  0.715

(10) **Comparison of SGT formation at 4 h.p.i. on hexacosanal treated slides and barley**

> summary(model)

Error: Batch
   Df Sum Sq Mean Sq F value Pr(>F)
Residuals  1 1.2470  1.2470

Error: Batch:Tray
   Df Sum Sq Mean Sq F value Pr(>F)
Residuals  1 2.3773  2.3773

Error: Batch:Tray:Rep
   Df Sum Sq Mean Sq F value Pr(>F)
Residuals  1 1.8591  1.8591

Error: Within
   Df Sum Sq Mean Sq F value Pr(>F)
Status  1 1.6876  1.6876  3.8852 0.05437
Residuals 49 21.2835 0.4344

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> 

(11) **Comparison of SGT formation at 4 h.p.i. on cuticle treated slides and barley**
> summary(model)

Error: Batch
  Df  Sum Sq  Mean Sq   F value Pr(>F)
Residuals 1 0.52915  0.52915

Error: Batch:Tray
  Df  Sum Sq  Mean Sq   F value Pr(>F)
Residuals 1 0.97005  0.97005

Error: Batch:Tray:Rep
  Df  Sum Sq  Mean Sq   F value Pr(>F)
Residuals 1 0.98217  0.98217

Error: Within
  Df Sum Sq  Mean Sq Pr(>F)
Status 1 2.6374  2.6374 0.01518 *
Residuals 49 20.4106  0.4165

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(12) Comparison of SGT formation at 4 h.p.i. on cuticle treated slides and wheat

> summary(model)

Error: Batch
  Df  Sum Sq  Mean Sq   F value Pr(>F)
Residuals 1 1.2567  1.2567

Error: Batch:Tray
  Df  Sum Sq  Mean Sq   F value Pr(>F)
Residuals 1 0.17078  0.17078

Error: Batch:Tray:Rep
  Df  Sum Sq  Mean Sq   F value Pr(>F)
Residuals 1 1.8681  1.8681

Error: Within
  Df  Sum Sq  Mean Sq Pr(>F)
Status 1 0.085  0.085 0.1283
Residuals 49 32.340  0.660

(13) Comparison of SGT formation at 4 h.p.i. on 16-hydroxyhexadecanol-treated slides and the hydrophilic gelbond

> summary(model)

Error: Batch
  Df  Sum Sq  Mean Sq  Pr(>F)
Residuals 1 0.55986  0.55986

Error: Batch:Tray

353
Df    Sum Sq    Mean Sq F value Pr(>F)
Residuals  1 6.8801e-08 6.8801e-08

Error: Batch:Tray:Rep
Df    Sum Sq    Mean Sq F value Pr(>F)
Residuals  1 0.26511 0.26511

Error: Within
Df    Sum Sq    Mean Sq F value Pr(>F)
Status  1 1.186e-05 1.186e-05 2.897e-05 0.9957
Residuals 49  20.0647  0.4095

(14) Comparison of non-germination on hydrophilic and hydrophobic Gelbond

> summary(model)

Error: Batch
Df    Sum Sq    Mean Sq F value Pr(>F)
Residuals  1 0.016154 0.016154

Error: Batch:Tray
Df    Sum Sq    Mean Sq F value Pr(>F)
Residuals  1 0.035513 0.035513

Error: Batch:Tray:Rep
Df    Sum Sq    Mean Sq F value Pr(>F)
Residuals  1 0.074219 0.074219

Error: Within
Df    Sum Sq    Mean Sq F value Pr(>F)
Status  1 20.1496 20.1496 99.665 2.128e-13 ***
GermState  7 2464.40 352.06 926.0094 < 2.2e-16 ***

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

9.3.5: Overall comparison of germination at 16 h.p.i on surfaces

Error: Batch
Df    Sum Sq    Mean Sq F value Pr(>F)
Residuals  2  3.711  1.8555

Error: Batch:Tray
Df    Sum Sq    Mean Sq F value Pr(>F)
Residuals  6  2.8049  0.46749

Error: Batch:Tray:Rep
Df    Sum Sq    Mean Sq F value Pr(>F)
Residuals 18  3.2592  0.18107

Error: Within
Df    Sum Sq    Mean Sq F value Pr(>F)
Status  6  8.60  1.43  3.7713 0.0009899 ***
GermState  7 2464.40 352.06 926.0094 < 2.2e-16 ***
(1) Comparison of primary to secondary germ tubes at 16 h.p.i in clean slides

(2) Comparison of primary to secondary germ tubes at 16 h.p.i. on monomer treated slides
(3) Comparison of primary to secondary germ tubes at 16 h.p.i. on hydrophilic Gelbond

Error: Batch
Df  Sum Sq  Mean Sq  F value  Pr(>F)
Residuals  2 3.4836  1.7418

Error: Batch:Tray
Df  Sum Sq  Mean Sq  F value  Pr(>F)
Residuals  6 6.2467  1.0411

Error: Batch:Tray:Rep
Df  Sum Sq  Mean Sq  F value  Pr(>F)
Residuals 18 5.1112  0.28395

Error: Within
Df  Sum Sq  Mean Sq  F value  Pr(>F)
GermState  1 6.0663  6.0663  17.953  0.0002514 ***
Residuals 26 8.7856  0.3379

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(4) Comparison of App formation at 16 h.p.i. between 1-hexacosanal treated slides and chromic acid cleaned slides

Error: Batch
Df  Sum Sq  Mean Sq  F value  Pr(>F)
Residuals  1 0.088991 0.088991

Error: Batch:Tray
Df  Sum Sq  Mean Sq  F value  Pr(>F)
Residuals  1 0.050502 0.050502

Error: Batch:Tray:Rep
Df  Sum Sq  Mean Sq  F value  Pr(>F)
Residuals  1 0.11709  0.11709

Error: Within
Df  Sum Sq  Mean Sq  F value  Pr(>F)
Status  1 30.702 30.7015  34.676  3.473e-07 ***
Residuals 49 43.384  0.8854

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(5) Comparison of App formation at 16 h.p.i. between cuticle extract and chromic acid treated slides

Error: Tray
Df  Sum Sq  Mean Sq  F value  Pr(>F)
Residuals  1 3.8857  3.8857

356
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Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

### (6) Comparison of secondary to appressorial germ tubes at 16 h.p.i. on cuticle extract treated slides

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<td>0.2186</td>
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### (7) Comparison of App formation at 16 h.p.i. between hydrophobic Gelbond and monomer

```r
> summary(model)
```

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>
**Comparison of secondary to appressorial germ tubes at 16 h.p.i. on monomer treated slides**

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**Comparison of App formation at 16 h.p.i between monomer and clean glass**

```r
> summary(model)
```

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**Comparison of App formation at 16 h.p.i. between hydrophobic Gelbond and clean glass**

```r
> summary(model)
```

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(11) Comparison of multiple germ tubes at 16 h.p.i. on hydrophilic Gelbond and appressorium induced by 1-hexacosanal

(12) Comparison of extra-long germ tubes at 16 h.p.i. on extract-treated slides and cleaned slides
<table>
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---

(13) **Comparison of branched germ tubes at 16 h.p.i. on hydrophobic Gelbond and hydrophilic gelbond**

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
9.4: Comparison of droplet diameter as an indicator of hydrophobicity

Test of Homogeneity of Variances

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<td>7</td>
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ANOVA

<table>
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Post Hoc Tests

Multiple Comparisons

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*. The mean difference is significant at the 0.05 level.
9.5: Comparison of contact angle as an indicator of hydrophobicity

Test of Homogeneity of Variances

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Robust Tests of Equality of Means

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Post Hoc Tests

Multiple Comparisons

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* The mean difference is significant at the 0.05 level.
### 9.6: Comparison of gene expression on treated surfaces

#### 9.6.1: EST C00148 expression on treated surfaces

**Test of Homogeneity of Variances**

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**ANOVA**

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**Post Hoc Tests**

**Multiple Comparisons**

**Tukey HSD**

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9.6.2: Comparison of EST D00189 expression on treated surfaces

Test of Homogeneity of Variances

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ANOVA

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Robust Tests of Equality of Means

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a. Asymptotically F distributed.

Post Hoc Test

Multiple Comparisons

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* The mean difference is significant at the 0.05 level.
### 9.6.3: Comparison of EST C00563 expression on treated surfaces

#### Test of Homogeneity of Variances

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**ANOVA**

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**Robust Tests of Equality of Means**

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<sup>a</sup> Asymptotically F distributed.

#### Post Hoc Tests

**Multiple Comparisons**

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9.6.4: Comparison of EST D00471 expression on treated surfaces

Test of Homogeneity of Variances

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ANOVA

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Robust Tests of Equality of Means

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\(^a\) Asymptotically F distributed.

Post Hoc Tests

Multiple Comparisons

Games-Howell

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| Wheat       | -.68933 | 1.45982 | 1.000 | -7.8206 - 9.1992 |</p>
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9.6.5: Comparison of EST C00606 expression on treated surfaces

Test of Homogeneity of Variances

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ANOVA

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Post Hoc Tests

Multiple Comparisons

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| Hydrophobic G.b. | Barley     | 1.21033 | .67324 | .683 | -1.1486 | 3.5693 |
| Hydrophobic G.b. | Wheat      | 1.72800 | .67324 | .265 | -.6309  | 4.0869 |
| Hydrophobic G.b. | Non-Treated glass | - .63967 | .67324 | .986 | -2.9986 | 1.7193 |
| Hydrophobic G.b. | Treated glass | -.08333 | .67324 | 1.000 | -2.4423 | 2.2756 |
| Hydrophilic G.b. | -.35000 | .67324 | 1.000 | -2.7089 | 2.0089 |
| Hydrophilic G.b. | Aldehyde   | -.10900 | .67324 | 1.000 | -2.4679 | 2.2499 |
| Hydrophilic G.b. | Extract    | 1.28167 | .67324 | .620 | -1.0773 | 3.6406 |
| Hydrophilic G.b. | Monomer    | .84533 | .67324 | .932 | -1.5136 | 3.2043 |

| Hydrophilic G.b. | Barley     | 1.56033 | .67324 | .381 | -.7986  | 3.9193 |
| Hydrophilic G.b. | Wheat      | 2.07800 | .67324 | .110 | -.2809  | 4.4369 |
| Hydrophilic G.b. | Non-Treated glass | - .28967 | .67324 | 1.000 | -2.6486 | 2.0693 |
| Hydrophilic G.b. | Treated glass | .26667 | .67324 | 1.000 | -2.0923 | 2.6256 |
| Hydrophilic G.b. | Hydrophobic G.b. | .35000 | .67324 | 1.000 | -2.0089 | 2.7089 |
| Hydrophilic G.b. | Aldehyde   | .24100 | .67324 | 1.000 | -2.1179 | 2.5999 |
| Hydrophilic G.b. | Extract    | 1.63167 | .67324 | .329 | -.7273  | 3.9906 |
| Hydrophilic G.b. | Monomer    | 1.19533 | .67324 | .696 | -1.1636 | 3.5543 |

| Aldehyde | Barley     | 1.31933 | .67324 | .586 | -1.0396 | 3.6783 |
| Aldehyde | Wheat      | 1.83700 | .67324 | .205 | -.5219  | 4.1959 |
| Aldehyde | Non-Treated glass | - .53067 | .67324 | .996 | -2.8896 | 1.8283 |
| Aldehyde | Treated glass | .02567 | .67324 | 1.000 | -2.3333 | 2.3846 |
| Aldehyde | Hydrophobic G.b. | .10900 | .67324 | 1.000 | -2.2499 | 2.4679 |
| Aldehyde | Hydrophilic G.b. | -.24100 | .67324 | 1.000 | -2.5999 | 2.1179 |
| Aldehyde | Extract    | 1.39067 | .67324 | .523 | -.9683  | 3.7496 |
| Aldehyde | Monomer    | .95433 | .67324 | .877 | -1.4046 | 3.3133 |

| Extract | Barley     | -.07133 | .67324 | 1.000 | -2.4303 | 2.2876 |
| Extract | Wheat      | .44633 | .67324 | .999 | -1.9126 | 2.8053 |
| Extract | Non-Treated glass | -.92133 | .67324 | .166 | -4.2803 | .4376 |
| Extract | Treated glass | -.36500 | .67324 | .545 | -3.7239 | .9939 |
| Extract | Hydrophobic G.b. | -.28167 | .67324 | .620 | -3.6406 | 1.0773 |
| Extract | Hydrophilic G.b. | -.63167 | .67324 | .329 | -3.9906 | .7273 |
| Extract | Aldehyde   | -.139067 | .67324 | .523 | -3.7496 | .9683 |
| Extract | Monomer    | -.43633 | .67324 | .999 | -2.7953 | 1.9226 |

| Monomer | Barley     | .36500 | .67324 | 1.000 | -1.9939 | 2.7239 |
| Monomer | Wheat      | .88267 | .67324 | .915 | -1.4763 | 3.2416 |
| Monomer | Non-Treated glass | -1.48500 | .67324 | .442 | -3.8439 | .8739 |
| Monomer | Treated glass | -.92867 | .67324 | .892 | -3.2876 | 1.4303 |
| Monomer | Hydrophobic G.b. | -.84533 | .67324 | .932 | -3.2043 | 1.5136 |
| Monomer | Hydrophilic G.b. | -1.19533 | .67324 | .696 | -3.5543 | 1.1636 |
| Monomer | Aldehyde   | -.95433 | .67324 | .877 | -3.3133 | 1.4046 |
| Monomer | Extract    | .43633 | .67324 | .999 | -1.9226 | 2.7953 |

* The mean difference is significant at the 0.05 level.
### 9.6.6: Comparison of EST C00506 expression on treated surfaces

#### Test of Homogeneity of Variances

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#### ANOVA

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#### Robust Tests of Equality of Means

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a. Asymptotically $F$ distributed.

#### Post Hoc Tests

### Multiple Comparisons

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<th>(J) VAR00001</th>
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### Post Hoc Tests

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* The mean difference is significant at the 0.05 level.
9.6.8: Comparison of EST D00972 expression on treated surfaces

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**ANOVA**

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**Robust Tests of Equality of Means**

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a. Asymptotically F distributed.

**Post Hoc Tests**

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9.6.9: Comparison of EST D00944 expression on treated surfaces

Test of Homogeneity of Variances

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Post Hoc Tests

**Multiple Comparisons**

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* The mean difference is significant at the 0.05 level.
9.6.10: Comparison of EST D00881 expression on treated surfaces

Test of Homogeneity of Variances

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Post Hoc Test

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* The mean difference is significant at the 0.05 level.
9.6.11: Comparison of EST D01260 expression on treated surfaces

Test of Homogeneity of Variances

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**9.6.12: Comparison of EST C00009 expression on treated surfaces**

**Test of Homogeneity of Variances**

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**ANOVA**

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**Post Hoc Test**

**Multiple Comparisons**

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- **Barley**
  - Wheat: \(4.76900\), .84393, .001, 1.9343, 7.6037
  - Non-Treated glass: -.08800, .84393, 1.000, -2.9227, 2.7467
  - Treated glass: .44633, .84393, .994, -2.3884, 3.2810
  - Hydrophobic G.b.: -.10400, .84393, 1.000, -2.9387, 2.7307
  - Hydrophilic G.b.: .68767, .84393, .959, -2.1470, 3.5224

- **Wheat**
  - Barley: \(4.76900\), .84393, .001, -7.6037, -1.9343
  - Non-Treated glass: \(4.85700\), .84393, .001, -7.6917, -2.0223
  - Treated glass: .432267, .84393, .003, -7.1574, -1.4880
  - Hydrophobic G.b.: \(4.87300\), .84393, .001, -7.7077, -2.0383
  - Hydrophilic G.b.: \(4.08133\), .84393, .004, -6.9160, 3.5224

- **Non-Treated glass**
  - Barley: .08800, .84393, 1.000, -2.7467, 2.9227
  - Wheat: \(4.85700\), .84393, .001, 2.0223, 7.6917
  - Treated glass: .53433, .84393, .986, -2.3004, 3.3690
  - Hydrophobic G.b.: -.01600, .84393, 1.000, -2.8507, 2.8187
  - Hydrophilic G.b.: .77567, .84393, .934, -2.0590, 3.6104

- **Treated glass**
  - Barley: -.44633, .84393, .994, -3.2810, 2.3884
  - Wheat: \(4.32267\), .84393, .003, 1.4880, 7.1574
  - Non-Treated glass: -.53433, .84393, .986, -3.3690, 2.3004
  - Hydrophobic G.b.: -.55033, .84393, .984, -3.3850, 2.2844
  - Hydrophilic G.b.: .24133, .84393, 1.000, -2.5934, 3.0760

- **Hydrophobic G.b.**
  - Barley: .10400, .84393, 1.000, -2.7307, 2.9387
  - Wheat: \(4.87300\), .84393, .001, 2.0383, 7.7077
  - Non-Treated glass: .01600, .84393, 1.000, -2.8187, 2.8507
  - Treated glass: .55033, .84393, .984, -2.2844, 3.3850
  - Hydrophilic G.b.: .79167, .84393, .929, -2.0430, 3.6264

- **Hydrophilic G.b.**
  - Barley: -.68767, .84393, .959, -3.5224, 2.1470
  - Wheat: \(4.08133\), .84393, .004, 1.2466, 6.9160
  - Non-Treated glass: -.77567, .84393, .934, -3.6104, 2.0590
  - Treated glass: -.24133, .84393, 1.000, -3.0760, 2.5934
  - Hydrophilic G.b.: -.79167, .84393, .929, -3.6264, 2.0430

* The mean difference is significant at the 0.05 level.
9.6.13: Comparison of EST C00482 expression on treated surfaces

Test of Homogeneity of Variances

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Post Hoc Test

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* The mean difference is significant at the 0.05 level.
## 9.6.14: Comparison of EST C01244 expression on treated surfaces

### Test of Homogeneity of Variances

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### Robust Tests of Equality of Means

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a. Asymptotically F distributed.

### Post Hoc Test

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* The mean difference is significant at the 0.05 level.
9.6.16: Comparison of EST C00059 expression on treated surfaces

Test of Homogeneity of Variances

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Post Hoc Test

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9.6.17: Comparison of EST PS11B04 expression on treated surfaces

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9.6.18: Comparison of EST C01157 expression on treated surfaces

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Post Hoc Test

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9.7: Unsequences related to ESTs.

All unsequences are taken directly from the COGEME (‘Consortium for the Functional Genomics of Microbial Eukaryotes’) hosted on http://cogeme.ex.ac.uk/

9.7.1: EST Clone Library Identifier D00972.

Unisequence:

1  CGATGAGAAG ATATATGGGA GGGCATCTTC TTACAAAAAG ACTCAGNGCG CCGAATCGGG
61  ACGGGTCTGA CCGCTAAATG TTAATTTACG GAGTCAAACG CAAAGTAGTAT
121  CACCCCTCAA GAATATATATA CCGGCTTTTG CCGGCTTTTG ACTCTAATGA TCAGGATAGC
181  TGGTGCTTAG GCATGATAGA CCGTGCACTC GAGTAACATA AATGTCTAGA AACATTGAAA
241  TTGGGAATCAA AGCTACCTAG TTTGCGCTGG TGTGATAAAT TTTGCTACAT
301  TAATTTTAGTC AGGACATTTC TCTGCTGAAAC ATCCGATACT GCACAACTAT CTCCTACACT
361  TCCATCTCTCA AAATGTTTACG TAGCTACTGT ATATGGTCTGT AAAAAGAAAA AAAAAAAA

9.7.2: EST Clone Library Identifier D00658.

Unisequence:

1  TAAAGTAACT TGATAGTACT TAATATGGGA TAATTGCGTA ACCTAAGGTG AAAATTATCA
61  TTGGGCGTAT CCTCGTATTT ATACATTATT ATTCCTTTTC CCTGTGCTTT TCTCCAATTC
121  TAACCCCTTC CCTAACGCCT GATTAAATAA TCAAAATATA GCTTTGCCAG AAATTATTTG
181  ATGTCTTTAT ACTGTGACTC TTCACACCTT GATCCACAAG CTTAAAACAC CCTTGGCGTA
241  CTCAAGAATT TCATCATCGC CAAGTTTGCT TTCTCCATAA AGGCGATCGA CGAATGTGAT
301  TGGACATCCT CACCGCTAGT ATCCCCATTGG CTTAGCTGTG GAAATGAGCT TGGACAGCG
361  AGGNTATACCT TTTACTTCTG GCACATCTTT TCAATGAGCT ATATTTATAT
421  AGCCGAAAC TTTCTG

9.7.3: EST Clone Library Identifier C00009.

Unisequence:

1  TAATAATGTG ATTACTAGTATA TAAAATGATC TATCATCATG CAGACATTCG GAGCAGTTTT
61  CGATGCCTCGGC AGCCGATCAG CAGCAGCTAC GAGCGATTGG GAGACCTGCG CAGGACCTGT
121  CAGTGATGTG AAGGCTGGAC AGCCATACAA GATAACATGG GAGAACGCCG CAGGACCTGT
181  TACAAATCTT TTTAGAAGATG GACACTACCT CAACTACACG ACCGTTTTCA ACATTCTCTC
241  CGCCCAACTCG GAAATCTTCG TCCAGCTGCC ACCCTCAAGA CCGATAAAAA
301  TGTTTGCGAA ATACCTCCTG CAGGAGGCG GAAATCTCTC GATACAGCTC CACACAGCG
361  TGAATCGGAC CGCCGACGCA TGCTTCTCGG TAATCCAGTC GCATGACAGA GAATGAGCT
421  CCGTGATCGC TCCGGATGTA AGGTCTAGG TCAACGCACA CTCCTAATAG
481  GACTGCTTAC CCACCTACCT GGGCGCTAGT ATACTGAGGA GGCCTGCTCA ACCACATCT
541  TCCCATGGTG TCACTTTCTG ATATAGCTAT CACACTTCCT GCAGATCCCG GCACAGCGG
601  AGTATCGCTG CATGAGATAGA AAGATGTG TCCAGCTGCA CACATCTTGA CCTGCGCTCT
661  GACTTTGATT GCTATTCTTC TATTTCTTTC TATTTAATAT CAGATCATAT
721  GCTTCAGATG ATATATACGT TCTTCCAGTG ATAAATTTAC TTTTCGCTGA CAGTTTCTT
781  AATATTTTTCG ATGTAGTGCT TGGAGGAGTT TGGGCGTATG ATGCTCGTGA
841  TTTCATGCAA CGGAATATCG ATTCACACGA TGGACAGCTG ATTATAGCTT ATTATGAAC
901  ATCCACAGCAT ATTTGTTATG AGAATTATAC CAAATTCATCA ACAATCATAT
961  AAAAAAAAA

401
9.7.4: EST Clone Library Identifier D00881.

**UniSequence:**

1  CTACAACATG CTTTCGTTCC GCCAGGCGTT GTTCTAGTTC TCACTGTACT
61  TTACTTTTAC ACACAATACCT ACCCCTATAG ATCCCGGCGA TCATCTGAAA GCCGAAACAC
121  CGGAGCAGCC CGAATGATT CAATTAATAT TACCGCCCTC CCGACTCCCA TAACGACCGA
181  TGGAACACAA GCCAGCTGGCT CTCAGAAGCT TTTAATCGAT ATGTCACAGG CGCCCCTCG
241  AGAAATACCT GCATATCATAT TTCCCTACGA CGTTGAGGCT AAGTTTTCCT GCCTACATAT
301  GGCAAAACCTG GAAATTTTACA CTCGACACCG GAGATTTTGA CGAAGAAATT CGGGCTTCT
361  GAGGCATATG GGAGTAAAAA ACATCTACT TTCT

9.7.5: EST Clone Library Identifier D00944.

**UniSequence:**

1  AGGATTCTAT GTGCTAATGG GTTCGGGCTC CGGACTCTGA TCGCGATAAA GATTTACCAA
61  CCATAAAAAAT TGGATGAGCCA ATGGAAATAG CAGCTACAGT GCTAGACAGC CAAATGATTA
121  TGGAAGAAAC CGTGAGAGATG AAGGAATATG CTTAGGGTG GTAATCAGGG GAATCTGCG
181  TATTTTCGCG GGAGGTGGGG AATCACTGGA AGCAGGAACC ATCTCATTTT GAGGAGCCAT
241  CTTTATAGCTG CATATACCTCC GTAATTTGTC GAAAGAGTTAA TGCCGAAGTA AGGGATTTTG
301  TGAATAAAATT CGACACACCG TATCATCATAT AAGAGATATG GCTGGAATGG ATCGGGTCCC
361  GGAGGACAGTT TGCGAATACCT ATCCCGAGTT CAGACAGCAA AAGCTGCTATG GTCAAATTAC
421  TAAAGTTGAG GAAATTTTGG CCTACACCGG CGGACCGCTA TTTGAGATG GCTCAAATTC
481  GTAAAGCTGTT CCGGATATAT TAGATTTAGT CCCACACCGG CGGACGGCTA TTTGGATGAT GTCAAATTAC

9.7.6: EST Clone Library Identifier PS11B04.

**UniSequence:**

1  TCCGATANTC TNAACTTTGC ACATCTCAAAT GACATTTTAT GTGCTTATAC TATATGGCCAA
61  TCACGAGCAAAGT CAGGGCTAAGC CAGCGCGCAGC CAGCGCGAAGT TCGCTGCGAA TCGCTGCGAA
121  ACCTACCTCT CAGGTGAACC GTTACAGGCA CAGGTAATAC TATATGGCCA GCACGAGCAGC
181  CATTAGAAATT CTTGGATGG ATAAAAGAGTT AGCCATATAC TCTTACTTCT GACGAGATATG
241  AGACCACTATG TCTGCTGCTA AATATGCTAT AGTTGCTATG GTGCTGCTATG GTGCTGCTATG
301  ANCACTTTATC TAGAGGGGTC TAGACTCTCT GAGGATATNC TGATTAATGC ACTANCTTTC
361  CTACTATATCT CGACCGACCG AGGANATATC GTGCTNACT CACCGCTAGT GATCAGGCT

9.7.7: EST Clone Library Identifier C00482.

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1  CCGAGGGCTC TACCTCCAGG CAGCGCGGCA ACTGCGGCTC ATGCTGCTGC CGTCGCGGCA
61  AAGGCTCGAG CATTGCTCTCG ACTCAGACAG CAGGCAAGCAG TGCTGCTGCT CCGGCGGCG
121  TGTTTGTTCGG CAGGACCGGG ATCAGGCTCT CAGATCTGCT CATATGGCC ATATGGGCGG
181  ACCTATATATC TATAGCTACAG CAGGCAAGGAA GATGAGCGCT TCAGGCTGCT
241  AAACAACTACAT TGGGACCAGA GATAACACGA GAGAAGCTTC CTCAACATTC ATACAGGCG
301  CTAACACACAT TTAGGAGTCC AATGGTACTC GCTGGCTACT CACCTCCTCTT AACCATGAC
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402
9.7.8: EST Clone Library Identifier D01244.

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61  TTAACACTTCCC CACATTGGCGGACTCTTTCAG TACTGCCGACTTTTCCAAA
121  AAAATTAAAC TCTCCTCTCTCCTCCTCA AGTACGTTGATTAC TGGCCTTACATGTTGATCCAA
181  GCATTCCCGAG AAGTGTGGGTC AAGCTTCTATTCTGCCGCTTCTGAACTCCAA
241  TGAATTTGTTGCTACGTTCACG AGGCGGCTTCCGAGGAAGGGTA GAGGATGATTTCCGCTTCTCT
301  ATACGGCGCTC TTTACGCTGCTG ACTACTTTC AAGACGACTACCTGTAATAG
361  GACCCGACCC AAGTGGTGGGTC AAGCTTCTATTCTGCCGCTTCTGAACTCCAA
421  TGAATTTGTTGCTACGTTCACG AGGCGGCTTCCGAGGAAGGGTA GAGGATGATTTCCGCTTCTCT
481  ATACGGCGCTC TTTACGCTGCTG ACTACTTTC AAGACGACTACCTGTAATAG
541  GACCCGACCC AAGTGGTGGGTC AAGCTTCTATTCTGCCGCTTCTGAACTCCAA
601  TGAATTTGTTGCTACGTTCACG AGGCGGCTTCCGAGGAAGGGTA GAGGATGATTTCCGCTTCTCT
661  ATACGGCGCTC TTTACGCTGCTG ACTACTTTC AAGACGACTACCTGTAATAG
721  TGAATTTGTTGCTACGTTCACG AGGCGGCTTCCGAGGAAGGGTA GAGGATGATTTCCGCTTCTCT
781  ATACGGCGCTC TTTACGCTGCTG ACTACTTTC AAGACGACTACCTGTAATAG
841  ATACGGCGCTC TTTACGCTGCTG ACTACTTTC AAGACGACTACCTGTAATAG
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9.7.9: EST Clone Library Identifier C01417.

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61  ACAAAGTGCC AATAATGGCG ATGATGCTGCTG ATGGTCCAAC TACGCCGGAT GCTGCTCTCA
121  TCAAGGGCTG CTCCGACCGCT TGCCCTCAAC TACGCCGGAT GCTGCTCTCA
181  TGAATTTGTTGCTACGTTCACG AGGCGGCTTCCGAGGAAGGGTA GAGGATGATTTCCGCTTCTCT
241  TGAATTTGTTGCTACGTTCACG AGGCGGCTTCCGAGGAAGGGTA GAGGATGATTTCCGCTTCTCT
301  ATACGGCGCTC TTTACGCTGCTG ACTACTTTC AAGACGACTACCTGTAATAG
361  ATACGGCGCTC TTTACGCTGCTG ACTACTTTC AAGACGACTACCTGTAATAG
421  ATACGGCGCTC TTTACGCTGCTG ACTACTTTC AAGACGACTACCTGTAATAG
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781  ATACGGCGCTC TTTACGCTGCTG ACTACTTTC AAGACGACTACCTGTAATAG
841  ATACGGCGCTC TTTACGCTGCTG ACTACTTTC AAGACGACTACCTGTAATAG
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9.7.10: EST Clone Library Identifier C01157.

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9.7.11: EST Clone Library Identifier C00148.

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403
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**UniSequence:**

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3. GACCCCTTCA CTGTGCTTGC ATACATTACAT CTGATAACAT TCTTCTCTAC CATACACTCA
4. CGGGCCGCC AATTTTTGCC CAGGTGCCCT CTCCGTCTTC CCAGTCCAGA AAAAACCACC
5. CTGTGTTGCC GACCCATAAA CCTTCT

9.7.13: EST Clone Library Identifier D00471.

**UniSequence:**

1. ATCTCGACTC TTCCAT
2. ATGGCAGCCC CTGCATCTGT TACTATCAAG GATCTTACAG GAAAATGGAC CATGGATAAA
3. AAGTTATCTA CACCACAGA GCTCTTCTTC ACTCTTGCAG GAGTCGGCTG GGTTAAGCGC
4. AAGGCAATCA CTAACGCCAC TATTGCTTTG GATATAAAAC AGTACCTCGA TGCATCTAAC
5. CTAACCCACA TTGATATCAA ACAAACCGCA AGCGGTATCC CAGGAAACAAC CGAAACAAC
6. TCAGACCTCT GATGGAAGAG TTATATTTTT TCTGCCATGG CAAGGAAATA CTATTGCAGG
7. GACCCCTTCA CTGTGCTTGC ATACATTACAT CTGATAACAT TCTTCTCTAC CATACACTCA
8. CGGGCCGCC AATTTTTGCC CAGGTGCCCT CTCCGTCTTC CCAGTCCAGA AAAAACCACC
9. CTGTGTTGCC GACCCATAAA CCTTCT

9.7.14: EST Clone Library Identifier C00506.

**UniSequence:**

1. TCAGACCTCT GATGGAAGAG TTATATTTTT TCTGCCATGG CAAGGAAATA CTATTGCAGG
2. TACCACAGAC GCACCGGAGCA GCATATCTCA CAACCCGGTA GCAGCGGAAG AGGATACGTGA
3. ATCTCGACTC TTCCAT
4. ATGGCAGCCC CTGCATCTGT TACTATCAAG GATCTTACAG GAAAATGGAC CATGGATAAA
5. AAGTTATCTA CACCACAGA GCTCTTCTTC ACTCTTGCAG GAGTCGGCTG GGTTAAGCGC
6. AAGGCAATCA CTAACGCCAC TATTGCTTTG GATATAAAAC AGTACCTCGA TGCATCTAAC
7. CTAACCCACA TTGATATCAA ACAAACCGCA AGCGGTATCC CAGGAAACAAC CGAAACAAC
8. TCAGACCTCT GATGGAAGAG TTATATTTTT TCTGCCATGG CAAGGAAATA CTATTGCAGG
9. GACCCCTTCA CTGTGCTTGC ATACATTACAT CTGATAACAT TCTTCTCTAC CATACACTCA
10. CGGGCCGCC AATTTTTGCC CAGGTGCCCT CTCCGTCTTC CCAGTCCAGA AAAAACCACC
11. CTGTGTTGCC GACCCATAAA CCTTCT

404
9.7.15: EST Clone Library Identifier D00563.

**UniSequence:**

- 1 CCAAATACGGT TACCGTCCTT GGTTAAGGTT GATAACATTC TTTTAGTCGA TCTCTATTTA
- 61 CCACGCATTT AACACCTCTTT GGTTAAGGTT GATAACATTC TTTTAGTCGA TCTCTATTTA
- 121 TTGCGACCAT ATACACTCAT ACCGCGCTTG TTGGTTGTTA ATTTAGGTGA CTCCAACTCG AGTCATATCA CATTTCAAAA TCAGCATGAT
- 181 GAGAAATCCC TTACTATTAT AATAATACAC ATACTTCGAC AGGAGCATCT AGAGCGTTAT
- 241 ATACCCGGATT ATACCGCCCT TTGGTTGTTA ATTTAGGTGA CTCCAACTCG AGTCATATCA CATTTCAAAA TCAGCATGAT
- 301 ACCCGCCATT CTTCTTTCGT CATGTCATGC TCTTT CACAA ACCAAGTTCT CGCACAGATT
- 361 GAGAAGCTAG AAGTGGGTGT ATACGTCCTT CCTAAGATTT TGGATGAAAC TGTAGCTTTA

9.7.16: EST Clone Library Identifier C00606.

**UniSequence:**

- 1 AAACCTCGGA GCTATGACGA CACAACCTTA ATAACTCCAT TTCCGGAGCT AAATTATAAG
- 61 CGTAAATTTCC TTACCACTTT GCACGATCAG TCTCCAGGTCA AATTGCTTGA TGCATATATTG
- 121 CACGCGCTTT ATACATCGGC GAGGACTGGT ACCGCGCTTG TTGGTTGTTA ATTTAGGTGA CTCCAACTCG AGTCATATCA CATTTCAAAA TCAGCATGAT
- 181 CATATGGTCC ATTTAGGTGA CTCCAACTCG AGTCATATCA CATTTCAAAA TCAGCATGAT
- 241 GAGAAGCTAG AAGTGGGTGT ATACGTCCTT CCTAAGATTT TGGATGAAAC TGTAGCTTTA
- 301 ACCCGCCATT CTTCTTTCGT CATGTCATGC TCTTT CACAA ACCAAGTTCT CGCACAGATT
- 361 GCCACCGATGC TTGCTGTTTT GCACGATCAG TCTCCAGGTCA AATTGCTTGA TGCATATATTG

9.7.17: EST Clone Library Identifier D01260.

**UniSequence:**

- 1 GCTCTACAGG GTATGGGTGC TTGGTTGCTTA ATACATCGGC GAGGACTGGT ACCGCGCTTG TTGGTTGTTA ATTTAGGTGA CTCCAACTCG AGTCATATCA CATTTCAAAA TCAGCATGAT
- 61 CAGGCTGCTG AGGTGCCCCT CAATACTCCAT TTCCGGAGCT AAATTATAAG
- 121 ATCAAGTCATT CTTCTTTCGT CATGTCATGC TCTTT CACAA ACCAAGTTCT CGCACAGATT
- 181 GAGAAGCTAG AAGTGGGTGT ATACGTCCTT CCTAAGATTT TGGATGAAAC TGTAGCTTTA
- 241 GAGAAGCTAG AAGTGGGTGT ATACGTCCTT CCTAAGATTT TGGATGAAAC TGTAGCTTTA
- 301 ACCCGCCATT CTTCTTTCGT CATGTCATGC TCTTT CACAA ACCAAGTTCT CGCACAGATT
- 361 CAGAAGCTAG AAGTGGGTGT ATACATCGGC GAGGACTGGT ACCGCGCTTG TTGGTTGTTA ATTTAGGTGA CTCCAACTCG AGTCATATCA CATTTCAAAA TCAGCATGAT

405
9.7.18: EST Clone Library Identifier D00403.

**UniSequence:**

1 CACGATCAGG CATTTTCTAA TAACACGGGG TTCTTGGCTT CACATATAAT TACTTACATA

61 CACACCTTGA CCGTGAACAT GCACCATAC TGCTCCTTAAG AACCCTGGCAT TACCTCCTTT

121 GMCGGSMSS SMSMTTGGCA GTCAAGCCA TTGGGTCTGGC CCAGGGGTC GTCACTCAAA

181 CTCAATTCGCC TCATATCGCC ACGGTATGTG TCAATTCATC CCAGAGATTA GCACCTCCCT

241 CTGCGCCCAC TATCCGCAAGG TTTCGATTAC GTCAACTGC ATATGATATA GATATGACTA

301 AATGGCAGCT CAGGACTACT AGGAAATGGG ATGCTCTCCG ATGCACTCAT CACGGAGAAG

361 ATGTTGTTGTT GGAGCAATAT TTCCGACCTC CATACACCAT CTATTACCCA TATCCATTGA

421 GCCCAATTTT GCCAGCTTCC GGGGCTGGCC TGAAGTCGCA AGTGGCAAAT ACTAGGAGAAG

481 AGCTGTGCAG ATGATCTGAA TGCTGAAATGG ATATGGCATG CATAGAAGA

541 TATGCTTCTTCC TATGCTTCGC CAGTACGCTA AAAGATGATG CATAGAAGA

601 CATAGAATG GCAGTTCTCT GGTGCAATGG CAGGACTCTG CAGGACTCAT CAGGACTCAT

661 CACAGCTGGA GTATTTTCTAA TAACACGGGG TTCTTGGCTT CACATATAAT TACTTACATA

721 TATTATCCCT AGGTCTCAGT TAATCCCTAA AAAGATGATG CATAGAAGA

781 TTTTCTAA TAACACGGGG TTCTTGGCTT CACATATAAT TACTTACATA

841 TTTTCTAA TAACACGGGG TTCTTGGCTT CACATATAAT TACTTACATA

ACGAA
9.8: Protein Sequences of Genes Exhibiting Significant Effects to Stimuli.

9.8.1: EST Clone Library Identifier C01417.

**Genome annotation:** >bgh01761_mRNA

**Amino acid sequence:** 148 residues

MFARYLVLAFLTVAIAAFNINLGAAYSPALVGGGEISFGGAEGEASAE
GIFNTLQSGTTGVAAGEAKGSAKGDDLTVDKKAAGKEATKVKAAAEK
RAEKDAMAGFTAAALNYADAALNKGPIIDLJTGGGGSGVGTVKPGVVA

**Predicted weight:** 14.5 kilodaltons

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9.8.2: EST Clone Library Identifier C00482.

**Genome annotation:** >bgh00086_mRNA

**Amino acid sequence:** 426 residues

MLYPRALLPAAVALASLVLAVPLEERQLAFDFNNQKVGRVNLGGWFVLPE
WITPSIFQWANGGVIDEYSYTAALGKDEAFTPRLNNHWAITEEDFAE
IASMGINHRIPIFYWALVAIPNDFYQQQLSYVDRUIDARKNGLKVML
DLHGAPGSQNGFNSGRTGTIAWQSGDNVPNTRAIQLAERYAPQTDVV
TAIELNELATWGNLQI7KFKYDGGWNGVRTQGQTAVTIHDAFLDPRSW
NGFMNSEXAVVNVLIDTHYQVFISNEVAMKPCAHQVTACSSIDKIKPTD
KWTIVEGWTAGQTDCAKMLNGLGGARYGDTGFLGHEGSGYGYSGCDKXYGT
VDSMLPVDTNLQYFVEAQLDAYESETGWFFWTWKESAPEWHFQNLTRA
GLIPQPPLLDSRKVFSQCGTSQCLVPGN

**Predicted weight:** 47.45 kilodaltons
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9.8.3: EST Clone Library Identifier C00009.

Genome annotation: >bgh05886_mRNA
Amino acid sequence: 217 residues
MQTGAVFVAAALIALSEAARLTNTADQNYVDKAGQFYPYKITTENAAAGPVTI
LKKNGPSTHLQVTSTIASQQTGSYETWTPPSTLTDKAFEITDSGEPNY
SVQFITTGDDPTPMSSQPRAITGTVPVPSGMSSYTSRVSATSTMG
TYPYSGGGYSSGFSNTTPVHVSSSTYMTSTIAASPSSTTVPADANNANGIS
SPLAVFSTFFAFIFLH
Predicted weight: 33.93 kilodaltons
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| TMMHMM   | tmhmm   | transmembrane_regions |
| Residue location | | T[1-18] NA |
| | | T[198-216] NA |

9.8.4: EST Clone Library Identifier D00881.

**Genome annotation:** >bgh02742_mRNA

**Amino acid sequence:** 369 residues

MLSFRQAVGAVLTVYFTTHTHPLVSRSSSRENTEADPNSINITA
LPTPITTDGKAASQKLLIDMSQAPLREKAYQFYDVEASFPAYWQT
WKFTPASGDFDEKRASEAWSKEHPTTFHVITDQVAHVHLIRHLASVP
EIILTAYNLPVPVLKADFFRYLLILLARGIGYSDTQALKSAEVWLPESV
PKKSIGLIFGIEADPDREDASWRSRLQFQWTILQSKPGHFILREVVAN
ITLTLQKSKDGSLNSRSVIEFTGPGDVTDFGMNDRSDGFDST
NGPITNWKEFTGISTKIGDGVVLPITFSFPGIHQMAGEYDSSALVHK
GFEGTWKPEDERPHIGIIE

**Predicted weight:** 41.38 kilodaltons

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| | | |

409
9.8.5: EST Clone Library Identifier D00944.

**Genome annotation:** >bgh00413.mRNA  
**Amino acid sequence:** 330 residues  
MVCLALLLYTGETAQLKDFRVTIFSEDNEPSYQSYEVNYQKLFPVDPDK  
SGIHSTSVKINGFGTLYTVCISKVS1KDLQEFVSDKLTAQVGLRGSF  
NDQGAEDECFNHVNLDRERTAIAKGHIQEIQAPEPDSFPVSNLIQSDQ  
CTKRLISLAPQRRLCLNGFGLRTLYR DKDLPTIKFDEPMIEATVLDS  
QMIRKTVKMKEMALGWNQKLGQVFSREVGNHWWQEPHSHEEPGLGWIS  
EFVRKGNAAVQRDFVNKDTRYHKEYASEWIGVTRTVSHGLETYRFRRH  
KLHVKLRKFSPTADGYLDVVLKRLKFGIY  
**Predicted weight:** 38.19 kilodaltons

**Interpro scan results:**

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9.8.6: EST Clone Library Identifier D00658.

**Genome annotation:** >bghT007426000001001  
**Amino acid sequence:** 123 residues  
GGYDVTSFYASGGGGSWSGDLKRLISRGANLFADTVLPRGVSRLTGSF  
RLYKYVVLK1ESTESKGYTFQMEMMVRAKAMYGVTAEVFTFDVRLYG  
ESKLGGSDILEYAOKGVLNSIKV  
**Predicted weight:** 19.08 kilodaltons

**Interpro scan results:**

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**410**
Paralogs to Protein sequence associated with EST D00658:

Genome annotation: >bghT008100000001001
Amino acid sequence: 228 residues
MKVTKDKYSVILPTYRNLPIITWLNHELIIIVDGGSPDTQIVAKQ
LVKYAPHVHLKPRAGKLGLGAYVHGQAQFGNGVIIMADFSHHPKFI
APMIAKQKTNFSGGYDITGTRYASGGVGSGDRLKRKISRGRNLFADTD
VLARPGVSDBLTHGSRLKYAVLKLKIIESTESKGYTFQMEMMVRAKAMGYTV
AEVPITFVDRLYGESKLGDEILEYAKG
Predicted weight: 25.28 kilodaltons

Interpro scan results:

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Genome annotation: >bgh00800_mRNA
Amino acid sequence: 210 residues
MNLDWELIIIVDGGSPDTQIVAKQLVKVYPAPHVHLKPRAGKLGKLGTAYVH
GLQFGNGVIIMADFSHHPKFIAPMIAKKTNFSGGYDITGTRYASGGVGSGDRLKRKISRGRNLFADTD
VLARPGVSDBLTHGSRLKYAVLKLKIIESTESKGYTFQMEMMVRAKAMGYTVAEVPITFVDRLYGESKLGDEILEYAKG
KGVLSLIWKV
Predicted weight: 23.05 kilodaltons

Interpro scan results:
**InterProScan (version: 4.2)**

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**Genome annotation:**

- >bgh00799 mRNA
- Amino acid sequence: 144 residues
- MSHHPKPAIPMAAIKPITSPGGYDIVTCTYASGGGSGLKLRKLISR
- GANLFAIYTLRPVRSDLTGSFRYYKAYVULKIIESTSKUGTFQMEMMVR
- AKAMGYTVAEVPITPVDRYGESKGDEILEYAKGVLSLWIKV

**Predicted weight:** 15.99 kilodaltons

**Interpro scan results:**

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The following protein sequences were generated by inserting the EST sequence into the ‘ORF Predictor software’ hosted on: http://proteomics.ysu.edu/tools/OrfPredictor.html. No hits were found when running the sequence on InterPro Scan.

9.8.7: EST Clone Library Identifier D00189.

**Amino acid sequence:** 80 residues
MSDATLWTSTAPVNFSFSPKATDEPTLRHLVHGRLNVRGPFASHDEIENW
RSIDNSLSTGMIQGPVSWMNMTIHSMVF

9.8.8: EST Clone Library Identifier C00606.

**Amino acid sequence:** 56 residues
MVHLGDSNSSHITFQNQHDEKSLTITIHILRQEHLYRIDSVTLVLGPDKYP

9.9: Expression of RT-qPCR Reference Gene

*(Relative expression of EST D00403 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)*

(The author declares that the graph contained herein is based on data collected by Dr. Maike Paramor).
9.10: Appendix D: Chapter 5 (Towards Agrobacterium-mediated Transformation)

(1) The complete DNA sequence for binary vector pCAMBIA-0380 (backbone of the Agrobacterium-mediated transformation vectors used in this study)

(NCBI Accession: AF234290). Features present include: nos (nopaline synthase) 3'-UTR (polyA signal) (61-313 bp); right border T-DNA repeat (351-376 bp); STA region from pVS1 plasmid (1417-2417 bp); replication origin from pVS1 (3010-4010 bp); bom site from pBR322 (4420-4680 bp); pBR322 origin of replication (4820-5100 bp); aadA (Kanamycin resistance) gene amplified from pIG121Hm (5391-6185 bp); left border T-DNA repeat from C58 (6610-6635 bp); pUC8 MCS (6737-6772 bp). T-DNA borders are highlighted in green. Kanamycin cassette is highlighted in yellow.

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181  acgttattta tgagatgggt ttttatgatt agagtcccgc aattatacat ttaatacgcg
241  atagaaaaca aaatatagcg cgcaaactag gataaattat cgcgcgcggt gtcatctatg
301  ttactagatc gggaattata ctatcagtgt ttgacaggat atattggcgg gtaaacctaa
361  gagaaaagag cgtttattag aataacggat aatttaaagg gcgtgaaaag gtttatccgt
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481  ccaaccccctc cgctgctata gtcgagtccg cttctgacgt tcagtgcagc ctgcttctga
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cgatgatca ataacaaat acgcaagggg aacgcatgaa ggttatcgct gtacttaacc
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cggatggagag ctggcgctgc tgtccgagcc ccttggggtc ggttctgtta gatgtgccgc
gagtacggtaga gctgctgcggc gccgctgccg tcgccgtgcg ccgcgccgc ccgccgacg
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2581 atcaagaaca accaggcacc gacgcgcggtt aatgcgcctat ttaatggagga aaggggcgggtt
2641 ggccaggctgt aagcggtctgg gttgtctgccc ggcctgtcaaa tggcactggg acccacaagc
2701 ccgagaatc ggcgtgacgg tccgaaccca tccggcccggt tacaactcgg cgccggcgtggt
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3001 gacgagcgtc ttcgctgtct atccgagcgt tgggacaggt cgcagcggctg gctcagcctcggcagaatcc
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3301 tttacacca cgcacggtgc catgcagcgt acgaagaagc ccaagaacgg ccgactgtggc
3361 acggtatccg agggtagacg cttgattgcg ccgtcagaaga tcgttaagag ccgaaccggg
3418 cggccggagt acatcagat cagagctagct gattggatgt accgcagagat cacagaaggc
3481 aagaacccgg acgtgctgac gtttcacccc gattactttt tgatcgatcc cggcatcggc
3541 cgttttctct accgccttgg acgcgccgccc gcaggcaagg cagaagccag atggttgttc
3601 aagacgtatct acgaacgcag tggcaagcgc ggagagttca aagacgtatct tttcacccttg
3661 cgcaagctga tcgggtcaaa tgacctgccg gagatcgatt tgaaggagga gcgggggagc
3721 gctggccgga tcctagctct gcgtacagag aacgtagctcg gggcaagttcc atccgccggt
3781 tcctaatgta cggacagat gcataggggcc attgcctctag caggggaaaa aggtcgaanaa
3841 ggtctctttcc ctgtgataag cacgcataat gggaaaccce aacgcataac cgggaacccgg
3901 aaccgtaca ttgggaaccc aaacgcctac attgggaacc ggtaacacat tgaagtgact
3961 gatataaaag agaaaaaaagg cagatattttt gcctaaact ctttaaact tattaact
4021 cttaaaaccc gccttgccagtc tgacaacttg ttggccagcc gcacagcccc agaagtgcnaa
4081 aacgcgccta cccctcgggt cgctgctgct caacgcctcc cggcttgcgg ccgctccttc
4141 gcgcgctggt gcgcgcdead aatggctggc ctacgccccg gcaatctacc agggcgcgga
4201 caacgcgcgc cgctgccact cgacgccccg cgccacactc aaggccacct gctccgagcg
4261 ttccggtgat gacggtgaac acctgagaca ctgcagctc ccggagacgg tcacagcttg
4321 tctgtaagcg gatgccccag gcagacaagc cgctcagggc gcgtcagcgg gcgtggtgccgg

418
4381  gtgtcggggc gcagccatga cccagtcacg tagcgatagc ggagtgtata ctggcttaac
4441  tatgcccgtat cagagcagat tgtactgaga tgtcaccata tgcgggttgta aataccgcac
4501  agatgctgtaa ggagaaata cccgacacgg ccgcctttccg ctccctctgc cactgacactc
gttctcgct  ggcggcgacg gtatcagctc actcaaaggc ggtatacccg
4561  ctggttcggg tgggctggct gcggcgagcg gtatcagccgt actcaaaggc ggtatacccg
4621  ttatccacag aatcagggga taacgcagga aagaacatgt gacgaaagcc ccagcaaaag
4681  ggcgagaccc gttaaaaggc cgcgttgcgg gtgttttttc ataggctcgc cccccctgac
4741  gagcattcaca aaaatcagcc ctcaagctcaagagttgagcgaa accgcagaccg actataaaaga
4801  taccaggcgt ttccccctgg gaagctccctc gtgcgccttc ctgttccgac cctgccaacctcgtc
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4921  ttgatgttct caatttcggt gtaggtcgttc cggcttaacc tggaactatc ttgtttggagt ccggccccgtc
4981  cccgccagc ccgacccgctg cgcccttatcc ggtaactatc gttcttgagtc caacccgaga
5041  acacacgagt tatacgccact ggcagccagcc actggtaaca ggtattagcag acgcgggtgaggtat
5101  gttagggggtgt ggtcaagggc tgagccatact acggctacac tagaaggaca
gttaccttcg gaaaaagagt tggtagctct
5161  gttattggta tctgctcttc gttgaagctg tggacctatac acggctacac tagaaggaca
gttaccttcg gaaaaagagt tggtagctct
5221  ggtttaagagat ctcagggcagcc ggtgtggatct ttggtgaaaaagagt tggtagctct
gttaccttcg gaaaaagagt tggtagctct
5281  acgcgcagaa aaaaaagatc tcaagaagat cctttgatct tttctacgaa gcagctcagcgt
6301 cagctttttc aaagttgttt tcaaagtggg cgtataacat agtatcagcg gacccgattt
6361 tgaaaccgcg gtgatcacag gcagcaacgc tctgctatcg ttacaatcaa catgctaccc
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6481 cggttaacatg agcaaaagtct gccgcctttc aacggctctt ccgctgacgc cgtcccgac
6541 tgtatgggtg cctgtatcga gtgggtatat tgtgcttgagc tcgcgcttgg ggaatgttg
6601 gcgtgctgg gccaggatat tttgtgtgtt aaactt aatttg acgttagac aacttaataaa
6661 cacattgcgg acgttttaaa tgtactgaat taacgccgaa ttaattccta ggccaccatg
6721 ttggcccggg cgcgcgggat tcccggggttg ccgctgacct gcagccaaag ttaagagga
6781 gtccaccatg gtatgtctga ctagtgttaa cg
(2) DNA sequence of Blumeria graminis f. sp. hordei aqu1 gene (encoding an aquaporin), exons 1-4

(NCBI ACCESSION: AJ544064). Numbers represent nucleotide position. Amplified terminator region is shown underlined and in Italics. Primer binding sites are shown highlighted (Blue for amplification primers, Yellow for sequencing primers) and in bold.

1  aacataatac tctgtgatcc aaatatcgtt taactgaag cccgccgtca atattgacct
61  aaacgctttt cgggggaatc cgggtcatatc ctgcttcca ttttgccttc ttttctccca
121  tctatctct gcgggccctg catgggtgag tagggggaa aggtatacgc gtacagtaat
181  gcagtttaaga tgacagtcc ctctcactaa acagagttta caagctacca catgagggcc
241  aggcacctac cctatataga ccaatccctc cagtctctgt ctataaaaa cgtcagcgtc
301  tgtagccacc tctatttcaa ctctcaggt ctcaagtttg acaaatctcg tcaactctctg
361  tctgccttac tggtggaatt ccaacctcat cgggacaatc tctctcacta aaacctcgtgta
421  aatacgaaag cgcacttagt gatgacaact tccgaaacca tgtcatagca gccatcgctgc
481  agttcctcgg gaccaccttg ttcctctttt atggattctct agcagcacag attatcaaca
541  gcggacccct caaatctccct gacgtcctca tgtactcctca gtaaatattc gttgcttctag
601  ccttggggtt cagtgagcgtctcag tctatttctta cgggtcagt ggaggccatt
661  taaacctgcg agtcggtgtgctac tcatccctaa accagagata tctatattgctgtagtac
actatcggct ttactctaat aggagctgta ccaagtcaact cagctttact tctcgtatcc
gttcagctac ttggttgtat attagctgca tctctagtat tagccggtta cccaggtct
cataatgttc agaccgcttt gggcaatgca acatccgtga agcaaggttt ctttttgag
tagattctca ctgccacccc ttgtttgaca gtgttttatgc tcgctgtaga gaaacaccgt
accactcctc tagacaccttt aggaatcgtt ctcattcttt tcctcgatgt cctcttaggt
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tggcggctca ttaaacccag cacgatccttt tggttcagct gtggctgaat tgaacttttc
gacataccat tggattttact gttccgacc aatcgccaggt gcagcttagg caggccgctat
cctcaagtt ctaaagtttc ttgcatacga gactgcaggt gctggacagg accatgatgg
actagacgctc taccggccta tggatgttat gtatccaga atcttttagt aacttaacta
aggtgttccat cgtttacaga tgcgggtggt gtgaagctcg atcgggaaat tcaacttgaat
tagaatcgtt aacgcagact aacgacataa tgcgatgatg cagcggagtta
tggtaaaac aacgcagact aacgacataa tgcgatgatg cagcggagtta
tggtaaaac aacgcagact aacgacataa tgcgatgatg cagcggagtta
tggtaaaac aacgcagact aacgacataa tgcgatgatg cagcggagtta
tggtaaaac aacgcagact aacgacataa tgcgatgatg cagcggagtta

Taq-For

Taq-Seq

agatctagaa atgtttaggg tatttagagc actaaaatgc tacatctata tgatcgcgg
agatctagaa atgtttaggg tatttagagc actaaaatgc tacatctata tgatcgcgg
agatctagaa atgtttaggg tatttagagc actaaaatgc tacatctata tgatcgcgg
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agatctagaa atgtttaggg tatttagagc actaaaatgc tacatctata tgatcgcgg
agatctagaa atgtttaggg tatttagagc actaaaatgc tacatctata tgatcgcgg
1681  taacctatg  aacttctaac  tgtcactatc  cacccaaaagg  tcgagataaa  gatattcttt

<--- Taq-Seq2

1741  cgtcatcttt  tcggtgaggt  cttcaaact  ctcaacgcagt  ttatgtctcc  gtaatatatg

1801  atcatagcct  atttgccccca  taaatatgag  gctaacgtct  caggttaaat  gttgtgtaga
<--- Taq-Rev

1861  agcccaact  aataatcatt  tccttgctct  tagagtgca
DNA sequence of Blumeria graminis f. sp. hordei H3 Histone Promoter, isolate CC146

attatgacgt tctttgtgact atttttggtc atatgcgtgc ttagactctg gg acgtattaca

agtctcgttg aaacaacaa catttacgcg acgtaggc gc aacaadccaa taggagtaaa

attgtgttag accttggcct gtcggcgtcag acatggagtc ctagagcgtat

tgaaagcgg atggccttaa ggcgcgtac agttgctgcc atcaattgcttg ttcgacgcat

gacaagcgtg tgcggcgc tggcggatct cagatcagc atcctctcttgcct taccatcgttc

cgtcgtccac aacctctatc actctatgcct tggaggaatca gatcgttcgtgct gtcgcggtc

gagatttgta gtctttgtac gtcggctgcag atctttcag gactacatcgt taccccttgac

cacataggtgc tgaagttactc aatcttttaa cttgagttatc atctgcacctg cccggtcatg

taatgcggtc catcttgccct agctctttactg atcttagctg gatcgttccttg gacaagcgca

gccttgcctgc acccaataca cggcggtgctt cttgagttactc atcttttaag atccccctgt

gttggttgtcg aaacttgga cttgagttactc atcttttaag atccccctgt

ttgaggtgcgcc cagcgtctac tggactcatc ttttgccctt gatacttatt tagaatcttattagct
1141 agtatgtaat caggggcccg ctcctattgc attcaaatac accggttaac ccagcctcac
1201 tcgcatacct caccacacac tcctctcttc cggctctcca tcaaatctca tcaaaacaca
1261 catataaccg catcaaaacc tcaccaaagtc agt gcctgaacca agcaaaccgc gcgcaaggta
1321 agtcataacaa tatatgtctgt gttcaccaaac ccctagcgtc tcacagtctc actagagtcgtcagagagcct
1441 ttactaataat acctccacgc tcgttaaagg gcaccctcaca ctggaggttgta aagaagaaccttgcagagagcct
1501 cacagtaagt gtatgtagaag tcaccttaataat ctcaagtcgc ggtacgctgta ctatgcagagagcct
1561 gatacaagcc cggtactgtt gccctccgag aaattcgctg ttaccagaaaa tccacccgaac
1621 tttaggtcgtg aaactctccc ttcacgctgc ttgtgtagtga aatgtcgcca gattttaaat
1681 ccgacccaag atttcagtcgc tcaagccgapc gacgcctcctca ggaatcggta gaggcctacc
1741 tcgcattacact attcgaggac acaa

(4) DNA sequence of eGFP

1 ATGGTAGAGA TGCTGCCAAC TGTCGCTGTG CTGGTCTTGG CAGTGTCCGT
51 GGTTGCCAAG GATAAACACCA CGCTGCAGGA ATTCGCCACC ATGGTGAGCA
101 AGGGCGAGGA GCTGTTCACC GGGGTGGTGAC CCATCCTGGT CGAGCTGGAC
151 GGCACGCTAA ACGGCAACAA GTTCACCGTG TCGGGCAGGG GCGAGGGCGA
201 TGCCACCTAC GCCAACGCGA CCCTGAAGTTC CATCTGGACC ACCCGACAAGC
251 TGCCCGTGCC CCCCGCCCGC CTCGTGACCA CCCTGACCTA CGGCGTGCA
301 TGCTTCAGCC GCATACCGGA CCACATGAAGTG CAGCACGACT TCTTCAAGAC
351 CGCCATGCCC GAAGGCTACCG TCCAGGAGCG CACCATCTTC TTCAAGGACG
401 ACGGCAACTA CAAGACCCGC GCCGAGGTGA AGTTCGAGGG CGACCGTCCG
451 GTGACGGCGCA TGCGACTGAA GGGCATCGAC TTCAAGGACG ACGGCAACAT
501 CTGGGGGCAC AAGGCTGAGT ACAACTACAA CAGCCACAAC GTCTATATCA
551 TGGCCGCAAC ACGGAAAGAC GCACCGGAC GAGTGAATTGA TGACTTTGCA GCACCGCCAC
(5) **Plasmid isolation solutions**

**2X Cracking Solution:**
0.2 N NaOH,  
0.5 % SDS,  
20 % Sucrose

**Loading Buffer:**
0.4M KCl  
1/6th volume of Loading Dye
(6) DNA sequence of Blumeria graminis f. sp. hordei CYP51 gene (encoding an eburicol 14α-demethylase), exons 1-3, isolate CC146

(NCBI accession number: AJ313155). Numbers represent nucleotide position. CYP51 sequencing primer binding sites are shown highlighted and in bold.

1   atgggaatat cagaaagctt tatttttcca taacttgcaac ctttgctcca actcggtttt
Primer cc146Seq1B

61 ggcatggcgt tggctagtgga aattttaaagt ttatatttac tggtaacctt cttgaacgta
Primer 146Seq2

121 ttgaagcagt tacttttcaa gaatccctga gtatcctttca tggattcct

181 atcattggaa gtacattttc atatggaagt aatcccttaca aatcttttca tgaatccca

241 gcxaaggtcag tttgccccacg cactctctctg aggaatacga tattaattga ggttaaagtac

301 ggaatatctc taacttgtggt aagaagacga cggatatatc agtcgcacag
Primer 146Seq3

361 ggaataatttt ttttctttcag tggaaactc agagacgtta atggccgaaga aatttatacg

421 gctttgcacat ctcctgtcttt cgggactgat gtaggttttg actgtcctaa ttgattaacttt

481 atggaaacag agaaggttttt aataatcatg gataactttt cagaactcaga ctctgtactt

541 tgcacagtactgagaagcgactc cctctgctttg cgggtgtcctt acgctcttatg tacctatcat

601 cccaaatgaa gtgaaaagcattc tatcgaaaa atgcgacgat tttcgaaaat caaaaggtat

661 catcaatatc catgcaagaa actgcttcac acacccctac
Primer 146Seq4

721 agggaagggaa gtccgcaaat ctatatattttt ggttatttttgact taccgtata
781 tatgggcttc accccaatca atttcagtct tcactgggca ccaccttccgc acaatcgagc
841 tcgtgatcat gcccaacgga cagtgcgaaa gatatacatg gagattatca acagccggtcg
901 gcgcagaaaa gaaactgatg attccaatatt agatataatg tggcaattaa tgcgctcttc
961 ctacaaagat ggcacgcccqg taccggataa agagattgca cacatgtatg tcgcgctcttc
1021 gatggctgag caacattctt cgtcgctact cagcacaatgg atcatgctgt ggcttgctgc
1081 tcgaccagac atcatctgaag aactctacca agaacaatga gaattatgg gtcgaagatt
1141 accccttcttc aaatatgaag atctctcgaa actttctttt catcaaaacg tattgaaaga
1201 ggtttctcctgt ctgca tcggtgctcct
1261 cgttccagga actagttatg taatactcaa gaccaatccc ctcttggcgg cccctgggtg
1321 gcgcagtcga gacgcctcat acttccccaa tccgcttaag tgggatccac atcgttggga
1381 cacgatgctc gagcgcctcat acctcccaaa tccgcttaag tgggatccac atcgttggga
1441 tcgcttgcgt cgggctttta taggcacgga tagggaggtg gaaaaattcg attatgggtta
1501 ctgcatagcc gazcagtttg gacgcaatgg cacatcctcgc tttggggccg gacggcatcg
1561 cagtttcaag tttcacaacc ttgacggaag gaatagcgtt gccgaaacgg attactcaag
1621 tatgtttttc cggccaatgg cacctgcac aatgcattg gagaagaggg aaaaaagga
1681 aacggagttg taa

Primer 146Seq5
1201 ggtttctcctgt ctgca tcggtgctcct
Primer 146Seq6
(7) *Agrobacterium tumefaciens* growth media and solutions

**Induction Medium Requires:**
K$_2$HPO$_4$: 3H$_2$O : 3H$_2$O/KH$_2$PO$_4$ (filter sterilise 1 M stock solution)
MgSO$_4$ (Filter sterilise 1 M stock solution)
MES’ (2-N-Morpholino ethanesulfonic acid) 400 mM Stock Solution

For approx 1 Litre:
NACl- 0.08 g
CaCl$_2$:2H$_2$O- 0.038 g
FeSO$_4$- 0.001 g
(NH$_4$)$_2$SO$_4$- 0.2675 g
Glucose- 0.963 g
(535 ml H$_2$O)

Add 60 ml 400 mM MES

Autoclave before adding 1.2 ml 100 mM acetosyringone (final concentration 200 μM)

1.2 ml 1 M MgSO$_4$
5 ml 1M K$_2$HPO$_4$: 3H$_2$O : 3H$_2$O/KH$_2$PO$_4$

**Minimal Media (1 litre).** 50 ml 20x Nitrate Salts, 10 g Sucrose, 1 ml Trace Elements, Fill to 1 litre with ddH$_2$O, 1 ml 1000x Vitamin Solution before use.
- **Conidial Extraction Solutions**

**Solution A**
0.35 M Sorbitol
0.1 M Tris pH 7.5
5 mM EDTA (ethylenediaminetraacetic acid)

**Solution B**
0.2 M Tris pH 7.5
50 mM EDTA (ethylenediaminetraacetic acid)
2 M NaCl

2 % CTAB (Cetyltrimethylammonium bromide)
Appendix E: Chapter 6 (CMEG 5'-regulatory region driven GFP expression in *M. oryzae*)

(1) Vector Sequences from pMJK27.2

**DNA sequence of the MPG1 promoter**

```plaintext
1 AACAAAAAGCT GGAGCTTACC CGCGTGGGGA GCTCTCCCAT ATGGTGGACC
51 TGCAGACGCA TGGTGGTGGA CAACCCACGC AGGCGCCGCC AACCTCAGTG
101 GATGACAGCC TCAATTCGTCGCC TCAATTCGCA ATGGGGGTAT GGGTGGATCG
151 GAATTCATGG ATCTGCTACG TGGGGTCTTT TAATTTGCTCA TCCACCGCCT
201 CGGAGGTGAA TGCAGGGGCC CTGCCACCTTC ATCTGCTGCA ATCTGCTGCA
251 TTGGAAACTG TATGACAGGT TATCAGACCA GGGGGGATGG CATCGCAGAT
301 AAGCGACAGCC TGGGATGAAA GGAGAGGAAA AAAAAAACAA AAAAAACACC
351 CCGAGCCAGG TATGACAGGT TATCAGACCA GGGGGGATGG CATCGCAGAT
401 GGGGTTGATG ATGGTTGGAAC AAAAAATCCCT ATGGGATTAG TCTGCTGCTG
451 GCCAAACAGG CAACCCCTGCA TCCCTGGAAA AAAGCTGCTC TCTCCCTGGG
501 TGCCGAGGCC CAGGCGAGGT CCCCTCAGAG ATGGTTGGAAC AAAAAATCCCT
551 CGATCGAGCC GCAGATGCGG GGGGTTGATG ATGGTTGGAAC AAAAAATCCCT
601 GAGGAGGTGAA TGCAGGGGCC CTGCCACCTTC ATCTGCTGCA ATCTGCTGCA
651 ATGACCGGCA AAACCGAAGG ACTCCACACT GTCACTTCATT TTAATTTTTT
701 TCTGCTGCTG TCTGCTGCTG TCTGCTGCTG TCTGCTGCTG TCTGCTGCTG
751 AAATATGGAT TCTGCAATTG TATGGGACAG TCTGCTGCTG TCTGCTGCTG
801 gg
```

**DNA sequence of the sGFP Gene.**

```plaintext
1 ATGGTGAGCA AGGGCGAGGA GCTGTTCACC GGGGTGGTGC CCATCCTGGT
51 CGAGCTTACC GGGCGAGGAA AGGAGCTGGCG ACATTTGCCAC GGGGTTGATG
101 GGCGAGGTGAA TGGGATGAAA GGAGAGGAAA AAAAAATCCCT ATGGGATTAG
151 ACCGGCAAGG TTGGCCTGCC CTGCCACCTTC ATCTGCTGCA ATCTGCTGCA
201 CGGAGGTGAA TGCAGGGGCC CTGCCACCTTC ATCTGCTGCA ATCTGCTGCA
251 TCTGCTGCTG TCTGCTGCTG TCTGCTGCTG TCTGCTGCTG TCTGCTGCTG
301 TTCAAGGAGC AGGGCGAGGA GCTGTTCACC GGGGTGGTGC CCATCCTGGT
351 CGACACCCCTG TGGGGGCCAAG GGCCAGCTGG GGGGTTGATG ATGGTTGGAAC
401 ACGGGCAAATG TGGGCTGGAC AAGGTGGGTG ACAACTACAA CAGCCACAAGC
451 GTCTATATATC TTGGGGACCAC ACAAGGAGGA GCTGGGATG ACAACTACAA CAGCCACAAGC
501 GATGCTGCTG CTGGGGGCCAAG GGCCAGCTGG GGGGTTGATG ATGGTTGGAAC
551 AGCAGGTGCT GTCCAGGAGT GCATCCGACG GACGACTGGC GAGACAGCTGG
601 DATGCTGCTG CTGGGGGCCAAG GGCCAGCTGG GGGGTTGATG ATGGTTGGAAC
651 TCAGGTGCTG CTGGGGGCCAAG GGCCAGCTGG GGGGTTGATG ATGGTTGGAAC
701 TGGGAGGAGC AGGAGCTGGCG ACATTTGCCAC GGGGTTGATG ATGGTTGGAAC
```

**DNA sequence of the trpC terminator.**

```plaintext
1 tccacttaacct gtactgaaa ctatcaataa gcttgacgaa tctggatata
```
DNA Sequence of the \textit{hph} (hygromycin) Resistance Gene.

\begin{verbatim}
51   agatcgttgg tgtcgatgct agctccggag ttgagacaaa tggtgttcag
101  gatctcgata agatacgttc atttgtccaa gcagcaaaga gtgccttcta
151  gtgatttaat agctccatgt caacaagaat aaaacgcgtt ttcgggttta
201  cctcttccag atacagctca tctgcaatgc attaatgca tgtactgcaac
251  ctagtaacgc cttncaggct ccggcgaaga gaagaatagc ttagcagagc
301  tattttcatt ttcgggagac gagatcaagc agatcaacgg tcgtcaagag
351  acctacgaga ctgaggaatc cgctcttggc tccacgcgac tatatatttg
401  tctctcaatg tattttgaca tttctacctt atagcttgac
451  tatgaaaatt ccgctaccag cncctgggtt cgcaaagata attgtcatg
501  tctctctttga actctcaacgc atcacagctca tctgcaatgc attaatgca
551  acctcggatat ccgctaccag cncctgggtt cgcaaagata attgtcatg
601  gtgtcctgag tgaatgcctcg tcacccgcgaa gaaacttttt tggtaggttct
651  caagacctgc ctgaaaccga actgcccgct gttctcgagc cggtcgcgga
701  tggcagatggat gttggcttgac gttggcttgac ctgagagctgg gcggatgggtc
751  gcccattcgg accgcaagga atcggtcaat acactacatg gcgtgatttc
801  atatgcgcga ttgctgatcc ccatgtgtat cactggcaaa ctgtgatgggta
851  cgacaccgtc atgtgcgtccg ttcgcgacgc ggtgatgtga ggtgatgtca
901  cggaggcatc cggagctttcg cggccggcag ccggccggcag ccggccggcag
gggaggttct gggaggttct gggaggttct gggaggttct gggaggttct
951  acctcggatat ccgctaccag cncctgggtt cgcaaagata attgtcatg
1001 gcaactgctcc gaggccaaag gaataag
\end{verbatim}

\textbf{(2) Gateway cassette A}

Cassette Schematic:

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{cassette_schematic}
\end{figure}

Reading Frame Cassette A: 1711 bp
\textbf{attr1} site: 4-128 bp
\textbf{Primer 1 site}: 163-185 bp
\textbf{Chloramphenicol resistance gene}: 237-896 bp
\textbf{ccdB gene}: 1238-1543 bp
\textbf{Primer 2}: 1444-1466 bp
**attR2:** 1584-1708 bp

**Sequence:**

1 ATCACAAGTT TGTACAAAA AGCTGAACGA GAAACGTAAA ATGATATAAA
51 TATCAATATA TTAATTAGA TTTTGCATAA AAAACAGACT ACATAATACT
101 GTAACACACA ACATATCCAG TCACTATGGC GGCCGCATTA GGCACCCCAG
151 GCCATATCCT TTATCGACAA GCTAAATCTG TTTGAGTTAG
201 GATCCGTCGA GATTTTCAGG AGCTAAGGAA GCTAAAATGG AGAAAAAAAT
251 CACCTTGTTT ACACCGTTT CCAATGCCTG ACTGAAACGT TTTCATCGCT
301 ATGAAGTTCGG CTGTTAACGT GAAACCCCTT CTATTTCCCC TAAAAGGTTT
351 ATGATGACTGATAT TCACACCCCA ATTTCCGAG GAGACCAAGTT
401 TTGAGGCATT TCAGTCAGGG CTATCGTTCT TTTATCTCAG GACCGTTCAG
451 TGGTATTACG TTGTATTACG TCGTATTACG GCGTACCGGT GCACCCGCGT
501 CTAATGTTTTC TGTCTTTTTT TCATTCCCGC GAGGCTTATC TTTGTCGCT
551 ATGATGACTGATAT TCACACCCCA ATTTCCGAG GAGACCAAGTT
601 ATGATGACTGATAT TCACACCCCA ATTTCCGAG GAGACCAAGTT
651 CACCTTGTTT ACACCGTTT CCAATGCCTG ACTGAAACGT TTTCATCGCT
701 TTATCGATTTA AAGCTGTTGCA ATATGGCAAA CTCTTTTCTG ACGATCCGG
751 ATACACACCA ATATGCTTACG CAAGCCGAGC AGGGTGCTATG GCGGTGTGGC
801 TATATCCTGGCT ATATGCTTACG CAAGCCGAGC AGGGTGCTATG GCGGTGTGGC
851 TATATCCTGGCT ATATGCTTACG CAAGCCGAGC AGGGTGCTATG GCGGTGTGGC
901 TATATCCTGGCT ATATGCTTACG CAAGCCGAGC AGGGTGCTATG GCGGTGTGGC
951 ATGATGACTGATAT TCACACCCCA ATTTCCGAG GAGACCAAGTT
1001 AAAAACAGGTT ATGCTATGAA GCAGCGTATT ACAGTGACAG TTGACACCGA
1051 CAGCTATCAG TTGCTCAGGC CAGTATGAGT GCTAAATCTG TTTGTCGCT
1101 AAGCACAACC ATGGACAGAG TACGCTGCTG TCTCGTGCAG GACGGCGAGC
1151 AAGCTGTTGCA ATATGCTTACG CAAGCCGAGC AGGGTGCTATG GCGGTGTGGC
1201 AAGCTGTTGCA ATATGCTTACG CAAGCCGAGC AGGGTGCTATG GCGGTGTGGC
1251 ATACACACCA ATATGCTTACG CAAGCCGAGC AGGGTGCTATG GCGGTGTGGC
1301 ATGATGACTGATAT TCACACCCCA ATTTCCGAG GAGACCAAGTT
1351 TCTCGTGCAG GACGGCGAGC AGGGTGCTATG GCGGTGTGGC
1401 AATAGCTGTTG CAAGCCGAGC AGGGTGCTATG GCGGTGTGGC
1451 CAGCTTTCATGC TATACTGTTG CAGTATGAGT GCTAAATCTG TTTGTCGCT
1501 AAGCTGTTGCA ATATGCTTACG CAAGCCGAGC AGGGTGCTATG GCGGTGTGGC
1551 CAGCTTTCATGC TATACTGTTG CAGTATGAGT GCTAAATCTG TTTGTCGCT
1601 ATGATGACTGATAT TCACACCCCA ATTTCCGAG GAGACCAAGTT
1651 ATGATGACTGATAT TCACACCCCA ATTTCCGAG GAGACCAAGTT
1701 AAAGTGGGTTA T

9.12: *Blumeria graminis* f. sp. *hordei* DH14 CMEG 5’-regulatory regions

(The author declares that the graphs contained herein are based on data collected by Dr. Maike Paramor).

9.12.1: Promoter D00146 (2)*:

Cloning Primer Forward: 5’-TGATTGCGTAAGCAGCTACC-3’
Cloning Primer Reverse: 5’-CGTGTCACAACCTAGTTATGCATGAAAGACCTTGGAGGA-3’
Possible Gene Function/Identity: retinal short chain dehydrogenase reductase

```
1  TGCTACAGAT TTTGATTCGG TAAGCAGCTA CCTAGCTATG ACACAATTAA
51  AGAGAAAAAG AAGACCAATTC ATGCCCGCGA TTCACTTATC AGGTAACCTT
101  GCGAAGTTAT GGTCACTTCT TCACCTAAGG CAGCTCTACA CCGTAGATAC
151  CCACCCAGGA AAATGTCACA GAAGCCCTGG GCTATCCAGA CATGCC
201  TCAATCCACTG AAATTTTTCC GCGTGAAATG TAGTCTTCGG TGTCTCCTTC
251  CCAAAGTGGAG TAGGTCCCTCA GCTACTCAA ACTATACGAG AAGACACCAC
301  CAGAAACCGG ACCACCACAC CTCTTCATCGT CAACCTATCT TTAATAGTAC
351  AGCTGCACCTC TTGAGGGGGA AGTCTGCCCG TGCACAGAGC TCACATGCC
401  AAACTCTTTTA CGGAATTGTA AAATGAAAGG CCGCTTCTAGT TTTGAGCTAT
451  CCTATACATC ATCTTGTGAA CTTTTCTCTGA ACCCTCTCAG GCCCATCCCA
501  AAGAACCCTGC TCCACACATT TGCGCAGGCG CTTTGCTTCC TGGACAGGCG
551  ATAAATATCTG GCGTGAGGTCA TAGGTCCCTA TGGCAGGATG TGGGCGTC
601  CTTGGAATATG AAGAGTCTCA TCTTATGATG ATGGGTTAGC TTGGAGGTCA
651  AGCTGCACTC TTGAGGGGGA AGTCTGCCCG TGCACAGAGC TCACATGCC
701  ATGTCGATGG CTGGCTAAGC TATGATAGTG TCACGTGCTT GTATCAACTA
```
9.12.2: Promoter C00750 (3):

Cloning Primer Forward: 5'–CATGTCGCACTCAAGACTGAA-3'  
Cloning Primer Reverse: 5’-CGTGTCAACTAGTTATTTTTCGCCATTAACTGTTGGT-3’

Possible Gene Function/Identity: cap20
9.12.3: Promoter C00222 (4):

- **Cloning Primer Forward:** 5’-GGGGATAAGGAACGAGCTTC-3’
- **Cloning Primer Reverse:** 5’-CGTGTCAACTAGTTACAGCCTGTGTGTAACCTATAGAATTT-3’
- **Possible Gene Function/Identity:** unknown

```
 1  AGGAGCACCC  TTTGTTGCGC  TGCATACTGG  TTACGTGATC  GGTCTACAAG
  51 CGAACACGCT  TGGGGATAAG  GAACGAGCTT  CATCCAACGG  TAACATAATA
 101 ATTATATATG  TAACCTTACT  TCTGCATATT  AATGGAGATA  TCTCTCACA
 151 AAATATCITTA  GCTGTCCACG  CGCAAGTGTA  GATTCCCAAA  CCTATAGAGG
 201 GCATGACCCT  CATCATATAT  TGGACAAATAT  TAGATATATC  AATATCTAAA
 251 TTCACTATTATACTTTAAGGC  TATTAGATA  GTTTTGACTG  TCTTCATTG
 301 TCATAGACTCC  GTATAGGACA  ATAAAAATTAA  ATATATATTT  ATCCGAAAGTC
 351 AAATATCTTTG  GTTTCTAGAT  CAGTTCTTGAG  ATTTCTTTGGG  CACAATCTCC
 401 TGCTGTTCAG  TGTTGGCGGC  CTAGTGTAG  TGGTCGCTCT  AGGGTCCGGCT
 451 TTGTCTACACC  CCTATAGAAA  TACCTTTCCT  TCTTCCATTG  TCTTCATTG
 501 CATTTGAGTTT  ATATAGGCTT  TCCTTCTCTCT  TAGGAAAACT  AGAACAATCC
 551 TCATTATTATTT  GTTCTCTATT  ATATATCATC  GACCCATCCC  CGTGAACATT
 601 AGGAGCAGGCG  AGGGGTGGCA  TGATAAGGCC  GACCCATCCC  GAACATCTGG
 651 ACAAGCCAAA  GGAATCTAAA  TACAAAGGA  TTAGTATGAA  ATAGGCTTGG
 701 AGCATGACGG  GATACCCGAA  CATCTCTACT  CCTATAGGCT  GATATCGGTA
 751 AGAGCTAACGC  TCGGCTGAG  TATTAATATC  CCTTACTCTC  CTGTAGCTG
 801 CTATGATCATG  CAGCTGAAGT  CAGGTCTCTGT  CCGGCTACTG  GGTCTTCTT
 851 TCATAGGATA  TATGATATTAT  TCTCTTTCCT  TCTCCTTCCT  GCTCTCCTCT
 901 AACAGTACCG  CCCCTCACA  GCCCTCATTT  CATCATACAC  ACAGGGACAT
 951 GCGTCAAGCA  TTTTTTATCT  TATTGGAAGA  TGGTATCATG  ATCTCATCTGG
1001 TCTTTTGCCC  CTAAGATGCC  CGGACAGACT  CTAATAGATGGA  GATCATGATA
1051 GACCCAAGTT  CAATAATGAA  CTCTGGAAAGG  TGGGCTACT  TGCGAGGCTG
1101 GACAGCTTTGT  GACCCACACA  TTTTTAACC  AGTGCACAAA  GCAAATATGG
1151 TGAGATGCTGT  GATATTATAGG  CAAAAGACAG  GATAAATAA  CACCTTAAACC
1201 CACCCAAAGT  GATTTACGTT  CGTGAACGG  TACATGCTAA  TGATCTAAATT
1251 CCAATGTCGG  TTTTCTTTAT  TATACTTAC  TTTGTTGTTA  TTTAAACAT
1301 ATTTTTGAA  TACCCCTGGG  CCGCATGTTG  GACACAAATT  CTGCTACATTT
1351 TGAGGTAGAT  CTCCTTCTCT  CAAAGAAGCA  TGAAGGCTCT  CATATGCTATG
```

(Relative expression of EST C00222 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)
9.12.4: Promoter D01230 (5):

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<th>Sequence</th>
<th>Description</th>
</tr>
</thead>
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<tr>
<td>1 1401 TGTCGCAAAT GTGCTGTGAT TGCGGCTGTT GGTTTAGAGA AGCGTCAGCC</td>
<td>Promoter D01230 (5): (Relative expression of EST D01230 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)</td>
</tr>
<tr>
<td>2 1451 TGACCTGAGG TGCTAAGGCC AGTGCGAGTT GTATGCTGAG AACTTACATG</td>
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<tr>
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<td>5 1601 CATGTCAGAA AAGAAACGCA GCAGACGGAA AATGTAACAA AATAATCTGT</td>
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<tr>
<td>6 1651 ATCGGTGTTT GGACACTCCA CTCTAGGTGT TTTACAGTGA TGTCTGAGGG</td>
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<td>7 1701 CATGTCAGAA AAGAAACGCA GCAGACGGAA AATGTAACAA AATAATCTGT</td>
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<tr>
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<td>10 1851 ATCGGTGTTT GGACACTCCA CTCTAGGTGT TTTACAGTGA TGTCTGAGGG</td>
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<td>11 1901 ATCGGTGTTT GGACACTCCA CTCTAGGTGT TTTACAGTGA TGTCTGAGGG</td>
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<tr>
<td>18 2251 ATCGGTGTTT GGACACTCCA CTCTAGGTGT TTTACAGTGA TGTCTGAGGG</td>
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</table>

**Cloning Promoter Forward:** 5’-TCGTATAGTATTTACAGATTTCTCAGCA-3’

**Cloning Promoter Reverse:** 5’-CGTGCAACTAGTAGTTATGCAGGTGAGATGAAAGGATG-3’

**Possible Gene Function/Identity:** ATP synthase protein 9, mitochondrial precursor
9.12.5: Promoter C00082 (6):

Cloning Promoter Forward: 5'-CCTGTGGAGCCACTGTTACC-3'
Cloning Promoter Reverse: 5'-TTGAAATGCTTTGTGCTTACTCCACCACGAC-3'
**Possible Gene Function/Identity:** gamma subunit of the F1 sector of mitochondrial F1F0 ATP synthase

```
1  CTACCTGGGG TGGTGCCTTG AAAAGCTTCA AGTCTTTAAA ACCTGTGGAG
51  CACAGTGTAC CTACATAA AAATCTAATGG AGTCTTCTTT TCGTATCCGG
101  AAAATAACTG TATTGGGCCG TTAAAGATC AACAGTTTCA ATTTGCAGTG
151  AGCCGTATCC AGAGGTACCG TGTGAATCCG CCGATATCGC
201  ATCAGATAAG TGTTTCAATT CGATGTATTT TTTGAGTATG
251  TTTGTCCTTT AATACCTGCTT TGGCAGGCAA GCATGACATA CTGGTAGCA
301  CCTGAGATAT TTCTTCTTCA GAAATACTG TATTTCGAGG AGGATGATGC
351  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
401  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
451  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
501  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
551  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
601  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
651  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
701  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
751  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
801  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
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2151  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
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2401  GCAGCCAGCC TCGGTAGCAG CGCGGCGGCTC TCTTTCTCGC CTGGTAGCA
2451  AGACTATGATT CTGGTGGCAT ATCAGATGCG CTGGTCAGTA
```
9.12.6: Promoter D00095 (8):

Cloning Primer Forward: 5’-GCAAATAAAAAGCCACGGA-3’
Cloning Primer Reverse: 5’-CGTGTCACCTAGTTATGCATGGAGAGGATTATGTG-3’
Possible Gene Function: 60S ribosomal subunit nuclear export factor

(Relative expression of EST D00095 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)
9.12.7: Promoter D00651 (10):

(Relative expression of EST D00651 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)

**Cloning Primer Forward:** 5'-GCCGCCCAGTATCTAGACG-3’

**Cloning Primer Reverse:** 5'-CGTGTCAGTTAATCTGTAATTTAATGACGAAG-3’

**Possible Gene Function:** Malate dehydrogenase

```
1 TTGTCTATTG TATCCAAATTT CGGTGGGTGT GGGCCCGTTC GGCGACAGTT
51 CGAGCGAAAT TCGGCAGGTT GCATGGTAGT ACCCTAGTTT TGGGCCGCCC
101 AGTATCTAGA GCTTTGTATG TAGTTGACAC TTGACCTCTG AGCGCCGCTA
151 ATATTTGACC AAGCAAAGAC TAGTGATTTT GTTTCTGAGG TGAGGCCCTA
201 TCCAAATTATA AGACAGGAGA GCTTTTTTTT GCTTGCCCAT TCCTCTATAG
251 CTTCCCTTGT TCGTGTCGGA CGATTGGCTGC TCAGGCGGCT GCCTCTCTGA
301 ATGTCTGCTG CTCTTCTTTG GTTTTGTCCG TCCCTGCTTT ATATTTATTC
351 AGTGGGCTAG AGTGGGCTAG ATGCTGGGAT ATGCTGGGAT GTGGGGCATG
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751 AGTGGGCTAG AGTGGGCTAG ATGCTGGGAT ATGCTGGGAT GTGGGGCATG
801 AGTGGGCTAG AGTGGGCTAG ATGCTGGGAT ATGCTGGGAT GTGGGGCATG
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1101 AGTGGGCTAG AGTGGGCTAG ATGCTGGGAT ATGCTGGGAT GTGGGGCATG
1151 AGTGGGCTAG AGTGGGCTAG ATGCTGGGAT ATGCTGGGAT GTGGGGCATG
1201 AGTGGGCTAG AGTGGGCTAG ATGCTGGGAT ATGCTGGGAT GTGGGGCATG
1251 AGTGGGCTAG AGTGGGCTAG ATGCTGGGAT ATGCTGGGAT GTGGGGCATG
1301 AGTGGGCTAG AGTGGGCTAG ATGCTGGGAT ATGCTGGGAT GTGGGGCATG
```

442
9.12.8: Promoter C00741 (11):

Cloning Primer Forward: 5'-ATCCAGTGCGGCGTCG-3'
Cloning Primer Reverse: 5'-CGTGTCAACTAGTTATAGGCTGACCTTCTCGTC-3'
Possible Gene Function/Identity: Signal recognition particle 54 kDa protein homolog
9.12.9: Promoter BG22203 (15):

Cloning Primer Forward: 5'-TTTCACCTTTTAATAATGTGGTGTTTT-3'
Cloning Primer Reverse: 5'-CGTGTAACGTAGGTTATCCAGTACTAGCGTGTTGTCT-3'
Possible Gene Function/Identity: Glutamate decarboxylase

1   CTAATTTCTG  GCTAATATTT  CACTTTTTAA  TTGTGGTTGT  TTAATCTTTT
51  CCAATAGATTG  TTGAGGGTTCA  TGCCCTTACC  AACACAGATA  TGTCGTACCT
101  TGAGGCCATG  AAAATTCAGC  CGATAACAGA  TCTACTTCGT  CGTCTCTTT
151  TACCTAGGAG  GATTCCGCTCT  GTGAGTCTAT  GTCAAGTACA  CTACTACTGT
201  GGAACACAGAT  CAGCATAGCA  CCGCTAATCC  ATTGCCCGGT  TTTTTCATTA
251  CCACTTGAGAT  TTGTAATACCT  TTGTGATTAC  TGTAAATCTA  GAACACATTG
301  AGATGAAGATG  AACGTGAAGAG  ACAATATCAT  AGCTCCTGCA  ACTAAAAGGT
351  ATGGTAATTG  AGGGTGAGAG  ATGTGGGGTT  TAAAATGAGG  ATTCGAATCC
401  CTGATAGTGG  CAGTGTTGGA  ATCATACCGT  AGCCCCAATT  TTGAAGCGGC
451  GGATAACACT  TGTTGCAATT  TCATTCCACT  TCAATGCTAT  TGTCGTCTTT
501  CGATTATGTC  AAGAAGGAGA  TGAAGGCCCA  AATACAGCAG  CGATGGCAAA
551  TAGCTATAAA  TAATCGTTTC  TAGTGATTCA  TCGCCACTCG  CATAAGCGGT
601  GATGTACCTC  TCTAATACGG  GTTTGAGGCA  CCACTTTATT  GATAAAATTTA
651  TATATTGAGA  GCATATCTAC  TCACCGATAG  AAAATCGGCA  TAACTATCGT
701  GTGGACAGCA  AGCCACCAGA  CAGTTGATGC  GTGAGATCG  AGACGAATA
751  TAGTATACAC  AGCTTTCGCTG  ACTCGACCCT  GGAACTGCTT  AAGCAGCCT
801  ACTGGCGGAT  TCACCATCA  ATATCAGAT  TCCAAAACTCC  ATATCAGCTG
851  TAGTACCGCA  GTTCCAAATC  ACCATGAGGT  CCAACCAATT  GCGATCAGGA
901  GAACACCGAG  GCCAACCAGA  CCACGGTACT  GAACCTGGAAG  AT
9.12.10: Promoter C01420 (16):

(Relative expression of EST C01420 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)

Cloning Primer Forward: 5'-GGAAACTCTCGGCAGCTTTA-3'
Cloning Primer Reverse: 5'-CGTGTCACCTAGTTTACTGACGCCGTTAGGATAC-3'
Possible Gene Function/Identity: Aconitase (carbohydrate metabolism)

1 GAGTGAGCGG GTAAGACGTT TACTGCCGAA CCCTTGACT CACTTTTCAT
51 TTGGAAACTC TCAGGACCTT TCAATAGCCA TTGAGCCATA TATCAAAAGC
101 TGGAAATTTAG TATATCTTAG ATCGGTAGGT AAGGCTCGAC ATCTGTTATC
151 CAAAAAATGG ATTTTACCAG GCCGATGAAG CTTTGTAGAC TTCCAACCAA
201 CACTAAAAAG TCACATCAAAA CTCGGGTAGT GTAFTACATA ACTATTTCAT
251 TCACACAAA TCACATCGCC AGTGTTACCA TTTCCTGATA TATCAAACTC
301 TAATCTCCAA GTTGTGGCAA GAGGAAAGGT CAAGACCTA GTATTACAG
351 CAGACTTATA TGATCTTCTA ACACAAACCC GCCAAAGGTT AGGTGACGG
401 TTATTTTGTCG CAAATCGGCG GCTTTCAAC GAAATTGAAG TGTGGACGA
451 GATGAGAGTA CTTACTCGGA TATTATATGT TTTATATAGT AGAAATTGTC
501 TTTCTTACGT GTAGACTGTG GAGGAGGTAC AAGTATACAG CTAATGTATG
551 TTTGGTCGTA CATTGACCTC ATCTCTGCTA TGTATTCGT AGAATTTGTC
601 CTATTTTACT CGAGGGGCT GGCGATACGT CGTGCAACTC CAGTATCACAT
651 CAAATAATTCA TCAGGCTGGCG ATATAGGCC ATTAAAAATA AGGATTTAAT
701 TCACCTGTGA GAGTGCTGGG AGGGATACAC TCCCGGAAGA TAAGGATATTCC
751 TGGACATGCT GCCGAAAGAG TCCCCCATAA TCTATCTGGT AGTTGATCTA
801 TTTGGGCTAG ATAGGGAAAT AACCTATTTA TCTGACTCTG GGTGAGATTAT
851 AGGGCGCTCT AGATATATTGC TATTAGAAC TGGCGACACT TATGATCTAAT
901 CCGAGACCTG TATGTCCGG TGCTTGTATG GTGCTACGG CACGTTTTGAT
951 TTTGGTCTACAT GATGCGGCTC GGTATGGAT GGTATGGATG CAAAATGTTTAC
1001 GTCCAAATTAT ATCCAAGCG TAAATGCAAC GCGATTTGA TTGCCCCTTC
1051 CTCCTGGAGC CGAAGCCGCC TGACGTACCT ATGTGAAACT CTTGCTCGAT
1101 AGCACTGTTGA TTATTTAAGAG CGCGATGACGC CTCTATGGCC ATTTCCCGCG
1151 CAGCACCACATCTTTCAATTTTTCTCTGTTAATC TCGACTACA AATTTGCT
1201 CGATTTGGAC ATCATGTTG AGTACCTACA GCGCGTACG GCCCTTCAGC
1251 GACGAATACT CGCGCTGCCG GCACCGCCGC GTATGGGCGAC A
9.12.11: Promoter C01157 (17):

Cloning Primer Forward: 5'-GGCCTCCTATAGGCCAAGAC-3'
Cloning Primer Reverse: 5’-CGTGTCAACTAGTTAAGCTCCACCAATAAGGCTGA-3'
Possible Gene Function/Identity: Protein disulfide isomerase precursor

1  GATGTGAAGCACTGATTAGAGCAACGGCCTCCTATAGGCCAAGACTTGAGC
51   TTTGTATATTGTGACCTAATTTCCTCCTTGCGGGTATACGTATCATCGTA
101  ATTTTGTAGTGGGAGATGGCCGATTTTTGA TGAGGTAAATGCCCGATTGC
151  AATTGGACTAACGTCATTTACGGGTATTTGAGGTTGGTGTTATTAAGAG
201  TTGAGATAGAGGGCTCGCGTGAGAATGATA GGTTAAACATTGTGTGAGA
251  ATGACTCTACTTGGCTATTCCAGATATGCAGAGCACTCTGAGCGCATCACCTG
301  GGCATCACTGGAAGGTAAAAATTCGTCTTTACTGACCCTCAATCCCTATTGTTGATAG
351  TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
401  CGACTCTACTTGGCTATTCCAGATATGCAGAGCACTCTGAGCGCATCACCTG
451  GGCATCACTGGAAGGTAAAAATTCGTCTTTACTGACCCTCAATCCCTATTGTTGATAG
501  TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
551  CGACTCTACTTGGCTATTCCAGATATGCAGAGCACTCTGAGCGCATCACCTG
601  GGCATCACTGGAAGGTAAAAATTCGTCTTTACTGACCCTCAATCCCTATTGTTGATAG
651  TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
701  CGACTCTACTTGGCTATTCCAGATATGCAGAGCACTCTGAGCGCATCACCTG
751  GGCATCACTGGAAGGTAAAAATTCGTCTTTACTGACCCTCAATCCCTATTGTTGATAG
801  TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
851  CGACTCTACTTGGCTATTCCAGATATGCAGAGCACTCTGAGCGCATCACCTG
901  TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
951  CGACTCTACTTGGCTATTCCAGATATGCAGAGCACTCTGAGCGCATCACCTG
1001 GATGTGAAGCACTGATTAGAGCAACGGCCTCCTATAGGCCAAGACTTGAGC
1051 TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
1101 TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
1151 TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
1201 TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
1251 TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
1301 TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
1351 TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
1401 TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG
1451 TCGATTTTCAGAGTGTGACTCTGTTAAGATATGC TTGAGGTTGCTTTCGACTTCGATCTGGATG

(Relative expression of EST C01157 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)

446
9.12.12: Promoter D00154 (18):

Cloning Primer Forward: 5’-TCGACAACTTCGCTCAATCC-3’
Cloning Primer Reverse: 5’-CCAGCTCCACCAGTCAACCA-3’
Possible Gene Function/Identity: plasma membrane H+-ATPase (pma) gene

(Relative expression of EST C01518 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)

Cloning Primer Forward: 5’-ACTCGCTTTTCTCCATCCCTT-3’
Cloning Primer Reverse: 5’-CGTGTCGACTAGGGGACACGCTGTGCCGTTAATAAT-3’
Possible Gene Function/Identity: unknown

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1 CAGAGGCCCA GCTCCGTGGA TGCCCTCGC ATGGAGGTGA CTCTAAGGTT
51 CCATTCGAC TCCTTCCTCT CCAATTCCTTC ATGCCCGTGG CGCCGTTGTC
101 GTGCCGCCGC AGAAGCTGAG TGTTTGGACC GGGCCCCAGG ACCCCCTCCGC
151 ACTTTCCGTC CCGAGTAGGC CAATTTCACT GAGGCCGGGT GGCCAGGATC
201 ACCTGGTTTT CCGATGACTC TGTAAACCAC CTGCAACACC CGCTCATCTA
251 CCACCACAGC AGGCAAGGCT GAAGCAGAAAG ACGTAACAGT GCGCTGGTAT
301 AAACGCTTGTG CCGTCTCTCTC AGGTCCTTTTG AGTTCGGTTT ACAAGTCTCA
351 ACATTCATTT CAAACTCCAC TATCATTTCT GACAGCAAGG TAAACGTGAT
401 CTTTACTTCT TGAACTGTTG GTCAGCCTTG CGCGTGATTC TGTCGACTTT
451 CTCCACACGC ACCACCTAC ACCACACGTC CGTAGACAAAG ACAATATTCG
501 AAATGTGTGGC TAGATATCTT GTACTGCTAT TTTAAACGAC CGTGCCATT
551 GCGCGGCGGC TAAACATAAA
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![Graph showing relative expression of EST D00014 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass.]

**Cloning Primer forward:** 5'-'CGCTTTTCTTCAGTTGCATT-3'

**Cloning Primer Reverse:** 5'-CGTGTCAACTAGTTACGTATCAGGCTTGACTGCTG-3'

**Possible Gene Function/Identity:** MepB (metalloproteinase) (protein modification)
9.12.15: Promoter D01317 (22):

(Relative expression of EST D01317 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)

Cloning Primer Forward: 5’-TTAGTGGAGACTCGTAGAGAGTAACG-3’
Cloning Primer Reverse: 5’-CGTGTCAACTAGTTACACATGAAATAGCCTCAAATCG-3’
Possible Gene Function/Identity: alternative oxidase gene

1 TTAGTGGAGA CTCGTAGAGA TAGTTGGGCA AGGACGAGTC
51 CGTGATCCTG ATTCATGAG AAGTGGGAA TGGCGTCTAG GATCTGAACT
101 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
151 CGAGTTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
201 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
251 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
301 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
351 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
401 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
451 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
501 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
551 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
601 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
651 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
701 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
751 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
801 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
851 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
901 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
951 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
1001 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
1051 GCCAGTGGTG AGTGTTAACG AGAAGTACG TTGGACTCG AAGTGGGCA
9.12.16: Promoter C00209 (25):

Cloning Primer Forward: 5’-CGCAGAGTTCCGTCAACTT-3’
Cloning Primer Reverse: 5’-CGTGTAAGTTATCGCTGAGACTAGAAGAGA-3’
Possible Gene Function/Identity: Mannosyl-oligosaccharide alpha-1 (mannosidase precursor; carbohydrate metabolism)

1 ACGCAGAGTT CCTGTAAGCT TATGCTCTAC TACGATCCTG GCTACTAGGC
51 TGTGAGATTAG CCCCAACGCT ATCTATCCCA TGGACAGGGC GAGACTAAGG
101 CTGACTAAGG ACAGCCGCAA CTTGGCCCTT CAAATTTTAT TAGAAAAAGG
151 AGGATTGCA ATTTGGATAG GTGGGGAAAA GGGGTACCTAA CCCATATAAA
201 CTAATACCTC TCCATCTCTC AGGACATCTT ACGCCGAAGC CTGCGCTTCA
251 GCCGTTTTTGT CTACCTCCTC CTGTTATATAA TACACATAGT CTCCTCTTTG
301 GTGCCTTTTCC GTCACTCCTG TACCTGCGCG AAAGGAATCA TCCACCTCCG
351 GCCATAATAT CCTTTCTCTC TTTTCAAAAA TGTATTATTT TTCTCTTCTA
401 GTGCCAGCGGA CATTATATGT TCTTCCGTA CAAACATGTC AAGCCC
9.12.17: Promoter C00606 (27):

Cloning Primer Forward: 5’-GAAACAATCGGGCAAGAAAC-3’
Cloning Primer Reverse: 5’-CGTGTAACCTAAGTTAACTAGTGCCGCTGTAAGACC-3’
Possible Gene Function/Identity: unknown

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1 GCCACCTGAA TCTTGCAGAT TTTGGAGATA TATGAAGGTT CAGGCTGAAA
51 CAATCGGGCA AGCAATCCATC GCTGCGAGAC ATGGATTACC TCGTCAGCTT
101 CCGGCCCAAA AGTCAGGGAG CCGTGTCTGC CATGGGCCTG CCCCTTCTCC
151 CGCGCGAGCT CAGACTCGGA CGAGTGTCTG GACACCATCA ACCAAGACGC
201 CAGTCACGAG AGGAATGGCG TGTCGCTCTC GCACAATCTA CAGAGTGGAT
251 GGTGCGTGGG CCGTGAGAGG ATTCGAGGAT GTGTCGGTAT TTCCGACCCT
301 TTCCGCAAGGT CAACGCGCAT GAAAGTGGGT CTCAAGATGG GCAGGGAGG
351 GTATTATATTT TTTCTCTGAA GTGTTGTGGG CCTCAGTTTA GCTTTTCTTG
401 CGTCTCCTCTT CTCTGGCTGG GCATCGACTC TCAAGATCTCC GCAGCGCATC
451 TAACGACCAAG CAGCCGGACAC CCACCACAGC ACCAAGACGC CCACATCAC
501 CAACATCAC ACCACCAACC CATCAAGGG CTCAAGGATC AACTGCGAGG
551 CCGTCAACAG GCACCGAGA GCATGGCCAT CTAAGATGGC TTTTTTCTCA
601 CCTCGGGCAG CCAGGCTCAT GAGCTGCATG ACTGCTGCTG TACCCGACAC
651 CGTCTCAAGGC CACCGAGAG GCCAGCCGCTA CTAGATCCG TTTTTTCTCA
701 AACCGCTCTC GCCACCTCCC GCAGCAAGAC ACACAGACTC GCCCTCCTAC
751 CTTCGCTCTC GTAATCCTAA GATCGGCCC GATCGGCCTC GCAAAGAAA
801 GCCGAGGCG ACTGAATAT TGCCCAATAT CGTTGCCC GCCTCCCACC
851 GCTTGCACAT CTGTGGCCACA CTGAGACTGT CTACAGATAT TCCATTCCAT
901 GCTCAGCTCT CCTGCGCTTT ACCAGTTCTG GCCGTCTGAG GCCGAATATA
951 TCTTCCGGCCG GTCAGTTTTC GATTTGTGTCC AAGCCGTCAC CAGTATCCT
1001 AACCATCCAGG ATGACTCCG AGTAGGTGCAG TCCGTAGAG AACAATCCTC
1051 GCTCAGCTTC TCTCAATCTG ACCGTTCCTT GCTCTTCTGC AGGCTAACAG
1101 GCCGACCCAT CAAAGGGCCG GCAGGCCGCC TACCTCTACC TCAAGGATCG
1151 GTTGCTCTTC CGCTCGTTTTACTGCTGGCAG ACGGAAGCCT CCTCAAGGG
1201 TCTTACAGCG CACAGGTGGG AACA
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Promoter C00010 (30):

Cloning Primer Forward: 5’-GTTCGACCACCAATTTTCC-3’
Cloning Primer Reverse: 5’-CGTGTCAAGCTAGTTAAAATATGGGAGAGATGGGTCG-3’
Possible Gene Function/Identity: Fructose biphosphate aldolase

(Relative expression of EST C00010 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)
9.12.19: **Promoter D00573 (31):**

![Graph of D00573](image)

(Relative expression of EST D00014 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)

**Cloning Primer Forward:** 5’-CGTTAGATGAGCTCTGGCAG-3’

**Cloning Primer Reverse:** 5’-CGTGTCAGCTAGTTACCATCAGCGACCAGAAAACGAG-3’

**Possible Gene Function/Identity:** small GTPase RanA

(Sequence not available)

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9.12.20: **Promoter D00451 (32):**

![Graph of D00451](image)

(Relative expression of EST D00014 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass)
Cloning Primer forward: 5’-GAATATTAGTATTGGATGCCAC-3’
Cloning Primer Reverse: 5’-CGTGCAACCTCAAGCTTAGTTATTTACCAGAAATACCTTGCCAC-3’
Possible Gene Function/Identity: unknown
(Sequence not available)

9.12.21: Promoter C00056 (35):

![Graph showing relative expression of EST C00056 at 0, 4, 8, and 16 h.p.i. on barley, wheat, cellulose and glass]

Cloning Primer Forward: 5’-GTCTCAACCTCAAGCTTAGTTATTTACCAGAAATACCTTGCCAC-3’
Cloning Primer Reverse: 5’-CGTGCAACCTCAAGCTTAGTTATTTACCAGAAATACCTTGCCAC-3’
Possible Gene Function/Identity: unknown
(Sequence not available)

**Cloning Primer Forward:** 5' - TGAGCGATTTCCTGACTAAA-3'

**Cloning Primer Reverse:** 5' - GTGTCAACTAGTTATTCAGATTATGTGTGTTGATGG -3'

**Possible Gene Function/Identity:** unknown

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1 TTGAAGTCCT GAGCGATTTC CCTGACTAAA CGTTGGAAAG GTAATTTCCG AATCAGGAGT
61 TCGGTAGATT TTTGTATCTT TCTGATTTCA CGTAGGGCGA CGGTACCGGG CTGTTATCTT
121 TTACATCATG TTTAGCTTTG TTCACAGTAA GATCTAAATG GCTTACTGTG AGGTTCTTT
181 ACTCTCTTGG TAGATGGAGC ACTTTTCCGT GCTGCGTTGG ATTAGTGTTT AATTGGTACT
241 CATAGCTGTG TTCTTTAACA GCCTTGAGAG CGAGTTGTCT CGGTAGGACT TTTCCCTCCAG
301 TGGACGTGCG TATACTTGTGT TAGTATGTTA TATTTGTGCT TCTCTAGGCT TCTCTAGGCT
361 TGTGGAAGAC TGGGACATTG AATTGGTGTG ATGTGTCGTT GCCTGTATCC TAGGTTGACT
421 TTGCAGGTGT TATGTCGATG ATGCATGTTA TTGTGTAGAT TACGGTGCCG ATTGTCAAGA
481 TGGGGAAGAA CACACCTTGA TAAGTACAAG AAATG
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1 TTGAAGTCCT GAGCGATTTC CCTGACTAAA CGTTGGAAAG GTAATTTCCG AATCAGGAGT
61 TCGGTAGATT TTTGTATCTT TCTGATTTCA CGTAGGGCGA CGGTACCGGG CTGTTATCTT
121 TTACATCATG TTTAGCTTTG TTCACAGTAA GATCTAAATG GCTTACTGTG AGGTTCTTT
181 ACTCTCTTGG TAGATGGAGC ACTTTTCCGT GCTGCGTTGG ATTAGTGTTT AATTGGTACT
241 CATAGCTGTG TTCTTTAACA GCCTTGAGAG CGAGTTGTCT CGGTAGGACT TTTCCCTCCAG
301 TGGACGTGCG TATACTTGTGT TAGTATGTTA TATTTGTGCT TCTCTAGGCT TCTCTAGGCT
361 TGTGGAAGAC TGGGACATTG AATTGGTGTG ATGTGTCGTT GCCTGTATCC TAGGTTGACT
421 TTGCAGGTGT TATGTCGATG ATGCATGTTA TTGTGTAGAT TACGGTGCCG ATTGTCAAGA
481 TGGGGAAGAA CACACCTTGA TAAGTACAAG AAATG

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456
9.13: **Midiprep extraction solutions**

**Solution I**: 50 mM glucose; 25 mM Tris-Hcl (pH 8.0); 10 mM EDTA (pH 8.0) (prepare 100 ml, autoclave and store at 4 °C; add RNase to 10 µg/ml final concentration- 1:3000 dilution of Sigma RNase stock if applicable).

**Solution II**: 0.2 N NaOH (ideally freshly diluted from a 5 or 10 N stock); 1 % SDS (stored at room temperature to prevent precipitation).

**Solution III**: 60 ml 5 M potassium acetate; 11.5 ml glacial acetic acid; 28.5 ml H₂O. (this leads to a resulting solution that is 3 M with respect to potassium and 5 M with respect to acetate).

9.14: **Magnaporthe oryzae growth media and solutions**

**Complete Media (1 Litre)**. 50 ml 20X Nitrate Salts, 1 ml Trace elements, 10 g D-glucose, 2 g Peptone, 1 g Yeast Extract, 1 g Casein amino acids (Casein Hydrolysate), 1 ml 1000x Vitamin Solution, ddH₂O to 1 litre. pH= 6.5 (using NaOH) (For solid medium: 15 g agar)

**20X Nitrate Salts (1 Litre)**. 120 g NaNO₃, 10.4 g KCl, 10.4 g MgSO₄.7H₂O, 30.4 g KH₂PO₄,

**1000x Trace Elements (100 ml)**. 80 ml ddH₂O (Hot), 2.2 g ZnSO₄.7H₂O, 1.1 g H₂BO₃, 0.5 g MnCl₂.4H₂O, 0.5 g FeSO₄.7H₂O, 0.17 g CoCl₂.6H₂O, 0.16 g CuSO₄.5H₂O, 0.15 g Na₂MoO₄.2H₂O, 5 g Na₄EDTA, (pH = 5.0, use KOH). Fill to 100 ml with ddH₂O. Filter if necessary.

**1000x vitamins (100 ml)**. 0.01 g Biotin, 0.01 g Pyridoxin, 0.01 g Thiamine, 0.01 g Riboflavin, 0.01 g PABA (p-amino benzoic acid), 0.01 g Nicotinic Acid. Fill to 100 ml with ddH₂O. Store at 4 °C.

**OM Buffer**. 1.2 M MgSO₄; 10 mM Na-PO₄ (pH 5.8) diluted from a 1.0 M stock of equimolar and dibasic. Add Lysing enzyme from *Trichoderma* spp. or Glucanex and then filter sterilise.
ST Buffer (500 ml). 0.6 M Sorbitol (54.66 g); 0.1 M Tris-HCL pH 7.0 (50 ml 1 M); (Autoclave).

STC Buffer (500 ml). 1.2 M Sorbitol (109.32 g); 10 mM Tris-HCL pH 7.5 (5 ml 1 M); 10 mM CaCl$_2$. (Autoclave).

PTC Buffer (100 ml). 60 % PEG 4000 (60 g); 10 mM Tris-HCl pH7.5 (1 ml 1 M); 10 mM CaCl$_2$ (1 ml 1 M).

YGS Buffer. 0.5 % yeast extract (0.5 g); 2 % Glucose (2 g); 1.2M Sorbitol (21.86 g)
9.15: The predicted sequence of C00879(s)

1 TGAGATCGTC GAGTTAGTTG TGTAGTACCG TGGCGATTGC ACAAATGGGG
51 AAGAACACGA CCTATTAAGT ACAGCAAATC GCGTTAGCGA GTGATCGCGG
101 GTGATTTATT TTCGCGAGTG CGCATTGAAC CACTTGAGAT ACATGTAACC
151 AATCAGGGTA CACTTACGTA AAGTTCTATC TTTGCTATGT GACTACCGAA
201 TACACCGTCT AGATACTTTT AACCATCTTT TCAACAATTT CTACTATAGA
251 TCATAGGTTG AAATTTTAAT CTGAGTTAA ATTTGATAGT GTATAACTCG
301 ACTTTTCTAG TGACTGCCCTA CTAATGGTTG CAAGTCTTTT TACAGCCTTG
351 ATGTGTACC CAGGAAATTT GAAAACGAAA TTTGTGTGTT CTAATTAGG
401 ATTAGCGTAA CATCCTTGTC GGTGATTCCT TGTGCCACGG ATACGGTGAT
451 TGCTACTAC TTATCCTACT CTGAAACAATC ACTCCTGCCG TGACAGAACC
501 ACATAAGTAA GCTTAGTATC CGACTGTATA TCGAAATTA CCAACGAAAC
551 ATCATACATC ATCATTA

9.15.5: Phylogenetic Analysis Ma (Performed by Dr. Yusanne Ma)

X-axis represents basepairs up stream from 3’-end of selected regulatory region. Y-axis represents conservation of sequences. Low values indicate conservation.
9.16: *M. oryzae* wild-type Strain developing on agar, barley, cellulose, and glass

Figure 9-1: Development of wild type *M. oryzae* on barley at 4-16 h.p.i.

A) Spore with developing appressoria on barley at 4 h.p.i. (inset picture shows spore body which was out of the plane of focus) B) Confocal fluorescence image of germling. C) Emission spectra showing weak returns for the selected regions of interest. D) Spore with mature appressorium at 8 h.p.i. E) Fluorescence image F) Emission Spectra for regions of interest exhibiting autofluorescence. This quality exhibits itself in the creation of noisy, multi maxima returns. G/J) Spore development at 16 h.p.i. on glass with apparent sub-apical structure. H/K) Confocal fluorescence image of the developing *M. oryzae* spore. I/L) Emission spectra. Inset pictures in images G and J indicate fungal structures at lower magnification. **Scale Bar** = A, B, D, E) 8µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 9-2: Development of wild type \textit{M. oryzae} on barley at 31 h.p.i.

M/P) Development of invasive internal hyphae at 31 h.p.i. N/Q) Fluorescence image of spore infection structures at 31 h.p.i. O/R) Emission Spectra demonstrating wither weak returns or maxima at multiple wavelengths. Inset pictures where present indicate fungal structures at lower magnification. \textbf{Scale Bar} = M, N, P, Q = 5 \textmu m. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.

Figure 9-3: Mycelial preparation of wild type \textit{M. oryzae} growing on hygromycin B selective complete medium.

A) Hyphae growing through agar. B) Confocal fluorescence image of hyphae. notation equates to the ‘Region of Interest’ selected for wavelength analysis. C) Emission spectrum showing weak returns suggestive of autofluorescence. \textbf{Scale Bar} = 5 \textmu m. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 9-4: Development of wild type *M. oryzae* on glass at 4-31 h.p.i.

Figure 9-5: Development of wild type *M. oryzae* on cellulose at 4 and 8 h.p.i.

Figure 9-6: Development of wild type *M. oryzae* on cellulose at 16 and 31 h.p.i.

**J-L** Spore with mature appressorium at 16 h.p.i. Emission Spectra suggest wavelengths other than that seen for GFP are responsible for fluorescence. **M-R** Spore with branched network also exhibiting autofluorescence at 16 h.p.i. **S-W** Germling with pronounced branched network. Fluorescence is strong in the region of the mature appressorium, although the accompanying emission spectra suggest autofluorescence is the cause. **Y-Zi** Germling at 31 h.p.i with heavily melanised appressorium, exhibiting weak fluorescence, again at wavelengths not specific to GFP Fluorescence (i.e. no lone maxima at, or in the vicinity of 507 nm range. **Scale Bar** = **J,K**) 15 µm, **M-T**) 5 µm; **V-W**) 2.5µm approx. **Y-Z**) 5 µm
9.17: *M. oryzae* pMJK27.2 Strain developing on agar, barley, cellulose, and glass

Figure 9-7: Mycelial preparation of *M. oryzae* transformed with pMJK27.2 growing on hygromycin B selective complete medium.

A) Hyphae growing through agar. B) Confocal fluorescence image of hyphae. Green notation equates to the ‘Region of Interest’ selected for wavelength analysis. C) Emission spectrum showing peak approximately 510 nm, which accords to the region associated with GFP fluorescence. D) Putative developing conidiophore. E) Confocal fluorescence image of developing conidiophores. F) Emission spectrum indicating a peak near 510 nm. G) Mature *M. oryzae* spore. H) Confocal fluorescence image of mature *M. oryzae* spore. This equates to the baseline activity of the pMJK27.2 promoter at 0 Hours on all surfaces. I) Emission spectra, with emission maxima at 507-510 nm, suggesting that the fluorescence in all compartments of the conidium is due to the presence of GFP. Scale Bar = 10 µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 9-8: Development of *M. oryzae* transformed with pMJK27.2 developing on barley leaf epidermis at 4, 8, 16 h.p.i.

A) Spore with developing appressorium on barley at 4 h.p.i. B) Confocal fluorescence image of germling, with 5 regions of interest highlighted. C) Emission spectra with peaks clustering at 510 nm. D) Spore with developing appressorium at 8 h.p.i. E) Fluorescence image. F) Emission spectra suggesting the presence of GFP in cellular compartments. G) Spore development with melanised appressorium and invasive hyphae present at 16 h.p.i. on barley. H) Confocal fluorescence image of the developing *M. oryzae* spore. I) Emission spectra with maxima at 508 nm approx suggesting GFP presence in the invasive hyphae. Scale Bar = A-B) 5 µm, D-H) 8 µm. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 9-9: Development of *M. oryzae* transformed with pMJK27.2 developing on barley leaf epidermis at 16 and 31 h.p.i.

Gii) Spore, featured in G at a different focal plane, showing development with melanised appressorium and invasive hyphae present at 16 h.p.i. on barley. J) Emission spectra with peaks clustering at 510 nm. L/O) Spore with developing appressorium and invasive hyphae at 31 h.p.i. M/P) Confocal fluorescence image of germling with regions of interest labelled. N/Q) Emission spectra showing weak, noisy peaks with maximums at approximately 510 nm. Scale Bar Gii/J = 8 µm, L-P) 5 µm. Inset pictures indicate fungal structures at lower magnification. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 9-10: Development of *M. oryzae* transformed with pMJK27.2 on cellulose at 4 and 8 h.p.i.

Figure 9-11: Development of *M. oryzae* transformed with pMJK27.2 on cellulose at 16 and 31 h.p.i.

N(Q) Spore with developing, branched germ tube on cellulose at 16 h.p.i. O(R) Confocal fluorescence image of germling. P(S) Emission spectra. T(W) Spore with elongated and branched germ tubes at 31 h.p.i. U(X) Fluorescence image V(Y) Emission spectra. Inset pictures indicate fungal structures at lower magnification. Scale Bar = N-R, U-X) 5 µm approx, T) 10 µm. Inset pictures indicate fungal structures at lower magnification. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 9-12: Development of *M. oryzae* transformed with pMJK27.2 on glass at 4 and 8 h.p.i.

A) Spore with developing germ tube on glass at 4 h.p.i. B) Confocal fluorescence image of germling. C) Emission spectra showing strong peaks at approximately 510nm. D) Spore with two germ tubes at 8 h.p.i. E/G) Fluorescence image showing weak returns F/H) Emission Spectra. Scale Bar = A, B) 8 µm D-G) 10 µm approx. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
Figure 9-13: Development of M. oryzae transformed with pMJK27.2 on glass at 16 and 31 h.p.i.

**I)** Spore with developing germ tubes on glass at 16 h.p.i.  
**J)** Confocal fluorescence image of germling.  
**K)** Emission spectra.  
**L/O)** Spore with long and elongated two germ tubes at 31 h.p.i. exhibiting strong fluorescence.  
**M/P)** Fluorescence image.  
**N/Q)** Emission Spectra. Inset pictures indicate fungal structures at lower magnification. Scale Bar = **I, J)** 8 µm **D-K)** 10 µm approx. Emission spectra axis: X-axis is wavelength (nm), Y-axis equates to relative fluorescence units.
9.18: Analysis of CMEG promoter driven GFP expression.

EST C00879 isolate ‘C’ Behaviour on Barley normalised against the \textit{M. oryzae} Actin Gene.

Test of Homogeneity of Variances

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ANOVA

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<th>Sig.</th>
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Robust Tests of Equality of Means

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\(^a\) Asymptotically F distributed.

Post Hoc Tests

Multiple Comparisons

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<th>(J) VAR00001</th>
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<th>Sig.</th>
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<th>Upper Bound</th>
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\(^*\) The mean difference is significant at the 0.05 level.
EST C00879 isolate ‘A’ Behaviour on Barley normalised against the *M. oryzae* Actin Gene

**Test of Homogeneity of Variances**

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**ANOVA**

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**Post Hoc Tests**

**Multiple Comparisons**

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* The mean difference is significant at the 0.05 level.
EST C00879 isolate ‘A’ Behaviour on Barley normalised against the *M. oryzae* homologue of the NADH Ubiquinone Oxidoreductase.

**Test of Homogeneity of Variances**

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**ANOVA**

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**Post Hoc Tests**

**Multiple Comparisons**

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<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
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<th>Upper Bound</th>
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* The mean difference is significant at the 0.05 level.

EST C00879 isolate ‘B’ Behaviour on Barley normalised against the *M. oryzae* Actin Gene.
### Test of Homogeneity of Variances

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### ANOVA

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### Robust Tests of Equality of Means

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a. Asymptotically F distributed.

### Post Hoc Tests

#### Multiple Comparisons

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EST C00879 isolate ‘C’ Behaviour on Cellulose normalised against the *M. oryzae* Actin Gene.

### Test of Homogeneity of Variances

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### ANOVA

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### Robust Tests of Equality of Means

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a. Asymptotically F distributed.

### Post Hoc Tests

#### Multiple Comparisons

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<th>95% Confidence Interval</th>
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*. The mean difference is significant at the 0.05 level.

EST C01420 isolate Behaviour on Barley normalised against the *M. oryzae* Actin Gene.
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### ANOVA

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### Robust Tests of Equality of Means

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<sup>a</sup> Asymptotically F distributed.

### Post Hoc Tests

#### Multiple Comparisons

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<td>.156</td>
<td>-9.3201 - 1.5981</td>
</tr>
<tr>
<td></td>
<td>8 h.p.i.</td>
<td>-.10867</td>
<td>1.77921</td>
<td>.998</td>
<td>-5.5678 - 5.3505</td>
</tr>
</tbody>
</table>
“The possibility of physical and mental collapse is now very real. No sympathy for the Devil, keep that in mind. Buy the ticket, take the ride.”

“There was only one road back to L.A. - U.S. Interstate 15. Just a flat-out high speed burn through Baker and Barstow and Berdoo. Then onto the Hollywood Freeway, and straight on into frantic oblivion. Safety. Obscurity. Just another freak, in the freak kingdom.”

- Fear and Loathing in Las Vegas
Sequences Homologous to the Protein of EST C00009 and ClustalW alignment

>bg05886 (BluGen annotation of the gene relating to EST C00009)
MQTFGAVFVAALALSEEALRLNTDAQYNDKGAQFNYKWTENAAGPVTILLLNGPSTHLQTVSTIAASGQT
GNSYEWTPSSTLTKDFYAEITDSAGNVSYQFTITGDDTPDSMTSSQPIRATGTGYPVSQGMSYTSRVNSATSTMTGTYPGTGSGYSQGSPNTTVPHVSSTYMTSTIAASPSSTTVPADANNANGLSSPLAVFSTFFAF
IFLH

>gi|310790835|gb|EFQ26368.1| hypothetical protein GLRG_01512 [Glomerella graminicola M1.001]
MKTFASVLVASFAALAQAVKLNTSNYDVEAQPFTITWSDAQPQVPLTLLKKNGPSTNLVTQVIPATGQSGT
SVWTPFTTLPQDAIEISDGTPQNYEQFSLATGVTASASASASASASASASASASVTATASASASA
SAATSAGGTATAPSTSGGSSSTSAEASSAVSAANSTATTISTSSARASTHHSDEPTAFTTVPS
DSVRLGSIALGLTVAMLFQP

>gi|209570398|emb|CAQ16265.1| hypothetical protein [Glomerella graminicola] MKTFASVLVASFAALAQAVKLNTSNYDVEAQPFTITWSDAQPQVPLTLLKKNGPSTNLVTQVIPATGQSGT
SVWTPFTTLPQDAIEISDGTPQNYEQFSLATGVTASASASASASASASASASASVTATASASASA
SAATSAGGTATAPSTSGGSSSTSAEASSAVSAANSTATTISTSSARASTHHSDEPT

>gi|302414918|ref|XP_003005291.1| predicted protein [Verticillium albo-atrum VaMs.102]
MKYSVAVTLAALVAYVQAKAEFTNTQADFAAISAGDFTLTWSGAEQGPVTILLKTGPSDVTETITTG
EGSFLTWSPFTTLVSGQYAFEIINDGTEPNYSEQFFPLVSQGTAASAPASTASATVTVSTVAEST
STAAESSASASETATSSAAESSATTTEASSSVVTTRTRSTTTAESAATGTAASVPDAGRLGSPVALIMTLAAMLFPH

>gi|154319131|ref|XP_001558883.1| hypothetical protein BC1G_02517 [Botryotinia fuckeliana B05.10]
MQFSTLFLAAAAATLASAVQLTNPSFEVTAGSFPNTWTADAEAGPVTLLLLKKNGPSTSLTVTSTGSGRTT
SYSWTPSLLSLHAYEIQDSTWNPQSFQFQVSGATAVASTVATSTASASSAGSTSAGSTSGSSGTSTS
GTSTGSGTGSTGTSTSSGTSSGTTSASSGPTASSGNTLSGSSSARSRTSSSSRTASSGGERGTT
TATSAOSSAEEFASPLAFILFALAAIVTLN

>gi|156063466|ref|XP_001597685.1| hypothetical protein SS1G_01851 [Sclerotinia sclerotiorum 1980]
MQFSNLFLAAAAATLASAVQLTNPSFEVTAGSFPNTWTADAEAGPVTLLLLKKNGPSTSLTVTSTGSGRTT
SYTWTPATLDSLALAIRSYPYSEQFFQVGAVAVASTVSTASSTSVSSSETASSTSSGTSTS
SESSSSSTSSAPSSSSEISSNSNSTATRSSSSSSSSSSSSSTSSSTRTTTATASRTSTSTTAGTS
SATSAOSSAEEFASPLAFILFALAAIVTLN

>gi|39974653|ref|XP_368717.1| hypothetical protein MGG_00527 [Magnaporthe oryzae 70-15]
MKFTLSACVAVLAAIEARVQFTPNSNFVVEAGKPFELTVSGATGPVTSLKGDPSNLRTVETVLSNAG
GKATVTLDASQLSGTYAFGIKDTGEPENYSQQFNIVGTGLASTTASGAASSTASSTAMSTLST
TARPTAANVSSTASSTANSSTAARTSATSTPASTTASSPNTGAGSGLQSSLALVFLGAAYFLN

>gi|258575749|ref|XP_002542056.1| predicted protein [Uncinocarpus reesii 1704]
MHFFKTLLAGAALVASTAAQARLAFTSFPSNVQGKPVVTWSSGIPTKPTVITLRLKGPSSDLKDVAVLT
STATGGFTTWPSVLVDGPDYALQISQGSEINYTLNFIPITGGSRTASPSTAAELSSLAVSTTVLTAAT
TSSHASRGTTLISRNSTIPTPTLTSTRAVTLTPATPTEPEVITPDAPNAAPAILSSPVALLLSALVAF
AYLH

>gi|320591205|gb|EFX03644.1| hypothetical protein CMQ_572 [Grosmannia clavigera kw1407]
MKFSIITAALASLAAAANKFVTVGKPVFTLWGSMTGVTIVFATGAFTDLTPVETIDKGDSG
SSFTWTCPADTSGYVAEITDSETTNYSVFSLPATSSSAAPVSSAATSTASKTASASSASSASK
SSASSAVSSAPASSAPASSAPASSAPASSAPASSAPASSASSASSASSASSASSATNTSSSHSATKSSSTTLS
TSKTSTASKTSTASATAGTSAAPTNSGMKESPLAFILVTAALMYFN

>gi|116180556|ref|XP_001220127.1| hypothetical protein CHGG_00906 [Chaetomium globosum CBS 148.51]
MKFSFGTVLALAAALIASTVAQGELAFTSFPSDVQGKPVVTWAGGDATKPFVITLRLKGPSSDLKDVAVLT
SSATGGSYTWPSLV kmDYGALQINQGSDINYLFSISGSGSAPSTDATLSATPSSTFTATSTT
IQLTTGVTTSHISRGTSLSISRNATISTPALSSTRAVTLTPTAAPTEPESAPTSTPNAAPAILSSPVAL
ALGGLAAAFVLYN

>gi|303323757|ref|XP_003071870.1| conserved hypothetical protein [Coccidioides posadasii C735 delta SOWp]
MHFFRTLLLAAALIASTVAQGELAFTSFPSDVQGKPVVTWAGGDATKPFVITLRLKGPSSDLKDVAVLT
SSATGGSYTWPSLV kmDYGALQINQGSDINYLFSISGSGSAPSTDATLSATPSSTFTATSTT
IQLTTGVTTSHISRGTSLSISRNATISTPALSSTRAVTLTPTAAPTEPESAPTSTPNAAPAILSSPVAL
ALGGLAAAFVLYN

>gi|119188539|ref|XP_001244876.1| predicted protein [Coccidioides immitis RS]
MHFFRTLLLAAALIASTVAQGELAFTSFPSDVQGKPVVTWAGGDATKPFVITLRLKGPSSDLKDVAVLT
SSATGGSYTWPSLV kmDYGALQINQGSDINYLFSISGSGSAPSTDATLSATPSSTFTATSTT
IQLTTGVTTSHISRGTSLSISRNATISTPALSSTRAVTLTPTAAPTEPESAPTSTPNAAPAILSSPVAL
ALGGLAAAFVLYN

>gi|76008496|gb|ABA38724.1| extracellular matrix protein [Coccidioides posadasii]
VAQGELAFTSFPSDVQGKPVVTWAGGDATKPFVITLRLKGPSSDLKDVAVLTSSATGGSYTWPSLV
DGDDYALQINQGSDINYLFSISGSGSAPSTDATLSATPSSTFTATSTT IQLTTGVTTSHISRGTSLSISRNATISTPALSSTRAVTLTPTAAPTEPESAPTSTP
extracellular matrix protein [Arthroderma gypseum CBS 118893]

MQLFKTLLAGALAAVAAEKLAFSFSGLTAGQFVTWTGNAADLPVTITLRHGPSDDLKDVILTSSATGGGSYTFSTSLVSGTDYALQ1SOSQGEIYTGLFTITGGHGFSPSTTPATTASSSSSSGEPKPVTPKPAVTSTSGSAMSMTSTNHMTVKTSTGTASMTATTHTSHRNMTMSSTTSLTSGSATSATLPTATPTGGAA
SLTALSSPLALIMAALVAFAYLN

hypothetical protein FG00523.1 [Gibberella zeae PH-1]

MKYTIATIAAATMALAPKLAPLNFALTEGKPTIRYSYGCDSCGTII1LQNGESTDLKDYKTLTSGAKGDSSTFTPSQLPSDYNFKITDSAGDINYSQAFTYEGSYDAPVSATSAAVETTAAQTEATTLASVTKPVEEATTAKIIPHTPVPPKATTP1ATPTKGGAGQQTGVAPVSGATRMSSLALIGAAMAMVYN

extracellular matrix protein [Arthroderma otae CBS 113480]

MHFFKTLLAGALAVAVQQCGKISFTSFSSLOQAGKPCIEITWTGGNGPAPAVTTILTGRKSTNLKDVAVLTSATGGGYTFSTSLWNGDYPALQLISQGGEINYTGFLTISSGGNGTPSSATTTTTSSAYSGEPSKPVTPKVPSSAPSMSTIQSANHMTYMTKTSAGGTASMGTTSHRRNTMATPLSFHSATLPTAPTNGASSLAAMSSPLALAMAALAFAYLN

extracellular matrix protein, putative [Penicillium marneffei ATCC 18224]

MAPVKYIFVSAGLLVSADVAYATEMPAAALVAGEPMRLRWWGGNGAPATITLRLQGQSTDLODQVQLSKDANNGEFSTPPTDLQNDDNYAFESIQGDNHYTGSMTGSGDTKPVENMGNLKTSTHESMSNSASTADTASKTAVTDSATGHTATETATGDSTSSGTTTSSEAATGTDATASTADATATTTGATASDDSSATTSGAETETETALASHMNTPFVQSHQSTSTATSSSVQXGARGVGIPLAEFLAGAGFLFLFV

extracellular matrix protein precursor [Fusarium oxysporum f. sp. lycopersici]

MKYTLATIAALAVALKAPFLNTEFLTEGKYRTIRYSYGCCDGDCTIVLQNGPSDLSDDYKTLTTSATGDSTFTPSREPDYFIMKIDKAVEVNYSAFQQKSYAAPSVTSATAATAETATKAATAASATVSKSASATTSVGKXIPIF1HPSKNTTAPAHHTPSTGASGGETSVPVPSGAAMTSSLALIGAVMANYLN

hypothetical protein PAAG_04829 [Paracoccidioides brasiliensis Pb01]

MHLYKALVASALLVATAAVAQGISFTSFPPDVQGVPVTWTTGGTGTAPVTTITLRGBREDLVRQVQLTSGKKGSFTWTPSDLANGNHYALQISQGTDVNYGSLFISGGSSNDKTTSASVTTQTSSSLRSARTPATTTTTTAHTGTGYSAPTASLANKTNTAGPRRTRTTTSVITTPSPGSAPPTPTETTGGAAVMSSFALIMGVLAAYLN

extracellular matrix protein [Ajellomyces capsulatus G186AR]

MKSLLASALLVAAAVAQGISFTSFEDVQGVKPVTVTWTGGTGETPVITKLKGPLDLDKEVEVLTTTGGD
GSFTWTPSSLVNGDDYALKVEQGDEINYTNQFDISGGADKPASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASASA
TPPPFNNGAGRVGAVGAALLAIAAAFAL

>gi|145253040|ref|XP_001398033.1| extracellular matrix protein [Aspergillus niger CBS 513.88]
MRFTEAIVFAACLACQVQIAQAAALAFHTWPSLDAGVPITLNLWTETDSDAPVTITLRRKAAALDLTVQLT
KDAGGGSYT_PKTDSLPAGSDYAFQIDQDGVQYNGLSLNDSQRVATTSTASSVSTQETTTTPSQTET
TPTPTPDTPAPRDEVNSLPGLNNATFNLNDHSSSNVSSKSAMAAMQNGGAPFQMVSLDLVLGI
MGFLYVC

>gi|302923014|ref|XP_003053586.1| hypothetical protein NECHADRAFT_102374 [Nectria haematococca mpVI 77-13-4]
MKYTFATIAAFASVALAQFAFLNSKFDVQEGKPTIKYSGGCTITLQNGKSTDRTKDEVEVTATAEAGD
SFTFTPNPLSDTNFKKNNEDGTINYYGQSFAYEGETGTGLPSTKEATSAATSAETSAATSAATT
EATLTTTVSKPLISTKEKTTEETHTIMHFIATKNNATPTIPPTTKATSTG8SSTGGGSESTAEATSAATT
SATTVPESGAARMTSSLALIGAVMAVMYVLN

>gi|295663867|ref|XP_002792486.1| GPI anchored serine-threonine rich protein [Paracoccidioides brasiliensis Pb01]
MKFSACIVAAAFAAAAYTPPDTKSPPSNIPISRPGLLELVFGQYPITWTQPSTPGVSLLLRRGSPSN
VKYLTALDVSNTGTGYIWTTPSLELDGESGYIQIVVEGTQGQSYSTQFQIKNDDKVPEPSDIYTPNKY
PTAKPTDNYPTAKPSDYPGTSSSSSSSYTVLPISTATITVCATVTACPQTMVPTSGTQGTVQPTVQ
YTASPKKTTTPPDGAAGRNGVAILGLVAAAAMFAL

>gi|320031823|gb|EFW13781.1| hypothetical protein CPSG_09648 [Coccidioides posadasii str. Silveira]
MHFFRTLLAAAIATSTAVQGELAFTSFPSDVQGVKPVTVWAVGDKPVIKSRTKIVMTCSSATTG
SYTWPSSSLVHDYALQINQGSDNTNLFSISGGSGSAIPSTDSATLSATPSSTTSTTQIQTG
VTTSHISRTGLSISRNATIFPAALSSTRAVTLTPTAAPTEFAPSTTPNAAPAILSRFVALAGGLA
AVFVLYN

>gi|225677966|gb|EEH16250.1| GPI anchored serine-threonine rich protein [Paracoccidioides brasiliensis Pb03]
MKFSACIVAFAAAAYTPPDTKPSPPSNISRPGLLELVFGQYPITWTQPSTPGVSLLLRRGSPSN
VKYLADTSINTGTSTSWPTSLVGEESGYGIQIVVEGTQGQSYSTQFQIKNDDKVPEPSDKNKYP
TDKYPNTAKPTDYPGTSSSSSSSYTVLPISTATITVCATVTACPQTMVPTSGTQGTVQPTVQ
QTVPQSASKPTTTPPDGAARNGVAILGLVAAAAMFAL

>gi|240279599|gb|EER43104.1| GPI anchored serine-threonine rich protein [Ajellomyces capsulatus H143]
MKFIATVAVAFAAAAYTSKPYLSLPNTPIALPGNLDIVPVQGVYITKQWPTTDGVEVSLILLRRGPN
VKPIGTIADSNTGSYEWPTSDLELDGTVHYGLMVEGTQGQSYSTQFQIKNDDHASPSVTGVTYPYV
TTYGRTLVPSTGTICPTTKPTPPTPFGFPFSGYGTGIFPSFSPPQGAARNGVAIGGVIFVVA
ALAIFAF
>gi|255931309|ref|XP_002557211.1| Pc12g03260 [Penicillium chrysogenum Wisconsin 54-1255]
MHFSKSVLAVTASLVLGLAALDPFSWPKPEPLEPGKPVTLWTGATPDEPVTILLRQGNAGNLQDVKP
ITGQAKGGTFTWTPDDSVKKAADTKYAFtIQKQDQTNYTALLKGGNSFAALPEAKDITSESGAATAAT
GTTTATATGGTGTATPNQFTGTTTtestQGTTTTSTASKALISSAASPGSFSSSAAPSSTDSLRAT
GTEIVHGKEASSTESATQGASIPQSYQLMGVMGVLALLYL

>gi|322708836|gb|EFZ00413.1| extracellular matrix protein precursor [Metarhizium anisopliae ARSEF 23]
MHGDVGSSPAWLSSLTRLSFAHFTVICRLPFFEMKYALVISALAALAAAAEKPKFLNSNFQITEGQSFDL
KFDGCEGGCTTILQDPNTLKDYKVISTSGSFGALVPEGVSGTKYAFITNANKEYNYQOOQSYL
TGASVTASAATGTGSSGATATASAATASSTVSITASESTESGATTTSTTTVSATTASGSSS
APSSATSHSSTRTTSTATTITVPNAGVTRPMFAVAGAVAALYL

>gi|226287287|gb|EEH42800.1| GPI anchored serine-threonine rich protein [Paracoccidioides brasiliensis Pb18]
MKFSACIVAAAFAAAAYTPDTSKPSGPNISRPGLLELVFVGQPYTITWQFSTPGVSLLLGPRSNN
VKYLAT.ADVSNTGTYTWPTTPSLVGEESGYGTVQVTSGFESPKNDKVEPESDKYTAEP
TDKYTPAKKDYPKPSDYPTGSSSSSSSYTLPFSTARITVCASTVLSRTNQDCYPQRNWSC
HRPANRSNLQR

>gi|169595724|ref|XP_001791286.1| hypothetical protein SNOG_00605 [Phaeosphaeria nodorum SN15]
MFAQTAIVALFAGLAAQAQPVEGPKNPIRPILELVPAFPPKEITWQPTTSNLSVLLGRTSVNP
ISTIVTGIANSGKYSWTPSSGLEADVTHYGLQLIDDVTGQYQYSTQFISKGAECSGVPASSAAYGG
GYPASSAASSSAPAGYPSVAVSSAPNATTIVKPSAASSSTGYPGVGQNSTIVMPTSMSV
SSLRPTSTGAANATRPGLESTASLSLQAGLSFAGVAAALML

>gi|226492549|ref|NP_001140975.1| hypothetical protein LOC100273054 [Zea mays]
MRFFSTALVSLALASAYTQPDSQSPGNAILTPFQIVPGKPEITWDPITTSNGSLVLLRGSPT
NVVPLETIVENIGNSGSSWTPTSTTEPDTHYIGLVEVGTGQYQSYQFGISPAYSSSSSSAATST
TAAAVSSSDASETSVIISKISTICPETATATADVKPTFTSVPVVGNNFSSFVVTASPAGSASLIRSSA
TPSGTPAASSSSSVVPFVTGAADRNAISLGAVGVAALSF

>gi|38256988|dbj|BAD01560.1| hypothetical protein [Aspergillus kawachii]
MRFFSTALVSLALASAYTQPDSQSPGNAILTPFQIVPGKPEITWDPITTSNGSLVLLRGSPT
NVVPLETIVENIGNSGSSWTPTSTTEPDTHYIGLVEVGTGQYQSYQFGISPAYSSSSSSAATST
TAAAVSSSDASETSVIISKISTICPETATATADVKPTFTSVPVVGNNFSSFVVTASPAGSASLIRSSA
TPSGTPAASSSSSVVPFVTGAADRNAISLGAVGVAALSF

>gi|225562786|gb|EEH11065.1| GPI anchored serine-threonine rich protein [Ajellomyces capsulatus G18AR]
MKFIATVAVAALAAASTKPDYQQLPTGNPIALPGMLNDIVFGQPYTIKWQPTTVGEVLSLILLRGFPSTN


VKPIGTIADSIANTGSYQWTPSTDLEGDVTHYGLMIVVEGTGQYQYSTQFGIKNDHASPSVTDGTVFPYV
TTTSTRTLVPISTGTITICPPTKTPTPTFSGFPVSGYPTGIFPSPSPPFPQEGAAGNGVAIGGVFVA
ALAIFAF

>gi|154279868|ref|XP_001540747.1| predicted protein [Ajellomyces capsulatus NAm1]
MKFIATVAVAAALAAASTEPQSDLTPGNYALPGLNDIVPVGQFYITWQFTTDDGEVSLIILLRPSNN
VKPIGTIADSIANTGSYQWTPSTDLEGDVTHYGLMIVVEGTGQYQYSTQFGIKNDHASPSVTDGTVFPYV
TTTSTRTLVPISTGTITICPPTKTPTPTFSGFPVSGYPTGIFPSPSPPFPQEGAAGNGVAIGGVFVA
ALAIFAF

>gi|145255109|ref|XP_001398865.1| extracellular serine-threonine rich protein [Aspergillus niger CBS 513.88]
MRFFTALVSALAALASAYTQPDYQSNPTGNAILTPELNVQVFAGKPEITWDPTTSGTVSLVLLRPST
NVVPQETVEDIDNSGSYSWPSSTTELPDTTHYGLILLVEGTGQYQYSTQFGISNPYGTYGSTSQSSSTSF
TATETKSSATEESSSSSSSTTKPETTTTTTTTTTTTTTTTTTTATTSSASASADTTTTTATTPTATTLVTQSTANVPTST
VVVSVFNSSAGSSGAGSPQSSFTPSPTLSGDRKAISLGAIVVGAFAVMF

>gi|70997816|ref|XP_753640.1| extracellular serine-threonine rich protein [Aspergillus fumigatus Af293]
MRRLIASVSSLAALAMAAATKPDYQPTGNAILKPGNLELVPAKPYITWDPTTSGTVSLVLLRPST
NVVPQETLEDIDSNVSWSWTFTSTLEPDTHYGLILLVEGTGQYQYSTQFGISNPYGTYGSTSQSSSTSF
TATETKSSATEESSSSSSSTTKPETTTTTTTTTTTTTTTTTTTATTSSASASADTTTTTATTPTATTLVTQSTANVPTST
VVVSVFNSSAGSSGAGSPQSSFTPSPTLSGDRKAISLGAIVVGAFAVMF

>gi|159126627|gb|EDP51743.1| extracellular serine-threonine rich protein [Aspergillus fumigatus Af293]
MRRLIASVSSLAALAMAAATKPDYQPTGNAILKPGNLELVPAKPYITWDPTTSGTVSLVLLRPST
NVVPQETLEDIDSNVSWSWTFTSTLEPDTHYGLILLVEGTGQYQYSTQFGISNPYGTYGSTSQSSSTSF
TATETKSSATEESSSSSSSTTKPETTTTTTTTTTTTTTTTTTTATTSSASASADTTTTTATTPTATTLVTQSTANVPTST
VVVSVFNSSAGSSGAGSPQSSFTPSPTLSGDRKAISLGAIVVGAFAVMF

>gi|67522150|ref|XP_659136.1| hypothetical protein AN1532.2 [Aspergillus nidulans FGSC A4]
MHPLRPALLWACLOQPVTVITLRQGFPAGNLRLKLVTHDAQQGSFTWPDELASPGDSYALQIEQDGIS
YSGLVTVLVDSQKPKQPSASKHPPSVSPPGARTSVQKNNGIPTLNSSASSRVMSTGKSTAHKTSNDG
VTFRYISAEMIALMAAVYFAA

>gi|67527334|ref|XP_661653.1| hypothetical protein AN4049.2 [Aspergillus nidulans FGSC A4]
MRFFSTGLISALVAMATAYTTPDYSVGPSGNAILAPGLQEQVPAKPYITWDPTTSEGVSVLVLLRPST
NVQPLYIAAENIGNSHYETFSTELEPDVTHYGLILLVESGPNKGAQWTSQFGISPYNYQQGESSSSA
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>gi|169771247|ref|XP_001820093.1| extracellular serine-threonine rich protein [Aspergillus oryzae RIB40]
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>gi|119497047|ref|XP_001265292.1| GPI anchored serine-threonine rich protein [Neosartorya fischeri NRRL 181]
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>gi|159130594|gb|EDP55707.1| GPI anchored serine-threonine rich protein [Aspergillus fumigatus A1163]
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>gi|70990528|ref|XP_750113.1| GPI anchored serine-threonine rich protein [Aspergillus fumigatus Af293]
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Aspergillus fumigatus

Talaromyces stipitatus

Penicillium chrysogenum

Botryotinia fuckeliana

Claviceps fusiformis

Aspergillus clavatus

Penicillium marneffei

Aspergillus niger

Aspergillus nidulans

Talaromyces stipitatus

Aspergillus oryzae

Botryotinia fuckeliana

Chaetomium globosum

Magnaporthe oryzae

Gibberella zeae

Sclerotinia sclerotiorum

Leptosphaeria maculans

Penicillium chrysogenum

Aspergillus fumigatus

Ajellomyces dermatitidis

Paracoccidioides brasiliensis

Coccidioides immitis

Coccidioides posadasii

Paracoccidioides brasiliensis

A. niger

A. fumigatus

A. kawachii

A. dermatitidis

A. capsulatus

A. dermatitidis

A. dermatitidis

A. capsulatus

A. dermatitidis

A. dermatitidis

P. brasiliensis

G. zeae

S. sclerotiorum

P. maculans

P. marneffei

A. fumigatus

A. kawachii

P. brasiliensis

G. zeae

S. sclerotiorum

P. maculans

P. marneffei

Aspergillus fumigatus

Aspergillus fumigatus

Aspergillus kawachii

AJellomyces dermatitidis

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AJellomyces dermatitidis

AJellomyces dermatitidis

AJellomyces dermatitidis

AJellomyces dermatitidis

AJellomyces dermatitidis

Gibberella zeae

Sclerotinia sclerotiorum

Leptosphaeria maculans

Penicillium marneffei
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### Organism Report

**Glomerella graminicola**
- M1.001 [ascomycetes] taxid 645133
  - gb|EFQ26368.1| predicted protein [Glomerella capsulatus NAm1] >gi|2504126
  - extracellular serine-threonine rich protein [Aspergillus fumigatus]

**Glomerella graminicola**
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  - fgi|49650771|emb|CAG7770 extracellular serine-threonine rich protein [Aspergillus fumigatus]

**Verticillium albo-atrum**
- VaMs.102 [ascomycetes] taxid 526221
  - ref|XP_003005291.1| predicted protein [Verticillium albo-atrum] >gi|108
  - extracellular serine-threonine rich protein [Arthroderma benhamiae]

**Botryotinia fuckeliana**
- B05.10 [ascomycetes] taxid 332648
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  - extracellular serine-threonine rich protein [Glomerella graminicola]

**Sclerotinia sclerotiorum**
- 1980 UF-70 [ascomycetes] taxid 665079
  - ref|XP_001597655.1| predicted protein [Sclerotinia sclerotiorum] >gi|108
  - extracellular serine-threonine rich protein [Glomerella graminicola]

**Magnaporthe oryzae**
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  - extracellular matrix protein [Magnaporthe oryzae]

**Magnaporthe grisea**
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Grosmania clavigera kw1407 [ascomycetes] taxid 655863
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Chaetomium globosum CBS 148.51 [ascomycetes] taxid 306901
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Arthroderma gypseum CBS 118893 [ascomycetes] taxid 535722
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Gibberella zeae PH-1 [ascomycetes] taxid 229533
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Penicillium marneffei ATCC 18224 [ascomycetes] taxid 441960
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Ajellomyces capsulatus G186AR \( \text{[ascomycetes]} \) taxid 447093

Ajellomyces capsulatus H88 \( \text{[ascomycetes]} \) taxid 544711

Ajellomyces capsulatus H143 \( \text{[ascomycetes]} \) taxid 544712

Paracoccidioides brasiliensis Pb18 \( \text{[ascomycetes]} \) taxid 502780

Paracoccidioides brasiliensis Pb03 \( \text{[ascomycetes]} \) taxid 482561

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Leptosphaerzia maculans (blackleg of crucifers fungus, ...) \( \text{[ascomycetes]} \) taxid 5022

Aspergillus terreus NIH2624 \( \text{[ascomycetes]} \) taxid 341663
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Talaromyces stipitatus ATCC 10500 [ascomycetes] taxid 441959
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Metarhizium acridum CQMa 102 [ascomycetes] taxid 655827
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Aspergillus niger CBS 513.88 [ascomycetes] taxid 425011
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Aspergillus niger [ascomycetes] taxid 5061
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Nectria haematococca mpV1 77-13-4 [ascomycetes] taxid 660122
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Aspergillus nidulans FGSC A4 [ascomycetes] taxid 227321
ref|XP_659136.1| hypothetical protein AN1532.2 [Aspergillus... 51 5e-05
gb|EAA64239.1| hypothetical protein AN1532.2 [Aspergillus ... 51 5e-05
ref|XP_661653.1| hypothetical protein AN4049.2 [Aspergillus... 50 1e-04
gb|EAA95920.1| hypothetical protein AN4049.2 [Aspergillus ... 50 1e-04
ref|XP_3919245.1| TPA: GPI anchored serine-threonine rich pr... 50 1e-04
tpe|BF74809.1| TPA: extracellular matrix protein, putat... 48 6e-04

Aspergillus clavatus NRRL 1 [ascomycetes] taxid 344612
ref|XP_001274310.1| extracellular serine-threonine rich pr... 50 1e-04
gb|EAW12884.1| extracellular serine-threonine rich protein... 50 1e-04
ref|XP_001269791.1| GPI anchored serine-threonine rich protein ... 36 2.8
gb|EAW08365.1| GPI anchored serine-threonine rich protein ... 36 2.8

Aspergillus oryzae RIB40 [ascomycetes] taxid 510516
ref|XP_001820093.1| extracellular serine-threonine rich pr... 50 2e-04
ref|XP_001826671.2| GPI anchored protein [Aspergillus oryz... 40 0.21

dbj|BAE58091.1| unnamed protein product [Aspergillus oryzae] 50 2e-04
dbj|BAE65538.1| unnamed protein product [Aspergillus oryzae] 40 0.23

Aspergillus oryzae [ascomycetes] taxid 5062

dbj|BAE38104.1| GPI anchored serine-threonine rich protein... 50 2e-04
gb|EAD55815.1| GPI anch...
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<td>. Chaetomium globosum CBS 148.51</td>
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<td>. Uncinocarpus reesi 1704</td>
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<td>. Ajellomyces capsulatus</td>
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Ajellomyces capsulatus G186AR .............. 2 hits 1 orgs
Ajellomyces capsulatus H88 ................. 2 hits 1 orgs
Ajellomyces capsulatus H143 ................ 2 hits 1 orgs
Ajellomyces capsulatus NAm1 ................ 2 hits 1 orgs
Ajellomyces dermatitidis .................... 8 hits 2 orgs
Ajellomyces dermatitidis ER ................... 2 hits 1 orgs
Ajellomyces dermatitidis SLH14081 ......... 6 hits 1 orgs
Trichocomaceae .................................. 58 hits 15 orgs [Eurotiales]
mitosporic Trichocomaceae ..................... 33 hits 10 orgs
Penicillium ........................................ 10 hits 2 orgs
Penicillium marneffei ATCC 18224 .......... 4 hits 1 orgs [Penicillium marneffei]
Penicillium chrysogenum Wisconsin 54-1255 6 hits 1 orgs [Penicillium chrysogenum complex; Penicillium chrysogenum]
Aspergillus ...................................... 23 hits 8 orgs
Aspergillus terreus NB2624 .................... 6 hits 1 orgs [Aspergillus terreus]
Aspergillus niger .................................. 4 hits 2 orgs
Aspergillus niger CBS 513.88 ................... 2 hits 1 orgs
Aspergillus kawachii ........................... 1 hits 1 orgs
Aspergillus clavatus NRRL 1 ................... 4 hits 1 orgs [Aspergillus clavatus]
Aspergillus oryzae ............................... 4 hits 2 orgs
Aspergillus oryzae RIB40 ....................... 2 hits 1 orgs
Aspergillus flavus NRRL3357 ................... 4 hits 1 orgs [Aspergillus flavus]
Talaromyces stipitatus ATCC 10500 .......... 4 hits 1 orgs [Talaromyces; Talaromyces stipitatus]
Neosartorya ....................................... 15 hits 3 orgs
Aspergillus fumigatus ......................... 9 hits 2 orgs [Neosartorya fumigata]
Aspergillus fumigatus AF293 ................. 6 hits 1 orgs
Aspergillus fumigatus AI163 .................... 3 hits 1 orgs
Neosartorya fischeri NRRL 181 .............. 6 hits 1 orgs [Neosartorya fischeri group; Neosartorya fischeri]
Aspergillus nidulans PGSC A4 .................. 6 hits 1 orgs [Emericella; Emericella nidulans; mitosporic Emericella nidulans]
Leptosphaeriaceae ................................. 10 hits 2 orgs [Leptosphaeriaceae; Leptosphaeria; Leptosphaeria maculans complex]
Phaeosphaeria nodorum SN15 .................. 4 hits 1 orgs [Phaeosphaeriaceae; Phaeosphaeria; Phaeosphaeria nodorum]
Pyrenophora ....................................... 3 hits 2 orgs [Pleosporaceae]
Pyrenophora tritici-repentis Pt-1C-BFP ........ 2 hits 1 orgs [Pyrenophora tritici-repentis]
Pyrenophora teres f. teres 0-1 ............... 1 hits 1 orgs [Pyrenophora teres; Pyrenophora teres f. teres]
Tuber .............................................. 10 hits 2 orgs [Pezizomycetes; Pezizales; Tuberaeae]
Tuber melanosporum ......................... 10 hits 2 orgs
Tuber melanosporum Mel28 ..................... 5 hits 1 orgs
Yarrowia .......................................... 2 hits 2 orgs [Saccharomycotina; Saccharomycetes; Saccharomycetales; Dipodascaceae]
Yarrowia lipolytica CLIB122 ................... 1 hits 1 orgs
Schizosaccharomyces japonicus yFS275 ........ 2 hits 1 orgs [Taphrinomycotina; Schizosaccharomyces; Schizosaccharomyces; Schizosaccharomyces japonicus]
Zea mays ......................................... 2 hits 1 orgs [Viridiplantae; Streptophyta; Streptophytina; Embryophyta; Tracheophyta; Euphyllophyta; Spermatophyta; Magnoliophyta; Liliopsida; commelinids; Poales; Poaceae; PACCAD clade; Panicoideae; Andropogoneae; Zea]
Sequences Homologous to the Protein of EST C00482 and ClustalW alignment

>bg00086 mRNA (BluGen annotation of the gene relating to EST C00482)
MLYPRALLPAALASLVALAVPLEERQLAFDNQQKVGRVNLGGGWLEFWITPSIFQWWANGDVIDEYSYTAALGKDEAFTRLNHWATWITEEFAIESAISGMLHVIRIPIGYVALAIPDPYVQQLSYVDRAIDWARKNLKVLMLHGAPSGONGFDNSGRGTGIATWSGDNVPNTLRAIQALERYAPQTDVTAIELLENFWGNDLSSQIKFFYDGNVRQRTQGQTAVTIHDFLDRSNGFMSEAGVNVIDTHIYQFNSQENVAMHPVQTCSSIDRKPTDKWITGEWQATDCAKWLNLGKARYGTGLQGSEGEGYSCDDKYYGTVDSMLPVDKTNLQYFVEAQLDAYEHTGWFFWTWKTESAPEWHFQNLRAGLIPPLLDSRKFVSQCGTSEQCLVPN

Previously identified Bgh Protein:

>gi|46395596|sp|Q96V64.1|EXG_BLUGR RecName: Full=Glucan 1,3-beta-glucosidase; AltName: Full=Exo-1,3-beta-glucanase; Flags: Precursor
MLYPRALLPAALASLVALAVPLEERQLAFDNQQKVGRVNLGGGWLEFWITPSIFQWWANGDVIDEYSYTAALGKDEAFTRLNHWATWITEEFAIESAISGMLHVIRIPIGYVALAIPDPYVQQLSYVDRAIDWARKNLKVLMLHGAPSGONGFDNSGRGTGIATWSGDNVPNTLRAIQALERYAPQTDVTAIELLENFWGNDLSSQIKFFYDGNVRQRTQGQTAVTIHDFLDRSNGFMSEAGVNVIDTHIYQFNSQENVAMHPVQTCSSIDRKPTDKWITGEWQATDCAKWLNLGKARYGTGLQGSEGEGYSCDDKYYGTVDSMLPVDKTNLQYFVEAQLDAYEHTGWFFWTWKTESAPEWHFQNLRAGLIPPLLDSRKFVSQCGTSEQCLVPN

Homologous Proteins:

>gi|156054378|ref|XP_001593115.1| glucan 1,3-beta-glucosidase [Sclerotinia sclerotiorum 1980]
MVSSRFTLIIIAACLVTTEATPLKKGGLSFDYNGDKVGRVNLGGGWLEFWITPSFSLFYGSWVEYTLQTGKSAQSQQILNNAHWTIQNDSNIEASVGLHNHRIPIGYVALNPGLPDYVQQLYLDQAIQWARQAGLKIILDWHAPGSQNFDSNGRKPITWUTQDTQTLLAIZQTQAYPATOMDVTVIGEELLENFPANWALDMGAVKQFYDGGWNVNRNANDTAVHDFLSPSNGFMNQSVGVDILDTHIYQIFSFAEVMKPCQHVQVACSQIGNLANTDKRWITVEGFSQAQTDCAKWNLNGFVGVSRYDSYPSARYSGPASYQSKTQDGTVGDLRAIDKVNLYAFMEOALDAYEASGWFVFWTWKTESAPEWHFQNLRAGLIPPLLDSRKFVSQCGTSEQCLVPN

>gi|154321217|ref|XP_001559924.1| hypothetical protein BC1G_01483 [Botryotinia fuckeliana B05.10]
MTEATPLKKGGLSFDYNGKSVRGVNLGGGWLEFWITPSFSLFYGSWVEYTLQTGKSAQSQQILNNAHWTIQNDSNIEASVGLHNHRIPIGYVALNPGLPDYVQQLYLDQAIQWARQAGLKIILDWHAPGSQNFDSNGRKPITWUTQDTQTLLAIZQTQAYPATOMDVTVIGEELLENFPANWALDMGAVKQFYDGGWNVNRNANDTAVHDFLSPSNGFMNQSVGVDILDTHIYQIFSFAEVMKPCQHVQVACSQIGNLANTDKRWITVEGFSQAQTDCAKWNLNGFVGVSRYDSYPSARYSGPASYQSKTQDGTVGDLRAIDKVNLYAFMEOALDAYEASGWFVFWTWKTESAPEWHFQNLRAGLIPPLLDSRKFVSQCGTSEQCLVPN

>gi|242802088|ref|XP_002483905.1| exo-beta-1,3-glucanase (Exg1), putative [Talaromyces stipitatus ATCC 10500]
MIFSQMNTLALATFMLAQAAPGRVRESDLAFNYNSDKVRGVLGGGWLEFWITPSFSLFEGAGE
>gi|312211387|emb|CBX91472.1| hypothetical protein [Leptosphaeria maculans]  
MSKRETILAQPSGNSEDEVDWGVYLTSGGGTLKRLWTNATDNVIADLTSMTMVGLIQVRLASFSLPPPFSHCPFPLIIFMPLICGLRLASEWHLNGIIPIRSGLIAYSTTHDYPSTRRLFTRYFLDMYLQVGTVECRYYA  
GLAATQDGSHISHSHGKAEVLTRKSLSFVKLCELEWAMYTRSQRSPHFLALKSLQVLALRRCVTGLGG  
KLSSLIVYGTGVGLDEPEYAKRPAEFANIVNMGWHTIAAVGILASIANAPSLHDINLRAPRFDFDQQQV  
VRGBVNTGGFWLEVLPITPSIEFGNNAVDEYTFCCQLGADAARSLQAHWNSWITQDDFNQMAAAGLNVFR  
IPIGYWSVIFPREGDFYTVGDAYKDLWASAGAVKLMDHTAPEQNSQFGDNSKGYNGVQTGDQSVQ  
HTIRVLNKIRDDHANHPAVASIENLPEIPLGNLDNMPQVFMCWAGNLRDNSVAVAFHDAFQGVTSSWN  
WGAGMWHLLLLDLTHYIEFDFDNPRMSSIDHVRTACDFGNNMQMAGKWTIAGETTGGITDCAKWLNGGBK  
ARYDTGYNGAAWTDGTCTGSTTVGLASEDGTKTNVRGFIEAQLDAYEKAASGWIFWFTWKTEGAPWDMQL  
LAAGIFPQPLTARKYPQCG

>gi|239609585|gb|EEQ86572.1| glucan 1,3-beta-glucosidase [Ajellomyces dermatisidid ER-3]  
MATLKFKLFLVACFDFLAWVMVASATPHAAAPHLASRENKEIYGVNLGGWLVLEPITPSVFENAGDRAV  
DEYTLSQVLAGNASRSLKSHWSWITAEDFKQIAAAGLTHVRIPIGYWAVAPKGEYVQVGVGMDKAL  
RAWARESLKVAIDLHGAPSGQNFSDNSGRGPFINWPFWGETVAGTLNAVRALAERYAHQTDVDSIEIINE  
PFVPGVPLSVQKRYHFHDGYKIVRNANRVVGAISDAFQDPFSSNGFLPSQNFHVQVDVHVFQDNA  
LVNFNVQDHVNLASCFLGREKLAKTDKTREVGWEATAAMTDCAYLONGLRGMGARFDKSHPGKSGACGGRY  
FGSVGRLPAQQKAEIRRFIEAQLDAYENCAGGWFFWFTWKTEGSPEDMDQDLLSAGLFQPQDFRDRKYGGCK

>gi|261196716|ref|XP_002624761.1| glucan 1,3-beta-glucosidase [Ajellomyces dermatisidid SLH14081]  
MATLKFKLFLVACFDFLAWVMVASATPHAAAPHLASRENKEIYGVNLGGWLVLEPITPSVFENAGDRAV  
DEYTLSQVLAGNASRSLKSHWSWITAEDFKQIAAAGLTHVRIPIGYWAVAPKGEYVQVGVGMDKAL  
RAWARESLKVAIDLHGAPSGQNFSDNSGRGPFINWPFWGETVAGTLNAVRALAERYAHQTDVDSIEIINE  
PFVPGVPLSVQKRYHFHDGYKIVRNANRVVGAISDAFQDPFSSNGFLPSQNFHVQVDVHVFQDNA  
LVNFNVQDHVNLASCFLGREKLAKTDKTREVGWEATAAMTDCAYLONGLRGMGARFDKSHPGKSGACGGRY  
FGSVGRLPAQQKAEIRRFIEAQLDAYENCAGGWFFWFTWKTEGSPEDMDQDLLSAGLFQPQDFRDRKYGGCK

>gi|169595726|ref|XP_001791287.1| hypothetical protein SNOG_00606 [Phaeosphaeria nodorum SN15]  
MVQGYLTAAVAVGLLASSASLPQVKRAPSFSPFYEQVKVQRGVNAGGWLVEPITPSVFENAGDAVR  
ELLSGDKAAKQHRSNWWSTFTKDFDFQAMAGLHVRIPGYWSVLPREGDPYVQAGYDQGLGALWAQE  
AGLKVMDILHGAPLSQNFSDNSQGYGRVQMTQDSVHVTLNVLNKRIDDHAPAVSAIQLNPELPGSL  
DMNVVRQFYMDGWNLKSNSEVAITFHDAFQGTVSNGNWAGWNLLILDDTHYIEFDMNGMVSDQNLHIGK  
ACDGFQGNMASTKGNIAEGTWGGLTDCALKWLGKDKGARYDGTLSQGVDCKAGYTGVSALSNSDKYY  
NIGRFIEAQLDAYEKAAGWIFWFTWKTEGAPWDMQDLLSAGLFQPQDFRDRKYGGCK

>gi|115391045|ref|XP_001213027.1| glucan 1,3-beta-glucosidase precursor [Aspergillus terreus NIEUS2624]  
MLYNLSLAVLSLVLASSADAAGIRLEKRASTFDEYETMVRGVCGLGGWLVLEPWLSPGFDAPGDVAVDE  
WTYTEILQGDEAKARLIGHWDFTITEQDFDFDIAAAGMNHRIPIGYWAVEALPGDPYVDQQLEYLRAIE  
WAGAAGLKIVLVLHAPGQSNGDNSRGRAQGAIQWQGDTLGQTVAIFKLAREYVPPSSDVTAIEAVNEP
>gi|294956574|sp|B0XN12.1|EXGA_ASPFC RecName: Full=Probable glucan 1,3-beta-glucosidase A; AltName: Full=Exo-1,3-beta-glucanase 1; Flags: Precursor (Aspergillus fumigatus A1163)
MIFKFSQKALVALYLVGLAEAVPSKRVSRSATFDYNIVGRVNGWLVLEPWTIPSDNAGDAAV
DEWTTLATLQDGAKALSQHWSFTIQDFDFQIAIQAGMNHVRIPIGYWAVSSLPDEYPVDQLEYLDNA
ISWAREAGKVIDLHGAQPNQGDNDSRKGPIAWQQGDTVSQTVDARLAERYLQPQSDVTAIEALN
EPNIPGGVSEAGLDRYYNQIADVVRQIDPSTSVLSDGFLSTESWNFGKTEDVVMDTHHYEMFDYNLIS
LDIDGHVSKACDFGQIEGSDKPVVVGEWSGTDCYKHLNGKGVSTRYQGEYANNVKGDCANTQGSV
ADLSQDERTDTRFIEAQLDAYEGKNGWLFWTKEAGPQDMQDLANGVFSPLTDRQFNPQCA

>gi|70990522|ref|XP_750110.1| exo-beta-1,3-glucanase (Exg1) [Aspergillus fumigatus Af293]
MIFKFSQKALVALYLVGLAEAVPSKRVSRSATFDYNIVGRVNGWLVLEPWTIPSDNAGDAAV
DEWTTLATLQDGAKALSQHWSFTIQDFDFQIAIQAGMNHVRIPIGYWAVSSLPDEYPVDQLEYLDNA
ISWAREAGKVIDLHGAQPNQGDNDSRKGPIAWQQGDTVSQTVDARLAERYLQPQSDVTAIEALN
EPNIPGGVSEAGLDRYYNQIADVVRQIDPSTSVLSDGFLSTESWNFGKTEDVVMDTHHYEMFDYNLIS
LDIDGHVSKACDFGQIEGSDKPVVVGEWSGTDCYKHLNGKGVSTRYQGEYANNVKGDCANTQGSV
ADLSQDERTDTRFIEAQLDAYEGKNGWLFWTKEAGPQDMQDLANGVFSPLTDRQFNPQCA

>gi|119497053|ref|XP_001265295.1| exo-beta-1,3-glucanase (Exg1), putative [Neosartorya fischeri NRRL 181]
MIFKFSQKALVALYLVGLAEAVPSKRVSRSATFDYNIVGRVNGWLVLEPWTIPSDNAGDAAV
DEWTTLATLQDGAKALSQHWSFTIQDFDFQIAIQAGMNHVRIPIGYWAVSSLPDEYPVDQLEYLDNA
ISWARDAGKVIDLHGAQPNQGDNDSRKGPIAWQQGDTVSQTVDARLAERYLQPQSDVTAIEALN
EPNIPGGVSEAGLDRYYNQIADVVRQIDPSTSVLSDGFLSTESWNFGKTEDVVMDTHHYEMFDYNLIS
LDIDGHVSKACDFGQIEGSDKPVVVGEWSGTDCYKHLNGKGVSTRYQGEYANNVKGDCANTQGSV
ADLSQDERTDTRFIEAQLDAYEGKNGWLFWTKEAGPQDMQDLANGVFSPLTDRQFNPQCA

>gi|325092725|gb|EGC46035.1| immunodominantigen Gp43 [Ajellomyces capsulatus H88]
MAPLKSLLAFVCLSLTWVISIAAATHVRHRQHSSKTSIRGWNIGWLVLEPWTIPSVFKAGDRAVEY
TSLQILAGNLSLRHWSWITADDKFQAIAGAALTHVRIPIGYWAPLKEPVQGVSYLDKAIKWA
RQSNLKVAIDLHGAQPNQGDNDSRKGPIAWQQGDTVSTOMLRAERYADQTDVSEILNEPFV
PPGVPLDEKVQFYHKGKVVRDINPVNIGAVSDAQDLRNSWGFMLPSKNFHNVLFAHAYQVFVDAFTS
FSVDQHVNLCMGRQGQAVKDTDFCVWNGSAMAAMTCALNRLMKGARFDKSNFGKSKGCRYGS
VKQLPQKVGRRFIEAQLDAYGLAGGWFWFTKEGSPQGMDRLLSAGLFPQPTDRKYGCK

>gi|240279598|gb|EER43103.1| immunodominantigen Gp43 [Ajellomyces capsulatus H143]
MAPLKLAVCLVLWTVASIAATHVVRQSTHHKSRIRGVLNWGLWVPWTFSVEKAGRDAVEY TLSQILAGNARSRLSHHNSWITADDQKIAAAGLTHVRIPIGYWAVAPLKEPYVQGQSYLYDKAIRWA RQSLKVAIDLHGAPSNGFDSGRSGSINWPQGTAVIQLRAAFYADQTVDVSDIEILNEPFV PGGVPLDEVQFYHQYKVKVRDINPVGAISDAPQTLRPSWNGFPLSKHNVFLDAHYQVFSDAFTS FSVQVINLACYSYREQVAKTDKKTFVGEWASAAMTDCAKLYNGRDKGARFDKSFNGRKSAGCGRYFGS VKQLDQQKVGRFIEAQALDAYLGLAGWGFWTWKTGESPGWDMRLSDALAGFQFPTFRKYGGCK

>gi|154279862|ref|XP_001540744.1| glucan 1,3-beta-glucosidase [Ajellomyces capsulatus NAm1]
MAPLKLAVCLVLWTVASIAATHVVRQSTHHKSRIRGVLNWGLWVPWTFSVEKAGRDAVEY TLSQILAGNARSRLSHHNSWITADDQKIAAAGLTHVRIPIGYWAVAPLKEPYVQGQSYLYDKAIRWA RQSLKVAIDLHGAPSNGFDSGRSGSINWPQGTAVIQLRAAFYADQTVDVSDIEILNEPFV PGGVPLDEVQFYHQYKVKVRDINPVGAISDAPQTLRPSWNGFPLSKHNVFLDAHYQVFSDAFTS FSVQVINLACYSYREQVAKTDKKTFVGEWASAAMTDCAKLYNGRDKGARFDKSFNGRKSAGCGRYFGS VKQLDQQKVGRFIEAQALDAYLGLAGWGFWTWKTGESPGWDMRLSDALAGFQFPTFRKYGGCK

>gi|225562785|gb|EEH11064.1| glucan 1,3-beta-glucosidase precursor [Ajellomyces capsulatus G158AR]
MAPLKLAVCLVLWTVASIAATHVVRQSTHHKSRIRGVLNWGLWVPWTFSVEKAGRDAVEY TLSQILAGNARSRLSHHNSWITADDQKIAAAGLTHVRIPIGYWAVAPLKEPYVQGQSYLYDKAIRWA RQSLKVAIDLHGAPSNGFDSGRSGSINWPQGTAVIQLRAAFYADQTVDVSDIEILNEPFV PGGVPLDEVQFYHQYKVKVRDINPVGAISDAPQTLRPSWNGFPLSKHNVFLDAHYQVFSDAFTS FSVQVINLACYSYREQVAKTDKKTFVGEWASAAMTDCAKLYNGRDKGARFDKSFNGRKSAGCGRYFGS VKQLDQQKVGRFIEAQALDAYLGLAGWGFWTWKTGESPGWDMRLSDALAGFQFPTFRKYGGCK

>gi|46395594|sp|Q8NKF9.1|EXG_CANOL RecName: Full=Glucan 1,3-beta-glucosidase; AltName: Full=Exo-1,3-beta-glucanase; Flags: Precursor (Candida oleophila)
MLLTFAPIFLLISSVAAAPTQLORQKLEGEDYQNDKIRGVLNWGLWVPWTFSVEKAGRDAVEY TLSQILAGNARSRLSHHNSWITADDQKIAAAGLTHVRIPIGYWAVAPLKEPYVQGQSYLYDKAIRWA RQSLKVAIDLHGAPSNGFDSGRSGSINWPQGTAVIQLRAAFYADQTVDVSDIEILNEPFV PGGVPLDEVQFYHQYKVKVRDINPVGAISDAPQTLRPSWNGFPLSKHNVFLDAHYQVFSDAFTS FSVQVINLACYSYREQVAKTDKKTFVGEWASAAMTDCAKLYNGRDKGARFDKSFNGRKSAGCGRYFGS VKQLDQQKVGRFIEAQALDAYLGLAGWGFWTWKTGESPGWDMRLSDALAGFQFPTFRKYGGCK

>gi|149244260|ref|XP_001526673.1| glucan 1,3-beta-glucosidase precursor [Lodderomyces elongisporus NRRL YB-4239]
MKVQFFAILAFIALLDIIKRFQEOGNSAISLDSANSNLKSRQWQRYNDITRGLNLGGLNWGLWVPWTFSVEKAGRDAVEY TLSQILAGNARSRLSHHNSWITADDQKIAAAGLTHVRIPIGYWAVAPLKEPYVQGQSYLYDKAIRWA RQSLKVAIDLHGAPSNGFDSGRSGSINWPQGTAVIQLRAAFYADQTVDVSDIEILNEPFV PGGVPLDEVQFYHQYKVKVRDINPVGAISDAPQTLRPSWNGFPLSKHNVFLDAHYQVFSDAFTS FSVQVINLACYSYREQVAKTDKKTFVGEWASAAMTDCAKLYNGRDKGARFDKSFNGRKSAGCGRYFGS VKQLDQQKVGRFIEAQALDAYLGLAGWGFWTWKTGESPGWDMRLSDALAGFQFPTFRKYGGCK
NQCNFNS

>gi|294956597|sp|Q5B5X8.2|EXGA_EMENI RecName: Full=Probable glucan 1,3-beta-glucosidase A; AltName: Full=Exo-1,3-beta-glucanase 1; AltName: Full=Exo-1,3-beta-glucanase A; Flags: Precursor (Aspergillus nidulans)
MFRFISQAAILASLLVACTSAATLAEKVRGVNLGGWLVLEPWITPSLFDEAGDEAVDEYTLTEVLGVEEAAARLSHEWNTFITEEDFAIAEAAGLYVRIPYGWAAALPDEGYPYVSQGQLEHDNAVAWARAHNLKVIVDLYQTA VTDHAPGSPQGNDNSGHRGPIGWQGTVEQDVTQITALFETLAQRYLADDDTVMIEALNPHEVPQINIQDQL KDYEEETLAVKRVKSNPEATLLLHDFGQTEQWNGFMTGENVMMDTHHYEVEFQGGQNAMSIEKHUDAACQL GRQHLQADKPVIGEWGTGALSDCTRYLNKIGIRYDGTGLGNTVAACGKSQGSGSVAQLSADEIANTR RFIEAQLDAFELRNGWVFWTKTEGAPGWDMQDLLANGVFPQLTDFRPNQCNF

>gi|145255120|ref|XP_001398868.1| glucan 1,3-beta-glucosidase A [Aspergillus niger CBS 513.88]
MFVESAKALLALILAAASAAQAVPRVRQGASSFDYKSIQIVRGVNLGGWLVTEPWITPSLFDYAGDEAVDE YTLTEVLYVEAAARLSEHWNFTITEEDFAIAEAAGLYVRIPYGWAAALPDEGYPYVSQGQLEHDNAVA WARAHNLKVIVDLYQTA VTDHAPGSPQGNDNSGHRGPIGWQGTVEQDVTQITALFETLAQRYLADDDTVMIEALNPHEVPQINIQDQL KDYEEETLAVKRVKSNPEATLLLHDFGQTEQWNGFMTGENVMMDTHHYEVEFQGGQNAMSIEKHUDAACQL GRQHLQADKPVIGEWGTGALSDCTRYLNKIGIRYDGTGLGNTVAACGKSQGSGSVAQLSADEIANTR RFIEAQLDAFELRNGWVFWTKTEGAPGWDMQDLLANGVFPQLTDFRPNQCNF

>gi|67527343|ref|XP_661656.1| hypothetical protein AN4052.2 [Aspergillus nidulans FGSC A4]
MGITGCGDARLRAFSQSSRDAATCKNAGVRKQRRAAFDQYKQIEYFRQLENHVRVRIYDEAVDE YTLTEVLYVEAAARLSEHWNFTITEEDFAIAEAAGLYVRIPYGWAAALPDEGYPYVSQGQLEHDNAVA WARAHNLKVIVDLYQTA VTDHAPGSPQGNDNSGHRGPIGWQGTVEQDVTQITALFETLAQRYLADDDTVMIEALNPHEVPQINIQDQL KDYEEETLAVKRVKSNPEATLLLHDFGQTEQWNGFMTGENVMMDTHHYEVEFQGGQNAMSIEKHUDAACQL GRQHLQADKPVIGEWGTGALSDCTRYLNKIGIRYDGTGLGNTVAACGKSQGSGSVAQLSADEIANTR RFIEAQLDAFELRNGWVFWTKTEGAPGWDMQDLLANGVFPQLTDFRPNQCNF

>gi|295663871|ref|XP_002792488.1| glucan 1,3-beta-glucosidase [Paracoccidioides brasiliensis Pb01]
MNLSLNLALASCVLAVSLASASSSHTSHIVFRQAKSAIAIYGVNLGGWLLEPWITPSVFEOAGSSAVIDE YTLKSNLNGSNAKTRLHWKSTFDAFQIAAAAALTHEVRIYIEAVSWPIKEVYIQGVEYDUALV AKNSNLKVVIDLHAPGSPQGNSGRGPGINQKGTDQITLAAIALNRYAIDENTVNEIFVNPVEPVRGVEDLTQFQFQVAGWSNAGPDARKFLYKNFVSWIDHTEYYVQDFFAKFTIDQHVKLACSPKDRSLGVDKPVIGEWGSGMTDCAKLYNRGRGRGAFDNYSPGSKFAGCARGSTGS SSKLSSAQKKKDETTRIEAFQLEAFVQGAWFVFWTKEGAPGWDMRDLKQELFPQFSPARKYGC

>gi|317144408|ref|XP_001820101.2| glucan 1,3-beta-glucosidase A [Aspergillus oryzae RIB40]
MFIKLLNKLALAVSLASASSHHTSHIVFRQAKSAIAIYGVNLGGWLLEPWITPSVFEOAGSSAVDE YTLKSNLNGSNAKTRLHWKSTFDAFQIAAAAALTHEVRIYIEAVSWPIKEVYIQGVEYDUALV AKNSNLKVVIDLHAPGSPQGNSGRGPGINQKGTDQITLAAIALNRYAIDENTVNEIFVNPVEPVRGVEDLTQFQFQVAGWSNAGPDARKFLYKNFVSWIDHTEYYVQDFFAKFTIDQHVKLACSPKDRSLGVDKPVIGEWGSGMTDCAKLYNRGRGRGAFDNYSPGSKFAGCARGSTGS SSKLSSAQKKKDETTRIEAFQLEAFVQGAWFVFWTKEGAPGWDMRDLKQELFPQFSPARKYGC
>gi|171848759|pdb|2PC8|A Chain A, E292q Mutant Of Exo-B-(1,3)-Glucanase From Candida Albicans In Complex With Two Separately Bound Glucopyranoside Units At 1.8 A
GGGHNVAWDYDNIRVGNLGGWFLEPYMTFSLEFPFQNGNDQSGVPVDEYHTQTLGKEAALRILQKH
WSTWITEQDFKQISNLGLNVFPRIGYWAFAQQLDNDPVQGQVYQYLEKALGWARKNNIRVWIDLHAGPSQ
QNGFDNSGLRDSYNFQNGDNTQVTLNVLNTIKKYGGNEYSDVIGIELNNEPLGPVLNMIDKLQFFLDG
YNSLRQGTSGVTVPIIHDADFQVFQGFYNWNFTLVAEGQWNVVDHHHYQVFSGGELSRLNINDHISVACNWGW
AKKESHNVAVQWTSAALTDCAKWLNGVNRGARYEGAYDNAPYGSCQPLLDISQWSDEHKDTDRRYIEAQ
LDAFYETGGWFSWKTEAPEWSFQTLYNGLFPFPVTRDFQPNQCGFH

>gi|171848757|pdb|2PBO|A Chain A, E27q Mutant Of Exo-B-(1,3)-Glucanase From Candida Albicans At 1.85 A
GGGHNVAWDYDNIRVGNLGGWFLEPQLPYMTFSLEFPFQNGNDQSGVPVDEYHTQTLGKEAALRILQKH
WSTWITEQDFKQISNLGLNVFPRIGYWAFAQQLDNDPVQGQVYQYLEKALGWARKNNIRVWIDLHAGPSQ
QNGFDNSGLRDSYNFQNGDNTQVTLNVLNTIKKYGGNEYSDVIGIELNNEPLGPVLNMIDKLQFFLDG
YNSLRQGTSGVTVPIIHDADFQVFQGFYNWNFTLVAEGQWNVVDHHHYQVFSGGELSRLNINDHISVACNWGW
AKKESHNVAVGEWSAALTDCAKWLNGVNRGARYEGAYDNAPYGSCQPLLDISQWSDEHKDTDRRYIEAQ
LDAFYETGGWFSWKTEAPEWSFQTLYNGLFPFPVTRDFQPNQCGFH

>gi|255730225|ref|XP_002550037.1| glucan 1,3-beta-glucosidase precursor [Candida tropicalis MYA-3404]
MQLSSLSSSSVILLVQLIDNAISNSFKSNGVEKGRRGAVWYENIDIVRGVLGGFVLEPYMNPSLFEE
FFKINGNDESVPVFDEYHTQTLGKETASKILEDHWAKWITEWDFQQMSNLGLNLVRIPIGYWAFQLLDND
PYVQGQVAFLEDEALWarHNNIKVWIDLHAPGSQNGFDNSGLRDSLEFQNGDNTQVTLNVLAEIQFQYG
TSDYDDVVGIELVNEPLGSPMDALDKKFMYDDGSSRTNEDSVPFDLYHDFAQVSGYWNFLTLVAGQ
WNVVLDDHHHYQVFSAGELSRDIDQHISVACNWGWAKNEYHWHTGWSAALTCDAYLWNGVRGARWEG
AYDGSPYGSCEFYLQFSSWTDHKTNNVRVRYIEAQLDAFYETGGWFSWKTEAIDDFQKLETNGIFPQ
QLDDLDRQFPNQCGFH

>gi|13399550|pdb|1EQP|A Chain A, Exo-B-(1,3)-Glucanase From Candida Albicans
AWDYDNIRVGNLGGWFLEPYMTFSLEFPFQNGNDQSGVPVDEYHTQTLGKEAASRILQKHWSTWIT
EQDFKQISNLGLNVFPRIGYWAFAQQLDNDPVQGQVYQYLEKALGWARKNNIRVWIDLHAGPSQNGFDN
SGLRDSYNFQNGDNTQVTLNVLNTIKKYGGNEYSDVIGIELNNEPLGPVLNMIDKLQFFLDGYNSLRC
TGVSPTVPIIHDADFQVFQGFYNWNFTLVAEGQWNVVDHHHYQVFSGGELSRLNINDHISVACNWGWDAK
KHESHNWAGWSAALTDCAKWLNGVNRGARYEGAYDNAPYGSCQPLLDISQWSDEHKDTDRRYIEAQ
LDAFYETGGWFSWKTEAPEWSFQTLYNGLFPFPVTRDFQPNQCGFH

>gi|306991909|pdb|3O6A|A Chain A, F144YF258Y DOUBLE MUTANT OF EXO-Beta-1,3-Glucanase From Candida Albicans At 2 A
GGHVNWADYNIRVGNLGGWFLEPYMTFSLEFPFQNGNDQSGVPVDEYHTQTLGKEAALRILQKHW
STWITEQDFKQISNLGLNVFPRIGYWAFAQQLDNDPVQGQVYQYLEKALGWARKNNIRVWIDLHAGPSQ
NGYDNSGLRDSYNFQNGDNTQVTLNVLNTIFKKYGGNEYSDVVIGIELLNEPLGPVLNMDKLKQFFLDG
YNSLRQTGSVTPVIIHDAFQVFGYWNNFLTVAEGQWNVVVDHHHYQVYSGGELSRNINDHISVACNWGD
A KKEHNSNVAGEWSAALTDCAKWNVLGNWRGYEAGYNAPIygscqplldisqwsdehkdtrrryeaql
DAFEYTGGWVFSWKTENAPESFWQTLYNGLFQPVTDRQPFPNCYGFR
>gi|171848779|pdb|2PF0|A Chain A, F258i Mutant Of Exo-B-(1,3)-Glucanase
From Candida Albicans At 1.9 A
GGHNVAVWDYDNVIRGVNLGWWFVLEPYTPTSLFEPFGSNIVFDEHYC
QTILGKDEASDLQKHKNWSTYEDDEEAISAGLNTVRIPIGHWAFVTDGEPYVQQKQYLDLALQANRN
HNLKVIDLHTAPFSGQNGDNLGQDQVQQODANITATLALTQNFNYGDEYKDVSVGIILLNEPLG
VTSMDNQLENYFQWAYKNRVSSTNNVIHDAFQFPNYDSFMQADGGYYNVLHDDHYQVFSGGELSRD
AIRNAHLSVCAWGSASSATESHWNVACCEPSAALTDCAVWLNGVRGARWSGYDNSPNCGSDMYNPDNWT
SDHKTNVRYIEAQLDAFEHTGGWIFWNWKCEDAIDWMSRLIDVGFQPLDPSRQYPGQCY
>gi|260942561|ref|XP_002615579.1| hypothetical protein CLUG_04461
[Clavispora lusitaniae ATCC 42720]
MKLLVSLASLATSVLAASEVLPSKRSSSWDYQNDKVRGVNLGWWFVLEPYITPSLFEPFGSNIVFDEHYC
QTILGKDEASDLQKHKNWSTYEDDEEAISAGLNTVRIPIGHWAFVTDGEPYVQQKQYLDLALQANRN
HNLKVIDLHTAPFSGQNGDNLGQDQVQQODANITATLALTQNFNYGDEYKDVSVGIILLNEPLG
VTSMDNQLENYFQWAYKNRVSSTNNVIHDAFQFPNYDSFMQADGGYYNVLHDDHYQVFSGGELSRD
INAHLSVCAWGSASSATESHWNVACCEPSAALTDCAVWLNGVRGARWSGYDNSPNCGSDMYNPDNWT
SDHKTNVRYIEAQLDAFEHTGGWIFWNWKCEDAIDWMSRLIDVGFQPLDPSRQYPGQCY
>gi|150868234|ref|XP_001385760.2| Glucan 1,3-beta-glucosidase precursor
(Exo-1,3-beta-glucanase) [Scheffersomyces CBS 6054]
MVQLTSIVSSILVLSQSLVASASINNPLDNNNLKCLKGTGASWDYQNDVIRGVNLGWWFVLEPYITP
SLFEQWENWGDQSQPVDEHYHTQKLGKLVAGQRDLTHWTQDEQFSDIAAALGQLFVRIPIGYWAFLQ
LONDYPVQVGGQVEYQLADGWANYKLKWLDLHAGPSQNGDNSLGRRTQYQQPNVQVTINLEQIF
EKYNGNEYNPSVNEPLGFPSVNNLNLFTQNGYNLRLRTQGSVPITVIIHDAFQAPGYDNDNFLTE
NGDYWSVIDHHHYQFSYGELARIDQHISVACNWDSKKEYHNSNVAGEWSAALTDCAKWLGVRGAR
RYAQQYDNSAYIGDCTPYLGLGTQDQYKTNVRYIEAQLDGFEQTGGWVFSWKTENAAVEWFKRTLAA
QLFSPLTDQFPFCYGFR
>gi|117572654|gb|ABK40520.1| exo-beta-1,3-glucanase [Wickerhamomyces anomalus]
MLISTIISLLSIALANPISRPRGQQFYKRGDYWDYQNDKIRGVNLGWWFVLEPYFTPSLFEAFENQGQ
DVPVFYDEHTAVGKLDAIERLQWRQHSWWIIAEFDQSIAGLNFVRIPIGYWAFLQLLONDYPVQQQESY
LDQALEWAKKYDIKVWIDLHAGPSQNGDNSLGRRTQYISKNYGGSDYGDVIG
IEILLNEPLGSVDMLGKNDPWQOOGYHNNLRNTGGSNVQIHDAFFQTWYNDKFTFHPDYWNVIDDHYYQ
VFSPGELRSVDEHYKVCAGWGANSTKENHNLWGCESAAMTDCTKWNLGVGRSSRQTDFDPSONQY
YGSCQCGSQDISTWDDDKNYSSYRNYEIAQLDAFEKRSGWIFWTKTETTELEDFQKLSYYIGFSPSLNR
QYPQCD
>gi|320580735|gb|EFW94957.1| Glucan 1,3-beta-glucosidase [Pichia angusta DL-1] MKFSSFLIASSLLSLVAAPVTLLKRDRSRWDYANDKITYGVNIGGWLVLEPPFITPSLFEAVSSDVDPVEHYTYEALGKEEAERKLQEHWSTWIREEDEFKGMANVGLNFRIPIGYWAFLAQELEDPFYVQQGQYELDKALEW CAYGLKAWVDLHGAPSGQNGFNSGKGKGE1GQWN TNTGYYDVTLTQLVDQIAASYKGGSNSYDVIIGEELNP EPLGSNLDFQLDVFYNYKGYQLRDNGA NAPVI IHDAI ADHTFDNVLNTEQDPNIEVEI VDVHHYQVDQ GSIQSIDHEVSTACGGWQGQSENETHEYSLCGEWTAALTDCAKWLNGAGRRGARYDATFQGGNYIGSCDQLY TANYDYFTPEVISNYRRYEVAEQMDSFLYKGNAGWFWFCWKTENTIEWDMQRLLLGLGIIAQPLDDRQYPNQ CGFD

>gi|241948457|ref|XP_002416951.1| exo-1,3-beta-glucanase I/II, putative; glucan 1,3-beta-glucosidase I/II precursor, putative [Candida dubliniensis CD36] MQLSFLTSSVFILLLEFVKASVINSF PKPNGNLKFKRRGGNHVAVWDYDKDVIRG VNLGGWVFVEFPYMTF SLEPEPONQDQSVGPDEYHTQTG1KDAQAQLQQHSTWITEQDFKIQSDLGLNFVRIPIGYWAFLQ LONDPYVQVQVEYELEGALGWARNHKLWDHAPGS QNGFNSGLRDSYEFQNGDNTQVALDVLQYSINKGGSYDGOVVI GIELLENPLGSVDLGRNLDFQGQYHNLRNTGSSQ NVIHDAPFQWDNDFKHFDPFYWDVWFNVDHHYQ VFSPEGOELSRVDEHKVACWGANSTHENHWNLCEGSAAMTDCTK WLNGVGRSGRYQTFDYDFSPQFONQ YIGSCQGSQDISTWDDDKSNNYRRYEIAQD AFKRSGWIFTWKTETTLEWDFQKLSYGYIFPS PLTSQRQYPQCD

>gi|46395631|sp|O93983.1|EXG2_HANAN RecName: Full=Glucan 1,3-beta-glucosidase 2; AltName: Full=Exo-1,3-beta-glucanase 2; Flags: Precursor (Wickerhamomyces anomalus) MLIFIISSLSIALAPNPISRGTQFYKRGDYWDYNKIRGVNLGGWVFLEPPFITPSLFEAFENOQQ DVPVDEHYTEKALGKDLAKERLDQWHSSWIEADFQSIAGAGLNFRIPIGYWAFLQLDNDPYVQQGE SY LDQALEWAKKYDIKWIDLHAPGSQNGFNSGLRDSYEFQNGDNTQVALDVLQYSINKGGSYDGOVVI GIELLENPLGSVDLGRNLDFQGQYHNLRNTGSSQ NVIHDAPFQWDNDFKHFDPFYWDVWFNVDHHYQ VFSPEGOELSRVDEHKVACWGANSTHENHWNLCEGSAAMTDCTKWLNGVGRSGRYQTFDYDFSPQFONQ YIGSCQGSQDISTWDDDKSNNYRRYEIAQD AFKRSGWIFTWKTETTLEWDFQKLSYGYIFPS PLTSRQYPQCD

>gi|46395581|sp|Q12626.1|EXG_PICAN RecName: Full=Glucan 1,3-beta-glucosidase; AltName: Full=Exo-1,3-beta-glucanase; Flags: Precursor (Pichia angusta) MLFPVLHLPKAMKFSSFLIASSLLSLVAAPVTLLKRDRSRWDYANDKITYGVNIGGWLVLEPPFITPSLFE AVSSDVDPVDEYHTEALGKEEAERKLQEHWSTWIREEDEFKGMANVGLNFRIPIGYWAFLAQELEDPFYVQQ GQYEYLDKALEWCAKYGLKA WDGLHAPGSQNGFNSGKRGE1GQWN TNTGYYDVTLTQLVDQIAASYKGGSNSYDVIIGEELNP EPLGSNLDFQLDVFYNYKGYQLRDNGA NAPVI IHDAI ADHTFDNVLNTEQDPNIEVEI VDVHHYQVDQGSIQSIDHEVSTACGGWQGQSENETHEYSLCGEWTAALTDCAKWLNGAGRRGARYDATFQGG GNYIGSCDQLYTANYDYFTPEVISNYRRYEVAEQMDSFLYKGNAGWFWFCWKTENTIEWDMQRLLLGLGIIAQPLDDRQYPNQCGFS
A Chain A, F229aE292S DOUBLE MUTANT OF EXO-Beta-1,3-Glucanase From Candida Albicans In Complex With Laminaritriose At 1.7 A

GGHNVAWDYDNNVIRGVNLGGWFVLEPYMTPSLFEFQGNDQSGVGPVEHYHTQTLGKEAARLQLKHW
STWITEQDFQISINLGNFVRIPIGYWAFLDNDPYYQGQQYLEKALGWARKNNINRWTDLHAGAPGSQ
NGFDNSLQRDSYNQDTQVNLTVFQKYGGNYEDSVVIGIENLENPGLFVMNMDQLQFNLDDG
NSLRQTGSVPVIIHDAQVQYYNWNVLTVAEQQWNVVDDHHHVGFGDNLRSNINDHSVACNWGWGDKR
KKESSHVNNWAGSWAALTDCAWLENGVAGRAYEGYDANAPYIGSCQPLDLISQSDEHKTDTRRYEUAL
DAFEYTGWVFWSKTEPAFQFTLYNLGFQPYQTDRQFPNCGFH

exobeta-1,3-glucanase (Exgl), putative
[Aspergillus clavatus NRRL 1]
MLSRLSQTALVALSMTVLEAVPSRMRIQTRDSAVYSEQEIVRGNLGGWLVEPYMTPSIFFENGGAADV
DETWLAELVGLKAKARILSQQHSSFFITQDDQFQIAQAAGMHRVPYGMVAYSAPEDEYVQDFLELQIAM
SWARAAGLKLMIDHLGGKSEQNFVQITEWDQAVETAPSGSNGFNSGKRPGFIAQWQODTAVTDFAKAL
AERYLPSDDVTAVIAEAVNPPIPNGGVNQGLKEYNYQVEVHVSNIPDAQVLSDFGFLATASWNGYANG
NVVMDTTHYHMFDLSLTRSLDINAHYRAACEFQNGQIKSGDKPVVGETALTDCTKHLNDDIPRYEQG
WANSPPYRDCGNKRQGSSSSLEQERSDTRRQFIAQLDAYSERGKNGWFLWTKEGAPGWMQDLANLFP
NPPTERQYGNQCA

hypothetical protein BC1G_12478
[Botryotinia fuckelianca B05.10]
MYFTNTVGTLLATLVASKPYQYRGVDFAYGSTFVRRGNLGGWLVEPYMTPSIFQGVDQSLISGIDVETLT
QKGLGEEAAALIJKPHDWSCTADDFFNQIANAFLNFTPRIPYWAGYSLDNEPYTGQAAAAYMDAIDWAR
GAGLKLWDLHGLPSQRNGFDSNGTHKSTSSPAAFGGQDSVKNTLSVLTNTEYKAKQYQDQVVGIIELENN
ANWKVNFVDFLSEQFYRDGQVQVRAVSDSIVVIHDAFLPSWNWNLSSNDANAYVGGVDHEQYVQFSDSL
AMSRAEHEYVCSNAGAYTGADKWVWVGEFTAMTDAYALNYGNYGDYPTGGSYSGSCGDKSIT
TWSDDFKTDKMNYSALAQLLSYETKANGWFEWNPKEGAHEWDAKLVEIDFPLRAGTPAICS

Major exo-1,3-beta-glucanase of the cell wall, involved in cell wall beta-glucan assembly [Pichia pastoris GS115]
MNLYLITLFLFASLCSAITLPKRDIDWYSESDKVINGNLGNLGGWLVEPYMTPSLFEAFGDDDVPEDEYTER
LQKSLALDLRLQHQWHSTFDEKDQIAAYGLNFRIPQYWAFLDQFQGEEYDLEKSAWRH
LKVIWDLHGAPGQNGFDSNGKRDSDWDFQGNNVQVTDLVKYSKGYTTDYDVIGIQIGLQNLPELGPI
LDMDNLQRQFYADGYDLVRDGNFVTVHDAFQYQAPEYWGDDFTSAEAGYNWVLDHHHYVQFDEADREQLSI
DEHIEAACWDGRDANKYHWNLCGEWSAALTDCPWLNVGKGTRYEGQLDNSPWSGSCNSQDPSKLSS
ERICEYRRYVEQALDFALHNGKSAGIFWCARKTEASLEDFKRGNLNWGMIFQPLDDQYPNQCGF

hypothetical protein SS1G_05775
[Sclerotinia sclerotiorum 1980]
MFFKNTVGSLLVALTVASKPYQYRKVDAYGTFVRFVRGNGGGLVLEPYMTPSLFEFQGNDQSLGQIDVETYTM
TEKLGTEAASAILQPHWSCTAVDFQKIAEDSGFNTVRIQPIGYWAYAHGYEPTQQGAAAYMDAIDWARS
AGLKLWDLHGLAPSQRNGFDSNCHTRTSSPAQTQDSVATLSVLTNQIITKQAYEQGFQVVGIEILNPE
AWIMDFEVELEQFYRDGYQVQVRAVSDSIVVHDFAYQPNTWNNLISPNDNAQGVDHHEQYVQFDALVA
MTPAEHVDVYCVSNAYHTGDKWVVFGEFTAMTDCAFALNYYGVGSKYRGDSYNFPSTYDSDCEGSKITT
immunodominant antigen Gp43 [Paracoccidioides brasiliensis]

YALI0F0530.1 [Yarrowia lipolytica]

EXG_DEBOC RecName: Full=Glucan 1,3-beta-glucosidase; AltName: Full=Exo-1,3-beta-glucanase; Flags: Precursor (Schwanniomyces occidentalis)

EXO-beta-1,3-glucanase [Williopsis saturnus]

immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
PLGPVLDMAKLNEFWETAYWNLRNSNSTQTVIHDFTAGYFNDFKQLNQGYWLVIDHHHYQVFSQEQVQRSDIEHEVEACQGGKDSKGENLNLNCGWESALATDCAKWLNGVKGARYDQTGFNSQYTGSCTNSQDI
STWSSDVKANYRRYIEAQLDAFEQRGGWFWCWKTEWAGDFQKLANYNVFQPLDDRQYPNQCGF

>gi|1150694|emb|CAA86952.1| exo-1,3-beta-glucanase/1,3-beta-D-glucanohydrolase [Yarrowia lipolytica]
MKLTKLVALAGAALAPIQLVPREGSFPLFGNYGSEKVCVHVNLGFWYLEPFTIPSLEAFGNNDAANPVPE
EYHTANLWGKEEAEKRLLTDEWNTTWIEYDIKAAIAENYKLNVRIPYGWFSSLPPDNYVQGEAYLDRA
LGWCRKYGVAKAVDVGPGSQQNFDNSGRLDHWDWPNADVQHSINVINYAGYGAPEYDINVVGIELVNEPLGPAIGMEVEIYKFQEGFWTVRHDGSTAVIHDAPQEKNYFNNFTQETFQFWNVLDDHHQYQVFS
PGELARNIDQHIAEVCNVRGQASTEYHWRIFGEWWSAltDDCTHWLNGVKGPSLDGSPFGSYQQSCQGRGDIQTWSEQDQKQESRYYEVAQLDWDWEHGDGGWYWTYKTENALEDFRRLVINGIFFPYWDROQFPNQCGF

>gi|8576322|gb|AAC49253.2| 43 kDa secreted glycoprotein precursor [Paracoccidioides brasiliensis]
MNFSNLALASCVLAWCLASASSHVASHIVPRQAGSAIYGNIVGGWLLLEFWISPSVEAGGGSSSVE
YTLSNKNLGRDKRHSKHWNTFTIETDFKNAIAAGLHNHRIPIGYAWNPIEGEPYVQQLDLYLDAKAVW
AKNSNLPRVVDLHGPGSQQNFDNSHGRAGINWQKGDITKQTILAIHTLAIRYANRTDVDSIELNVKPS
IPGGVQVQSLKEYYEDGYHIVRhDIIDSTGVWASDASLPPRTWNGFLAPKTVKYNVYDLRTYHQVFDFDFRFTIDQHVKLACSLPHDRLRAGDKPILKVEWSGMTDCAMYLRGRGISRFDGSFGPSGACGARSKGS
SSELSAQKDKTDLRYIEAQLDAFEVAGWFYWFTWKEGAPGWDMQDLLQQKLFQPIWARKYGGCIR

>gi|11496173|gb|AAG36668.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSNLALASCVLAWCLASASSHVASHIVPRQAGSAIYGNIVGGWLLLEFWISPSVEAGGGSSSVE
YTLSNKNLGRDKRHSKHWNTFTIETDFKNAIAAGLHNHRIPIGYAWNPIEGEPYVQQLDLYLDAKAVW
AKNSNLPRVVDLHGPGSQQNFDNSHGRAGINWQKGDITKQTILAIHTLAIRYANRTDVDSIELNVKPS
IPGGVQVQSLKEYYEDGYHIVRhDIIDSTGVWASDASLPPRTWNGFLAPKTVKYNVYDLRTYHQVFDFDFRFTIDQHVKLACSLPHDRLRAGDKPILKVEWSGMTDCAMYLRGRGISRFDGSFGPSGACGARSKGS
SSELSAQKDRDTDRYIEAQLDAFEVAGGWWYWTWKEGAGPQWDMDQDLLQQKLFQPIWARKYGGCIR

>gi|11496175|gb|AAG36669.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSNLALASCVLAWCLASASSHVASHIVPRQAGSAIYGNIVGGWLLLEFWISPSVEAGGGSSSVE
YTLSNKNLGRDKRHSKHWNTFTIETDFKNAIAAGLHNHRIPIGYAWNPIEGEPYVQQLDLYLDAKAVW
AKNSNLPRVVDLHGPGSQQNFDNSHGRAGINWQKGDITKQTILAIHTLAIRYANRTDVDSIELNVKPS
IPGGVQVQSLKEYYEDGYHIVRhDIIDSTGVWASDASLPPRTWNGFLAPKTVKYNVYDLRTYHQVFDFDFRFTIDQHVKLACSLPHDRLRAGDKPILKVEWSGMTDCAMYLRGRGISRFDGSFGPSGACGARSKGS
SSELSAQKDRDTDRYIEAQLDAFEVAGGWWYWTWKEGAPGQWDMDQDLLQQKLFQPIWARKYGGCIR

>gi|11496191|gb|AAG36677.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSNLALASCVLAWCLASASSHVASHIVPRQAGSAIYGNIVGGWLLLEFWISPSVEAGGGSSSVE
>gi|11496227|gb|AAG36694.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSSLNLALASCVLAWCLASASSHVASIVHRPQAGSAIYGVNIGGWLLLEPISPSVFEEAGGSSVDE
YTLSKSLRGDARKLSKWHTDFITEEDFKNIAAAAGLNHRIPIGYAVNPIEGEPYVQQLDLKAVW
AKNSNLRVVIDLHGVPQSNGFDNSGHRGAVNWQKGDTRQTLIAIHTLAIYANRDTVDSIELVNKPS
IPGGVQVSSLKEYEDGYHIVRIDSTVGVSISADSPRTWNGFLAPKTYKNYVLDTYHNQVFDDIFRT
FTIDQHVKLACSLPHDRLGADKPLIVKESGMATDCAMYLNRGIGSFDFSGPSGACGARSKGS
SSELSAQKQKDTRYLAIEQLDAFEVGAGWYFTWTEKAPGWDMDQDLLNQLFPQPIWARKYGGCR

>gi|11496198|gb|AAG36680.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSSLNLALASCVLAWCLASASSHVASIVHRPQAGSAIYGVNIGGWLLLEPISPSVFEEAGGSSVDE
YTLSKSLRGDARKLSKWHTDFITEEDFKNIAAAAGLNHRIPIGYAVNPIEGEPYVQQLDLKAVW
AKNSNLRVVIDLHGVPQSNGFDNSGHRGAVNWQKGDTRQTLIAIHTLAIYANRDTVDSIELVNKPS
IPGGVQVSSLKEYEDGYHIVRIDSTVGVSISADSPRTWNGFLAPKTYKNYVLDTYHNQVFDDIFRT
FTIDQHVKLACSLPHDRLGADKPLIVKESGMATDCAMYLNRGIGSFDFSGPSGACGARSKGS
SSELSAQKQKDTRYLAIEQLDAFEVGAGWYFTWTEKAPGWDMDQDLLNQLFPQPIWARKYGGCR

>gi|11496229|gb|AAG36695.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSSLNLALASCVLAWCLASASSHVASIVHRPQAGSAIYGVNIGGWLLLEPISPSVFEEAGGSSVDE
YTLSKSLRGDARKLSKWHTDFITEEDFKNIAAAAGLNHRIPIGYAVNPIEGEPYVQQLDLKAVW
AKNSNLRVVIDLHGVPQSNGFDNSGHRGAVNWQKGDTRQTLIAIHTLAIYANRDTVDSIELVNKPS
IPGGVQVSSLKEYEDGYHIVRIDSTVGVSISADSPRTWNGFLAPKTYKNYVLDTYHNQVFDDIFRT
FTIDQHVKLACSLPHDRLGADKPLIVKESGMATDCAMYLNRGIGSFDFSGPSGACGARSKGS
SSELSAQKQKDTRYLAIEQLDAFEVGAGWYFTWTEKAPGWDMDQDLLNQLFPQPIWARKYGGCR

>gi|11496193|gb|AAG36678.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSSLNLALASCVLAWCLASASSHVASIVHRPQAGSAIYGVNIGGWLLLEPISPSVFEEAGGSSVDE
YTLSKSLRGDARKLSKWHTDFITEEDFKNIAAAAGLNHRIPIGYAVNPIEGEPYVQQLDLKAVW
AKNSNLRVVIDLHGVPQSNGFDNSGHRGAVNWQKGDTRQTLIAIHTLAIYANRDTVDSIELVNKPS
IPGGVQVSSLKEYEDGYHIVRIDSTVGVSISADSPRTWNGFLAPKTYKNYVLDTYHNQVFDDIFRT
FTIDQHVKLACSLPHDRLGADKPLIVKESGMATDCAMYLNRGIGSFDFSGPSGACGARSKGS
SSELSAQKQKDTRYLAIEQLDAFEVGAGWYFTWTEKAPGWDMDQDLLNQLFPQPIWARKYGGCR

>gi|11496185|gb|AAG36674.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSSLNLALASCVLAWCLASASSHSHASHIVPRQAGSAIYGVNIGWWLLEPWSPSVPFEAGGSSSDEVDE YTLSKNLRDZAKRLSKWHDFDTIEDDFKNAIAAGLNVHRPIGYWANPIEGEPYVQQGLDYLKDVALW AKNLSNLRVVIDLHVGPSQNGFNSNHGAINWQKGDITRQTLIAHTLARYANRVDVDSIELVNPS IFPGGVQSVLLKEYEDYHIVRVIDGTVGSISDASLPPRTWNGFLAPKTYKNYVITYHNYQVFDDFFRT FTIQDHVKLACSLPHGLRAGDKPLVKEWSGAMTDACMLNHRGIGSRFDGFSFPSKPSGACGARKSGS SSELASQKDKDTLRYIEAQLDAFEVGAVGYFWTDATRAPGWMDQLNLQNKLFPQPIWARKYGGC

>gi|296811334|ref|XP_002846005.1| glucan 1,3-beta-glucosidase [Arthroderma otae CBS 113480]
MRFYFGQLLSLISVSAPVSTWKNSPRATAQDFIRGVNIGGWLVLEPWITPSIFEEGDSAVDEWTSQ ALGERAHDLRLKHWNTYDIQDNFNRIDVGLTHVRIPLYDIVAIPEGPVQEQVDMALLADWASHG LSVMIDLDAGPSQNGFDSNSGLGAPWANGKQGTDVATLKDFLIQNYSHQGVGVHSGLINEPPQAGI QVEPLKEYFKGAKVSSPNPLAVISAFMPSQKNWYDLGAKTIDYHYEFPSLEVWTQHVK AACDFGTNLTSSLSPVGEWCGMTDACYLVNHRGEGARYDGHSGKSNHDTAPVNGCVCSEGSISGF SDEEKANTRRYIEAQLDSLSRGVFFWTDATRAPGWDLILNKVPMIDTRTPYQQCH

>gi|71003403|ref|XP_756382.1| hypothetical protein UM00235.1 [Ustilago maydis 521]
MLLKHTLLALASASSLQRTTASPIQGAVPFPNGTDPVIRADTSGKLYDSLLEQOARIFFEP RQPNGFAGSVGKVRVGNNIGWLVAPWMTPSLDFNGSVIDWETFGFQYASNAYNRLNHWATFYTED FAQIAAAALNHRPIGYWAFTDAGEPYVRNSQADYLERAIQNSRNHKLVIDLHAGPSQNGFDSNG RSKVNWPDANDNNRAAVIGTIAARYAQYDGTTSIQLLNEPAGVGGNILDYTNYMNGYGAARSR FGNAASIMHDFQPLYSWNGFMQPQQFQVQLDLTHIYQVFSPAENRDNETFICMAGNGLASSKNL WTEGEWTDATDACNLNHRGEGARYDGSPGSYYVGFSCSDKTGDGSNFSDAYĐTLMFETQISVE RASVGFMTQWTKTEQAADWDYQRGLNRGYTNLDSFRPNAR

>gi|11496200|gb|AAG36681.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSSLNLALASCVLAWCLASASSHSHASHIVPRQAGSAIYGVNIGWWLLEPWSPSVEAGGSSSDEVDE YTLSKNLRDZAKRLSKWHDFDTIEDDFKNAIAAGLNVHRPIGYWANPIEGEPYVQQGLDYLKDVALW AKNLSNLRVVIDLHVGPSQNGFNSNHGAINWQKGDITRQTLIAHTLARYANRVDVDSIELVNPS IFPGGVQSVLLKEYEDYHIVRVIDGTVGSISDASLPPRTWNGFLAPKTYKNYVITYHNYQVFDDFFRT FTIQDHVKLACSLPHGLRAGDKPLVKEWSGAMTDACMLNHRGIGSRFDGFSFPSKPSGACGARKSGS SSELASQKDKDTLRYIEAQLDAFEVGAVGYFWTDATRAPGWDQMDQLNLQNKLFPQPIWARKYGGC

>gi|11496187|gb|AAG36675.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSSLNLALASCVLAWCLASASSHSHASHIVPRQAGSAIYGVNIGWWLLEPWSPSVEAGGSSSDEVDE YTLSKNLRDZAKRLSKWHDFDTIEDDFKNAIAAGLNVHRPIGYWANPIEGEPYVQQGLDYLKDVALW AKNLSNLRVVIDLHVGPSQNGFNSNHGAINWQKGDITRQTLIAHTLARYANRVDVDSIELVNPS IFPGGVQSVLLKEYEDYHIVRVIDGTVGSISDASLPPRTWNGFLAPKTYKNYVITYHNYQVFDDFFRT FTIQDHVKLACSLPHGLRAGDKPLVKEWSGAMTDACMLNHRGIGSRFDGFSFPSKPSGACGARKSGS SSELASQKDKDTLRYIEAQLDAFEVGAVGYFWTDATRAPGWDQMDQLNLQNKLFPQPIWARKYGGC
>gi|11496181|gb|AAG36672.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSSLNLALASCVLAWCLASASSHVASHIVPRQAGSAIYGVNIGGWLLLEPFWISPVFAGGSSSVDE
YTL SKNLGRDAKRHLKSHWDFITEDDFKNAIAAGLNNHVPIGYAVPINEPYQVQOQLDYLKDCAWL
AKNSNLVRVIDHLVPGSNGFDSNGHRGAINQKDQGTDIRQTLSIAHTLAIRYANRTDVDSLIELVNPKS
IPGGVQVSSLKEYYEDIHYIVRVIDSTVGVISIDASLPPRTWNGFAPKTYNKYIDTHYNQVFDFFIFRT
FTIDQHVKLCASLPHRGRGLDGKPILVKEWSGAMTDCAILNGRGIARGSFRDFSFGSFKSGACGARSGKS
SSELQAQQKKTDLRYIEAQQLDAFEVAGYGNFTWKTGEPGWDMQDLLNQKLFQQFIPWARYGGCR

>gi|11496189|gb|AAG36676.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSSLNLALASCVLAWCLASASSHVASIVPRQAGSAIYGIVNGGWLLLEPFWISPVFAGGSSSVDE
YTL SKNLGRDAKRHLKSHWDFITEDDFKNAIAAGLNNHVPIGYAVPINEPYQVQOQLDYLKDCAWL
AKNSNLVRVIDHLVPGSNGFDSNGHRGAINQKDQGTDIRQTLSIAHTLAIRYANRTDVDSLIELVNPKS
IPGGVQVSSLKEYYEDIHYIVRVIDSTVGVISIDASLPPRTWNGFAPKTYNKYIDTHYNQVFDFFIFRT
FTIDQHVKLCASLPHRGRGLDGKPILVKEWSGAMTDCAILNGRGIARGSFRDFSFGSFKSGACGARSGKS
SSELQAQQKKTDLRYIEAQQLDAFEVAGYGNFTWKTGEPGWDMQDLLNQKLFQQFIPWARYGGCR

>gi|11496208|gb|AAG36685.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
SLNLALASCVLAWCLASASSHVASIVPRQAGSAIYGVNIGGWLLLEPFWISPVFAGGSSSVDEYT
KLGRDAKRHLKSHWDFITEDDFKNAIAAGLNNHVPIGYAVPINEPYQVQOQLDYLKDCAWL
AKNSNLVRVIDHLVPGSNGFDSNGHRGAINQKDQGTDIRQTLSIAHTLAIRYANRTDVDSLIELVNPKS
IPGGVQVSSLKEYYEDIHYIVRVIDSTVGVISIDASLPPRTWNGFAPKTYNKYIDTHYNQVFDFFIFRT
FTIDQHVKLCASLPHRGRGLDGKPILVKEWSGAMTDCAILNGRGIARGSFRDFSFGSFKSGACGARSGKS
SSELQAQQKKTDLRYIEAQQLDAFEVAGYGNFTWKTGEPGWDMQDLLNQKLFQQFIPWARYGGCR

>gi|114962183|gb|AAG36673.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
MNFSSLNLALASCVLAWCLASASSHVASIVPRQAGSAIYGVNIGGWLLLEPFWISPVFAGGSSSVDE
YTL SKNLGRDAKRHLKSHWDFITEDDFKNAIAAGLNNHVPIGYAVPINEPYQVQOQLDYLKDCAWL
AKNSNLVRVIDHLVPGSNGFDSNGHRGAINQKDQGTDIRQTLSIAHTLAIRYANRTDVDSLIELVNPKS
IPGGVQVSSLKEYYEDIHYIVRVIDSTVGVISIDASLPPRTWNGFAPKTYNKYIDTHYNQVFDFFIFRT
FTIDQHVKLCASLPHRGRGLDGKPILVKEWSGAMTDCAILNGRGIARGSFRDFSFGSFKSGACGARSGKS
SSELQAQQKKTDLRYIEAQQLDAFEVAGYGNFTWKTGEPGWDMQDLLNQKLFQQFIPWARYGGCR

>gi|189197111|ref|XP_001934893.1| glucan 1,3-beta-glucosidase precursor [Pyrenophora tritici-repens Pt-1c-BFP]
MIFSRTIALSLSVAAALAPTEKRGVAFNWGTDKVRGNIVNGGWLLLEPFWITPSIFDNADNANNQKDIVDE
YTLQKGLSGAAAGSLRSHDTSWTDENKNKIQAGFNIVRIPSFLAYDTPGAYVSYSGCQVYDAAVDW
SRSLGKIDLHAGPSSNQNGFSNGKQMPQWQKGDYQTLQVLNTOQYKQAESQYQDVLIGqli
EPAEYNLNLDLQKYFIRQYQTRASDPTVHLHDFNNTWNGFLTPSNDENAYVMDDNYQVFQ
TLLKMSPAQHTSYVCSNSGTWSDGKWTIIEGTSAMTDCAILNGRGIARGSFRDFSFGSFKSGACGARSGKS
SSELQAQQKKTDLRYIEAQQLDAFEVAGYGNFTWKTGEPGWDMQDLLNQKLFQQFIPWARYGGCR

>gi|189197111|ref|XP_001934893.1| glucan 1,3-beta-glucosidase precursor [Pyrenophora tritici-repens Pt-1c-BFP]
MIFSRTIALSLSVAAALAPTEKRGVAFNWGTDKVRGNIVNGGWLLLEPFWITPSIFDNADNANNQKDIVDE
YTLQKGLSGAAAGSLRSHDTSWTDENKNKIQAGFNIVRIPSFLAYDTPGAYVSYSGCQVYDAAVDW
SRSLGKIDLHAGPSSNQNGFSNGKQMPQWQKGDYQTLQVLNTOQYKQAESQYQDVLIGqli
EPAEYNLNLDLQKYFIRQYQTRASDPTVHLHDFNNTWNGFLTPSNDENAYVMDDNYQVFQ
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_Candida_oleophila
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Schwanniomyces_occidentalis
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Cleistococcum nitidum_CBS_427
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Pichia_pastoris_GS115
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Aspergillus clavatus
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Coprinopsis cinerea okayama71
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Candida_oleophila
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2 Ajellomyces dermatitidis SLH
1 Ajellomyces capsulatus H88
Trichophyton verrucosum KRI 05
Arthroderma benhamiae CBS 1123
Arthroderma ota CBS 114893
Talomyces stipitatus ATCC 10
Penicillium marneffei ATCC 182
Penicillium chrysogenum
Neosartorya_fischeri NRRL 181
Aspergillus clavatus
Aspergillus clavatus NRRL 1
Aspergillus clavatus NRRL 3357
Aspergillus niger CBS 513.88
Aspergillus terreus NH2624
Aspergillus nigrum FGSC A4
2 Botryotinia fuckelliana B05.1
2 Sclerotinia sclerotiorum 198
2 Pyrenophora triticiana-repentis
2 Pyrenophora teres f. teres 0
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2 Phaeosphaeria nodorum SN19
2 Leptosphaeria maculans
2 Penicillium chrysogenum
Neosartorya_fischeri NRRL 181
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Penicillium marneffei ATCC 182
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Aspergillus_terreus_NH2624  PFPFEDRWFPKQCG-------
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Sporisorium_reilianum  TRNLNSRPNARC--------
Agaricus_bisporus  PQQNTERQFFGICG-------
Laccaria_bicolor_S238N-H82  PQDPTNFIYFKICN-------
Coprinopsis_cinerea_okayama7#1  PKNPTDRQYFPNIDC--------
Schizophyllum_commune_H4-8  PWNPTDIQNRATCG--------
NCBI Generated Lineage Report for EST C00482

Dikarya (fungi)
  > Ascomycota [ascomycetes]
  > Pezizomycotina [ascomycetes]
  > Leotiomycetes [ascomycetes]
  > Ustilaginomycotina [ascomycetes]
  > Biomeria graminii (grass mildew) ----------------------------- 886 2 hits [ascomycetes] RecName: Full=Glucon 1,3-beta-glucosidase; AltName: Full=Ex
  > Botrytis Fuckeliana B05.10 ------------------------------- 590 4 hits [ascomycetes] hypothetical protein BCIG14133 [Botrytis Fuckeliana B05]
  > Pyrenophora teres f. teres 0-1 - 426 2 hits [ascomycetes] hypothetical protein PFR14084 [Pyrenophora teres f. teres]
  > Pyrenophora tritici-repentis Pt-10-CPP ------------------ 423 4 hits [ascomycetes] hypothetical protein [Pyrenophora tritici-repentis]
  > Leptosphaeria maculans (blackleg of crucifers ..) - 420 2 hits [ascomycetes] glucon 1,3-beta-glucosidase [Leptosphaeria maculans]
  > Ajellomyces dermatitidis SUn14081 ------------------------------- 409 2 hits [ascomycetes] glucon 1,3-beta-glucosidase [Ajellomyces dermatitidis]
  > Ajellomyces dermatitidis EC3 ------------------------------- 409 1 hit [ascomycetes] glucon 1,3-beta-glucosidase [Ajellomyces dermatitidis]
  > Paecilomyces nubeculosus MHi ------------------------------- 405 3 hits [ascomycetes] hypothetical protein SNB50089 [Paecilomyces nubeculosus]
  > Aspergillus terreus NHM224 ---------------------------------- 405 3 hits [ascomycetes] glucon 1,3-beta-glucosidase [Aspergillus terreus]
  > Aspergillus fumigatus ATCC10516 ------------------------------- 404 4 hits [ascomycetes] RecName: Full=Probable glucon 1,3-beta-glucosidase A; AltNa
  > Aspergillus fumigatus AFB27 ------------------------------- 404 2 hits [ascomycetes] glucon 1,3-beta-glucosidase [Aspergillus fumigatus]
  > Aspergillus fumigatus AspA ------------------------------- 404 3 hits [ascomycetes] glucon 1,3-beta-glucosidase [Aspergillus fumigatus]
  > Neosartorya fischeri NRRL181 ---------------------------------- 401 3 hits [ascomycetes] immunodominant antigen Gp43 [Paracoccidioides brasiliensis]
  > Ajellomyces capsulatus H84 ------------------------------- 399 1 hit [ascomycetes] glucon 1,3-beta-glucosidase [Ajellomyces capsulatus]
  > Ajellomyces capsulatus H17 ------------------------------- 399 1 hit [ascomycetes] glucon 1,3-beta-glucosidase [Ajellomyces capsulatus]
  > Ajellomyces capsulatus H41 ------------------------------- 395 3 hits [ascomycetes] glucon 1,3-beta-glucosidase [Ajellomyces capsulatus]
  > Paracoccidioides brasilensis Pb61 ------------------------------- 387 2 hits [ascomycetes] glucon 1,3-beta-glucosidase [Paracoccidioides brasilensis]
  > Aspergillus oryzae R1840 ---------------------------------- 387 1 hit [ascomycetes] glucon 1,3-beta-glucosidase [Aspergillus oryzae]
  > Aspergillus flavus NHM1357 ---------------------------------- 387 3 hits [ascomycetes] glucon 1,3-beta-glucosidase [Aspergillus flavus]
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  > Paracoccidioides brasilensis Pb12 ------------------------------- 388 3 hits [ascomycetes] glucon 1,3-beta-glucosidase [Paracoccidioides brasilensis]
  > Paracoccidioides brasilensis Pb3 ------------------------------- 387 2 hits [ascomycetes] glucon 1,3-beta-glucosidase [Paracoccidioides brasilensis]
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  > Trichophyton verrucosum KRT 0017 ------------------------------- 351 2 hits [ascomycetes] glucon 1,3-beta-glucosidase [Trichophyton verrucosum]
  > Cochliobolus carbonum ------------------------------- 350 1 hit [ascomycetes] glucon 1,3-beta-glucosidase [Cochliobolus carbonum]
  > Arthroderma gypseum CBS 118932 ------------------------------- 344 2 hits [ascomycetes] glucon 1,3-beta-glucosidase [Arthroderma gypseum]
  > Tuber melanosporeus Mel128 ------------------------------- 385 1 hit [ascomycetes] glucon 1,3-beta-glucosidase [Tuber melanosporeus Mel128]
  > Candida oleophila ------------------------------- 393 2 hits [ascomycetes] RecName: Full=Glucon 1,3-beta-glucosidase; AltName: Full=Ex
  > Lecanicillium lecanii VR-19398 ------------------------------- 392 2 hits [ascomycetes] glucon 1,3-beta-glucosidase [Lecanicillium lecanii]
  > Debaryomyces hansenii CBS7959 ------------------------------- 386 1 hit [ascomycetes] hypothetical protein [Debaryomyces hansenii CBS7959]
  > Candida albicans SC5314 ------------------------------- 386 4 hits [ascomycetes] hypothetical protein [Candida albicans SC5314]
  > Candida albicans WC1 ------------------------------- 383 1 hit [ascomycetes] hypothetical protein [Candida albicans WC1]
  > Candida tropicalis NYA-3404 ------------------------------- 380 2 hits [ascomycetes] glucon 1,3-beta-glucosidase [Candida tropicalis NYA-3404]
  > Clavispora lusitaniae ATCC 45270 ------------------------------- 379 2 hits [ascomycetes] hypothetical protein [Clavispora lusitaniae ATCC 45270]
Organism Report

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gb|EO047928.1| glucan 1,3-beta-glucosidase precursor [Pyre... 423 3e-116
ref|XP_001934863.1| glucan 1,3-beta-glucosidase precursor ... 357 2e-95
gb|EO047487.1| glucan 1,3-beta-glucosidase precursor [Pyre... 357 2e-95

Leptosphaeria maculans [blackleg of crucifers fungus, ... [ascomycetes] taxid 5022
cmb|CEK29141.1| hypothetical protein [Leptosphaeria maculans] 420 3e-115
cmb|CEK297550.1| hypothetical protein [Leptosphaeria maculans] 481 4e-91

Ajellomyces dermatitidis EDH14081 [ascomycetes] taxid 559298
ref|XP_002570611.1| glucan 1,3-beta-glucosidase [Ajellomy... 409 4e-112
gb|EDU43728.1| glucan 1,3-beta-glucosidase precursor [Pyre... 409 4e-112

Ajellomyces dermatitidis ER-3 [ascomycetes] taxid 559297
gb|EEQ25787.1| glucan 1,3-beta-glucosidase [Ajellomyces de... 409 4e-112

Phaeosphaeria nodorum SN15 [ascomycetes] taxid 321614
ref|EF001791287.1| hypothetical protein [Phaeospha... 405 5e-111
gb|EF001791287.1| hypothetical protein [Phaeospha... 405 5e-111
ref|XP_001791287.1| glucan 1,3-beta-glucosidase [Phaeospha... 405 4e-93

gb|EAT92101.1| hypothetical protein [Phaeosphaer... 405 4e-93

Aspergillus fumigatus A1163 [ascomycetes] taxid 451804
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gb|EDP55704.1|exo-beta-1,3-glucanase (Exg1), putative [As... 404 1e-110

Aspergillus fumigatus A1293 [ascomycetes] taxid 330879
ref|XP_750110.1|exo-beta-1,3-glucanase (Exg1) [Aspergillu... 404 2e-110
gb|EAL88072.1|exo-beta-1,3-glucanase (Exg1), putative [As... 404 2e-110

Aspergillus fumigatus [ascomycetes] taxid 5085
sp|B0XN12.1|EXGA_ASPFC RecName: Full=Probable glucan 1,3-b... 404 1e-110

Neosartorya fischeri NRRL 181 [ascomycetes] taxid 33117
ref|XP_001523025.1|exo-beta-1,3-glucanase (Exg1) [Aspergillu... 401 9e-110
sp|ALD452.1|EXGA_NF RecName: Full=Probable glucan 1,3-b... 401 9e-110

gb|FAW22939.1|exo-beta-1,3-glucanase (Exg1), putative [Ne... 401 9e-110

Ajellomyces capsulatus H88 [ascomycetes] taxid 544711
gb|EKZ46035.1|immunodominantigen Gp43 [Ajellomyces capsul... 399 3e-109

Ajellomyces capsulatus H143 [ascomycetes] taxid 544712
gb|EEA43103.1|immunodominantigen Gp43 [Ajellomyces capsul... 399 4e-109

Ajellomyces capsulatus N461 [ascomycetes] taxid 339724
ref|XP_001540744.1|glucan 1,3-beta-glucosidase [Ajellomy... 395 6e-108
gb|EDN08074.1|glucan 1,3-beta-glucosidase [Ajellomyces ca... 395 6e-108

Ajellomyces capsulatus G186AR [ascomycetes] taxid 447093
gb|EEH11064.1| glucan 1,3-beta-glucosidase precursor [Ajel... 395 6e-108

Candida oleophila [ascomycetes] taxid 45573
sp|Q8NNP4.1|EXG_CANOL RecName: Full=Glucan 1,3-beta-glucos... 393 3e-107
gb|AAM21469.1| 1,3-Beta-glucanase [Candida oleophila] 393 3e-107

Lodderomyces elongisporus NRRL YB-4239 [ascomycetes] taxid 379508
ref|XP_00126673.1| glucan 1,3-beta-glucosidase precursor ... 392 6e-107
gb|EDK43323.1| glucan 1,3-beta-glucosidase precursor [Lodd... 392 6e-107

Emericella nidulans [ascomycetes] taxid 162425
sp|Q8S5X8.2|EXGA_EMENI RecName: Full=Probable glucan 1,3... 392 6e-107

Aspergillus niger CBS 513.88 [ascomycetes] taxid 425011
ref|XP_001398868.1| glucan 1,3-beta-glucosidase A [Aspergi... 389 5e-106
sp|A2RAR6.1|EXGA_ASPNC RecName: Full=Probable glucan 1,3-... 389 5e-106

Aspergillus oryzae RIB40 [ascomycetes] taxid 5061
emb|CAR45212.1| unnamed protein product [Aspergillus niger] 389 5e-106

Aspergillus niger [ascomycetes] taxid 227321
ref|XP_661456.1| hypothetical protein AN4552.2 [Aspergi... 388 8e-106
sp|EAA25523.1| hypothetical protein AN4552.2 [Aspergillus ... 388 8e-106
tp|EFPF74801.1| TPA: beta-1,3-exoglucosidase (Eurofung) [A... 388 8e-106

Paracoccidioides brasiliensis Pb01 [ascomycetes] taxid 502779
ref|XP_000792488.1| glucan 1,3-beta-glucosidase [Paracoccidioides brasiliensis Pb01] 387 1e-105
gb|EEE84724.1| glucan 1,3-beta-glucosidase [Paracoccidioides brasiliensis Pb01] 387 1e-105

Aspergillus oryzae RIB40 [ascomycetes] taxid 510516
ref|XP_001820101.2| glucan 1,3-beta-glucosidase A [Aspergillus oryzae RIB40] 387 2e-105

Aspergillus clavatus [ascomycetes] taxid 5057
sp|AICR00.2|EXGA_ASPCL RecName: Full=Probable glucan 1,3-... 387 2e-105

Aspergillus flavus NRRL3357 [ascomycetes] taxid 332952
ref|XP_002743416.1| exo-beta-1,3-glucanase (Exg1), putative [Aspergillus flavus NRRL3357] 387 2e-105
sp|BBN1513.1|EXGA_ASPYR RecName: Full=Probable glucan 1,3-... 387 2e-105
gb|EED55554.1| exo-beta-1,3-glucanase (Exg1), putative [Aspergillus flavus NRRL3357] 387 2e-105

Aspergillus oryzae [ascomycetes] taxid 5062
sp|CAB74460.1|EXGA_ARAZ RecName: Full=Probable glucan 1,3-beta-gluc... 387 2e-105
cbi|CAZ84341.1| unnamed protein product [Aspergillus oryzae] 387 2e-105

Debaryomyces hansenii CBS767 [ascomycetes] taxid 284592
ref|XP_458827.2| DEHA2D07426p [Debaryomyces hansenii CBS767] 386 3e-105

Debaryomyces hansenii [ascomycetes] taxid 4959
emb|CAG6973.2| unidentified protein [Debaryomyces hansenii] 386 3e-105

Tuber melanosporum Mal120 [ascomycetes] taxid 656061
ref|XP_002840150.1| hypothetical protein [Tuber melanosporum Mal120] 385 8e-105

Tuber melanosporum (French truffle, ...) [ascomycetes] taxid 39416
emb|CAE44341.1| unnamed protein product [Tuber melanosporum] 385 8e-105
**Candida albicans** SC5314 [ascomycetes] taxid 237561
gb|EAW08371.1| hypothetical protein Ca019.10507 [Candida ... 382 3e-104
db|EAL02409.1| hypothetical protein Ca019.10507 [Candida a... 382 3e-104
ref|XP_721488.1| hypothetical protein Ca019.2990 [Candida ... 382 4e-104
db|EAL026990.1| hypothetical protein Ca019.2990 [Candida a1... 382 4e-104

**Candida albicans** WD-1 [ascomycetes] taxid 294748
gb|IEQ42862.1| glucan 1,3-beta-glucosidase precursor [Cand... 383 3e-104

**Candida albicans** [ascomycetes] taxid 5476
sp|F29717.1|EXG CANAL RecName: Full=Glucan 1,3-beta-gluco... 382 4e-104
gb|CAA19826.1| glucan 1,3-beta-glucosidase [Candida albic... 382 4e-104
gb|CAA19826.1| beta-glucosanase [Candida albicans] 382 5e-104
pdb|1FB1A| Chain A, Exo-B-(1,3)-Glucanase From Candida Alb... 382 6e-104
pdb|1FC8A| Chain A, Exo-B-(1,3)-Glucanase From Candida Alb... 382 7e-104
pdb|1EQTJ| Chain A, Exo-B-(1,3)-Glucanase From Candida Alb... 382 7e-104
pdb|1FC8A| Chain A, E27q Mutant Of Exo-B-(1,3)-Glucanase ... 381 1e-103
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pdb|1EQTJ| Chain A, E27q Mutant Of Exo-B-(1,3)-Glucanase F... 381 1e-103
pdb|1F56A| Chain A, F144YF258Y DOUBLE MUTANT OF EXO-Beta-1... 381 3e-103
pdb|1F56A| Chain A, F258I Mutant Of Exo-B-(1,3)-Glucanase ... 379 3e-103
pdb|1F56A| Chain A, F258I Mutant Of Exo-B-(1,3)-Glucanase ... 377 2e-102

**Candida tropicalis** MYA-3404 [ascomycetes] taxid 294747
ref|XP_001269797.1| glucan 1,3-beta-glucosidase precursor [Cand... 382 1e-103
gb|IEQ42862.1| glucan 1,3-beta-glucosidase precursor [Cand... 382 1e-103

**Clavispora lusitaniae** APDC 42720 [ascomycetes] taxid 306902
ref|XP_002615579.1| hypothetical protein CLUG_04461 [Clavispora... 379 4e-103
gb|IEQ41033.1| hypothetical protein CLUG_04461 [Clavispora... 379 4e-103

**Schiffersomyces stipitis** CBS 6054 [ascomycetes] taxid 322104
ref|XP_001857602.2| Glucan 1,3-beta-glucosidase precursor ... 379 7e-103
gb|IEQ41033.1| Glucan 1,3-beta-glucosidase precursor [Exo-... 379 7e-103

**Wickerhamomyces anomalus** [ascomycetes] taxid 4927
gb|BAE40550.1| exo-beta-1,3-glucanase [Wickerhamomyces anom... 378 9e-103
pdb|1E22A| Chain B, exo-beta-1,3-glucanase [Wickerhamomyces anom... 377 1e-102
pdb|1E22A| Chain B, exo-beta-1,3-glucanase [Wickerhamomyces anom... 377 1e-102

**Pichia angusta** DL-1 [ascomycetes] taxid 871575
gb|IEQ42862.1| glucan 1,3-beta-glucosidase [Pichia angusta... 377 1e-102

**Candida dubliniensis** CD6 [ascomycetes] taxid 573826
ref|XP_002475851.1| exo-1,3-beta-glucanase I/II, putative; ... 377 1e-102
gb|CAK44537.1| exo-1,3-beta-glucanase I/II, putative [Cam... 377 1e-102

**Pichia angusta** [ascomycetes] taxid 4905
sp|Q12626.1|EXG CANAL RecName: Full=Glucan 1,3-beta-gluco... 377 2e-102
gb|CAK44537.1| exo-1,3-beta-glucanase I/II, putative [Cam... 377 2e-102

**Aspergillus clavatus** NRRL 1 [ascomycetes] taxid 344612
ref|XP_001269797.1| exo-beta-1,3-glucanase (Exg1), putativ... 375 7e-102
gb|IEQ42862.1| exo-beta-1,3-glucanase (Exg1), putativ... 375 7e-102
**Pichia pastoris** GS115 [ascomycetes] taxid 644223
ref|XP 002491361.1| Major exo-1,3-beta-glucanase of the cell wall [Pichia pastoris] 364 1e-98

gb|CAT69982.1| Major exo-1,3-beta-glucanase of the cell wall [Pichia pastoris] 364 1e-98

**Pichia pastoris** [ascomycetes] taxid 4922
gb|AA129969.1| exo-beta-1,3-glucanase [Pichia pastoris] 364 1e-98

**Paracoccidioides brasiliensis** [ascomycetes] taxid 121759
gb|AAC66689.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 362 8e-98
gb|AAC66689.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 2e-97
gb|AAC66690.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 9e-97
gb|AAC66691.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 1e-96
gb|AAC66691.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 2e-96
gb|AAC66692.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 9e-96
gb|AAC66692.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 1e-96
gb|AAC66693.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 9e-96
gb|AAC66693.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 2e-96
gb|AAC66694.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 2e-96
gb|AAC66695.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 9e-96
gb|AAC66696.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 2e-96
gb|AAC66697.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 9e-96
gb|AAC66699.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 2e-96
gb|AAC66699.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 1e-96
gb|AAC66700.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 9e-96
gb|AAC66700.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 2e-96
gb|AAC66701.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 1e-96
gb|AAC66702.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 9e-96
gb|AAC66703.1| immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 2e-96
gb|AAC66711.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66712.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66713.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66714.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66715.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66716.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66717.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66718.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66719.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66720.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66721.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66722.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66723.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66724.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95
gb|AAC66725.1| truncated immunodominant antigen Gp43 [Paracoccidioides brasiliensis] 357 3e-95

**Schwanniomyces occidentalis** [ascomycetes] taxid 27300
sp|GI37225.2|EXO YARIL RecName: Full=Gluca 1,3-beta-glucos... 361 1e-97

**Yarrowia lipolytica** [ascomycetes] taxid 284591
ref|XP 002491361.1| YAL10705390p [Yarrowia lipolytica] 361 1e-97

**Yarrowia lipolytica** [ascomycetes] taxid 4952
sp|GI37225.2|EXO YARIL RecName: Full=Gluca 1,3-beta-glucos... 361 1e-97

**Yarrowia lipolytica CLIB122** [ascomycetes] taxid 284591
ref|XP 002491361.1| YAL10705390p [Yarrowia lipolytica] 361 1e-97

**Yarrowia lipolytica** [ascomycetes] taxid 4952
sp|GI37225.2|EXO YARIL RecName: Full=Gluca 1,3-beta-glucos... 361 1e-97

**Schwanniomyces occidentalis** [ascomycetes] taxid 27300
sp|GI37225.2|EXO YARIL RecName: Full=Gluca 1,3-beta-glucos... 361 1e-97

**Paracoccidioides brasiliensis Pb03** [ascomycetes] taxid 482561
gb|EER16293.1| glucan 1,3-beta-glucosidase [Paracoccidioides... 360 2e-97
**Williopsis saturnus** [ascomycetes] taxid 4906  
**Paracoccidioides brasiliensis Pb18** [ascomycetes] taxid 502780  
**Arthroderma otae CBS 113480** [ascomycetes] taxid 554155  
**Ustilago maydis 521** [basidiomycetes] taxid 237631  
**Trichophyton verrucosum HKI 0517** [ascomycetes] taxid 663202  
**Cochliobolus carbonum** [ascomycetes] taxid 5017  
**Agaricus bisporus** (common mushroom, ...) [basidiomycetes] taxid 5341  
**Ashbya gossypii ATCC 10895** [ascomycetes] taxid 284811  
**Kluyveromyces lactis NRRL Y-1140** [ascomycetes] taxid 284590  
**Sporisorium reilianum** [basidiomycetes] taxid 72558  
**Schizophyllum commune H4** [basidiomycetes] taxid 578458

Williopsis saturnus [ascomycetes] taxid 4906
ICP71252.21 exo-beta-1,3-glucanase [Williopsis saturnus] 360 3e-97

Paracoccidioides brasiliensis Pb18 [ascomycetes] taxid 502780
GB|EEA2795.1| glucan 1,3-beta-glucosidase [Paracoccidioides brasiliensis Pb18] 357 1e-96

Arthroderma otae CBS 113480 [ascomycetes] taxid 554155
Ref|XP_002848052.1| glucan 1,3-beta-glucosidase [Arthroderma otae CBS 113480] 355 5e-96
GB|EEQ23055.1| glucanase [Arthroderma otae CBS 113480] 355 5e-96

Ustilago maydis Pb18 [ascomycetes] taxid 237631
Ref|XP_002848052.1| N Eighteran protein UNO235.1 [Ustilago maydis Pb18] 355 6e-96
GB|EEH42795.1| glucan 1,3-beta-glucosidase [Paracoccidioides brasiliensis Pb18] 357 1e-96

Arthroderma otae CBS 113480 [ascomycetes] taxid 554155
Ref|XP_002848052.1| N Eighteran protein UNO235.1 [Ustilago maydis Pb18] 355 6e-96

Trichophyton verrucosum HKI 0517 [ascomycetes] taxid 663202
Ref|XP_002848052.1| N Eighteran protein UNO235.1 [Ustilago maydis Pb18] 355 6e-96

Cochliobolus carbonum [ascomycetes] taxid 5017
dc|ATAF5310.1| N Eighteran protein UNO235.1 [Ustilago maydis Pb18] 355 6e-96

Agaricus bisporus (common mushroom, ...) [basidiomycetes] taxid 5341
eUn|CAE32536.1| exo-1,3-beta-glucanase [Agaricus bisporus] 350 2e-94

Lachancea kluyveri [ascomycetes] taxid 4934
db|CAM12381.1| exo-1,3-beta-glucanase/1,3-beta-D-glucanase [Lachancea kluyveri] 350 2e-94

Arthroderma benhamiae CBS 112371 [ascomycetes] taxid 663331
Ref|XP_003017585.1| glucanase, putative [Arthroderma benhamiae CBS 112371] 346 3e-93
GB|EE441220.1| glucanase, putative [Trichophyton verrucosum HKI 0517] 351 1e-94

Kluyveromyces lactis NRRL Y-1140 [ascomycetes] taxid 284590
Ref|XP_452437.1| hypothetical protein [Kluyveromyces lactis NRRL Y-1140] 347 2e-93

Sporisorium reilianum [basidiomycetes] taxid 72558
Emb|CBG67637.1| probable EXG1-exo-beta-1,3-glucanase [Sporisorium reilianum] 345 7e-93

Schizophyllum commune H4-8 [basidiomycetes] taxid 579458
Ref|XP_003073234.1| glycoside hydrolase family 5 protein [Schizophyllum commune H4-8] 345 1e-92
GB|EEQ33055.1| glycoside hydrolase family 5 protein [Schizophyllum commune H4-8] 345 1e-92
<table>
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<tr>
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<td>Kluyveromyces lactis</td>
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<td>Kluyveromyces lactis NRRL Y-1140</td>
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<td>Lachancea kluyveri</td>
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<td>Yarrowia</td>
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<td>1 orgs [Ustilago; Ustilago maydis]</td>
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<tr>
<td>Sporisorium reilianum</td>
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<td>1 orgs [Sporisorium]</td>
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<td>Agaricales</td>
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<td>4 orgs [Agaricomycotina; Agaricomycetes; Agaricomycetidae]</td>
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<td>Agaricus bisporus</td>
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<td>1 orgs [Agaricaceae; Agaricus]</td>
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<tr>
<td>Coprinopsis cinerea okayama74130</td>
<td>2</td>
<td>1 orgs [Psathyrellaceae; Coprinopsis; Coprinopsis cinerea]</td>
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<tr>
<td>Schizophyllum commune H4-8</td>
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<td>1 orgs [Schizophyllaceae; Schizophyllum; Schizophyllum commune]</td>
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<tr>
<td>Laccaria bicolor S23BN-H82</td>
<td>2</td>
<td>1 orgs [Tricholomataceae; Laccaria; Laccaria bicolor]</td>
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Sequences Homologous to the Protein of EST C01417 and ClustalW alignment

>Bgh01761 (BluGen annotation of the gene relating to EST C01417)
MFARYLVLAFLTTVAIAAPLNINLGAYSPALLVGDGEISFGGAEGESAEGIFNTLQGSGTGTTGAAGEAEKSGAKG
DGLDTVKKAKGKEKTKVAAAASERAERQMDGFAAGTVAASEPAKIL

>gi|116206490|ref|XP_001229054.1| hypothetical protein CHGG_02538 [Chaetomium globosum CBS 140.51]
MVRYAIVAFGLAAAPLNINLGAYSPALLVGDGEISLGSTASASELMATLASSAAAGGGGAKPAAAE
AAAAPRAKLRAIDILGKRANAPIAKREPVESKMDAVEEMQWIKRDLAGFNAALGYAKEALKDQP
PKVEMGTENAGVIGIVNPGVNVPGASAAAGGGKKEKRDEVVEEEDAPKMTLVAITEV

>gi|311325484|gb|EFQ91509.1| hypothetical protein PTT_11632 [Pyrenophora teres f. teres 0-1]
MLTQNVLFALVATVAAAPLNNLGAYSPALLVGDGEISLGSTASASELMATLASSAAAGGGGARPAAAE
AAAAPRAKLRAIDILGKRANAPIAKREPVESKMDAVEEMQWIKRDLAGFNAALGYAKEALKDQP
PKVEMGTENAGVIGIVNPGVNVPGASAAAGGGKKEKRDEVVEEEDAPKMTLVAITEV

>gi|189207925|ref|XP_001940296.1| predicted protein [Pyrenophora tritici-repentis Pt-1C-BFP]
MLAQNVLFALVATVAAAPLNNLGAYSPALLVGDGEISLGSTASASELMATLASSAAAGGGGARPAAAE
AAAAPRAKLRAIDILGKRANAPIAKREPVESKMDAVEEMQWIKRDLAGFNAALGYAKEALKDQP
PKVEMGTENAGVIGIVNPGVNVPGASAAAGGGKKEKRDEVVEEEDAPKMTLVAITEV

>gi|156064069|ref|XP_001597956.1| hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
MAAMLAISIAIPLPNINLGAYSPALLVGDGEISLGSTASASELMATLASSAAAGGGGARPAAAE
AAAAPRAKLRAIDILGKRANAPIAKREPVESKMDAVEEMQWIKRDLAGFNAALGYAKEALKDQP
PKVEMGTENAGVIGIVNPGVNVPGASAAAGGGKKEKRDEVVEEEDAPKMTLVAITEV

>gi|312218923|emb|CBX98868.1| hypothetical protein [Leptosphaeria maculans]
MLPQHVLIALVATVAAAPLNNLGAYSPALLVGDGEISLGSTASASELMATLASSAAAGGGGARPAAAE
AAAAPRAKLRAIDILGKRANAPIAKREPVESKMDAVEEMQWIKRDLAGFNAALGYAKEALKDQP
PKVEMGTENAGVIGIVNPGVNVPGASAAAGGGKKEKRDEVVEEEDAPKMTLVAITEV

>gi|169614726|ref|XP_001800779.1| hypothetical protein SNOG_10510 [Phaeosphaeria nodorum SN15]
MPSPTFSHPHKLAMSAHPSPADHHDGTSRSSRSPFGLPRLARTWSRLISSWLRTKTVSSSKPIAGLAWHLAQR
GARAAMCLRAGMASKFXLFHSWQATLGCSRSACNIKALIPYPPTTIKMLPQSVLLALVATVAAP
LNINLGAYSPALLVGDGEISILGSSAESALEMATLASSAAGAAAAGGWRGHDKVQEQGQPGPKLP
NKLKNPLEEAARKPNLRLRAISDFISKRAPAVQQDDKIAAIEAATAWIKRDLQGFQASLAYAREAMK
DSPKVGLSGPNAGIVVGNVPAGSAAGTGPSAEHVKRDEVSDEVSDAETPKMTLVAITEV

>gi|302416909|ref|XP_003006286.1| conserved hypothetical protein [Verticillium albo-atrum VaMs.102]
MLHSYHYILAAAGLVGNNLALPPINLGLSVSALVVDGEIFGSKDSVNLNVLEGAANVAGV ANGQPPPAQAAVQASDKPQVQVIAPPAAKQQSLSQDQQEAQQAISFLGIDQKDPRAGPAATGKIDG TTAKRDLGSFDRALRYAEALVGPQVQLGTAEGSVGIIVDDNNQQAAAPGRGTEEGGCYWRRR RG

>gi|154298898|ref|XP_001549870.1| predicted protein [Botryotinia fuckeliana B05.10]
MAMATISIALPLNINGLGSPALVVGDGEISFGKAADVSNLNAVLEGAAAVGSAATNGAAAPPQQAA APAAAAAPATAAVGATEDKTEAQTALGQMEIAPRPRVAKLERSDVRADKRDQIDTSAALNYAAGAC
KTSPEVQLGTEESGVGLVAKGATTGNTAAAGAAGTAGGAKMGKRDSEPNIAPKTVKVTMMFIRGPAQQ

>gi|39975467|ref|XP_369124.1| hypothetical protein [Magnaporthe oryzae 70-15] (labelled 1_Magnaporthe oryzae 70-15)
MVSQSYLLRLVAFCAVVNAVPLPNINGLGAYSPALVVGDGEIFGSKDSVQLMNVLEGAANVAGV AAAEAGAAQPANQQAAAAPQQGLGLGAKVSDILPLAPGAPGAPVSEEVEDDEDENEQ SASAPLQKRQNAAGFQAALNFAESAALTGPKIQGTPEAVGVIIVDNTASSGRAAAA

>gi|145612209|ref|XP_362575.2| hypothetical protein [Magnaporthe oryzae 70-15] (labelled 2_Magnaporthe oryzae 70-15)
MVNYRFATLIGLAGMAVAVPPNLINGLGAYSPALVVGDGEIFGSKDSVAQPLMSVLEGAANVAGV GKKAGAAAASAGFAGAAAAAPVSNAAQQPAQPATADAAAPAPAFLLEQAAAPQTHMGKAIAPRQEGEAAMETEPEEKTARTQDGATDAEAEEDDEAVAKLRDLASQAAALAFAEKLTGPIIS LGTGEQSKAGVGVVNNPAGAAKAKKERKCRDLGVQGKVKTMTVYRSGIPASLRSNELVDIAIP VGVPSIPRLFSDZDASDZNLVYDGLMTFVETITDADADIESAE

>gi|310792500|gb|EFQ28027.1| hypothetical protein GLRG_03171 [Glomerella graminicola M1.001] (labelled 1_Glomerella graminicola M1.001)
MVNCYIFALAAQLAPNLINGLGAYSPALVVGDGEISFGSKDSVQLMNVLEGAANVAGV ANGQGGAGRAAPATAAPAAPAAAPAAAPATAAAPTSTATICQIQNOAQVQSVADIQGATVIAISSDNQ INEAIARRAAALQMKDIAPQVQQLSKSEHTAGKDRLADGFDARYAEVTLGFKIDLGTVGGESGIVK DNQGQGAQAGAGAGAAASLARKADEAQQPFRRAKTMVYRSGIPVAGEMASDZKVRPSAPVAVQ VPATSNVVKRAQEEELASRGVSGSAITDZNLYDGSVESGVTMTFVETITDAEVDEQQ

>gi|310797936|gb|EFQ32829.1| hypothetical protein GLRG_07973 [Glomerella graminicola M1.001] (labelled 2_Glomerella graminicola M1.001)
MQINTLMTALLAMGINAPNLINGLGAYSPALVVGDGALTGFGETAAAPAGAGGAGGAQVRQPLARRQDDK KVEKRQSGFDRALTFAEAALTGPDDELGTGEQAGVQIKIDNNPKPAAGGAAGAGRE

>gi|164426855|ref|XP_961512.2| hypothetical protein NCU03736 [Neurospora crassa OR74A]
**Organism Report**

**Chaetomium globosum CBS 148.51**
- [ascomycetes] PREDICTED: similar to PDZ domain containing 8 [Taeniopygia guttata]
- taxid:306901
- hypothetical protein CHGG_02538 [Chaetomium globosum CBS 148.51]
- hypothetical protein PPT_11632 [Pyrenophora teres f. teres]
- hypothetical protein NEIELOOT_02234 [Neisseria elongata subsp. glycolytica ANCC 29315]

**Pyrenophora teres f. teres**
- [ascomycetes] hypothetical protein CHGG_02538 [Chaetomium globosum CBS 148.51]
- taxid:861557
- hypothetical protein PPT_11632 [Pyrenophora teres f. teres]
- hypothetical protein CHGG_02538 [Chaetomium globosum CBS 148.51]

**Pyrenophora tritici-repentis**
- [ascomycetes] hypothetical protein PPT_11632 [Pyrenophora teres f. teres]
- taxid:426418
- hypothetical protein PPT_11632 [Pyrenophora tritici-repentis]
- hypothetical protein CHGG_02538 [Chaetomium globosum CBS 148.51]

**Sclerotinia sclerotiorum 1980**
- [ascomycetes] hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
- taxid:65079
- hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
- hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]

**Leptosphaeria maculans**
- [ascomycetes] hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
- taxid:5022
- hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
- hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]

**Phaeosphaeria nodorum SN15**
- [ascomycetes] hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
- taxid:321614
- hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
- hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]

**Verticillium albo-atrum VaM**
- [ascomycetes] hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
- taxid:720221
- hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
- hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]

**Botrytis fuckeliana B05.10**
- [ascomycetes] hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
- taxid:332648
- hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
- hypothetical protein SS1G_00042 [Sclerotinia sclerotiorum 1980]
Neurospora crassa [ascomycetes] taxid 5141
emb|CAD01419.1| putative protein [Neurospora crassa] 77 4e-13

Maganpore oryzae 70-15 [ascomycetes] taxid 242507
ref|XP_362575.2| hypothetical protein [Magnaporthe oryzae ... 48 3e-04
ref|XP_362575.2| hypothetical protein [Magnaporthe oryzae ... 47 9e-08
ref|XP_362575.2| hypothetical protein [Magnaporthe oryzae ... 47 9e-08
ref|XP_362575.2| hypothetical protein [Magnaporthe oryzae ... 46 9e-08
ref|XP_362575.2| hypothetical protein [Magnaporthe oryzae ... 46 9e-08

Glomerella graminicola M1.001 [ascomycetes] taxid 645133
gb|EFQ28027.1| hypothetical protein GLRG_03171 [Glomerella... 57 5e-07
gb|EFQ32829.1| hypothetical protein GLRG_07973 [Glomerella... 57 7e-07

Neurospora crassa OR74A [ascomycetes] taxid 367110
ref|XP_961512.2| hypothetical protein NCU03736 [Neurospora... 53 1e-05
gb|EAA32276.2| hypothetical protein NCU03736 [Neurospora ... 53 1e-05
ref|XP_961512.2| hypothetical protein NCU03736 [Neurospora... 53 1e-05
ref|XP_961512.2| hypothetical protein NCU03736 [Neurospora... 53 1e-05

Sordaria macrospora [ascomycetes] taxid 5147
eb|CBI51638.1| unnamed protein product [Sordaria macrospora] 53 1e-05

Taeniopygia guttata (zebra finch) [birds] taxid 59729
ref|XP_002187195.1| PREDICTED; similar to PDZ domain conta... 37 0.53

Neisseria elongata subsp. glycolytica ATCC 29315 [b-proteobacteria] taxid 56263
gb|EFQ44944.1| hypothetical protein NEIELOOT_02234 [Neisser... 37 4.1

Taxonomy Report
cellular organisms ................................. 33 hits 15 orgs [root]
  . Fungi/Metazoa group ................................. 30 hits 14 orgs [Eukaryota]
    . Leotiomycota ........................................ 24 hits 9 orgs
      . Sordariomycota .................................. 20 hits 7 orgs
      . . Sordariomycetidae ................................ 15 hits 5 orgs
      . . . Sordariales ................................... 9 hits 4 orgs
      . . . . Chaetomium globosum CBS 148.51 ...... 7 hits 3 orgs
      . . . . . Neurospora crassa ...................... 5 hits 2 orgs [Neurospora]
      . . . . . Neurospora crassa OR74A .......... 4 hits 1 orgs
      . . . . . . Sordaria macrospora ............... 2 hits 1 orgs [Sordaria]
      . . . . . . . Magnaporthe oryzae 70-15 ....... 6 hits 1 orgs [Magnaporthales; Magnaportaceae; Magnaportaceae oryzae]
      . . . . . . . . Verticillium albo-atrum VaMs.102 ....... 2 hits 1 orgs [Sordariomycetes incertae sedis; Phylloclorales; mitosporic Phylloclorales; Verticillium; Verticillium albo-atrum]
      . . . . . . . . . Glomerella graminicola M1.001 .......... 3 hits 1 orgs [Hypocreomycetidae; Hypocreomycetidae incertae sedis; Glomerellaceae; Glomerella; Glomerella graminicola]
      . . . . . . . . . Sclerotinia sclerotiorum 1980 UP-70 ....... 2 hits 1 orgs [Sclerotinia; Sclerotinia sclerotiorum]
      . . . . . . . . . Botrytis fuckeliana B05.10 .......... 2 hits 1 orgs [Botrytis; Botrytis fuckeliana]
      . . . . . . . . . Pleosporinae ................................ 6 hits 4 orgs [dothideomycota; Dothideomycetes; Pleosporaomycetidae; Pleosporales]
      . . . . . . . . . Pyrenophora ................... 3 hits 2 orgs [Pleosporales]
      . . . . . . . . . . Pyrenophora teres f. teres 0-1 ........ 1 hits 1 orgs [Pyrenophora teres; Pyrenophora teres f. teres]
Neisseria elongata subsp. glycolytica ATCC 29315
Sequences Homologous to the Protein of EST D00658 and ClustalW alignment

>bght007426000001001  (BluGen annotation of gene relating to EST D00658)
GGYDIVTGRYASGGGVSGLWKLXIRSGANLFAVDTLPRPGVSDLTGSLKLYAFLKIERSHKESKGYTQME
MMVRAKAMGVTAVAEVFIDFRGYSEKLGSDGDEILEYAKGVNLWLVK

>gi|156053279|ref|XP_001592566.1| mannose phospho-dolichol synthase
[Sclorella sclerotiorum 1980]
MAPKATKDKYGILPITYNERNRNLLITWLLNRTFTEQGLDWEELIIVDDGDSPGTQPITQMVANQLAKAYSPHV
LVKARAGKLGLTAYVHLQFVTNYVIMIMADDFSHPKFISQMIAKQKTLLSTNGYDVTGTTRYAGDGGV
FGWDLKRLVSRGANLFAVDTLPRPGVSDLTGSLKLYAFLKIERSHKESKGYTQMEMMVRAGMCTVE
AEVFISFDVRYSEKLGSDGDEILEYAKGVNLWLVK

>gi|154314666|ref|XP_001556657.1| hypothetical protein BC1G_04042
[Botryotinia fuckeliana B05.10]
MAPKATKDKYGILPITYNERNRNLLITWLLNRTFTEQGLDWEELIIVDDGDSPGTQPITQMVANQLAKAYSPHV
LVKARAGKLGLTAYVHLQFVTNYVIMIMADDFSHPKFISQMIAKQKTLLSTNGYDVTGTTRYAGDGGV
FGWDLKRLVSRGANLFAVDTLPRPGVSDLTGSLKLYAFLKIERSHKESKGYTQMEMMVRAGMCTVE
AEVFISFDVRYSEKLGSDGDEILEYAKGVNLWLVK

>gi|11181548|gb|AAG32629.1|AF102883_1 mannose phospho-dolichol synthase
[Hypocrea jecorina]
MAPKSSNDKYGILPITYNERNRNLLITWLLNRTFTEQGLDWEELIIVDDGDSPGTQPITQMVANQLAKAYSPHV
LVKTRSGKLGLTAYVHLQFVTNYVIMIMADDFSHPKFISQMIAVQKKNIDVTGTTRYAGNGGFG
DLKFKVSRSRGALFADTLRPGVSDLTGSLKLYAFLKIERSHKESKGYTQMEMMVRAGMCTVE
AEVFISFDVRYSEKLGSDGDEILEYAKGVNLWLVK

>gi|189205270|ref|XP_001938970.1| dolichol-phosphate mannosyltransferase
[Pyrenophora tritici-repentis Pt-1c-BFP]
MAPTELNKNKYSVPLLTYNERNRLPIITWLLNRTFTEQGLDWEELIIVDDGDSPGTQEAIAQLQKVYTPER
IQIRARAKGLGLGTYAVHLQFATGFIQFIAMIALQKTKNYDVTGTTRYAGDGGVFG
WDLKRFVSRSRGALFADTLRPGVSDLTGSLKLYKSTKIEKTRQVIRSKESKGYTQMEMMVRAGMCTVAE
AEVFISFDVRYSEKLGSDGDEILEYAKGVNLWLVK

>gi|311330160|gb|EFQ94636.1| hypothetical protein PTT_07599 [Pyrenophora teres f. teres 0-1]
MAPTEVNKNKYSVPLLTYNERNRLPIITWLLNRTFTEQGLDWEELIIVDDGDSPGTQEIAAQQLQKVYTPER
IQIRARAKGLGLGTYAVHLQFATGFIQFIAMIALQKTKNYDVTGTTRYAGDGGVFG
WDLKRFVSRSRGALFADTLRPGVSDLTGSLKLYKSTKIEKTRQVIRSKESKGYTQMEMMVRAGMCTVAE
AEVFISFDVRYSEKLGSDGDEILEYAKGVNLWLVK

>gi|312219981|emb|CBX99923.1| similar to dolichol-phosphate (beta-D)
mannosyltransferase 1 [Leptosphaeria maculans]
MDADDFSHPKFIMAPMIALQKTKNYDVTGTTRYAGDGGVFG
WDLKRFVSRSRGALFADTLRPGVSDLTGSLKLYKSTKIEKTRQVIRSKESKGYTQMEMMVRAGMCTVAE
AEVFISFDVRYSEKLGSDGDEILEYAKGVNLWLVK
>gi|322699920|gb|EFY91678.1| mannose phospho-dolichol synthase [Metarhizium acridum CQMa 102]
MAPAKASKNKSVLPTYNERRNLPITMWWLNNRTFERNLPIGSKLDWEIIIVDDGSPDGTEVANQLV
KAYSHPHVLKTSRSGKGLGATAYHLQFVGTGQFVIMADDSHHPKFIPQMVQMAEGSNNYDIVLTGTRYAG
NGVYGVWDLKRFVSQRANLFADTVLRPGVSRLTGSRFRYYKKVSLKEVISSTESKGYTFQMEMVMVRACKAM
GCTVAEPISFVDRLYGGEKLGSEIEVEYAKGVFLNLWLV

>gi|169610129|ref|XP_001798483.1| hypothetical protein SNOG_08158 [Phaeosphaeria nodorum SN15]
MAPTPKKDKYSVLLPTYNERRNLPIITWLLNTENNLDWEIIIVDDGSPDGTEVAQQQLKAYSPSRI
QIRARAGKLGTLAYHHQFQATGNYVIIMADDSHHPKFIPQMVQMAEGSNNYDIVLTGTRYAGNGVYGVW
DLKRFVSQRANLFADTVLRPGVSRLTGSRFRYYKKVSLKEVISSTESKGYTFQMEMVMVRACKAM

>gi|322703034|gb|EFY94650.1| mannose phospho-dolichol synthase [Metarhizium anisopliae ARSEF 23]
MAPAKASKNKSVLPTYNERRNLPITMWWLNNRTFERNLPIGSKLDWEIIIVDDGSPDGTEVANQLV
KAYSHPHVLKTSRSGKGLGATAYHLQFVGTGQFVIMADDSHHPKFIPQMVQMAEGSNNYDIVLTGTRYAGNGVYGVW
DLKRFVSQRANLFADTVLRPGVSRLTGSRFRYYKKVSLKEVISSTESKGYTFQMEMVMVRACKAM

>gi|302886581|ref|XP_003042180.1| glycosyltransferase family 2 [Nectria haematococca mpVI 77-4]
MASKANKYSVILPTYNERKNLPIITMWWLNNRTFERNLPIGSKLDWEIIIVDDGSPDGTEVANQLV
KAYSHPHVLKTSRSGKGLGATAYHLQFVGTGQFVIMADDSHHPKFIPQMVQMAEGSNNYDIVLTGTRYAGNGVYGVW
DLKRFVSQRANLFADTVLRPGVSRLTGSRFRYYKKVSLKEVISSTESKGYTFQMEMVMVRACKAM

>gi|310795806|gb|EFQ31267.1| glycosyl transferase family 2 [Glomerella graminicola M1.001]
MAPKNSVLPTYNKRNLPIITMWWLNNRTFERNLPIGSKLDWEIIIVDDGSPDGTEVANQLV
KAYSHPHVLKTSRSGKGLGATAYHLQFVGTGQFVIMADDSHHPKFIPQMVQMAEGSNNYDIVLTGTRYAGNGVYGVW
DLKRFVSQRANLFADTVLRPGVSRLTGSRFRYYKKVSLKEVISSTESKGYTFQMEMVMVRACKAM

>gi|46116404|ref|XP_384220.1| hypothetical protein FG04044.1 [Gibberella zeae PH-1]
MAPKNSVLPTYNKRNLPIITMWWLNNRTFERNLPIGSKLDWEIIIVDDGSPDGTEVANQLV
KAYSHPHVLKTSRSGKGLGATAYHLQFVGTGQFVIMADDSHHPKFIPQMVQMAEGSNNYDIVLTGTRYAGNGVYGVW
DLKRFVSQRANLFADTVLRPGVSRLTGSRFRYYKKVSLKEVISSTESKGYTFQMEMVMVRACKAM

>gi|46116404|ref|XP_384220.1| hypothetical protein FG04044.1 [Gibberella zeae PH-1]
MAPKNSVLPTYNKRNLPIITMWWLNNRTFERNLPIGSKLDWEIIIVDDGSPDGTEVANQLV
KAYSHPHVLKTSRSGKGLGATAYHLQFVGTGQFVIMADDSHHPKFIPQMVQMAEGSNNYDIVLTGTRYAGNGVYGVW
DLKRFVSQRANLFADTVLRPGVSRLTGSRFRYYKKVSLKEVISSTESKGYTFQMEMVMVRACKAM

>gi|46116404|ref|XP_384220.1| hypothetical protein FG04044.1 [Gibberella zeae PH-1]
MAPKNSVLPTYNKRNLPIITMWWLNNRTFERNLPIGSKLDWEIIIVDDGSPDGTEVANQLV
KAYSHPHVLKTSRSGKGLGATAYHLQFVGTGQFVIMADDSHHPKFIPQMVQMAEGSNNYDIVLTGTRYAGNGVYGVW
DLKRFVSQRANLFADTVLRPGVSRLTGSRFRYYKKVSLKEVISSTESKGYTFQMEMVMVRACKAM
>gi|296421374|ref|XP_002840240.1| hypothetical protein [Tuber melanosporum Mel28]
MAPRQSTKSRKDITYIIILPTYNERKNLPVTWTLLLEKTFETHKLDWEVIIVDDASPDGTQEVAKQLISVYG
ADR1VLKPRAGKLLGTAYVHLQFATGNFVIMADAFSSHHPKFIADFIALQKTKDIDVTGTRYAGNGG
VYGWDFKRLVSARGANLASSVVLVPNVSGLTSGFRLYKRVKVLTVISQTESKGYTFQMEMVRARGLYG
VAEVPISFVDRIYGDSDKLGDEIVEYAKGVNLWLKV

>gi|303317642|ref|XP_003068642.1| dolichol-phosphate mannosyltransferase, putative [Coccidioides posadasii C735 delta SOwgp]
MNPRGPRYRPSLEVHLQNSVLELHETRQPkPHIMAPPNKYSVILPTYNERKNLPICWLIEKTFRENNLN
WEVIIIVDDASPDGTQEIQAQLQGLWGEDHILKWARAGKLGTLATYHVHLQFVGTNFVIIMADAFSSHHPKFI
PEMKIKQTGCDTGTGRYANRDLHLHGVPYLWDLRKRLTSRGANLIAVDMPLMPVSDLGTSGFRLYKQ
VLEKVISTKESKGYTFQMEMVRAMGYKVEECPIFVDRILYGESKLGEEIVEYLGVFSLWKKV

>gi|119187095|ref|XP_001244154.1| hypothetical protein CIMG_03595
[Coccidioides immitis RS]
MAPPNKYSVILPTYNERKNLPICWLIEKTFRENNLDWEVIIIVDDASPDGTQEVAKQLQSLWPEHIVL
RPEQKLLGTAYVHLQFVQSNFVIMADAFSSHHPKFIPEMKIKQTGCDTGRYANRDHLHGVPYLWDLRTKL
GSLRGANLIAVDMPLMPVSDLGTSGFRLYKQVLEKVISTKESKGYTFQMEMVRAMGYKVEECPIFVDRILYG
ESKLGEEIVEYLGVFSLWKKV

>gi|154284546|ref|XP_001543068.1| dolichol-phosphate mannosyltransferase [Ajellomyces capsulatus NAm1]
MSSQNNKYSVILPTYNERKNLPICWLIEKTFRENNLDWEVIIIVDDASPDGTLEVAKQLQSLWPEHIVL
RPEQKLLGTAYVHLQFVQSNFVIMADAFSSHHPKFIPEMKIKQTGCDTGRYANRDHLHGVPYLWDLRTKL
GSLRGANLIAVDMPLMPVSDLGTSGFRLYKQVLEKVISTKESKGYTFQMEMVRAMGYKVEECPIFVDRILYG
ESKLGEEIVEYLGVFSLWKKV

>gi|295670878|ref|XP_002795986.1| dolichol-phosphate mannosyltransferase [Paracoccidioides brasiliensis Pb01]
MIPFRQRYRPTLQVDQNSVQSFNPSSVVHHNKMTSONKYSVILPTYNERKNLPICWLIEKTFRENN
LDWEVIIIVDDASPDGTQVAKQLQSLWPFQHIILKPREQKLLGTAYVHLKFTTNFVIIMADAFSSHHP
KFIPEMIKIQKTGCDTGRYASQNGNLRRGGYVGWLVRKLSRGANLIADMMLPMGVSGLTSGFRLYKQ
VLEKVISTKESKGYTFQMEMVRAMGFVKVEECPIFVDRILYGESKLGDEIVEYLGVFSLWKKV

>gi|68476637|ref|XP_717611.1| potential ER dolichol phosphate mannose synthase [Candida albicans SC5314]
MTQNYSVILPTYNERKNLPILYLTLNNKFTTANKLDWEVIIIVDDASPDGTQEIAKKLIDIFGPEHQLRP
RAGKLLGTAYVHLQFVTNFVIIMADAFSSHHPFIEPAFIAKPSQDDIVTGVRYAGGFGCWDKFR
KLISRGANFLASVVLRPSVLGTSDTGFRYKTIDVLLKIIIDVTQSKGYVFQMEMVRAMGFVGEVIPS
VDRLYGESKLGDEIVEQYAKGVNLWLTVS
>gi|225681605|gb|EEH19889.1| dolichol-phosphate mannosyltransferase [Paracoccidioides brasiliensis Pb03] MIPRGPFRPFLQVQFNSQFQFSPCMSVQVQFMKTSQNKVSILPTYNERLPIICWLEKTFRENN LDDEVIIIVDDGDGDGTLEVAQLQQLGLQFQHILFKPREKGLGLGTAYHLGKTFGTFNFIIMADAFSHHPKFIPEMIKIQTESCDIVTGRYASRNLRGGVYGWDLRKTSRGKANLIAADMLMMPGFSLTSGRFYK KPVLEKVIKSTESKGTYTFQMEMVRAKAMGFKECVCECPITFVDRLLYGESKLGDEIVEYLGKITWLKV

>gi|241949361|ref|XP_002417403.1| dolichol-phosphate mannose synthase, putative; dolichol-phosphate mannosyltransferase, putative; dolichyl-phosphate beta-D-mannosyltransferase, putative; mannosase-P-dolichyl synthase, putative [Candida dubliniensis CD36] MTQNKYSVILPTYNERNPILYLLNKTFTANKLDEVIIIVDDNSDPQTQEIAKLIDIFGPEHIQLRP RAGKLGLGTAYHLQFPVTGFNFIIMADAFSHHPFIAPIFIAKQQSDYDVTGTRYAGDGGVYGWDFKR KLISRGANFLASVLRPVSITSFTSRFLYKTEILKIIDVTQSKGYPFQMEMVRAKAMGFVGEVPISF VDRLLYGESKLGDEIVEYAKGVWLFTNV

>gi|261191254|ref|XP_002622035.1| dolichol-phosphate mannosyltransferase [Ajellomyces dermatitidis SLH14081] MSSQNTKSILPTYNERLPIICWLEKTFRENNLDDEVIIIVDDGDGDGTLEVAQLQQLGLQFQHILFK PREKGLGLGTAYHLGLQFTPFTGFNFIIMADAFSHHPKFIPEMIKIQTESCDIVTGRYASRNLRGGVYG WDLRKTSRGKANLIAADMLMMPGFSLTSGRFYK KPVLEKVIKSTESKGTYTFQMEMVRAKAMGFKECVCECPITFVDRLLYGESKLGDEIVEYLGKITWLKV

>gi|226288749|gb|EEH44260.1| dolichol-phosphate mannosyltransferase [Paracoccidioides brasiliensis Pb18] MTSQNKYSVILPTYNERNLPIICWLEKTFRENNLDDEVIIIVDDGDGDGTLEVAQLQQLGLQFQHILFK PREKGLGLGTAYHLGLQFTPFTGFNFIIMADAFSHHPKFIPEMIKIQTESCDIVTGRYASRNLRGGVYG WDLRKTSRGKANLIAADMLMMPGFSLTSGRFYK KPVLEKVIKSTESKGTYTFQMEMVRAKAMGFKECVCECPITFVDRLLYGESKLGDEIVEYLGKITWLKV

>gi|260944008|ref|XP_002616302.1| hypothetical protein CLUG_03543 [Clavispora lusitaniae ATCC 42720] MGDKYSIILPTYNEKKNPILVHDLKTFKEILDEVIIIVDDGDGDGTQDIAKLIDIFGPEHIQLRP AGKGLGLGTAYHLQFPVTGFNFIIMADAFSHHPKFIPEMIKIQTESCDIVTGRYASRNLRGGVYG WDLRKTSRGKANLIAADMLMMPGFSLTSGRFYK KPVLEKVIKSTESKGTYTFQMEMVRAKAMGFKECVCECPITFVDRLLYGESKLGDEIVEYLGKITWLKV

>gi|240281486|gb|EER44989.1| dolichol-phosphate mannose synthase [Ajellomyces capsulatus capsulatus H143] MTSQNKYSVILPTYNERNLPIICWLEKTFRENNLDDEVIIIVDDGDGDGTLEVAQLQQLGLQFQHILFK PREKGLGLGTAYHLGLQFTPFTGFNFIIMADAFSHHPKFIPEMIKIQTESCDIVTGRYASRNLRGGVYG WDLRKTSRGKANLIAADMLMMPGFSLTSGRFYK KPVLEKVIKSTESKGTYTFQMEMVRAKAMGFKECVCECPITFVDRLLYGESKLGDEIVEYLGKITWLKV
>gi|50427173|ref|XP_462199.1| DEHA2G15136p [Debaryomyces hansenii CBS767]
MSTDKYSVILPTYNEKRNLPIVLVYLLATKQFAHELDEWYIIIIVDDNPDTQIIAKELVINPFGEKHIQLRA
RAGKGLGLGATYVHLQFVTGNYVIIIMADAFSHHPEAPIQFIAKQKEQDYDVTGTRYAGDGGVYGWDLKR
KLGVRGANFLAATVLRPGVSDTLGSRFRLYKVLKVIKIDTEKSKGTYVFQMEMMEMRALKAGYNVEEVPSF
VDRLGYESGKLGDIEVYLGKVGWNLFTSV

>gi|255729550|ref|XP_002549700.1| dolichol-phosphate mannosyltransferase [Candida tropicalis MYA-3404]
MTNNKYSVILPTYNEKRNLPIYLNLKFTANKLDEWYIIIIVDDNPDTQEVAKKLIDIFGSEHQLRP
RAGKGLGATYVHLQFVTGNYVIIIMADAFSHHPEAPIQFIAKQKEQDYDVTGTRYAGDGGVYGWDLKR
KLGVRGANFLAATVLRPGVSDLTGSFRLYKVLKVIKIDTEKSKGTYVFQMEMMEMRALKAGYNVEEVPSF
VDRLGYESGKLGDIEVYLGKVGWNLFTSV

>gi|315055499|ref|XP_003177124.1| dolichol-phosphate mannosyltransferase [Arthroderma gypseum CBS 118893]
MPGRGPRYRPTLEAVDQAEOFQTEQPIPSHLKMSPTNKYSVILPTYNERKNLPIICWLEIKTFRENKL
WEIVIIIVDDGSPDTIEVAKQLQAYEQHIVLKPREGKGLGATYVHLKPFATGNFIIIIMADAFSHHPKF
IPEMIKIQESTKADIVTGTTRYASRNGLRGGVYGWDILRLKTSRGANLINADVALMPGVSDLTGSRFLYKKP
VLEKVIKTESKGYTFQMEMMEMRALKAGYNVKEECPITFVDRVYGSKLGGEIEVYLGKVFLWLV

>gi|39940326|ref|XP_359700.1| conserved hypothetical protein [Magnaporthe oryzae 70-15]
MAPAAKTSGGKDVILSVPFTNERQNLPIVTWLLNKTFTENNINWELIIIVDDGSPDTQDVAKQLVEVFK
PHVVLQTRTKGLGILATYVHLQFQAKNGYIIIMADAFSHHPKFIPQMIERQKSADYDIVTGTTRYAPGGGV
HGWDLKRMRMTSKGANILADTLLRPGVSDLTGSFRLYKRKNVLEKLFETTDVRGFSQMAMLAVTAKAMYSI
AEVPITFVDRVYGSKLGGEIEVYAKGVFLW

>gi|320583585|gb|EFW97798.1| dolichol-P-mannose synthesis [Pichia angusta DL-1]
MDKYSVILPTYNERKNLPITLIAKHFEGAKGLNEWIIIVDDASPDGTQDVAKQLLINLYGADHIQLRAR
GKLGLGATYHGLQVATGNFIIIMADAFSHHPSIPEIFKKEQGDKDYDIVTGTTRYAGDGGVYGWDLKR
KLGSRANFLATVLRHPSDLGGFLRKYKDDVLAIIGATKSKGTYVFQMEMMEMRARAMFCHIAEVPSFVD
RLGYESGKGDDEIIYLGKGVWNFLTSV

>gi|302659260|ref|XP_003021322.1| hypothetical protein TRV_04566 [Trichophyton verrucosum HKI 0517]
MSPANKYSVILPTYNERNLPIICWLIEKTREKLNWEIVIIIVDDGSPDTLEIARKQLQAYEQAHIQLV
KPREGLGGLGATYVHLKPFATGNFIIIMADAFSHHPKFIPMKIEQESTKADIVTGTTRYASRNGLRGGVYG
WDLVRKLTSRGANLINADVALMPGVSDLTGSRFLYKPVLEKVIKTESKGYTFQMEMMEMRALKAGYNVKEE
CPITFVDRVYGSKLGGEIEVYLVGTVFLWLV

>gi|296824402|ref|XP_002850652.1| dolichol-phosphate mannosyltransferase [Arthroderma otae CBS 113480]
MAPANKYSVILPTYNERNLPIICWLIEKTFREKLNWEIVIIIVDDGSPDITLEIARKQLQAYEQAHIQLV
>gi|121705564|ref|XP_001271045.1| dolichol-phosphate mannosyltransferase, putative [Aspergillus clavatus NRRL 1]
MGATKMKNSYVILPTYRNRLPICIICWLLERTFRENNDWELWEIVIVDDGSPDQTEVAKQLQALWGEHINL
KPRAGKMLGTAYVHGQYVRGNYVIIMADASFSHPKFIPEMIKIQESTKADIVTGTRYASRDLGSGGVY
WDLIRKLTSRGANLIADVALMPGVSLSLGRLYKKPVLEKVIKSTQSKGYSFQMEMVRRAKAMGYKVEE
CPITFVDRYGESKLGGHGEIYELKGVFTLWLVK

>gi|302508251|ref|XP_003016086.1| hypothetical protein ARB_05483 [Arthroderma benhamiae CBS 112371]
MSPANYSVILPTYRNRLPNICWLLIKTEKFLRENKLNWEVIIIVDDGSPDQTEIISKQLAAYGQHIVLK
PREKGLGGLTAYVHGQYVRGNYVIIMADASFSHPKFIPEMIKIQESTKADIVTGTRYASRDLGSGGVY
WDLIRKLTSRGANLIADVALMPGVSLSLGRLYKKPVLEKVIKSTQSKGYSFQMEMVRRAKAMGYKVEE
CPITFVDRYGESKLGGHGEIYELKGVFTLWLVK

>gi|149247152|ref|XP_001528001.1| dolichol-phosphate mannosyltransferase [Lodderomyces elongisporus NRRL YB-4239]
MTANKVSVILPTYRNRLPNLIVALYLLNKTFTETKLEWIIIVVDGSPDQTEVAWLIDYSGKHQHPRT
RAGKMLGTAYVHGQYVRTQFVIIIMADASFSHPKFIPEMIKIQESTKADIVTGTRYASRDLGSGGVY
WDLIRKLTSRGANLIADVALMPGVSLSLGRLYKKPVLEKVIKSTQSKGYSFQMEMVRRAKAMGYKVEE
CPITFVDRYGESKLGGHGEIYELKGVFTLWLVK

>gi|37798713|gb|AAR03724.1| dolichol-phosphate mannose synthase [Paracoccidioides brasiliensis]
MTSQNKNSVILPTYRNRLPIICWLLIKLEKFLRENKLNWEVIIIVDDGSPDQTEVAWLIDYSGKHQHPRT
PREKGLGGLTAYVHGQYVRGNYVIIMADASFSHPKFIPEMIKIQESTKADIVTGTRYASRDLGSGGVY
WDLIRKLTSRGANLIADVALMPGVSLSLGRLYKKPVLEKVIKSTQSKGYSFQMEMVRRAKAMGYKVEE
CPITFVDRYGESKLGGHGEIYELKGVFTLWLVK

>gi|171676958|ref|XP_001903431.1| hypothetical protein [Podospora anserina S mat+]
MAPAKSSSTGGKDMYSVILPTFNERQNPITIWLLNLRTFTENQLNDLWELVIVDDGSPDQTEVAWLIYKLAYPS
PHIQRLPRTGKGLGGLTAYVHGQYVRGNYVIIMADASFSHPKFIPEMIKIQESTKADIVTGTRYASRDLGSGGVY
WDLIRKLTSRGANLIADVALMPGVSLSLGRLYKKPVLEKVIKSTQSKGYSFQMEMVRRAKAMGYKVEE
CPITFVDRYGESKLGGHGEIYELKGVFTLWLVK

>gi|119491845|ref|XP_001263417.1| dolichol-phosphate mannosyltransferase, putative [Neosartorya fischeri NRRL 181]
MTDKNKNSVILPTYRNRLPIICWLLERTFRENNDWELWEIVIVDDGSPDQTEVAWLIDYSGKHQHPRT
KPRAGKMLGTAYVHGQYVRGNYVIIMADASFSHPKFIPEMIKIQESTKADIVTGTRYASRDLGSGGVY
WDLIRKLTSRGANLIADVALMPGVSLSLGRLYKKPVLEKVIKSTQSKGYSFQMEMVRRAKAMGYKVEE
CPITFVDRYGESKLGGHGEIYELKGVFTLWLVK
ECPITFVDRYLGSGKEHIVEYLGVLNLWLKV

>gi|70999724|ref|XP_754579.1| dolichol-phosphate mannosyltransferase
Aspergillus fumigatus Af293
MTDTKNKSVLPIY3NLIPICWLLERTFRENNDLDEIVYDDGSPODGTLTEVAQLQTLNGSEHILN
KPRAGKLGLGTAYVHGLQYVQRGNYVIIMADFSHHPFKIPEMIRIQTGDADIVTGRYASRDGIGKVGVY
GWDLFRKFTSRATANLVIADMVMPGSDLTGSRFLYKSVLKEVHIHSTQSKGYSFQEMMVRKAMGFKVA
ECPITFVDRYLGSGKEHIVEYLGVLNLWLKV

>gi|145252462|ref|XP_001397744.1| dolichol-phosphate mannosyltransferase
Aspergillus niger CBS 513.88
MGAAENKYSVLPIY3NLPIIWLLERTFRENNDLDEIVYDDGSPODGTLTEVAQLQTLNGSEHILN
HPRAGKLGLGTAYHGLQQTGNYVIIMADFSHHPFKIPEMIRIQTGDADIVTGRYASRDGIGKVGVY
GWDLFRKFTSRATANLVIADMVMPGSDLTGSRFLYKSVLKEVHIHSTQSKGYSFQEMMVRKAMGFKVA
ECPITFVDRYLGSGKEHIVEYLGVLNLWLKV

>gi|255936845|ref|XP_002559449.1| Pc13g10270
Penicillium chrysogenum Wisconsin 54-1255
MAAAKNKYSVLPIY3NLPIIWLLERTFRENNDLDEIVYDDGSPODGTLTEVAQLQTLNGSEHILN
KPRAGKLGLGTAYVHGLQYVQRGNYVIIMADFSHHPFKIPEMIRIQTGDADIVTGRYASRDGIGKVGVY
GWDLFRKFTSRATANLVIADMVMPGSDLTGSRFLYKSVLKEVHIHSTQSKGYSFQEMMVRKAMGFKVA
ECPITFVDRYLGSGKEHIVEYLGVLNLWLKV

>gi|50550011|ref|XP_502478.1| YALI0D06281p
Yarrowia lipolytica
VMWLLHTFTENNNDLDEIVYDDGSPODGTLTEVAQLQTLNGSEHILN
KPRAGKLGLGTAYHGLQQTGNYVIIMADFSHHPFKIPEMIRIQTGDADIVTGRYASRDGIGKVGVY
GWDLFRKFTSRATANLVIADMVMPGSDLTGSRFLYKSVLKEVHIHSTQSKGYSFQEMMVRKAMGFKVA
ECPITFVDRYLGSGKEHIVEYLGVLNLWLKV

>gi|258563706|ref|XP_002582598.1| dolichol-phosphate mannosyltransferase
Uncinocarpus reesii 1704
MAPDKYSVLPY3NLPIICWLIEKFTRENNDLDEIVYDDGSPODGTLTEVAQLQTLNGSEHILN
KPRAGKLGLGTAYHGLQQTGNYVIIMADFSHHPFKIPEMIRIQTGDADIVTGRYASRDGIGKVGVY
GWDLFRKFTSRATANLVIADMVMPGSDLTGSRFLYKSVLKEVHIHSTQSKGYSFQEMMVRKAMGFKVA
ECPITFVDRYLGSGKEHIVEYLGVLNLWLKV

>gi|320587712|gb|EFX00187.1| dolichol-phosphate mannosyltransferase
Grosmannia clavigera kw1407
MATVAKDSTRNFLPIIICWLIEKFTRENNDLDEIVYDDGSPODGTLTEVAQLQTLNGSEHILN
KPRAGKLGLGTAYHGLQQTGNYVIIMADFSHHPFKIPEMIRIQTGDADIVTGRYASRDGIGKVGVY
GWDLFRKFTSRATANLVIADMVMPGSDLTGSRFLYKSVLKEVHIHSTQSKGYSFQEMMVRKAMGFKVA
ECPITFVDRYLGSGKEHIVEYLGVLNLWLKV
>gi|67537554|ref|XP_662551.1| hypothetical protein AN4947.2 [Aspergillus nidulans FGSC A4]
MAKDNKYSVILPTYNERNLPIICWLLERTFRENKLDWEVIIVDDGSPDGLDVAKQLQNQWAGADHLVLRK
PRAGKLGTLGAYVHLQFTGTNGVIMIDAFDSHHPKFIPEMVRIQETADIVVTGRAASYRGGGTVY
WDLFRKFTSTANLIADVMLPGVSLTSFLRYSKSVLEKVIISTQSKGYSFQMEMMRVAKAMGKV
VAA CFITFVDRLYEGLLGSEGGEISEYELKGVFNLWLV

>gi|85106102|ref|XP_962098.1| dolichol-phosphate mannosyltransferase [Neurospora crassa OR74A]
MAPTKitTGDVYSVILPTFNERQNPLPITIWLLNRTFSEQNIDWELVIVDDGSPTQDVDAVQLVKLYAPH
VQLQTRTGTKGLGTAYVHLQFAKGYIIIMADAFSHHPKFIQPMIAKQAKNYIDVNTGRYAGDDGVY
WDLFRKFTSTANLIADVMLPGVSLTSFLRYSKSVLEKVIISTQSKGYSFQMEMMRVAKAMGKV
VAA CFITFVDRLYEGLLGSEGGEISEYELKGVFNLWLV

>gi|169770571|ref|XP_001819755.1| dolichol-phosphate mannosyltransferase [Aspergillus oryzae RIB40]
MGAANKNTVILPTYNERKNLPIICWLLERTFRENKLDWEVIIVDDGSPDGLDVAKQLQNQWAGADHLVLRK
PRAGKLGTLGAYVHLQFTGTNGVIMIDAFDSHHPKFIPEMVRIQETADIVVTGRAASYRGGGTVY
WDLFRKFTSTANLIADVMLPGVSLTSFLRYSKSVLEKVIISTQSKGYSFQMEMMRVAKAMGKV
VAA CFITFVDRLYEGLLGSEGGEISEYELKGVFNLWLV

>gi|289619294|emb|CBI54171.1| unnamed protein product [Sordaria macrospora]
MAPTKitTGDVYSVILPTFNERQNPLPITIWLLNRTFSEQNIDWELVIVDDGSPTQDVDAVQLVKLYAPH
VQLQTRTGTKGLGTAYVHLQFAKGYIIIMADAFSHHPKFIQPMIAKQAKNYIDVNTGRYAGDDGVY
WDLFRKFTSTANLIADVMLPGVSLTSFLRYSKSVLEKVIISTQSKGYSFQMEMMRVAKAMGKV
VAA CFITFVDRLYEGLLGSEGGEISEYELKGVFNLWLV

>gi|242782420|ref|XP_000247995.1| dolichol-phosphate mannosyltransferase, putative [Talaromyces stipitatus ATCC 10500]
MAKTNKYSVILPTYNERKNLPIICWLLERTFRENKLDWEVIIVDDGSPDGLDVAKQLQNQWAGADHLVLRK
PRAGKLGTLGAYVHLQFTGTNGVIMIDAFDSHHPKFIPEMVRIQETADIVVTGRAASYRGGGTVY
WDLFRKFTSTANLIADVMLPGVSLTSFLRYSKSVLEKVIISTQSKGYSFQMEMMRVAKAMGKV
VAA CFITFVDRLYEGLLGSEGGEISEYELKGVFNLWLV

>gi|126133424|ref|XP_001383237.1| dolichol-phosphate mannosyltransferase [Scheffersomyces stipitis CBS 6054]
MGSDKYSVILPTYNERKNLPIICWLLERTFRENKLDWEVIIVDDGSPDGLDVAKQLQNQWAGADHLVLRK
PRAGKLGTLGAYVHLQFTGTNGVIMIDAFDSHHPKFIPEMVRIQETADIVVTGRAASYRGGGTVY
WDLFRKFTSTANLIADVMLPGVSLTSFLRYSKSVLEKVIISTQSKGYSFQMEMMRVAKAMGKV
VAA CFITFVDRLYEGLLGSEGGEISEYELKGVFNLWLV

>gi|212527048|ref|XP_002143681.1| dolichol-phosphate mannosyltransferase, putative [Penicillium marneffei ATCC 18224]
MAKQDKYSVILPTYNERKNLPIICWLLERTFRENKLDWEVIIVDDGSPDGLDVAKQLQNQWAGADHLVLRK
PRAGKLGTLGAYVHLQFTGTNGVIMIDAFDSHHPKFIPEMVRIQETADIVVTGRAASYRGGGTVY
WDLFRKFTSTANLIADVMLPGVSLTSFLRYSKSVLEKVIISTQSKGYSFQMEMMRVAKAMGKV
VAA CFITFVDRLYEGLLGSEGGEISEYELKGVFNLWLV
>gi|299755265|ref|XP_001828559.2| dolichol-phosphate mannosyltransferase [Coprinopsis cinerea okayama?#130] MQGKFNTPDTHIKSVILPTYNERKNNLKPVIWLLLAKTFQDNDLAWEEIIVVDDASPDTQFIAQKLANVGEY DKIVLKPRAKGLGTLATIHGLNYVTGDFVIIMDADFSHPKFIQPQIRLQKAHNLDIVTGRYSTSTP YTTRAPQGGVGWDLKRLKVSARANFLAATVLYNVGDLLGTSGSFLRYLPLVRLHIISETTVSKVGVFQEMMEM VRARALGYTVGEPITVFVRIGESKLGADEIVSYAKGVWTLFSTV

>gi|260798376|ref|XP_002594176.1| hypothetical protein BRAFLDRAFT_117614 [Branchiostoma floridae] MASKGSDKYSVIILPTYNERDENLPLIVWLLVLRAFQESGHDIFEEIIVDDGDSPDGLTEVAQQLEKIQYKDI VLPRAKAKLLGTYAHIHMKNHATGNYIIMADADLSHPKFIPEFISSQKEKNDVSGTGYRSGGGVGYW DLKRLISRGANLIPQVLLPRGASDLTGSFRLYKAVLEKLVESCVSKVGVFQEMMINARQLGIFTGEV PITFVRIGESKLGGNEVISFAKGGLYLFFT

>gi|170100326|ref|XP_002918831.1| glycosyltransferase family 2 protein [Laccaria bicolor S238N-H82] MNPFDSASLKMGEFNSSDTKSVILPTYNERKNNLKPVIWLLANMFQKLAWEIIIVVDDASPDTQE IAKQLAKYVEDIRLVRPSKLLGLATIHGLNFVTGDFVIIMDADFSHPKFIQPQIRLQKAHNLDIV TGTRYRSSTTYPADATPGVGWSDLKRLKVSARANFLAAVLTSPIGDSLTGSFLRYLPLVRLHIISETTV SKGVVFQEMMINARALGYTVGEPITVFVRIGESKLGADEIVSYAKGVWTLFSTV

>gi|481010103|ref|XP_392640.1| PREDICTED: similar to Probable dolichol-phosphate mannosyltransferase (Dolichol-phosphate mannosyl synthase) (Dolichyl-phosphate beta-D-mannosyltransferase) (Mannose-P-dolichyl synthase) (MFD synthase) (MPD synthase) [Apis mellifera] MIEREQTKDIKELTKNKDYSIILPTYNEIENPIIIWLLIKYMDESDL/DDIEIIVDDGDSPDGLMDMK QLQNVYENKIVLRPSPEKLLGTAYMHIKIHATGNYIIMADADLSHPKFIPEFIQQRYLDDLADSVGTV YAQGGVYGWDFKRLISRGANLIPQLRPRGASDLTGSFRLYKAVLEKLVESCVSKVGVFQEMMINARQLGIFTGEV RQKFTIGEVPTVFVRIGESKLGGSEIFQFAKGLLYLFATT

>gi|321251850|ref|XP_003192200.1| GPI anchor biosynthesis-related protein [Cryptococcus gattii WM276] MSASYAINMPFVAQQTAPSTDKSVILPTYNERKNNLKPVIWLLAKTFESAGINWIEIIVVDDASPDTQE IAKQLAGIYGDKILPRAKGLGTLATIHGLNYCTGNFVIIMDADFSHPKFIQPQIRLQKLNLDIV TGTRYSSHPSTPTASSPISILPGGGYVGWDLKRLKVSARANLYADTVLYNVGDLLGTSGSFLRYLKLHIVKDI ISRCTSGKGVQFEMIIIRARALGYTVGEPITVFVRIGESKLSGNEIVGYAKGVASLWSV

>gi|302673740|ref|XP_003026556.1| glycosyltransferase family 2 protein [Schizophyllum commune H4-8] MQGPQHTADTHKSVILPTYNERKNNLPVMVWLLARVFEENELAWEEIIVVDDASPDTQFVARQLGAVGE
DKIVLKPRSGKLGTLAYIHGLNFCTDFVIIDADASHHPKFIPQFIRQQKAHNFDIVTGTRYRSTAKP
AMVIDQKGPGVFDLRKLVLTSRGANFLATMLNFGVSDTGFRLPVLRLHIITVETSGKYVFQMEMM
VRAKAKGVSSHGTVFVRDFGEGSKLADEVSYAKGWGLFTGV

>gi|57525735|ref|NP_001003596.1| dolichol-phosphate mannosyltransferase [Danio rerio]
MARSRRASKRDKPGKSVMPLPETYNERNLPIIPLVWLLKVFEGSENYEIIIVIDDSGPDTQLIAEQQLQK
IYGADKILLRFAAEKLGLGTAYIHGIHGKHTGNFVIIMADADSHPKFIPQFIEKQEGGYDDLVSFTYG
DGGYGWDLRKLISRGANFVTQVLLRPGASDLGSRFLYKKEVLEKLCVEQCVSKGKYVFQMEMVRARQL
GYTIGEVPIFVDFVRGEGSKLGGNEISFLKGLLTFATT

>gi|148230567|ref|NP_001089465.1| dolichyl-phosphate mannosyltransferase 1, catalytic subunit [Xenopus laevis]
MATSIGNKRSGDKYSVMPLPETYNERNLPIIPLVWLLLVRDRDGSNEIIEIIIVIDDSGPDTLEVAQQLQKIY
GSDKILLRPRAAKKLGLGTAYHVHGMQHATGNFIIIMADADLSHPKFPEFIRQKFGSVDYVISFTYAGNNG
VYGWDLKRKLISRGANFVTQVLLRPGASDLGSRFLYKKEVLEKLCVEQCVSKGKYVFQMEMVRARQNYT
IGEVPISFVDFVRGEGSKLGGNEISFLKGLLTFATT

>gi|260099743|gb|ACX31360.1| MIP13910p [Drosophila melanogaster]
RFLRTITMPTNHKSYLILMPLPETYNEKDNPIIPLVWLLKVFEGSENYEIIIVIDDSGPDTLDVAKDLQKIY
GEDKIVLRPRGSKLGTLAYIHGIHGKHTGNFIIIMADADLSHPKFPEFIFIKLQEGQNYDVSTYAGNNG
GVGFWDSDKRKLISRGANFQVLLRPNASDLGSRFLYKKEVLEKLCVEQCVSKGKYVFQMEMVRARQHY
TIAEVPISFVDFVRGEGSKLGGTEIIQFAKNLLLYFATT

>gi|195345131|ref|XP_002039129.1| GM17002 [Drosophila sechellia]
MPTNHKSYLILMPLPETYNEKDNPIIPLVWLLKVFEGSENYEIIIVIDDSGPDTLDVAKDLQKIYGEDKIVL
RPRGSKLGTLAYIHGIHGKHTGNFIIIMADADLSHPKFPEFIFIKLQEGQNYDVSTYAGNGGTVGFWD
RKRLISRGANFQVLLRPNASDLGSRFLYKKEVLEKLCVEQCVSKGKYVFQMEMVRARQHYTGIAEVP
ITFVDFVRGEGSKLGGTEIIQFAKNLLLYFATT

>gi|72076181|ref|XP_793909.1| PREDICTED: hypothetical protein [Strongylometrurus purpuratus]
MPRSADKSYLILMPLPETYNEKDNPIIPLVWLLKVFEGSENHFEIIIVIDDSGPDTLDVAKDLQDIYGDKIVL
RPRGSKLGTLAYIHGIHGKHTGNFIIIMADADLSHPKFPEFIFIKLQEEQCDVSFTYAGNGGTVGFWD
LRKRLISRGANFQVLLRPNASDLGSRFLYKKEVLEKLCVEQCVSKGKYVFQMEMVRARQHYTGIAEVP
ITFVDFVRGEGSKLGGSEIIQFAKNLLLYFATT

>gi|24585265|ref|NP_009980.1| CG10166 [Drosophila melanogaster]
MPTNHKSYLILMPLPETYNEKDNPIIPLVWLLKVFEGSENYEIIIVIDDSGPDTLDVAKDLQKIYGEDKIVL
RPRGSKLGTLAYIHGIHGKHTGNFIIIMADADLSHPKFPEFIFIKLQEGQNYDVSTYAGNGGTVGFWD
RKRLISRGANFQVLLRPNASDLGSRFLYKKEVLEKLCVEQCVSKGKYVFQMEMVRARQHYTGIAEVP
ITFVDFVRGEGSKLGGTEIIQFAKNLLLYFATT
>gi|148674597|gb|EDL06544.1| dolichol-phosphate (beta-D) mannosyltransferase 1, isoform CRA_b [Mus musculus]
FYLVFLYCFYFKALLRPREKGLGLGTAYIHGIKHATGNYVIIMDADLSHHPKFIPFEFIRKQKEGNFDIV
SGTRYKNGNGGVSGLDLRKRREVRANFITQiILLRPASDLTGFSRFLYKKEVLQKLIEKCVSQKGVQFQMEM
IVRARGVQNYTGEVIPFSFVDRVYESKLGGNEIVSFKLGLLTLFATT

>gi|58263533|ref|XP_569172.1| GPI anchor biosynthesis-related protein [Cryptococcus neoformans var. neoformans JEC21]
MSESYAINMPSVAQTAPSPTDKYSVILPTYNERKNLFPVIVWLLAKTFETDGINWEIVVIDADSDPGTQE
IAKQLAGYGEDKIVLPRAGKLGLGTAYVHGLNYCTGNNFIIMDADFSHHPKFIPFEFIRKQKEGNFDIV
TGTRYKSSHPKPTASSLSILGPGGGVYGLDLRKRREVRANFITQILLRPASDLTGFSRFLYKKEVLQKLIEKCVSQKGVQFQMEM
IVRARGVQNYTGEVIPFSFVDRVYESKLGGNEIVSFKLGLLTLFATT

>gi|193718481|ref|XP_001952221.1| PREDICTED: similar to Probable dolichol-phosphate mannosyltransferase (Dolichol-phosphate mannose synthase) (Dolichyl-phosphate beta-D-mannosyltransferase) (Mannose-P-dolichol synthase) [Acyrthosiphon pisum]
MPPKYSILLPYTNEKERENLPLIVVLLVKSFSESAINYEIIIIDDSPGDTLEVAQKLETIYGDKILLRFR
GKKLGLGTAYIHGKHTAGDFIIIMDADLSHHPKFILDLKQAEPQDYDVTVGYSYINGGGVSGWDFFKR
LVSRGANNVQSLIILRPASDLTGFSRFLYKKPVLEKILIESCISKGVQFQMEMIVRARGVQNYTGEVIPFSFVDRVYESKLGGNEIVSFKLGLLTLFATT

>gi|6753670|ref|NP_034202.1| dolichol-phosphate mannosyltransferase [Mus musculus]
MASTGASRSLAAASPQGGRSQRQDKYSVLLPTYNERENLPILWLLVKSFSAINYEIIIIIDDSPGD
TREVAEQLAAEIYGPUILLRPREKKLGLGLGTAYIHGIKHATGNYVIIMDADLSHHPKFIPFEFIRKQKENG
DIVSGTRYKNGGGVYGLDLRKRREVRANFITQIILLRPASDLTGFSRFLYKKEVLQKLIEKCVSQKGVQFQMEM
IVRARGVQNYTGEVIPFSFVDRVYESKLGGNEIVSFKLGLLTLFATT

>gi|195484495|ref|XP_002090719.1| GE12644 [Drosophila yakuba]
MPTNGHKYSILLPYTNEKDNLPILWILVVKYSMAGLEYEVIIIIDDSGPDGTLEVAQKLETIYGDKILLRFR
RPGSKSLGLYTAEIHGIKHATGDFIIIDDSHLHHHFIPFEFIRKQKEGNFDIVSGTRYAEGGGSQVDVRK
LRRKIEKRANFQSLVQILRPNASDLTGFSRFLYKKDVLEKICASVSKGVQFQMEMIVRARGVQNYTGEVIPFSFV
DRVYESKLGGNEIVSFKLGLLTLFATT

>gi|196004372|ref|XP_002112053.1| hypothetical protein TRIADDRAFT_23616 [Trichoplax adhaerens]
MAQNKYSILLPYTNERENLPILWLINKTLSGISDFEIIIIDDSGPDGLDVAQKQFGEKIIILRP
REKKLGLGTAYIHGIKHATGNYIIIMDADLSHHPKFIPFEFIRKQKEGNFDIVSGTRYAGGGSQVDVRK
LRRKLIEKRANFQSLVQILRPNASDLTGFSRFLYKKDVLEKICASVSKGVQFQMEMIVRARGVQNGTAYGIAE
VFSFVDRVYESKLGGNEIVSFKLGLLTLFATT

>gi|324522398|gb|ADY48055.1| Dolichol-phosphate mannosyltransferase [Ascaris suum]

MMAGDARPLYSILLPTNEKDLNLPLCVFLEIKYLKEGTGFTEVYI1IDDNSPDGTLDVAAKQLQNEFGDHV
ILRPRAKGKLGTAYTHGQARSQGFIVLMDADLSSHHPKFIPQMIALQRDHNYDIVTGTRYALGGVGAV
DKRKISRANFLAQFALQPQVSDLTGSRFLYRKEIIAKLIAISISKGYVFQMEMFRAKLGKYKVGE
PITFVDFFGESKLGGQEIVDYIKGLLYLPTFWV

>gi|148674596|gb|EDL06543.1| dolichol-phosphate (beta-D)
mannosyltransferase 1, isoform CRA_a [Mus musculus]
MLTSPTMKQKEGNFDIVSGTRYKGNGGVYGWDLKRKIIISRGANFITQIILLRGASDTLGSFRLYRKEVLQ
KLIKCVSKGKYVFQMEMIVRARQMNITYGEVFISFVDRVYGEKSLGGNEIVSLKGLLLTFATT

>gi|312068168|ref|XP_003137087.1| dolichol Phosphate Mannosyltransferase
family member [Loa loa]
MSKDGSHPNYTULLPTNEKNERNLPLVCIWLLLKVYKRMKAEFSYEVYIIDDNSPDGTMVARKLEDEFGSDKI
ILRPRARKLGLGTAYAHQPQFARGDYVILMDADLSSHHPKFIPQMIKLQQRKNYDITGTRYAGGGCGW
DLRKFVSRRANFLAQFPLLRRPGASDTLGSFRLYRKEVLQKLIKCVSKGKYVFQMEMIVRARQMDYTVGEFISFVDRF
GESKLGGQEIVDYIKGLLYLIFV

>gi|157818409|ref|NP_001100014.1| dolichol-phosphate mannosyltransferase
[Rattus norvegicus]
MASTGASRSLAASQRPPGSSRQDKYSVLLPTNERENLPILWLLVKSFSASEAINYEIIIDDGSRO
TREVNALEKIGPDRILLRPREKKGGLGTAYIHGIKNAIYIIMADLS
HHKPIPEFIRKQEGNFDIVSGTRYKNGGGVYGWDLKRKKIIISRGANFITQIILLRGASDTLGSFRFLYRKE
EVLKLIKCVSKGKYVFQMEMIVRARQMNITYGEVFISFVDRVYGEKSLGGNEIVSLKGLLLTFATT

>gi|123286707|emb|CAM22976.1| dolichol-phosphate (beta-D)
mannosyltransferase 1 [Mus musculus]
MTAINYIEEEIIIDDGSPDTREVAELQAELIGYPEDRILLRPREKKGGLGTAYIHGIKNYIIMADLS
HHKPIPEFIRKQEGNFDIVSGTRYKNGGGVYGWDLKRKIIISRGANFITQIILLRGASDTLGSFRFLYRKE
EVLKLIKCVSKGKYVFQMEMIVRARQMNITYGEVFISFVDRVYGEKSLGGNEIVSLKGLLLTFATT

>gi|224078689|ref|XP_002186864.1| PREDICTED: putative dolichol-phosphate
mannosyltransferase variant 2 [Taeniopygia guttata]
MATVRTQVGIDFEIIIDDGSPDTREVAELQAELIGYPEDRILLRPREKKGGLGTAYIHGMKYATGNFIVI
MDADLSSHHPKFIPQMIKLQQRKNYDITGTRYKNGGGVYGWDLKRKKIIISRGANFITQIILLRGASDTLGS
FRLYRKEVLKLIKCVSKGKYVFQMEMIVRARQMNITYGEVFISFVDRVYGEKSLGGNEIVSLKGLLLTFATT

>gi|12842797|dbj|BAB25735.1| unnamed protein product [Mus musculus]
MASTGASRSLAASPRPPQGRSSQDKYLSVLLPTNERENLPILWLLVKSFSASEAINYEIIIDDGSPDTRE
VAELQAELIGYPEDRILLRPREKKGGLGTAYIHGIKNYIIMADLS
HHKPIPEFIRKQEGNFDIVSGTRYKNGGGVYGWDLKRKIIISRGANFITQIILLRGASDTLGSFRFLYRKE
EVLKLIKCVSKGKYVFQMEMIVRARQMNITYGEVFISFVDRVYGEKSLGGNEIVSLKGLLLTFATT
>gi|14250108|gb|AAH08466.1| Dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit [Homo sapiens]
MASLEVSRGPRRSEQRELEVRSPRQNKYSVLLPTYNERENLPLIIVWLLVKSFSESGINYEIIIIDDDSGPDG
TRDVAEQLEKIGYSDRILLRPEKCLLGLGTAYIHMHKAGTVNYIIMDADLSHHPHFIEFIRKQEGNF
DIVGTRKYNNGGVGDRLKRIISRGANFTQILLRPGASDLTGSFRLYRKEVLKIECVSKGYVFQ
MEMIVRARQMNYTGEVPIFSDVDRYGESKLGNEGNEIVSFKLGLLTLFATT

>gi|149042799|gb|EDL96373.1| rCG32280, isoform CRA_h [Rattus norvegicus]
MDADLSHHPHFIEFIRKQEGNFDIVGTRSYKGNGGVGDRLKRIISRGANFTQILLRPGASDLTGS
FRLYRKEVLKIECVSKGYVFQMEMIVRARQMNYTGEVPIFSDVDRYGESKLGNEGNEIVSFKLGLLTLFATT

>gi|58332670|ref|NP_001011407.1| dolichyl-phosphate mannosyltransferase 1 [ Xenopus (Silurana) tropicalis]
MAASGNKRKSGDKYSVLLPTYNERENLPLIIVWLLVRCFSDSYEIIIVIDDDSGPDGTLEQAQLQKYG
SDKILLRPRAKKLGLGTAYVHGMIHATGNFIIIMDADLSHHPHFIEFIRKQEGNFIVGTRYSYSNGGY
VGYGDRLKRIISRGANFTQILLRPGASDLTGSFRLYRKEVLKIECVSKGYVFQMEMIVRARQMNYTGEVPIFSDVDRYGESKLGNEGNEIVSFKLGLLTLFATT

>gi|149042792|gb|EDL96366.1| rCG32280, isoform CRA_b [Rattus norvegicus]
MPQETMSSLWQTSRMTQEGNFIVGTRYSYSNGGYGDRLKRIISRGANFTQILLRPGASDLTGS
FRLYRKEVLKIECVSKGYVFQMEMIVRARQMNYTGEVPIFSDVDRYGESKLGNEGNEIVSFKLGLLTLFATT

>gi|297259564|ref|XP_001094366.2| PREDICTED: dolichol-phosphate mannosyltransferase isoform 2 [ Macaca mulatta]
MHKSCIFVIEPHNSRRTSAQSRSSHSPASEFTVRPVFLRAALRNSASGVWLSAMASLEVRRSPPMSRR
QLEVRSPRDKYSVLLPTYNERENLPLIIVWLLVKSFSESGINYEIIIIDDDSGPDGTIRVAELQLEKYSGD
RIIILKPREKCLLGLGTAYIHMHKAGTVNYIIMDADLSHHPHFIEFIRKQEGNFIVGTRYSYSNGGY
GDRLKRIISRGANFTQILLRPGASDLTGSFRLYRKEVLKIECVSKGYVFQMEMIVRARQMNYTGEVPIFSDVDRYGESKLGNEGNEIVSFKLGLLTLFATT

>gi|149042791|gb|EDL96365.1| rCG32280, isoform CRA_a [Rattus norvegicus]
MGDSLGLHRNTPETIKSEYSPENTLRRKAKSPCSCIYWPRQVTAINEYIIMDDSGPDGTREAVEQ
LEKICYGDRILLRPEKCLLGLGTAYIHMHKAGTVNYIIMDADLSHHPHFIEFIRKQEGNFIVGTRYSYSNGGY
GDRLKRIISRGANFTQILLRPGASDLTGSFRLYRKEVLKIECVSKGYVFQMEMIVRARQMNYTGEVPIFSDVDRYGESKLGNEGNEIVSFKLGLLTLFATT

>gi|126303258|ref|XP_001378527.1| PREDICTED: similar to dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit, [Monodelphis domestica]
MASQSDIPNSPGQRGKVATATTGTGDYSDKSYLLPTYNERENLPLIIVWLLVKSFSESINYEIIIIDDDSGPDGTLEVAELQKTYSGDRIIILKPREKCLLGLGTAYIHMHKAGTVNYIIMDADLSHHPHFIEFIRKQEGNFIVGTRYSYSNGGY
GDRLKRIISRGANFTQILLRPGASDLTGSFRLYRKEVLKIECVSKGYVFQMEMIVRARQMNYTGEVPIFSDVDRYGESKLGNEGNEIVSFKLGLLTLFATT
YVFQMEMIVRARQLNYTIGEVPIFSFVDRVYGESKLGGNEIVSFGBKLLLFATT

>gi|197128067|gb|ACH44565.1| putative dolichol-phosphate mannosyltransferase variant 2 [Taeniopygia guttata] MAARGPGVRVSSLTYERDNPLLVWLVRTRFSSGIDFEIIIIDDGSPDTKVEVQAQLKLYGSDKI LLRPARKLGLGTATYIHMKGYATGNFIVIMADLHSHHKPKFIEFIRKFQEGGFIVDSGRTRYKGGVGYGWDL RKRKLISRGANFTQVLLRPAGSDDLTGSFRFLYKRKEVLEKMEKCVMKSVKGYVFQMEMIVRARQLGFTVGEVP ISFVDRVYGESKLGGNEIVSFGBKLLLFATT

>gi|322784295|gb|EFZ11300.1| hypothetical protein SINV_10884 [Solenopsis invicta] NDKYSILLPTYNEVENLPIIWLIVKYMEESEAYEIVIIDGSPDGLTDACKQLCGLYGDDKVKLPRE KKLGLGTAYHIKGYATGNFIVIMADLHSHHKPKFIEFIRKFQEGGFIVDSGRTRYKGGVGYGWDL RKRKLISRGANFTQVLLRPAGSDDLTGSFRFLYKRKEVLEKMEKCVMKSVKGYVFQMEMIVRARQLGFTVGEVP ISFVDRVYGESKLGGSEIFQFAKGLLYLFTATT

>gi|50759086|ref|XP_417511.1| PREDICTED: similar to dolichol-phosphate mannose synthase [Gallus gallus] MAARGGNKFSVLLPTYNERENLPLIVWLLVRTRFRESGTDFEIIIIDDGSPDGTQEDVQELEGKLYGSDKILLRPARKLGLGTATYIHMKGYATGNFIVIMADLHSHHKPKFIEFIRKFQEGGFIVDSGRTRYKGGVGYGWDL RKRKLISRGANFTQVLLRPAGSDDLTGSFRFLYKRKEVLEKMEKCVMKSVKGYVFQMEMIVRARQLGFTVGEVP ISFVDRVYGESKLGGNEIVSFGBKLLLFATT

>gi|297707334|ref|XP_002830465.1| PREDICTED: dolichol-phosphate mannosyltransferase-like [Pongo abelii] MYKSRIFLIEPRNPRRTDQSHRSSEPGTFQVFLFRASLRNSASGILSSAMASLEVSRSFRSSRR ELEVRSRPRQNKYSVLLPTYNERENLPLIVWLLVRTRFSEGTDYIIIIDDGSPDGTQEDVQELEGKLYGSDKILLRPARKLGLGTATYIHMKGYATGNFIVIMADLHSHHKPKFIEFIRKFQEGGFIVDSGRTRYKGGVGYGWDL RKRKLISRGANFTQVLLRPAGSDDLTGSFRFLYKRKEVLEKMEKCVMKSVKGYVFQMEMIVRARQLGFTVGEVP ISFVDRVYGESKLGGNEIVSFGBKLLLFATT

>gi|119596014|gb|EAW75608.1| dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit, isoform CRA_b [Homo sapiens] MYKSRIFLIEPRNPRRTDQSHRSSEPGTFQVFLFRASLRNSASGILSSAMASLEVSRSFRSSRR ELEVRSRPRQNKYSVLLPTYNERENLPLIVWLLVRTRFSEGTDYIIIIDDGSPDGTQEDVQELEGKLYGSDKILLRPARKLGLGTATYIHMKGYATGNFIVIMADLHSHHKPKFIEFIRKFQEGGFIVDSGRTRYKGGVGYGWDL RKRKLISRGANFTQVLLRPAGSDDLTGSFRFLYKRKEVLEKMEKCVMKSVKGYVFQMEMIVRARQLGFTVGEVP ISFVDRVYGESKLGGNEIVSFGBKLLLFATT

>gi|194879477|ref|XP_001974240.1| GG21624 [Drosophila erecta] MPTNGHKYSILLPTYNEKDNLPIIIIWLVKYMAGSEYEVIVIIDGSPDGLTDVAKDLQKYGEDRIVL KPRGSKLGLGTDAYIHIKHAGZTQDFIVIIMADLHSHHKPKFIEFILQKQEGNFDIVSGTRYKGNNGVGYGWDF KRKLISRGANFTQVLLRPASD LTSGFLYKDDVLEKCIASCVMKSVKGYVFQMEMIVRARQHGYSAEVPI TFVDRYGETSKLGGTEIQFACKNLLLFATT
>gi|114682631|ref|XP_001168321.1| PREDICTED: similar to dolichol-phosphate-mannose synthase isoform 6 [Pan troglodytes]
MASLEVSRSPRRELEVRSPQNKYSVLLPTYNERENLPLIVWLLVKSFCESGINYEIIIIDDGDSPDG
TRDVAEQLKGYGDRRPREEKGLGTAYHGMKHATGNYIIIIMADLSSLHHPKFIPEFIRQKEGNF
DIVSGTRYKNGGGVYGWDLKRIISRGANFLTQIILLRPAGSLTGFLRLYKEVLKIEKCVKSKGYVFQ
MEMIVRARQLNTIEGPISFVDRVYGEKLLGNEIVSFKGLLLTLFATT

>gi|4503363|ref|NP_003850.1| dolichol-phosphate mannosyltransferase [Homo sapiens]
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TRDVAEQLKGYGDRRPREEKGLGTAYHGMKHATGNYIIIIMADLSSLHHPKFIPEFIRQKEGNF
DIVSGTRYKNGGGVYGWDLKRIISRGANFLQIILLRPAGSLTGFLRLYKEVLKIEKCVKSKGYVFQ
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>gi|195434485|ref|XP_002065233.1| dolichol monophosphate mannose synthase [Drosophila willistoni]
MPSNGHKYSILLPTYNEKDNPIIIWILVKYMASGTYEYEIVIDDDGDSPDGLDVAKDLQKYYGEDRIVL
RPRATKDLGTYHIGKHTGDFIIIIMADLSSLHHPKFIPEFIELQAKGYDIVSGTRYAGDDGGVGDWDF
KRKLISRGANFLSQILLRPASNLTSFRLSFLKDELCIAASCVKSKGYVFQMMEMIVRARQHGTIAEVPI
TFVDRYIGTSKLGGTEIVQFAKNLLYLFATT

>gi|170592929|ref|XP_001901217.1| dolichol monophosphate mannose synthase [Brugia malayi]
MFNEILLGLGTAYTHGQLFARGDYVILMDADLSSLHHPKFIPEMIKLLQOHKNTIVGTRYARGGVSVDWDL
KRKFVSRGANFLAQFLRLRPGVSDLTGFLRKYKDVMLARLIADSVSKGYVFQMEMMFRASKLYRIEVEPI
SFIDRFYGESQLSQEYVGITFYLFLFIFV

>gi|148674602|gb|EDL06549.1| dolichol-phosphate (beta-D) mannosyltransferase 1, isoform CRA_g [Mus musculus]
NEWVFIRGSRHDWLETVTISGSGFTTLGRRSAEPFPCSEIHYGFCRMTAINYEIIIIIDDGDSPDGETEVA
EQLAEIYGFRILLPRKELGLGTAYHGKHTGNYIIMADLSSLHHPKFIPEFIRQKEGNFDIVSG
TRFYKGHGGVYGWDLKRIISRGNFLTQIILLRPAGSLTGFLRLYKEVLKIEKCVKSKGYVFQMEMIMIV
RARQMNITYGEVPISFVDRVYGEKLLGNEIVSFKGLLLTLFATT

>gi|46329779|gb|AAH68840.1| LOC414689 protein [Xenopus laevis]
HASGWSLEGTVLAMMATSNKRKNKSGDXYVLLPTYNERENLPLIVWLLVRCFRDGSNGYNYEIIIDD
GSFPDGLTEVAQQLKGYGDKILLRFRAQGLGLGTAYHGMGHATGNYIIIIIMADLSSLHHPKFIPEFIRQ
KEGSYDIVSGTRYSGNGGTVGWDLKRIISRGANFLTQLLRLRPGASLTGFLRKYKDLQVCLVERC
SKGYVFQMEMIVRARQMNITYGEVPISFVDRVYGEKLLGNEIVSFKGLLLTLFATT

>gi|57104284|ref|XP_534456.1| PREDICTED: similar to Dolichol-phosphate mannosyltransferase (Dolichol-phosphate mannose synthase) (Dolichyl-phosphate beta-D-mannosyltransferase) (Mannose-P-dolichol synthase) (MPD synthase) (DFP synthase) isoform 1 [Canis familiaris]
MASEEASGRSSRSRREPEGRAPRQDKYSVLLPTYNERENLPLIVWLLVKSFSESGINYEIIIDDGSPDG
TRDAEQLKEYIGSDKILLRPREKCLGLGTYAIHGMMKATGNYIIIMDADLSSHHPFIFEPFIRKQKEGNF
DIVSGTRYKNGGGVGWLKRKIIISRGANFTQILLRPAGSDLTSFLYRKEVLQKLIKECVSKGYVFQ
MEMIRARQLNYTIGEVPISFVDRVYGESKLGNGEIVSFKGLLLTFATT

>gi|262072949|dbj|BAI47782.1| dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit [Sus scrofa]
MASEEASGRSSRSRREPEGRAPRQDKYSVLLPTYNERENLPLIVWLLVKSFSESGINYEIIIDDGSPDG
TRDAEQLKEYIGSDKILLRPREKCLGLGTYAIHGMMKATGNYIIIMDADLSSHHPFIFEPFIRKQKEGNF
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MEMIRARQLNYTIGEVPISFVDRVYGESKLGNGEIVSFKGLLLTFATT

>gi|301754367|ref|XP_002913023.1| PREDICTED: dolichol-phosphate mannosyltransferase-like [Ailuropoda melanoleuca]
MASEEASGRSSRSRREPEGRAPRQDKYSVLLPTYNERENLPLIVWLLVKSFSESGINYEIIIDDGSPDG
TRDAEQLKEYIGSDKILLRPREKCLGLGTYAIHGMMKATGNYIIIMDADLSSHHPFIFEPFIRKQKEGNF
DIVSGTRYKNGGGVGWLKRKIIISRGANFTQILLRPAGSDLTSFLYRKEVLQKLIKECVSKGYVFQ
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>gi|20138078|sp|Q9WU83.1|DPM1_CRIGR RecName: Full=Dolichol-phosphate mannosyltransferase; AltName: Full=Dolichol-phosphate mannose synthase;
Short=DPM synthase; AltName: Full=Dolichyl-phosphate beta-D-mannosyltransferase; AltName: Full=Mannose-P-dolichol synthase; Short=MPD synthase (Cricetulus griseus)
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TRDAEQLKEYIGSDKILLRPREKCLGLGTYAIHGMMKATGNYIIIMDADLSSHHPFIFEPFIRKQKEGNF
DIVSGTRYKNGGGVGWLKRKIIISRGANFTQILLRPAGSDLTSFLYRKEVLQKLIKECVSKGYVFQ
MEMIRARQLNYTIGEVPISFVDRVYGESKLGNGEIVSFKGLLLTFATT

>gi|167001855|ref|NP_001095290.1| dolichol-phosphate mannosyltransferase [Sus scrofa]
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DIVSGTRYKNGGGVGWLKRKIIISRGANFTQILLRPAGSDLTSFLYRKEVLQKLIKECVSKGYVFQ
MEMIRARQLNYTIGEVPISFVDRVYGESKLGNGEIVSFKGLLLTFATT

>gi|149734192|ref|XP_001488101.1| PREDICTED: similar to dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit [Equus caballus]
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TRDAEQLKEYIGSDKILLRPREKCLGLGTYAIHGMMKATGNYIIIMDADLSSHHPFIFEPFIRKQKEGNF
DIVSGTRYKNGGGVGWLKRKIIISRGANFTQILLRPAGSDLTSFLYRKEVLQKLIKECVSKGYVFQ
MEMIRARQLNYTIGEVPISFVDRVYGESKLGNGEIVSFKGLLLTFATT
CLUSTAL W (1.81) multiple sequence alignment

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Cryptococcus_neofor making_var._n
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Yarrowia_lipolytica
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Schizobacteria_commune_H4-8
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Penicillium_chrysogenum_Wiscon
Tuber_melanosporum_Mel28
Yarrowia_lipolytica
Xenopus_Silurana_tropicalis
2_Xenopus_laevis
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**1. Xenopus laevis**
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**3. Rattus norvegicus**
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**5. Mus musculus**
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**4. Mus musculus**
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**6. Mus musculus**
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**Cricetulus griseus**
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**Pongo abellii**
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**Pan troglodytes**
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**Canis familiaris**
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**Ailuropoda melanoleuca**
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**Monodelphis domestica**
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**Galimus gallus**
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**Danio rerio**
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**Branchiostoma floridae**
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**Strongylocentrotus purpuratus**
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**Drosophila sechellia**
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**Loa loa**
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**Brugia malayi**
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**Ascaris suum**
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*: * **.*:** **:** : * : .: *:
NCBI Generated Lineage Report for EST D00658

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            Metarhizium anisopliae ARSEF 23  216 1 hit [ascomycetes] mannose-phospho-dolichol synthase [Metarhizium anisopliae A]
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                  Pyrenophora teres f. teres 1-0  218 1 hit [ascomycetes] hypothetical protein PTT-07599 [Pyrenophora teres f. teres]
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                    Coccidioides posadasi str. Silveira  197 1 hit [ascomycetes] dolichol-phosphate mannosyltransferase, putative [Coccidioid
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                    Trichophyton verrucosum HKI 0517  189 2 hits [ascomycetes] hypothetical protein TRV_04566 [Trichophyton verrucosum HKI
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Organism Report

**Sclerotinia sclerotiorum 1980 UF-70** [ascomycetes] taxid 665079
ref|XP 001592566.1| mannose phospho-dolichol synthase [Sclerotio... 226 9e-58
gb|EOD004324.1| mannose phospho-dolichol synthase [Sclerot... 226 9e-58

**Botrytina fuckeliana B05.10** [ascomycetes] taxid 332648
ref|XP 001556657.1| hypothetical protein BC1G_04042 [Botry... 223 5e-57
gb|EDN23857.1| hypothetical protein BC1G_04042 [Botryotini... 223 5e-57

**Hypocrea jeconina** [ascomycetes] taxid 51453
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**Pyrenophora tritici-repentis Pt-1C-BFP** [ascomycetes] taxid 426418
ref|XP 001938970.1| dolichyl-phosphate mannosyltransferase... 218 2e-55
gb|EDU51557.1| dolichyl-phosphate mannosyltransferase [Pyre... 218 2e-55

**Pyrenophora teres f. teres 0-1** [ascomycetes] taxid 861557
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**Leptosphaeria maculans** (blackleg of crucifers fungus, ...) [ascomycetes] taxid 5022
emb|CBX99923.1| similar to dolichol-phosphate (beta-D) man... 218 3e-55

**Metarhizium acridum CQMa 102** [ascomycetes] taxid 655827
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**Phaeosphaeria nodorum SN15** [ascomycetes] taxid 321614
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**Metarhizium anisopliae ARSEF 23** [ascomycetes] taxid 655844
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**Nectria haematococca mpV1 77-13-4** [ascomycetes] taxid 660122
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gb|EU36467.1| glycosyltransferase family 2 [Nectria haema... 216 9e-55

**Glomerella graminicola M1.001** [ascomycetes] taxid 645133
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**Gibberella zeae PH-1** [ascomycetes] taxid 229533
ref|XP_384220.1| hypothetical protein FG04044.1 [Gibberell... 211 3e-53

**Tuber melanosporum Mel28** [ascomycetes] taxid 656061
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**Tuber melanosporum** (French truffle, ...) [ascomycetes] taxid 39416
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**Coccidioides posadasi str. Silveira** [ascomycetes] taxid 443226
gb|EF20516.1| glycosyltransferase [Coccidioides posadasi... 197 3e-49

**Coccidioides immitis RS** [ascomycetes] taxid 246410
ref|XP_001244154.1| hypothetical protein CIMG_03595 [Cocci... 196 7e-49

**Ajellomyces capsulatus NAm1** [ascomycetes] taxid 339724
ref|XP_001543068.1| dolichol-phosphate mannosyltransferase... 195 1e-48
gb|DN2250.1| dolichol-phosphate mannosyltransferase [Aje... 195 1e-48
Ajellomyces capsulatus G186AR [ascomycetes] taxid 447093
dolichol-phosphate mannose synthase [Ajello...  195 1e-48

Paracoccidioides brasiliensis Pb01 [ascomycetes] taxid 502779
dolichol-phosphate mannosyltransferase [Par...  195 2e-48

Candida albicans SC5314 [ascomycetes] taxid 237561
potential ER dolichol phosphate mannose synthase [Can...  195 2e-48

Candida albicans WO-1 [ascomycetes] taxid 294748
dolichol-phosphate mannosyltransferase [Can...  195 2e-48

Paracoccidioides brasiliensis Pb03 [ascomycetes] taxid 482561
dolichol-phosphate mannosyltransferase [Par...  195 2e-48

Candida dubliniensis CD36 [ascomycetes] taxid 573826
dolichol-phosphate mannose synthase, putat...  194 2e-48

Ajellomyces dermatitidis SLH14081 [ascomycetes] taxid 559298
dolichol-phosphate mannosyltransferase [Aje...  194 4e-48

Ajellomyces dermatitidis ER-3 [ascomycetes] taxid 559297
dolichol-phosphate mannosyltransferase [Aje...  194 4e-48

Paracoccidioides brasiliensis Pb18 [ascomycetes] taxid 502780
dolichol-phosphate mannosyltransferase [Par...  193 5e-48

Clavispora lusitaniae ATCC 42720 [ascomycetes] taxid 306902
hypothetical protein CLUG_03543 [Clavi...  193 6e-48

Ajellomyces capsulatus H143 [ascomycetes] taxid 544712
dolichol-phosphate mannose synthase [Ajello...  192 1e-47

Ajellomyces capsulatus H88 [ascomycetes] taxid 544711
dolichol-phosphate mannose synthase [Ajello...  192 1e-47
Debaryomyces hansenii CBS767 [ascomycetes] taxid 284592
ref|XP_462199.1| DEHA2G15136p [Debaryomyces hansenii CBS767]  192 2e-47

Debaryomyces hansenii [ascomycetes] taxid 4959
emb|CAG90691.1| DEHA2G15136p [Debaryomyces hansenii]  192 2e-47

Candida tropicalis MYA-3404 [ascomycetes] taxid 294747
ref|XP_003177124.1| dolichol-phosphate mannosyltransferase [Can...  190 5e-47
gb|EFQ98172.1| dolichol-phosphate mannosyltransferase [Can...  190 5e-47

Arthroderma gypseum CBS 118893 [ascomycetes] taxid 535722
ref|XP_003177124.1| dolichol-phosphate mannosyltransferase [Ar...  190 5e-47
gb|EFQ98172.1| dolichol-phosphate mannosyltransferase [Art...  190 5e-47

Magnaporthe oryzae 70-15 [ascomycetes] taxid 242507
ref|XP_003177124.1| conserved hypothetical protein [Magnapor...  190 5e-47
gb|EDJ95325.1| conserved hypothetical protein [Magnaporthe...  190 5e-47

Pichia angusta DL-1 [ascomycetes] taxid 871575
gb|EFW97798.1| dolichol-P-mannose synthesis [Pichia angust...  189 7e-47

Trichophyton verrucosum HKI 0517 [ascomycetes] taxid 663202
ref|XP_003177124.1| hypothetical protein TRV_04566 [Tricho...  189 8e-47
gb|EFE40704.1| hypothetical protein TRV_04566 [Trichophyto...  189 8e-47

Arthroderma otae CBS 113480 [ascomycetes] taxid 554155
ref|XP_003177124.1| dolichol-phosphate mannosyltransferase [Arth...  189 8e-47
gb|EDJ95325.1| dolichol-phosphate mannosyltransferase [Art...  189 8e-47

Aspergillus clavatus NRRL 1 [ascomycetes] taxid 344612
ref|XP_001271045.1| dolichol-phosphate mannosyltransferase...  189 8e-47
gb|EAW09619.1| dolichol-phosphate mannosyltransferase, put...  189 8e-47

Arthroderma benhamiae CBS 112371 [ascomycetes] taxid 663331
ref|XP_003160686.1| hypothetical protein ARB_05483 [Arthro...  189 8e-47
gb|EFE35441.1| hypothetical protein ARB_05483 [Arthroderma...  189 8e-47

Lodderomyces elongisporus NRRL YB-4239 [ascomycetes] taxid 379508
ref|XP_001528001.1| dolichol-phosphate mannosyltransferase...  189 8e-47
gb|EDK42343.1| dolichol-phosphate mannosyltransferase [Lod...  189 8e-47

Paracoccidioides brasiliensis [ascomycetes] taxid 121759
gb|AAR03724.1| dolichol-phosphate mannose synthase [Paracon... 189 1e-46

Podospora anserina S mat+ [ascomycetes] taxid 515849
ref|XP_001903431.1| hypothetical protein [Podospora anseri... 189 1e-46
emb|CAP61206.1| unnamed protein product [Podospora anserin... 189 1e-46

Neosartorya fischeri NRRL 181 [ascomycetes] taxid 331117
ref|XP_001263417.1| dolichol-phosphate mannosyltransferase... 189 1e-46
gb|EAW21520.1| dolichol-phosphate mannosyltransferase, put... 189 1e-46

Aspergillus fumigatus AF293 [ascomycetes] taxid 330879
ref|XP_754579.1| dolichol-phosphate mannosyltransferase [A... 189 1e-46
gb|EAL92541.1| dolichol-phosphate mannosyltransferase, put... 189 1e-46

Aspergillus fumigatus A1163 [ascomycetes] taxid 451804
gb|EDP52706.1| dolichol-phosphate mannosyltransferase, put... 189 1e-46

Aspergillus niger CBS 513.88 [ascomycetes] taxid 425011
ref|XP_001397744.1| dolichol-phosphate mannosyltransferase... 188 2e-46

Aspergillus niger [ascomycetes] taxid 5061
emb|CAK46850.1| unnamed protein product [Aspergillus niger] 188 2e-46

Penicillium chrysogenum Wisconsin 54-1255 [ascomycetes] taxid 500485
ref|XP_002559449.1| Pc13g10270 [Penicillium chrysogenum Wi... 187 3e-46
emb|CAP92096.1| Pc13g10270 [Penicillium chrysogenum Wiscon... 187 3e-46

Yarrowia lipolytica CLIB122 [ascomycetes] taxid 284591
ref|XP_502478.1| YAL1D06281p [Yarrowia lipolytica] 187 3e-46

Yarrowia lipolytica [ascomycetes] taxid 4952
emb|CAG80666.1| YAL1D06281p [Yarrowia lipolytica] 187 3e-46

Uncinocarpus reesii 1704 [ascomycetes] taxid 336963
ref|XP_002582598.1| dolichol-phosphate mannosyltransferase... 187 5e-46
gb|EFX00187.1| dolichol-phosphate mannosyltransferase [Unc... 187 5e-46

Grosmannia clavigera kw1407 [ascomycetes] taxid 655863
gb|EEX00187.1| dolichol-phosphate mannosyltransferase [Gro... 187 5e-46

Aspergillus nidulans FGSC A4 [ascomycetes] taxid 227321
ref|XP_662551.1| hypothetical protein AN4947.2 [Aspergillus... 186 7e-46
gb|EAA61025.1| hypothetical protein AN4947.2 [Aspergillus ... 186 7e-46
Neurospora crassa OR74A [ascomycetes] taxid 367110
dolichol-phosphate mannosyltransferase [Neu... 186 8e-46

taloromyces stipitatus ATCC 10500 [ascomycetes] taxid 441959
dolichol-phosphate mannosyltransferase,... 182 2e-44
	nesidium sinuosum ATCC 18224 [ascomycetes] taxid 441960
dolichol-phosphate mannosyltransferase... 181 2e-44

Penicillium marneffei ATCC 18224 [ascomycetes] taxid 441960
dolichol-phosphate mannosyltransferase... 181 2e-44

Coprinopsis cinerea okayama7#130 [basidiomycetes] taxid 240176
dolichol-phosphate mannosyltransferase... 178 2e-43

Branchiostoma floridae [lancelets] taxid 7739
hypothetical protein BRAFLDRAFT_117614... 177 5e-43

Laccaria bicolor S238N-H82 [basidiomycetes] taxid 486041
glycosyltransferase family 2 protein [Lacca... 176 7e-43
Apis mellifera (bee, ...) [bees] taxid 7460
ref|XP_392640.1| PREDICTED: similar to Probable dolichol-phosphate mannosyltransferase [Apis mellifera] RefSeq: XP_392640.1
176 1e-42

Cryptococcus gattii WM276 [basidiomycetes] taxid 367775
gb|ADV20413.1| GPI anchor biosynthesis-related protein [Schizobryopsis] RefSeq: EF191653.1
176 1e-42

Cryptococcus gattii WM276 [basidiomycetes] taxid 367775
gb|ADV20413.1| GPI anchor biosynthesis-related protein [Schizobryopsis] RefSeq: EF191653.1
176 1e-42

Schizopyllum commune H-8 [basidiomycetes] taxid 578458
gb|EFF91653.1| glycosyltransferase family 2 protein [Schizopyllum commune] RefSeq: XP_003206556.1
175 1e-42

Danio rerio (zebra fish, ...) [bony fishes] taxid 7955
gb|AAH77113.1| Dolichyl-phosphate mannosyltransferase poly... RefSeq: XP_001003596.1
175 2e-42

Cryptococcus gattii WM276 [basidiomycetes] taxid 367775
gb|ADV20413.1| GPI anchor biosynthesis-related protein [Schizobryopsis] RefSeq: EF191653.1
176 1e-42

Xenopus laevis (common platanna, ...) [frogs & toads] taxid 8355
gb|AAH88840.1| LOC414689 protein [Xenopus laevis] RefSeq: XP_003026556.1
175 2e-42

Drosophila melanogaster [flies] taxid 7227
gb|ACX31360.1| MIP13910p [Drosophila melanogaster] RefSeq: NP_001003596.1
174 2e-42

gb|AAH77113.1| Dolichyl-phosphate mannosyltransferase poly... RefSeq: XP_003026556.1
175 2e-42

Drosophila sechellia [flies] taxid 7238
ref|XP_002079944.1| GD21748 [Drosophila simulans] RefSeq: XP_002079944.1
174 3e-42

gb|EDX05529.1| GD21748 [Drosophila simulans] RefSeq: XP_002079944.1
174 3e-42

Drosophila simulans [flies] taxid 7240
gb|EDW55775.1| GM17002 [Drosophila sechellia] RefSeq: XP_002079944.1
174 3e-42

Strongylocentrotus purpuratus (purple urchin, ...) [sea urchins] taxid 7668
ref|XP_793909.1| PREDICTED: hypothetical protein [Strongylocentrotus purpuratus] RefSeq: XP_793909.1
174 3e-42

Mus musculus (mouse) [rodents] taxid 10090
sp|O70152.1| DPM1_MOUSE RecName: Full=Dolichyl-phosphate mannosyltransferase [Mus musculus] RefSeq: NP_0609980.1
174 3e-42

Mus musculus (mouse) [rodents] taxid 10090
sp|O70152.1| DPM1_MOUSE RecName: Full=Dolichyl-phosphate mannosyltransferase [Mus musculus] RefSeq: NP_0609980.1
174 3e-42

Mus musculus (mouse) [rodents] taxid 10090
sp|O70152.1| DPM1_MOUSE RecName: Full=Dolichyl-phosphate mannosyltransferase [Mus musculus] RefSeq: NP_0609980.1
174 3e-42
Cryptococcus neoformans var. neoformans JEC21 [basidiomycetes] taxid 214684
ref|XP_569172.1| GPI anchor biosynthesis-related protein [... 174 3e-42
gb|AAW41865.1| GPI anchor biosynthesis-related protein, pu... 174 3e-42

Cryptococcus neoformans var. neoformans B-3501A [basidiomycetes] taxid 283643
ref|XP_777090.1| hypothetical protein CNBB3220 [Cryptococ... 174 3e-42
gb|EAL22443.1| hypothetical protein CNBB3220 [Cryptococcus... 174 3e-42

Acyrthosiphon pisum [aphids] taxid 7029
ref|XP_001952221.1| PREDICTED: similar to Probable dolicho... 174 3e-42

Drosophila yakuba [flies] taxid 7245
ref|XP_002090719.1| GE12644 [Drosophila yakuba] 174 4e-42
gb|EDW90431.1| GE12644 [Drosophila yakuba] 174 4e-42

Trichoplax adhaerens [placozoans] taxid 10228
ref|XP_00210253.1| hypothetical protein TRIADDBRATT.23616 ... 174 4e-42
gb|EDV26020.1| hypothetical protein TRIADDBRATT.23616 [Tric... 174 4e-42

Ascaris suum [nematodes] taxid 6253
gb|ADY48055.1| Dolichol-phosphate mannosyltransferase [Asc... 174 4e-42

Loa loa [nematodes] taxid 7209
ref|XP_003137087.1| Dolichol Phosphate Mannosyltransferase... 174 4e-42
gb|EDV26985.1| Dolichol Phosphate Mannosyltransferase fami... 174 4e-42

Rattus norvegicus (brown rat, ...) [rodents] taxid 10116
ref|XP_001100014.1| dolichol-phosphate mannosyltransferase... 174 4e-42
gb|EDL96369.1| rCG32280, isoform CRA_e [Rattus norvegicus] 174 4e-42
gb|EDL96373.1| rCG32280, isoform CRA_h [Rattus norvegicus] 174 5e-42
gb|EDL96374.1| rCG32280, isoform CRA_h [Rattus norvegicus] 174 5e-42
gb|EDL96376.1| rCG32280, isoform CRA_h [Rattus norvegicus] 174 5e-42
Taeniopygia guttata (zebra finch) [birds] taxid 59729
ref|XP_002186864.1| PREDICTED: putative dolichol-phosphate...

Homo sapiens (man) [primates] taxid 9606
gb|AAH08466.1| Dolichyl-phosphate mannosyltransferase poly...
gb|EAW75608.1| dolichyl-phosphate mannosyltransferase poly...
ref|NP_003850.1| dolichyl-phosphate mannosyltransferase [H... spi|O60762.1|DPM1_HUMAN RecName: Full=Dolichol-phosphate ma...

synthetic construct [other sequences] taxid 32630
gb|ADQ31633.1| dolichyl-phosphate mannosyltransferase poly...

Xenopus (Silurana) tropicalis [frogs & toads] taxid 8364
ref|NP_001011407.1| dolichyl-phosphate mannosyltransferase poly...

Macaca mulatta (rhesus macaque, ...) [primates] taxid 9544
ref|XP_001094366.2| PREDICTED: dolichyl-phosphate mannosyl...

Monodelphis domestica [marsupials] taxid 13616
ref|XP_001378527.1| PREDICTED: similar to dolichyl-phospha...

Solenopsis invicta (red imported fire ant, ...) [ants] taxid 13686
gb|EFZ11300.1| hypothetical protein SINV_10884 [Solenopsis... 

Gallus gallus (buntam, ...) [birds] taxid 9031
ref|XP_417511.1| PREDICTED: similar to dolichyl-phosphate-...

Pongo abelii (Orang-utan, ...) [primates] taxid 9601
ref|XP_002830465.1| PREDICTED: dolichyl-phosphate mannosyl...
**Drosophila erecta** [flies] taxid 7220
ref|XP_001974240.1| GG21624 [Drosophila erecta] 172 e-41
gb|EDV54640.1| GG21624 [Drosophila erecta] 172 e-41

**Pan troglodytes** [primates] taxid 9598
ref|XP_001168321.1| PREDICTED: similar to dolichol-phospha... 172 e-41
gb|EDW76219.1| 172 e-41

**Drosophila willistoni** [flies] taxid 7260
ref|XP_002065233.1| GK14776 [Drosophila willistoni] 172 e-41
gb|EDW76219.1| GK14776 [Drosophila willistoni] 172 e-41

**Brugia malayi** (agent of lymphatic filariasis) [nematodes] taxid 6
ref|XP_001901217.1| dolichol monophosphate mannose synthas... 172 e-41
gb|EDP29897.1| dolichol monophosphate mannose synthase, pu... 172 e-41

**Canis lupus familiaris** (dogs) [carnivores] taxid 9615
ref|XP_534456.1| PREDICTED: similar to Dolichol-phosphate ... 172 e-41

**Sus scrofa** (wild boar, ...) [even-toed ungulates] taxid 9823
dbj|BAI47782.1| dolichyl-phosphate mannosyltransferase pol... 172 e-41
ref|NP_001095290.1| dolichyl-phosphate mannosyltransferase... 172 e-41
spl|A5GFZ5.1|DPM1_PIG RecName: Full=Dolichol-phosphate mann... 172 e-41
dbj|BAI47782.1| dolichyl-phosphate mannosyltransferase pol... 172 e-41

**Ailuropoda melanoleuca** [carnivores] taxid 9646
ref|XP_002913023.1| PREDICTED: dolichol-phosphate mannosyl... 172 e-41

**Cricetulus griseus** (Chinese hamsters) [rodents] taxid 10029
sp|Q9WU83.1|DPM1_CRIGR RecName: Full=Dolichol-phosphate ma... 172 e-41
gb|AAD30975.1|AF121895_1 dolichol-phosphate-mannose syntha... 172 e-41

**Equus caballus** (equine, ...) [odd-toed ungulates] taxid 9796
ref|XP_001488101.1| PREDICTED: similar to dolichyl-phospha... 172 e-41

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**Taxonomy Report**
root ........................................................... 196 hits 101 orgs
. Fungi/Metazoa group ........................................... 195 hits 100 orgs [cellular organisms; Eukaryota]
  . Dikarya ......................................................... 109 hits 68 orgs [Fungi]
  . . saccharomyceta ................................................. 97 hits 62 orgs [Ascomycota]
Pezizomycotina ......................................... 77 hits 50 orgs
Leotiomycetes ........................................... 75 hits 48 orgs
Sclerotiniaceae ........................................... 19 hits 13 orgs
Sclerotinia sclerotiorum 1980 UF-70 ............... 2 hits 1 orgs [Sclerotinia; Sclerotinia sclerotiorum]
Botryotinia fuckeliana B05.10 ...................... 2 hits 1 orgs [Botryotinia; Botryotinia fuckeliana]
Sordariomycetes .......................................... 15 hits 11 orgs
Hypocreomycetidae ..................................... 7 hits 6 orgs
Hypocreales ............................................. 6 hits 5 orgs
Metarhizium ............................................. 2 hits 2 orgs [Hypocreaceae; Hypocrea]
Metarhizium acridum CQMa 102 .............. 1 hits 1 orgs [Metarhizium acridum]
Metarhizium anisopliae ARSEF 23 ............... 1 hits 1 orgs [Metarhizium anisopliae]
Nectriaceae ............................................. 3 hits 2 orgs
Nectria haematococca mpVI 77-13-4 ........ 2 hits 1 orgs [Nectria; Nectria haematococca complex; Nectria haematococca; Nectria haematococca mpVI]
Gibberella zeae PH-1 ......................... 1 hits 1 orgs [Gibberella; Gibberella zeae]
Glomerella graminicola M1.001 ............... 1 hits 1 orgs [Hypocreomycetidae incertae sedis; Glomerellaceae; Glomerella; Glomerella graminicola]
Sordariomycetidae ................................... 8 hits 5 orgs
Magnaporthe oryzae 70-15 ..................... 2 hits 1 orgs [Magnaporthales; Magnaporthaceae; Magnaporthaceae]
Sordariales ......................................... 5 hits 3 orgs
Podospora anserina S mat+ ................... 2 hits 1 orgs [Lasiosphaeriaceae; Podospora; Podospora anserina]
Sordariaceae ............................................. 3 hits 2 orgs
Neurospora crassa OR74A ..................... 2 hits 1 orgs [Neurospora; Neurospora crassa]
Sordaria macrospora ......................... 1 hits 1 orgs [Sordaria]
Grosmannia clavigera kw1407 ........ 1 hits 1 orgs [Ophiostomatales; Ophiostomataceae; Grosmannia; Grosmannia clavigera]
Pleosporineae .......................................... 6 hits 4 orgs [dothideomyceta; Dothideomycetes; Pleosporomycetidae; Pleosporales]
Pyrenophora ............................................. 3 hits 2 orgs [Pleosporales]
Pyrenophora tritici-repentis Pt-IC-BFP ........ 2 hits 1 orgs [Pyrenophora tritici-repentis]
Pyrenophora teres f. teres 0-1 .............. 1 hits 1 orgs [Pyrenophora teres; Pyrenophora teres f. teres]
Leptosphaeria maculans ......................... 1 hits 1 orgs [Leptosphaeriaceae; Leptosphaeria; Leptosphaeria maculans complex]
Phaeosphaeria nodorum SN15 ............... 2 hits 1 orgs [Phaeosphaeriaceae; P. nodorum; Phaeosphaeria; Phaeosphaeria nodorum]
Eurotiomycetidae ....................................... 50 hits 31 orgs [Eurotiomycetes]
Onygenales ............................................. 27 hits 18 orgs
mitosporic Onygenales ......................... 9 hits 7 orgs
Coccidioides ........................................... 4 hits 3 orgs
Coccidioides posadasii ...................... 3 hits 2 orgs
Coccidioides posadasii C735 delta SOW gp .. 2 hits 1 orgs
Coccidioides posadasii str. Silveira ....... 1 hits 1 orgs
Coccidioides immitis R5 ....................... 1 hits 1 orgs [Coccidioides immitis]
Paracoccidioides ..................................... 5 hits 4 orgs
Paracoccidioides brasiliensis ............... 5 hits 4 orgs
Paracoccidioides brasiliensis Pb01 ...... 2 hits 1 orgs
Paracoccidioides brasiliensis Pb03 ....... 1 hits 1 orgs
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<th>Organism</th>
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<td>Paracoccidioides brasiliensis Pb18</td>
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<td>Ajellomyces</td>
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<td>Trichophyton verrucosum HKI 0517</td>
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<td>Trichocomaceae</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>Saccharomycetales</td>
<td>20</td>
<td>12</td>
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<tr>
<td>Candida</td>
<td>9</td>
<td>4</td>
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<tr>
<td>Candida albicans SC5314</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>
Candida albicans WO-1 ............................     1 hits    1 orgs
Candida dubliniensis CD36 ..........................     2 hits    1 orgs [Candida dubliniensis]
Candida tropicalis MYA-3404 ........................     2 hits    1 orgs [Candida tropicalis]
Clavispora lusitaniae ATCC 42720 .....................     2 hits    1 orgs [Metschnikowiaceae; Clavispora; Clavispora lusitaniae]
Debaryomyces .........................................     2 hits    2 orgs
Debaryomyces hansenii ................................   2 hits    2 orgs [Debaryomyces hansenii var. hansenii]
Lodderomyces elongisporus NRRL YB-4239 .............     2 hits    1 orgs [Lodderomyces; Lodderomyces elongisporus]
Scheffersomyces stipitis CBS 6054 .....................     2 hits    1 orgs [Scheffersomyces; Scheffersomyces stipitis]
Pichia angusta DL-1 ....................................     1 hits    1 orgs [Saccharomycetaceae; Pichia; Pichia angusta]
Yarrowia .............................................     2 hits    2 orgs [Dipodascaceae]
Yarrowia lipolytica ................................     2 hits    2 orgs
Yarrowia lipolytica CLIB122 ..........................     1 hits    1 orgs
Agaricomycotina ........................................ 12 hits    6 orgs [Basidiomycota]
Agaricales ............................................. 6 hits    3 orgs [Agaricomycetes; Agaricomycetidae]
Coprinopsis cinerea okayama?#130 .....................     2 hits    1 orgs [Psathyrellaceae; Coprinopsis; Coprinopsis cinerea]
Laccaria bicolor S238N-H82 ............................     2 hits    1 orgs [Tricholomataceae; Laccaria; Laccaria bicolor]
Schizophyllum commune H-4-8 ............................     2 hits    1 orgs [Schizophyllaceae; Schizophyllum; Schizophyllum commune]
Cryptococcus neoformans species complex .......... 6 hits    3 orgs [Tremellomycetes; Tremellales; Tremellaceae; Filobasidiella]
Cryptococcus gattii WM276 ............................     2 hits    1 orgs [Cryptococcus gattii]
Cryptococcus neoformans var. neoformans .......... 4 hits    2 orgs [Cryptococcus neoformans]
Cryptococcus neoformans var. neoformans JEC21 .....     2 hits    1 orgs
Cryptococcus neoformans var. neoformans B-3501A ....     2 hits    1 orgs
Metazoa .................................................. 86 hits    32 orgs
Bilateria ................................................ 84 hits    31 orgs [Eumetazoa]
Coelomata ............................................. 79 hits    28 orgs
Deuterostomia .......................................... 62 hits    19 orgs
Chordata ................................................ 60 hits    18 orgs
Branchiostoma floridanae ............................... 2 hits    1 orgs [Cephalochordata; Branchiostomi; Branchiostomata]
Euteleostomi .......................................... 58 hits    17 orgs [Crania; Vertebrata; Gnathostomata; Teleostomi]
Danio rerio ............................................. 2 hits    1 orgs [Actinopterygii; Actinopteri; Neopterygii; Teleostei; Elopoecephala; Clupeocephala; Otocephala; Otocephalii; Otophysi; Cypriniphi; Cypriniformes; Cyprinoidea; Cyprinidae; Danio]
Tetrapoda ............................................. 56 hits    16 orgs [Sarcopterygii]
Xenopus ................................................. 5 hits    2 orgs [Amphibia; Batrachia; Anura; Mesobatrachia; Pipidae; Pipidae; Xenopodinae]
Xenopus laevis .......................................... 3 hits    1 orgs [Xenopus]
Xenopus (Silurana) tropicalis .......................... 2 hits    1 orgs [Silurana]
Amniota .................................................. 51 hits    14 orgs
Theria .................................................... 48 hits    12 orgs [Mammalia]
Eutheria .................................................. 47 hits    11 orgs
Euarchontoglires ....................................... 39 hits    7 orgs
Muroidea ................................................. 24 hits    3 orgs [Glires; Rodentia; Sciurognathi]
Murinae .................................................. 22 hits    2 orgs [Muridae]
<table>
<thead>
<tr>
<th>Organism</th>
<th>Hits</th>
<th>Orgs</th>
<th>[taxonomic information]</th>
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<tbody>
<tr>
<td>Mus musculus</td>
<td>13</td>
<td>1 orgs</td>
<td>[Mus; Mus]</td>
</tr>
<tr>
<td>Rattus norvegicus</td>
<td>9</td>
<td>1 orgs</td>
<td>[Rattus]</td>
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<td>Cricetulus griseus</td>
<td>2</td>
<td>1 orgs</td>
<td>[Cricetidae; Cricetinae; Cricetulus]</td>
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<tr>
<td>Catarrhini</td>
<td>15</td>
<td>4 orgs</td>
<td>[Primates; Haplorrhini; Simiiformes]</td>
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<tr>
<td>Hominiidae</td>
<td>14</td>
<td>3 orgs</td>
<td>[Hominoida]</td>
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<tr>
<td>Hominaea</td>
<td>13</td>
<td>2 orgs</td>
<td></td>
</tr>
<tr>
<td>Homo sapiens</td>
<td>10</td>
<td>1 orgs</td>
<td>[Homo]</td>
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<tr>
<td>Pan troglodytes</td>
<td>3</td>
<td>1 orgs</td>
<td>[Pan]</td>
</tr>
<tr>
<td>Pongo abelii</td>
<td>1</td>
<td>1 orgs</td>
<td>[Ponginae; Pongo]</td>
</tr>
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<td>Macaca mulatta</td>
<td>1</td>
<td>1 orgs</td>
<td>[Cercopithecoida; Cercopithecidae; Cercopithecinae; Macaca]</td>
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<tr>
<td>Laurasiatheria</td>
<td>8</td>
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<td></td>
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<td>Caniformia</td>
<td>2</td>
<td>2 orgs</td>
<td>[Carnivora]</td>
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<td>Canis lupus familiaris</td>
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<td>1 orgs</td>
<td>[Canidae; Canis; Canis lupus]</td>
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<td>Ailuropoda melanoleuca</td>
<td>1</td>
<td>1 orgs</td>
<td>[Ursidae; Ailuropoda]</td>
</tr>
<tr>
<td>Sus scrofa</td>
<td>5</td>
<td>1 orgs</td>
<td>[Cetartiodactyla; Suina; Suidae; Sus]</td>
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<tr>
<td>Equus caballus</td>
<td>1</td>
<td>1 orgs</td>
<td>[Perissodactyla; Equidae; Equus; Equus subg. Equus]</td>
</tr>
<tr>
<td>Monodelphis domestica</td>
<td>1</td>
<td>1 orgs</td>
<td>[Metatheria; Didelphimorphia; Didelphidae; Didelphinae; Monodelphis]</td>
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<tr>
<td>Neognathae</td>
<td>3</td>
<td>2 orgs</td>
<td>[Sauropsida; Sauria; Archosauaria; Dinosauria; Saurischia; Theropoda; Coelurosauria; Aves]</td>
</tr>
<tr>
<td>Taeniopygia guttata</td>
<td>2</td>
<td>1 orgs</td>
<td>[Passeriformes; Passeroidea; Estrildidae; Estrildinae; Taeniopygia]</td>
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<tr>
<td>Gallus gallus</td>
<td>1</td>
<td>1 orgs</td>
<td>[Galliformes; Phasianidae; Phasianinae; Gallus]</td>
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<tr>
<td>Strongyluncentrotus purpuratus</td>
<td>2</td>
<td>1 orgs</td>
<td>[Echinodermata; Eleutherozoa; Echinoida; Echinidea; Echinoidea; Echinacea; Echinida; Strongylocentrotidae; Strongylocentrotus]</td>
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<tr>
<td>Neoptera</td>
<td>17</td>
<td>9 orgs</td>
<td>[Protostomia; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota]</td>
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<tr>
<td>Endopterygota</td>
<td>16</td>
<td>8 orgs</td>
<td></td>
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<tr>
<td>Aculeata</td>
<td>2</td>
<td>2 orgs</td>
<td>[Hymenoptera; Apocrita]</td>
</tr>
<tr>
<td>Apis mellifera</td>
<td>1</td>
<td>1 orgs</td>
<td>[Apoidae; Apidae; Apiniae; Apini; Apis]</td>
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<tr>
<td>Solenopsis invicta</td>
<td>1</td>
<td>1 orgs</td>
<td>[Vespoidae; Formicidae; Myrmicinae; Solenopsidina; Solenopsis]</td>
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<tr>
<td>Sophophora</td>
<td>14</td>
<td>6 orgs</td>
<td>[Diptera; Brachycera; Muscomorpha; Eremoneura; Cyclorrhapha; Schizophora; Acalyptratea; Ephydroidea; Drosophilidae; Drosophilinae; Drosophilina; Drosophilina; Drosophilidi; Drosophila]</td>
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<tr>
<td>Drosophilidae</td>
<td>4</td>
<td>1 orgs</td>
<td>[melanogaster group]</td>
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<tr>
<td>Drosophila melanogaster</td>
<td>12</td>
<td>5 orgs</td>
<td>[melanogaster group]</td>
</tr>
<tr>
<td>Drosophila sechellia</td>
<td>2</td>
<td>1 orgs</td>
<td></td>
</tr>
<tr>
<td>Drosophila simulans</td>
<td>2</td>
<td>1 orgs</td>
<td></td>
</tr>
<tr>
<td>Drosophila yakuba</td>
<td>2</td>
<td>1 orgs</td>
<td></td>
</tr>
<tr>
<td>Drosophila erecta</td>
<td>2</td>
<td>1 orgs</td>
<td></td>
</tr>
<tr>
<td>Drosophila willistoni</td>
<td>2</td>
<td>1 orgs</td>
<td>[willistoni group; willistoni subgroup]</td>
</tr>
<tr>
<td>Acyrtosiphon pismum</td>
<td>1</td>
<td>1 orgs</td>
<td>[Paraneoptera; Hemiptera; Sternorrhyncha; Aphidiformes; Aphidomorpha; Aphidoidea; Aphididae; Aphidinae; Macrosiphin; Acyrtosiphon]</td>
</tr>
<tr>
<td>Chromadorea</td>
<td>5</td>
<td>3 orgs</td>
<td>[Pseudocoelomata; Nematoda]</td>
</tr>
<tr>
<td>Ascaris suum</td>
<td>1</td>
<td>1 orgs</td>
<td>[Ascaridida; Ascaridoidea; Ascarididae; Ascaris]</td>
</tr>
<tr>
<td>Onchocercida</td>
<td>4</td>
<td>2 orgs</td>
<td>[Spirurida; Filarioidea]</td>
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<tr>
<td>Loa loa</td>
<td>2</td>
<td>1 orgs</td>
<td>[Loa]</td>
</tr>
<tr>
<td>Brugia maltayi</td>
<td>2</td>
<td>1 orgs</td>
<td>[Brugia]</td>
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</table>
... Trichoplax adhaerens ........................................ 2 hits 1 orgs [Placozoa; Trichoplax]
  synthetic construct ........................................... 1 hits 1 orgs [other sequences; artificial sequences]
Sequences Homologous to the Protein of EST D00881 and ClustalW alignment

>bgh02742
MLSRFQAFVGAVLVLTVYFTTTHPLVSRSSESRNEADPNSINITALPTTPITTDGNAASQKLLID
MSQAFLAPREAKAYFQYDVESAPAYIQWTKFPTASGDFDEKRFRASEASWEKHTPFVEHITIQVAVHLI
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PKKSIGLIGEADPREDWSASWYSWRRIRFCQWTLQSKPQHPILREIVANITLTTQLKSKDGGLLSNFRS
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>gi|156058294|ref|XP_001595070.1| hypothetical protein SS1G_03158
[Sclerotinia sclerotiorum 1980]
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ASLPEVLDAYNLPLVPKADFFRFLYLLILLARGIGYSDIDTQALSKADEVLEPSVPREAGILVGIEADPD
RPDNWAEPYSRRIQCQWTLQSKPQHPILREIVANITEQTLSMKESGLKHFDNKNVVEFTGPALRTDTV
DFLNDERYFDMTTSKGNITKWEFTGIFSQAKVGVDPVLPLTIFSFGVGGMQGAEYD
YDDPMAFVKHDFEGTWKFERHIGIIE

>gi|154309328|ref|XP_001553998.1| hypothetical protein BC1G_07558
[Botryotinia fuckeliana B05.10]
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EVITEDEVAVHLLKLYASVPEVLEAYNSLMNPVLPDKADFFRFLYLLILLARGIGYSDIDTQALSKADEVLEPSV
PREAGILVGIEADPDQFCQWTLQSKPQHPILREIVANITEQTLSMKESGLKHFDNKNVVEFTGPALRTDTV
DFLNDERYFDMTTSKGNITKWFTGIFSQAKVGVDPVLPLTIFSFGVGGMQGAEYD
YDDPMAFVKHDFEGTWKFERHIGIIE

>gi|85074763|ref|XP_965749.1| hypothetical protein NCU00609 [Neurospora
craspa OR74A]
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GQRIPQDMRSTLTLEYKLAYQFPYDVEKVFDPAYIQWTKWTAPGEHNFCDREATWTQHGFPFHEVTDK
VAVHILLLYASVPEVLEAYNMLPLVPKADFFRFLYLLILLARGIGYSDIDTQALSKADEVLEPSV
PREAGILVGIEADPDQFCQWTLQSKPQHPILREIVANITEQTLSMKESGLKHFDNKNVVEFTGPALRTDTV
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YDDPMAFVKHDFEGTWKFERHIGIIE

>gi|310797741|gb|EFQ32634.1| glycosyltransferase sugar-binding region containing DXD domain-containing protein [Glomerella graminicola MI.001]
MLTFRRAILVAAFFTIFLYISSSSNPSTTTTFAPKTDADVETAKHASTTATDSSGSAGNNQQNFQ
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VAVHILLLYASVPEVLEAYNMLPLVPKADFFRFLYLLILLARGIGYSDIDTQALSKADEVLEPSV
PREAGILVGIEADPDQFCQWTLQSKPQHPILREIVANITEQTLSMKESGLKHFDNKNVVEFTGPALRTDTV
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>gi|310797741|gb|EFQ32634.1| glycosyltransferase sugar-binding region containing DXD domain-containing protein [Glomerella graminicola MI.001]
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PREAGILVGIEADPDQFCQWTLQSKPQHPILREIVANITEQTLSMKESGLKHFDNKNVVEFTGPALRTDTV
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YDDPMAFVKHDFEGTWKFERHIGIIE
>gi|46135819|ref|XP_389601.1| hypothetical protein FG09425.1 [Gibberella zeae PH-1]
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DVANLRLYATVPEVEAYRSLPMVPVLKADFFYRLILFARGGYSDDTYAIQSVVKLPEQISRTDI
GLVIGIEADPDRFDWQAWSRSRIIFCQWITQAIQGHPALRDIINIRITKILDKEKGTLETFVDRNWVEF
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AYVKHDDEFGTMKFEREHMGQPQEEQAPLQQEQGQPQGGQPQGQQGPQGQQGQPQGQQGQPQGQQGQPPAAQ

>gi|322704557|gb|EFY96151.1| initiation-specific alpha-1,6-mannosyltransferase [Metarhizium anisopliae ARSEF 23]
MANSRRRAAVAVLFLVFLLTRSHSSPAQSSDSPFPAADATGAADTQAAAAPAPNDFPDQPPPEKPKEMEVKERRFRPDMSGMTTEYKLEYAYFVDTKSPQIWTQKQPQDPDQFQKDHGSSREWHPGVEHVD
DVANLRLYATVPEVEAYRSLPMVPVLKADFFYRLILFARGGYSDDTYAIQSVVKLPEQISRTDI
GLVIGIEADPDRFDWQAWSRSRIIFCQWITQAIQGHPALRDIINIRITKILDKEKGTLETFVDRNWVEF
TGPAVWTDAILDYFNDGRFDFMSQSKGTIDYKNTGMESTKRVDVVLPITSFSPGVQGMSKGKCPDDPM
AYVKHDDEFGTMKFEREHMGQPQEEQAPLQQEQGQPQGGQPQGQQGPQGQQGQPQGQQGQPQGQQGQPPAAQ

>gi|302895689|ref|XP_003046725.1| hypothetical protein NECHADRAFT_10591 [Nectria haematococa mpVI 77-13-4]
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DVANLRLYATVPEVEAYRSLPMVPVLKADFFYRLILFARGGYSDDTYAIQSVVKLPEQISRTDI
GLVIGIEADPDRFDWQAWSRSRIIFCQWITQAIQGHPALRDIINIRITKILDKEKGTLETFVDRNWVEF
TGPAVWTDAILDYFNDGRFDFMSQSKGTIDYKNTGMESTKRVDVVLPITSFSPGVQGMSKGKCPDDPM
AYVKHDDEFGTMKFEREHMGQPQEEQAPLQQEQGQPQGGQPQGQQGPQGQQGQPQGQQGQPQGQQGQPPAAQ

>gi|322693624|gb|EFY85478.1| initiation-specific alpha-1,6-mannosyltransferase [Metarhizium acridum CQMa 102]
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GLVIGIEADPDRFDWQAWSRSRIIFCQWITQAIQGHPALRDIINIRITKILDKEKGTLETFVDRNWVEF
TGPAVWTDAILDYFNDGRFDFMSQSKGTIDYKNTGMESTKRVDVVLPITSFSPGVQGMSKGKCPDDPM
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>gi|320034350|gb|EFW16295.1| hypothetical protein CFGS_07345 [Coccidioides posadasii str. Silveira]
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DVANLRLYATVPEVEAYRSLPMVPVLKADFFYRLILFARGGYSDDTYAIQSVVKLPEQISRTDI
GLVIGIEADPDRFDWQAWSRSRIIFCQWITQAIQGHPALRDIINIRITKILDKEKGTLETFVDRNWVEF
TGPAVWTDAILDYFNDGRFDFMSQSKGTIDYKNTGMESTKRVDVVLPITSFSPGVQGMSKGKCPDDPM
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>gi|46135819|ref|XP_389601.1| hypothetical protein FG09425.1 [Gibberella zeae PH-1]
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DVANLRLYATVPEVEAYRSLPMVPVLKADFFYRLILFARGGYSDDTYAIQSVVKLPEQISRTDI
GLVIGIEADPDRFDWQAWSRSRIIFCQWITQAIQGHPALRDIINIRITKILDKEKGTLETFVDRNWVEF
TGPAVWTDAILDYFNDGRFDFMSQSKGTIDYKNTGMESTKRVDVVLPITSFSPGVQGMSKGKCPDDPM
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PEIIIEAFSEMPLFVLAkadYFRLILLARGYISIDTFKATEWVPAAVDRTTIGGIGIADPRKD
WQTWYRSRRQFCQWITQSKPCHPILRVDVANITEALRMKSEGKLKKMKDKITVEFTGPANWTSDFRYP
FNNPYNFDGMRRDGNTSEIGYKHTGMVAQKAVGDVVLPLITSFSPGVRQMGAEAEPEHPMAFVKHNFQG
TLYTVLGLLSLRSRFN

MISFRRCILIAATFLAMYILHLSGSGSTRQPEGNSAGPDFKLSSVDSGLQFQOQIPLEDLARKPLRE
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PEIIIEAFSEMPLFVLAkadYFRLILLARGYISIDTFKATEWVPAAVDRTTIGGIGIADPRKD
WQTWYRSRRQFCQWITQSKPCHPILRVDVANITEALRMKSEGKLKKMKDKITVEFTGPANWTSDFRYP
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TLYTVLGLLSLRSRFN

MISFRRCILIAATFLAMYILHLSGSGSTRQPEGNSAGPDFKLSSVDSGLQFQOQIPLEDLARKPLRE
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PEIIIEAFSEMPLFVLAkadYFRLILLARGYISIDTFKATEWVPAAVDRTTIGGIGIADPRKD
WQTWYRSRRQFCQWITQSKPCHPILRVDVANITEALRMKSEGKLKKMKDKITVEFTGPANWTSDFRYP
FNNPYNFDGMRRDGNTSEIGYKHTGMVAQKAVGDVVLPLITSFSPGVRQMGAEAEPEHPMAFVKHNFQG
TLYTVLGLLSLRSRFN

MISFRRCILIAATFLAMYILHLSGSGSTRQPEGNSAGPDFKLSSVDSGLQFQOQIPLEDLARKPLRE
RLRYQFYDQSKFPFYIQWTKYTPASGKKEELRPEASFSTWEPHEGPVFHVQVITDDGLIYVVKYLAFF
PEIIIEAFSEMPLFVLAkadYFRLILLARGYISIDTFKATEWVPAAVDRTTIGGIGIADPRKD
WQTWYRSRRQFCQWITQSKPCHPILRVDVANITEALRMKSEGKLKKMKDKITVEFTGPANWTSDFRYP
FNNPYNFDGMRRDGNTSEIGYKHTGMVAQKAVGDVVLPLITSFSPGVRQMGAEAEPEHPMAFVKHNFQG
TLYTVLGLLSLRSRFN

MISFRRCILIAATFLAMYILHLSGSGSTRQPEGNSAGPDFKLSSVDSGLQFQOQIPLEDLARKPLRE
RLRYQFYDQSKFPFYIQWTKYTPASGKKEELRPEASFSTWEPHEGPVFHVQVITDDGLIYVVKYLAFF
PEIIIEAFSEMPLFVLAkadYFRLILLARGYISIDTFKATEWVPAAVDRTTIGGIGIADPRKD
WQTWYRSRRQFCQWITQSKPCHPILRVDVANITEALRMKSEGKLKKMKDKITVEFTGPANWTSDFRYP
FNNPYNFDGMRRDGNTSEIGYKHTGMVAQKAVGDVVLPLITSFSPGVRQMGAEAEPEHPMAFVKHNFQG
TLYTVLGLLSLRSRFN

MISFRRCILIAATFLAMYILHLSGSGSTRQPEGNSAGPDFKLSSVDSGLQFQOQIPLEDLARKPLRE
RLRYQFYDQSKFPFYIQWTKYTPASGKKEELRPEASFSTWEPHEGPVFHVQVITDDGLIYVVKYLAFF
PEIIIEAFSEMPLFVLAkadYFRLILLARGYISIDTFKATEWVPAAVDRTTIGGIGIADPRKD
WQTWYRSRRQFCQWITQSKPCHPILRVDVANITEALRMKSEGKLKKMKDKITVEFTGPANWTSDFRYP
FNNPYNFDGMRRDGNTSEIGYKHTGMVAQKAVGDVVLPLITSFSPGVRQMGAEAEPEHPMAFVKHNFQG
TLYTVLGLLSLRSRFN

MISFRRCILIAATFLAMYILHLSGSGSTRQPEGNSAGPDFKLSSVDSGLQFQOQIPLEDLARKPLRE
RLRYQFYDQSKFPFYIQWTKYTPASGKKEELRPEASFSTWEPHEGPVFHVQVITDDGLIYVVKYLAFF
PEIIIEAFSEMPLFVLAkadYFRLILLARGYISIDTFKATEWVPAAVDRTTIGGIGIADPRKD
WQTWYRSRRQFCQWITQSKPCHPILRVDVANITEALRMKSEGKLKKMKDKITVEFTGPANWTSDFRYP
FNNPYNFDGMRRDGNTSEIGYKHTGMVAQKAVGDVVLPLITSFSPGVRQMGAEAEPEHPMAFVKHNFQG
TLYTVLGLLSLRSRFN

MISFRRCILIAATFLAMYILHLSGSGSTRQPEGNSAGPDFKLSSVDSGLQFQOQIPLEDLARKPLRE
RLRYQFYDQSKFPFYIQWTKYTPASGKKEELRPEASFSTWEPHEGPVFHVQVITDDGLIYVVKYLAFF
PEIIIEAFSEMPLFVLAkadYFRLILLARGYISIDTFKATEWVPAAVDRTTIGGIGIADPRKD
WQTWYRSRRQFCQWITQSKPCHPILRVDVANITEALRMKSEGKLKKMKDKITVEFTGPANWTSDFRYP
FNNPYNFDGMRRDGNTSEIGYKHTGMVAQKAVGDVVLPLITSFSPGVRQMGAEAEPEHPMAFVKHNFQG
TLYTVLGLLSLRSRFN

MISFRRCILIAATFLAMYILHLSGSGSTRQPEGNSAGPDFKLSSVDSGLQFQOQIPLEDLARKPLRE
RLRYQFYDQSKFPFYIQWTKYTPASGKKEELRPEASFSTWEPHEGPVFHVQVITDDGLIYVVKYLAFF
PEIIIEAFSEMPLFVLAkadYFRLILLARGYISIDTFKATEWVPAAVDRTTIGGIGIADPRKD
WQTWYRSRRQFCQWITQSKPCHPILRVDVANITEALRMKSEGKLKKMKDKITVEFTGPANWTSDFRYP
FNNPYNFDGMRRDGNTSEIGYKHTGMVAQKAVGDVVLPLITSFSPGVRQMGAEAEPEHPMAFVKHNFQG
TLYTVLGLLSLRSRFN

MISFRRCILIAATFLAMYILHLSGSGSTRQPEGNSAGPDFKLSSVDSGLQFQOQIPLEDLARKPLRE
RLRYQFYDQSKFPFYIQWTKYTPASGKKEELRPEASFSTWEPHEGPVFHVQVITDDGLIYVVKYLAFF
PEIIIEAFSEMPLFVLAkadYFRLILLARGYISIDTFKATEWVPAAVDRTTIGGIGIADPRKD
WQTWYRSRRQFCQWITQSKPCHPILRVDVANITEALRMKSEGKLKKMKDKITVEFTGPANWTSDFRYP
FNNPYNFDGMRRDGNTSEIGYKHTGMVAQKAVGDVVLPLITSFSPGVRQMGAEAEPEHPMAFVKHNFQG
TLYTVLGLLSLRSRFN
>gi|76008510|gb|ABA30873.1| mannosyltransferase-like protein [Coccidioides posadasii]
TRQPEGNSAPEDKLLSSVDGLSQUALPQPOQIFFILEDARKPLRERLRYQFYQFYDKFQFAYIQWTQKYTYPASG
KFEELRPEASWTEHHPGFVHVQVITDGGILYIVKYLAFFFEIIIEAFESMPLVFLKADYFRYLLLLARG
GIYSDDTFLAKFATEWVPFAVDRRTTIQLIGIEADPDKRDWQTWYSSRIQFCWVTIQSKPQHIKLRDVV
ANITEEALRMKSEGKLKNKMKDTI韦ETGPAWVTSDISIFYRNPNPYDGMGRDGNDSTEGYKHTFGMV
AQKAVGVDVILPISTSFPGVQQMEAEPEHPMAFVKHHNFQG

>gi|258570739|ref|XP_002544173.1| conserved hypothetical protein [Uncinocarpus reesi 1704]
MISFRRCVILAVSLSPTFILHLSHTSNGAHQPFKPSDLLEKEPSGLDKRERQLFQPIMALQKPLRER
LRYQFPYDQLSKFQPAYIQWTQKYAPSSGHEELRPEASWTEAHPGFHVQVVTDAFGMIYIVKLYAAPF
EIIEAFESMPLVFKLADFRRYLLLLARGGIYDTDDTDLWKAIRWPKSVDSISLGIVIEAHPREDW
QDNAWYQSDQFRQGTVQSQLPHGKPLFQVANDVNEALRMKSHGKLKNKMKDTI韦ETGPAWVTDAVFY
NNPAYFDGVNDIHHTREDYFYTVGIGAQQVVGVDVILPISTSFPGVQQMEAEPEHPMAFVKHHNFQVA
SEEFIFRDSDRSLSERPLPSSFDLSDGSLSTFREYFVFVFPFAQRRKMWSTKEF

>gi|115398736|ref|XP_001214957.1| hypothetical protein ATEG_05779 [Aspergillus terreus H12624]
MITFRKSLIAAVFFVTIFIFLLRSSHSSPSYEPSTYPEATVENQDQAGTEERSFPQKDAVQQLPQPSA
PLRERLNYTFYDNLTKFQPAQIWTQTVKTIPASLMFTADLRAASWTEHLPFHEVQFPQADTTQHRLKI
YLSVPVEVEADFADLPLLVLADFRRYLLLLARGGIYQDDTALPKLASEWLPDTSVBDISTGIVIEADP
DRPDWHDWYRSRIQFCWTVQSSKHPQHIKFRDIVYITEEALRMKKGILKMKMKDTI韦ETGPAWATDA
VRYFYNNPFEYFNIQDNSKNITYEDFTNQEEYRKGVDVILPISTSFPGVQQMEAEPEHPMAFVKHDFD
GSKWDDPSL

>gi|145237860|ref|XP_001391577.1| alpha-1,6-mannosyltransferase subunit (Och1) [Aspergillus niger CBS 513.88]
MLTRFRKSLIALLITFITVYLRSSHSTASSLPSPDTSSAGHLNYQDYDGHADNERKGGTRDVTQQQLPTFP
PSAPLRLRDLRYHFFDYDEAKFAPIAQWTQKYAPSSMFSESLRDFESSWELHPGFHVQFVDPDRQHLI
KYLYGAVDPVDVFEAYDAMLPLVLADFRRYLLLARGGIYDDTTALPKASPDLWPAELDLATVAVVIGI
ADPDORPDWDWYARRQFCWTVQSKPGHIPMIRDIVYITEETLRMKKGILKMKMKDTKMEYTGPAW
TDAFYRFYNDPEYFNIQDNSKLSTNYEDFTQGQEGYKKGVDVILPISTSFPGVQQMEAEPEHPMAFVKH
HFEGTWDDPSL

>gi|225554546|gb|EEH02843.1| alpha 1,6 mannosyltransferase [Ajellomyces capsulatus G186A8]
MITIFRSLIALLITFITVYLRSSHSTASSLPSPDTSSAGHLNYQDYDGHADNERKGGTRDVTQQQLPTFP
PSAPLRLRDLRYHFFDYDEAKFAPIAQWTQKYAPSSMFSESLRDFESSWELHPGFHVQFVDPDRQHLI
KYLYGAVDPVDVFEAYDAMLPLVLADFRRYLLLARGGIYDDTTALPKASPDLWPAELDLATVAVVIGI
ADPDORPDWDWYARRQFCWTVQSKPGHIPMIRDIVYITEETLRMKKGILKMKMKDTKMEYTGPAW
TDAFYRFYNDPEYFNIQDNSKLSTNYEDFTQGQEGYKKGVDVILPISTSFPGVQQMEAEPEHPMAFVKH
HFEGTWDDPSL

GDPMAFVLHQFEGSWKGEAAEDEDEDKELHQNAADIIAQSALDIIRRTKNGQRRKTE
GDPMAFVLHQFEGGL

>gi|226286994|gb|EEH42507.1| initiation-specific alpha-1,6-
mannosyltransferase [Paracoccidioides brasilienis Pb18]
MITFRRSLVAVLFLSCLIVLLHSHSNPSHTSSKPISPQKIQQKEKSPSSALEQNPPTPPLHSTD
ASKPLRDRLRYQFYDPYDLPSKFAYIQWTKNTPASDFLEHFRASEASWTTTHPSFIHQVITD6MDPLL
RYLYAPFPEIETYRSLPVLKADFDRFYLILLARGGYSDITYALKPADVWLPQHLQRHISTYVGLVQIE
ADTDREDWAQWFSRRLQFCQWTTIQAKPSGFPLVRDVTATIDALRMKAAGILKGMMDKSVMTGPAWV
TAIFRYPYPNLYFETARPPTGRNLTAQDFSGYKEHRQLGDVVLPLITSFSPPGIMGADDDPMAFVK
HQSNGSWKAEAGRPAPVILEE

>gi|159126485|gb|EDP51601.1| alpha-1,6-mannosyltransferase subunit (Och1), putative [Aspergillus fumigatus A1163]
MLTFRKSLAIAVVLITFVULLRSAHSSPSAEPAVLNTETTAHDTSQAADHEHTDQKDIQQQPLKPPTA
PLRERLYQVFYLDERFLNFAYIQWTNKTPASMWFSEDLRPAEASWTELHPFGVEVHPIDQDDTRHLVYL
YGVSVEVFYAEYDMPLVahkanzyiyillisrgyisidtyahkpadwhlgdelaratvfgiveaDAP
DRPDPNHWSYRRIQFCQWTIQAQPSPHELRLDIVIEALMRKGKGLKEGKMDKTEVFTGPAWINTA
VFRYFNNPEYPSIEPSGHTHNYTEDFNTQGKYK>VGVDDVVLPLITSFSPPGMAGAGDDDDPMAVHFD
SGWKTDPAL

>gi|119479597|ref|XP_001259827.1| alpha-1,6-mannosyltransferase subunit (Och1), putative [Neosartorya fischeri NRRL 181]
MLTFRKSLAIAVVLITFVULLRSAHSSPSAEPAVLNTETTAHDTSQAADHEHTDQKDIQQQPLKPPTA
PLRERLYQVFYLDERFLNFAYIQWTNKTPASMWFSEDLRPAEASWTELHPFGVEVHPIDQDDTRHLVYL
YGVSVEVFYAEYDMPLVahkanzyiyillisrgyisidtyahkpadwhlgdelaratvfgiveaDAP
DRPDPNHWSYRRIQFCQWTIQAQPSPHELRLDIVIEALMRKGKGLKEGKMDKTEVFTGPAWINTA
VFRYFNNPEYPSIEPSGHTHNYTEDFNTQGKYK>VGVDDVVLPLITSFSPPGMAGAGDDDDPMAVHFD
SGWKTDPAL

>gi|70998096|ref|XP_753779.1| alpha-1,6-mannosyltransferase subunit (Och1) [Aspergillus fumigatus Af293]
MLTFRKSLAIAVVLITFVULLRSAHSSPSAEPAVLNTETTAHDTSQAADHEHTDQKDIQQQPLKPPTA
PLRERLYQVFYLDERFLNFAYIQWTNKTPASMWFSEDLRPAEASWTELHPFGVEVHPIDQDDTRHLVYL
YGVSVEVFYAEYDMPLVahkanzyiyillisrgyisidtyahkpadwhlgdelaratvfgiveaDAP
DRPDPNHWSYRRIQFCQWTIQAQPSPHELRLDIVIEALMRKGKGLKEGKMDKTEVFTGPAWINTA
VFRYFNNPEYPSIEPSGHTHNYTEDFNTQGKYK>VGVDDVVLPLITSFSPPGMAGAGDDDDPMAVHFD
SGWKTDPAL

>gi|225683379|gb|EEH21663.1| initiation-specific alpha-1,6-
mannosyltransferase [Paracoccidioides brasilienis Pb03]
MITFRRSLVAVLFLLSCLIVLLHSHSNPSHTSSKPISPQKIQQKEKSPSSALEQNPPTPPLHSTD
ASKPLRDRLRYQFYDPYDLPSKFAYIQWTKNTPASDFLEHFRASEASWTTTHPSFIHQVITD6MDPLL
RYLYAPFPEIIETYRSLPLPVKADFFRYLILLARGGIYSDIDTYVLKPADQWLPQHINRSTVGKLVVGIE
ADTDREDWAQWFSRRLQFCQWTIQAQPKGHPLVRDVFVTITEDALRMKAAAGILLIQORMDKSVEFTGPANW
TDAIFQYFNNPLYFETARPPTGRNLTAQDFSGKEHRQLGDVVLPITSFSFGIGHMGAGDADPPMAFV
HQFSGWKAEEAERPAPVILEE

>gi|296816695|ref|XP_002848684.1| alpha 1,6 mannosyltransferase
[Arthroderma otae CBS 113480]
MISFRKCVFAFLISGFGFLHALAHAKPVSANVEVETTHQRSEQPLRTSTAKLESEPEPEPKVEDPEP
EPEPEFPLRLGRSLKHYFPFGYVATARPFGYIQMTWKYTPASGDFDPTLRPLAESWTELHPGFHVQVDDESA
LYFLKLYASPEIVEAYSLPLPVKADFFRYLILLARGGIYSDIDTYVLKPADQWLPQHINRSTVGLVVGIE
ADTDREDWAQWFSRRLQFCQWTIQAQPKGHPLVRDVFVTITEDALRMKAAAGILLIQORMDKSVEFTGPANW
TDAIFQYFNNPLYFETARPPTGRNLTAQDFSGKEHRQLGDVVLPITSFSFGIGHMGAGDADPPMAFV
HQFSGWKAEEAERPAPVILEE

>gi|121713164|ref|XP_001274193.1| alpha -1,6 mannosyltransferase subunit
(Och1), putative [Aspergillus clavatus NRRL 1]
MLTFRKSLIAAVILIFVVLRLSAHSGPHSTSVIPKPDFAVFDVQAASNEHSTEQKKEAQQAPLKPPT
APLDRDLRQYFFDVLNPENQIPWQMTWYTPSWVFEDLRPAEASWTTLHPGFHVQVDDESA
LYGLSPEFVEAYDSMLPVLKADFFRYLILLARGGIYSDIDTYVLKPADQWLPQHINRSTVGLVVGIE
ADTDREDWAQWFSRRLQFCQWTIQAQPKGHPLVRDVFVTITEDALRMKAAAGILLIQORMDKSVEFTGPANW
TDAIFQYFNNPLYFETARPPTGRNLTAQDFSGKEHRQLGDVVLPITSFSFGIGHMGAGDADPPMAFV
HQFSGWKAEEAERPAPVILEE

>gi|212534636|ref|XP_002147474.1| alpha -1,6 mannosyltransferase subunit
(Och1), putative [Penicillium marneffei ATCC 18224]
MKDLESRYYPARTSTMARRIAIIFALVSIILLFRFSASTYIDSPLILLIPQQARGATGRFESQLTIGD
TITYPTLLEKLRHQYPDLDISKFPAPWQTWKSYSVESSSFQPQHRYLESTWTDNPEFVHEVISDESARHL
VQYLGSMPEFVEAASNLPAPVAKLDDRFLRYLILLARGGIYSDIDTYEALKFVVDLPDMSPSVGLVIGI
EADPDRPDWQYRWSIRIQFCWTTIQAQKPHGPHVLRDVTIVEDALRMKGGKILMTSLKDSVMETFGPAV
WTDAIFQYFNNPDYFISESTGNVTVEQFTLTIQKKGDVALLPLITSFSFGVDQMGAKLGPADDPLAFVSHRF
FGSWNPQNERIY

>gi|317150328|ref|XP_001823952.2| alpha -1,6 mannosyltransferase subunit
(Och1) [Aspergillus oryzae RIB40]
MLTYRKSLIAALIFVFLRSLSASSAPPPAPAHPDEVAYNTVEEHLGQKEIAIPQOQLKPSFSAPRERELYRFHLD
DKKFPAVYMTWKTYPDPWSFQGLEAASEWTELHPFHVQVPDDQTQYL
IKLYLSLPDDVFEAYELSLPLPVKLDRFYLILLARGGIYSDIDTYVLKPADQWLPQHINRSTVGLVVGIE
ADTDREDWAQWFSRRLQFCQWTIQAQPKGHPLVRDVFVTITEDALRMKAAAGILLIQORMDKSVEFTGPANW
TDAIFQYFNNPDYFNYPNDPSESNHNTYEFDNSQDKWRKGDVVLPITSFSFGVMQAGDYDDPMAFVK
HDFGSWKTDPSE

>gi|242790710|ref|XP_002481608.1| alpha -1,6 mannosyltransferase subunit
(Och1), putative [Talaromyces stipitatus ATCC 10500]
MKEIESQYPAKNPTMAPIKAIIFAFALVGIIFLFRPFSTSDAAYRGFSQKPLRIPQQARGATG
HDFGSWKTDPSE
QSQLPVGETAYPTLEELKRLRYQYPYDFDSDKFPAYIQWTQKDSVSNNSNFDPDFHLRPLEASWTENRFEPFHEVI
SDEAAADHLVQYLYGSPVEPEVAYNSLPAPVLFKADLFLRYLLARGGIYSIDTEALKPAIDLWLPDMPS
SVGLVIGIEADPRDPDHWEYRSRRISQFCQWUTIQAKPGHPVLDRDVVATIVEDTLRMRKKGVLTSKMDKSI
VEFTGPAVWMTDVFYRNFPDFYLSLEGTRNVTYEDEFTGLTRQKKVGVDDVLPITSFSGVQGMAQGPEPED
PLAFVSHLFEGSKPFESERIS

>gi|67537092|ref|XP_662320.1| hypothetical protein AN4716.2 [Aspergillus
nidulans FGSC A4]
MLTFRKALIVAAIFITLILFLRSSHGSPESAAAAASPEDIAYEISQETKGKTSSESSDSTQQQPLKP
ATAPLRRLRLRYHFPYNEAKFPAYIQWTQKDYTPSVWSQDALLRPEASWTENPSFVQHVQIPDRTLHHL
KYLYGSIPEVLEAFNMPVDMKADFFRYLLARGGVYSDITDALKPVDWLPSLDSLSTIFVVGIE
ADPRDPDHWEYRSRRISQFCQWUTIQSKPGHPILPDIYITIQEALRMKAGILKGGKMDKTIQETFGPAW
TDAVFRYFNDPEYFPEVGTTRNITYEDFNTQVEYQKVDVVLPLITSFSGPVQMAGAGYDDMAFVKH
NFSGTWFKTDPSL

>gi|238499555|ref|XP_002381012.1| alpha-1,6-mannosyltransferase subunit
(Och1), putative [Aspergillus flavus NRRL3357]
MLTYRKSLIAALFLITFVVLLRSS
HSASSPSPPAPAHLPDEVAYNTEEELHSQGKKEAIPOQQPLKP
SPSAPLRRLRLRYHFPYNEAKFPAYIQWTQKDYTPSVWSQDALLRPEASWTENPSFVQHVQIPDRTLHHL
KYLYGSIPEVLEAFNMPVDMKADFFRYLLARGGVYSDITDALKPVDWLPSLDSLSTIFVVGIE
ADPRDPDHWEYRSRRISQFCQWUTIQSKPGHPILPDIYITIQEALRMKAGILKGGKMDKTIQETFGPAW
TDAVFRYFNDPEYFPEVGTTRNITYEDFNTQVEYQKVDVVLPLITSFSGPVQMAGAGYDDMAFVKH
NFSGTWFKTDPSL

>gi|295666458|ref|XP_002793779.1| initiation-specific alpha-1,6-
mannosyltransferase [Paracoccidioides brasilienis Pb01]
MITFRRSLVAALFLLLCILLLHSNSASHKPLKPSHTSSKPIPNRKQNQEQEKSPSSAEILQPPKSLPSPDL
ARKTLRDLRLYQFPIDSLPSKFPAYIQWTQKNTASPDPFYEHRFTNEASWTIAHPFHFIQVITDIDDMDPLL
RYLYAPFPEEIETYRLSVLPVLFKADFFRYLLARGGIYSIDTDFVYLLKAPEWLRPHTRNTSGVITLGIVGIE
ADTDREDWAQWFSRQLFCQWUTIQAKPGHPVLDRDVVATTEDALRMKEAGILRGMMDKSMEETFPGAVW
TDAAFRYFNPNFLEYQIFQPNGHLTAHDFSGVKHERQQLGVDVVLPLITSFSGPVGHMGAGADDDMAFVKH
HHFSGKWSEAGRPVPVLE

>gi|255949602|ref|XP_002565568.1| Pc22g16540 [Penicillium chrysogenum
Wisconsin 54-1255]
MITPRKVLSSLCLCVTVFLILIOTFRDFEVPAGPGPYRFVKISQEEEDEVTSNLAKTTPQGRAQLPLGASP
TAPLRRLRYQFYENKFPAYIQWTQKDYTPSFNDEELRGEASETMHFGFHEVIPDETQKHLLIK
YLYGSIPEVFEAYDPSLAVMKADFFRYLLARGGIYSIDTDLARPAHTLWEELDSTRIGVIEA
DFPRDDOWHWDYRSRRISQFCQWUTLVKSGPHILRDMWAYTEHALRMRKAGILKVGKMDKTIQETFGPAW
DSVFRYFNPNAPFNIQPGDKKNITYEDFSHQHTTHKKDGVDVVLPITSFSGPVGQMGADDDMAFVKH
NFSGTWFKTDPSL

>gi|302505858|ref|XP_003014886.1| glycosyl transferase, putative
[Arthroderma benhamiae CBS 112371]
VTFSFGVDFQGAKDTPPLAFVKHLFSWSKFPEDERMNQNQPQVNQ

>gi|302420661|ref|XP_003008161.1| initiation-specific alpha-1,6-mannosyltransferase [Verticillium albo-atrum VaMs.102]
MLTPRRLARGGGCLSHPNAAAALVFKADFFYLLILLARGGYSIDTAYAIRSLDAWDVPDPRTTSIGLV
IGIEADPDRPBDWHDWPRESSQFQWTTIQAKGPVFLVRLDIVARITEETLRRVKAQGLSKVMMDNVVEFTGF
AVWDTDVFYLNDLARYFMDAHSKPIDWNRTFGMEQSKKVGDVVLPITFSFPGVQQMGAKDDDDPMAFV
KHEFEGLTKEAFAHRHNEGND

>gi|190348963|gb|EDK41524.2| hypothetical protein PGUG_05622 [Meyerozyma guilliermondii ATCC 6260]
MKQKQVKIAVVVLAVFYLLYTFHTSTTPKHAATFHEHSNTAKKLREHERHTWNKLYGFTQSFNNRAQLP
KITTQRQLAQFQQPYEPEKPFERNIQWTKVDLLENEFSPSKYRFQQTWLVDNPTKYHVPDEACEELV
NQLSFSVPDPVARAYIMPKSLKADFFYRLIFARGGYSIDTVSLKPIDVWMSANETLYGQPNNPGLV
VGIEADPDRDWDAYARRQRFCWTTIQAKKGHMRLREIVALITEITLERKEKNQILHKVLGKDEGGDVMV
WTGPGIWTDSVFAYMNALQSPENFAAKKHDEIVTKFTGKMPIAIDDVLVLPITSFSPDVNQMAGSK
SKDPMAYAKHFMGAGSKFPEDER

>gi|146413262|ref|XP_001482602.1| hypothetical protein PGUG_05622 [Meyerozyma guilliermondii ATCC 6260]
MKQKQVKIAVVVLAVFYLLYTFHTSTTPKHAATFHEHSNTAKKLREHERHTWNKLYGFTQSFNNRAQLP
KITTQRQLAQFQQPYEPEKPFERNIQWTKVDLLENEFSPSKYRFQQTWLVDNPTKYHVPDEACEELV
NQLSFSVPDPVARAYIMPKSLKADFFYRLIFARGGYSIDTVSLKPIDVWMSANETLYGQPNNPGLV
VGIEADPDRDWDAYARRQRFCWTTIQAKKGHMRLREIVALITEITLERKEKNQILHKVLGKDEGGDVMV
WTGPGIWTDSVFAYMNALQSPENFAAKKHDEIVTKFTGKMPIAIDDVLVLPITSFSPDVNQMAGSK
SKDPMAYAKHFMGAGSKFPEDER

>gi|33331887|gb|AAQ11191.1| putative mannosyltransferase [Pichia angusta]
MKINTVKRTLLLSTVSIAVVLVILRRLSDDSTFDHQLVSVPVSIDSQQDDQGRGGRGGRSGGGLSNGKS
NSEELIARKNLTEKLNNEQDVOLRKLEKQDQRLEKQLSELRRPNPEATLRELAYMPFDQNRKFFA
YIQWSKYGLNANDNFPGYRKEEGEQWALKKNPGFHVLEFNNTDNSNAIVHYLHMEFVKAYELLPHVLK
MDFFRYLFAGGYYADVTLPLQFIPNWIENVPDSEPINGMIIIGIQSDPDFTDRKQFARRLQFANWV
QAKPQHILREVIATEETLKRAGEKTLDFFDAASDFSIMEWTGAGVVTDFVFYFNDVYLSGISFKVTW
KDFKTLDVKLVSDVLVLPIFTCFSPGIGKMGAMDADHPLAVKHYSEHRFQ

>gi|294656033|ref|XP_002770210.1| DEHA2C13508p [Debaryomyces hansenii CBS767]
MIKKRIEVIVKSVIALYLIYLFCSRSFVPYKSKTFETHNTLDSIKELEHTNWKLKYGFHASNARNLPN
EHATLRQQLSFQPYEPAKPFPQKNIQWTKWPIEPFHNFPPKKYKKYQATWDDKNGKYKHYVPEDEQELQI
NQLYATVDPVARAYIMPKSLKADFFYLLILAIKGLYTDTISIKPIDTWSMTVEDSGPSMAGLV
VGIEADPREDWDAYARRQRFCWTTIQAKRGHMRLREISRTIDTTLTREKKQQLNKILGKDAGGDIRM
WTPGIPWTVFVEYMNINISQPFPDNFKKTKYDDINVSVEVTGTMIPVAIQVDLVLPITFSFSPDVQMGAKD
TSHPMAYAHFMGAGSKFPEDERKIINT
>gi|15086372|ref|XP_001383155.2| membrane-bound alpha-1,6-
mannosyltransferase Initiation-specific [Scheffersomyces stipitis CBS 6054]
MINKRVRGLGLAVLVLVLYLLYGFFRTFSVPFGSSHRTESSSSKQLQLKRELEIHSNWAKTGLNFQPNNKARLP
IETTVRQQLSOFQFYESKSFPFQKNIQWTQKVALDDSSFLKRYRTQSTWDKDPNFYKHYVVAADVCEELI
SQLYSTVDPVARAYNMPKSLKADFFRILYILARYARGVYSDITDVLKPIKDWSNTTLVYDPINPGLV
VGIEADPDRPDAEWYARRIQFCQWITIQAKGGHMLRELIAEITEKTLTRARGQLKKVLGDKEGDIM
WGGPGIWTDINAFNYMNNVLQPSEPQKZKYKDEITWIKFMTGESAIADDVLVLPLITSFSPDVCGMAGK
MNDDMPYAKHMFSGWSKHDIEITKPNGV

>gi|17978673|gb|AAL49987.1| putative mannosyltransferase [Candida albicans]
MLQLREPQVMHKLKLAVLGVIVVIFTTYFIISSSSPTSTHKTEYNPSKLQAKELELSNWKELGLNFQP
PNKKYSLPDESLRQLLSQFYPREKSFKNWIPWTKVGVDEKSFKRKLYQQQTWDKDPFDKYHYVVP
DKQCDDLLIEQLYSQVDPVAKAYRIMPKSIKADFFRYLLIFARGVYTDITDVLKPEWDIESNSEMILE
KKNRSLVVGIEADPDRPDWADNYARRIQFCQWITIQSKRGHMLREIAKIDITIDITLTTRHKGQLKKVLGK
NEGGDIMNWTPGPIFTDITFYEYMNNIQLSPVFKNKKWATIIDWKLFTGMEQPIAADDDVFLPLITSFSP
DVQNGAGKDSHPAYAKHMFSGWSKDDGMPEMQ

>gi|68478727|ref|XP_716632.1| mannosyltransferase Ochlp [Candida albicans sc5314]
MLQLREPQVMHKLKLAVLGVIVVIFTTYFIISSSSPTSTHKTEYNPSKLQAKELELSNWKELGLNFQP
PNKKYSLPDESLRQLLSQFYPREKSFKNWIPWTKVGVDEKSFKRKLYQQQTWDKDPFDKYHYVVP
DKQCDDLLIEQLYSQVDPVAKAYRIMPKSIKADFFRYLLIFARGVYTDITDVLKPEWDIESNSEMILE
KKNRSLVVGIEADPDRPDWADNYARRIQFCQWITIQSKRGHMLREIAKIDITIDITLTTRHKGQLKKVLGK
NEGGDIMNWTPGPIFTDITFYEYMNNIQLSPVFKNKKWATIIDWKLFTGMEQPIAADDDVFLPLITSFSP
DVQNGAGKDSHPAYAKHMFSGWSKDDGMPEMQ

>gi|241953569|ref|XP_002419506.1| initiation-specific alpha-1,6-
mannosyltransferase, putative; mannosyltransferase of the cis-Golgi
apparatus, putative [Candida dubliniensis CD36]
MLQLREPQVMHHRKLKLAVLGVIVVIFTTYFIISSLSSTTHKTGEYNPSKLQAKELELSNWKELGLNFQP
PNKKYSLPDESLRQLLSQFYPREKSFKNWIPWTKVGVDEKSFKRKLYQQQTWDKDPFDKYHYVVP
DKQCDDLLIEQLYSQVDPVAKAYRIMPKSIKADFFRYLLIFARGVYTDITDVLKPEWDIESNSEMILE
KKNRSLVVGIEADPDRPDWADNYARRIQFCQWITIQSKRGHMLREIAKIDITIDITLTTRHKGQLKKVLGK
NEGGDIMNWTPGPIFTDITFYEYMNNIQLSPVFKNKKWATIIDWKLFTGMEQPIAADDDVFLPLITSFSP
DVQNGAGKDSHPAYAKHMFSGWSKDDGMPEMQ

>gi|255726062|ref|XP_002547957.1| hypothetical protein CTRG_02254 [Candida tropicalis MYA-3404]
MRSDKDLRLVLVLGFTITSVTIFYLSTTFGSNSNRTSINISKLQFIRELESNPNWFKQLQKLFQDE
SIIIQQQLSKRFYDNPLSFKPNWQWTKVGLNDTFSRPFYKFSQSWDKKNDKHYIIPDECNELIE
QLYEIEIPDVSKAYKIMPKSIKADFFRILIFARGVYTDITDVLKPEWDIESNNEKYLNNKNNLLVY
GIEADPDRPDWADNYARRIQFCQWITIQSKSHGMLRELITKITEITLNQVENKQLKVLGDKEGDIM
WNGFTDITFYMNAILQSEPITGKYKWEITIDWKLFTGMEQPIAADDDVFLPLITSFSPDVQNGMSK
SKDPLAYAKHMFSGWSKDDGMPEMED
>gi|260947804|ref|XP_002618199.1| hypothetical protein CLUG_01658
[Clavispora lusitaniae ATCC 42720]
MVGPRRKLRIGIISIVLLFLLYTFVSTVSPRHVTKESTNTKLKARELEKNSNWKKTGLNFHTNKAA
LPALSTLRRQLSFSQFYFPEYQSGFQNIQWTWKVLNDETFPPTYGQETWAEALSNGYKHHVIPDDEC
MVKQLFASVPDVAQAWSLMFKNLKADFFRYLYARGVYSDIDTKCLKPIDTWSDKNQTTYIGIDNHAG
LVQIEADPDRPDWAEYARYRIFCQFWTIQAKKGMRLRELAKITLQRKNGKLEKKMGGSGG
MNWTPGPGIWTDVFEMYMNILQPGQENFERKCYDEIVTWKLFSGMEMPMAEDVLILPITSFPDVPNQMG
AQSHELAYAKHLFSGSWKDNGFSE

>gi|254564971|ref|XP_002489596.1| Mannosyltransferase of the cis-Golgi
apparatus [Pichia pastoris GS115]
MAKADGSLLLYYNHPFPRRYFYMAFAVSVICVLYGPSQQLSSPKIDYPILRLSDLKLTLEAPSLS
GTVEDNLRRQLEFHFPYRSPEFPQHQIWTWKVSPSDSFKPSKRDGLWQLGQSPNYDFVFIPDDAAW
LIHHYERVYEVLEALESPLKLAIDFFRYLYILFARGGYLEMDTMLKPIESWLTFNETIGVVMNAG
LVQIEADPDRPDWAEYARYRIFCQFWQIQKSRPHALRELIRVSTTLREKSGYNMVEKGRGDSV
MDWTPGPGIFTDTLDFYTMTNVNTGHSQGIGAGSAAYNALSLEERDALSARMGEMLEKVPQKYG
AQVVWEQFTNFLRPKLDIDILPITSFPGIGHSGADLNNHILAYIRHTEGSWKD

>gi|97973864|dbj|BAE94371.1| alpha-1,6-mannosyltransferase [Pichia minuta
var. minuta]
MNYHDLYDDSKRQLMKRKAEMNKKLVLVVVILTMYVSRLSAGSTQKESIFPGTMKESELEVNFK
FGMSLQKRPNELPAASATLREKLSFYFDFEKPVNPQIWWTWVDINDKSFPRHRFRFQETWPLQNS
YHLPIDSVDEFMRSFLANFEPVEIAAYMNMLPNKLADFFRYLVIFARGYSDTIDCLKPVNWFATF
EQTVISHYLKNGKTSQLPEDPSTRKPIGLTIGIEADPDRPDWHEYARIQFCQFWTIQAKKGMPLR
ELIIREIQTFQKEAMNLKKEVSDGQMSDFQISDDPRTTQGMLVQVYHLPEVQKQEDQIRRLDE
FQKREKLRQFQAIWQPTQQEYKDQFQTEQKRMHEHDSFQIQ

>gi|45825280|gb|AAS77488.1| Och1p [Pichia angusta]
MVYFLNFSITNPYVLPKRARRLYMATNRMLVVVVVLIVWVQVWTSVSPGTRDLAQVDKIAAEILNLH
TGFAQLRHLLRNPSARSATLREKLTFTYFPEKPVNFQIWQWTWKVLDELFNFFKQRYRFQETFQEM
VYHLIPSVIEFDVASYLANPVEVVAYQLPKNNKADFFRYLVHARGYSDMDTVCPLIKPDWAE
DRLHIAADKADLSQIDPEARPTVPVGLVIGIEADPDRPDWHEFPSRRTQFWTIQAIKGHPLRELI
RIVETFRKQHQMGVLRKEVGKHDAGIMQWTGPGIGFTDTLDFYLVNVSDGGLKLDGTVGSV
LKYRKHGYKLKTEINKNLEPLHSEQDINRSLTMNKDFPKIMGDVMVLPITSFSPVGQMSHSPDHPLAF
VMFQGWSKPD

>gi|45198953|ref|NP_985982.1| AFR435Wp [Ashbya gossypii ATCC 10895]
MMARRIKASHRYYVFFAPFLSLGLRLFRLFQVSNKLDLQTVLSLPTARLSLDLSTKRDMG
FQMFEEVVRVKQOCQRIIRLDRREKALEKCLQLKRPASHSTLRQQALLAVHEYGTFTKFPAYWQ
TMYSGAD
DRMDNRRLDQYEQKWWKNGFVHEIVVNDTDALKALVHYLASYAPFEVAENLPSKDRADFFKLYL
LARGGVYADTNPHQPVNPWIPENVSPTKIGMIIGIENDAKRDRWSSSFIRRLQFATWQVIQAKPGHP
IREV
VAKITEETLRLKDGDLSNLNRDQDNMSWTGSGAWTVFTVYMNDYVQSGILQKITWKFHFKIRPKLV
LVFPEVSFNPQADSDTDKQTLNSALHFATHTMKSWKNQQKLRGYK

>gi|68478501|ref|XP_716752.1| potential mannosyltransferase Hoc1p [Candida albicans SC5314]
MTKYTTTSARRGFRHIPIIIILILGLLVFTFFGFSFSGKAKDIQEVISNLNPKDNLGLSNLILTEN
KNQGELLKIEKLHNYKDYEQEERRKIEQNEILQIEKILKSSPPQHASIREKLFVYFDADVRFPAY
IWQTKHGNDEKDEFDEKREGERQWAYKNGFVHELFNDDTAHTMIKLYRQIPFVINAFAELPEVI
DDFRYRILIFAKGGVYADIDTFPIQIPNPWIENPVSPLDIGILVGVESDSNPSNWRSESVRRLQL
SKPGHPILREIAIQVLYTKELEPENLGNPNNAKAIAMKWTGSGRFTDVVPQYLNDYILSSYESINWQ
HLHNLEVPKLGDVLVLPVRSFSADEKKNLSFVKHYGDKIYKQV

>gi|241956410|ref|XP_002420925.1| alpha-1,6-mannosyltransferase, putative; glycosyltransferase, putative [Candida dubliniensis CD36]
MSKTYTTSARRGFRHIPIIIILILGLLVFTFFGFSFSGKAKDIQEVISNLNPKDNLGLSNLILTEN
KNQGELLKIEKLHNYKDYEQEERRKIEQNEILQIEKILKSSPPQHASIREKLFVYFDADVRFPAY
IWQTKHGNDEKDEFDEKREGERQWAYKNGFVHELFNDDTAHTMIKLYRQIPFVINAFAELPEVI
DDFRYRILIFAKGGVYADIDTFPIQIPNPWIENPVSPLDIGILVGVESDSNPSNWRSESVRRLQL
SKPGHPILREIAIQVLYTKELEPENLGNPNNAKAIAMKWTGSGRFTDVVPQYLNDYILSSYESINWQ
HLHNLEVPKLGDVLVLPVRSFSADEKKNLSFVKHYGDKIYKQV

>gi|260940971|ref|XP_002615325.1| hypothetical protein CLUG_04207 [Clavispora lusitaniae ATCC 4279]
MNQTSMGTRKRPIVFTVVLVAILLIVRLSTASQSRDALAIDNFGISVFSSVSENDDKDQDEFLKKE
EFVTSQDELRALERQNYLIEQVRQLIPQSMWIKLRFKLJLMFVYPDVKKAFPYQIWQKSWK
HLNQILFQAVSNPQVRPQISAILRILEQIKVQYAEYSLPEVI
DDFRYRILIFAKGGVYADIDTFPIQIPNPWIENPVSPLDIGILVGVESDSNPSNWRSESVRRLQL
SKPGHPILREIAIQVLYTKELEPENLGNPNNAKAIAMKWTGSGRFTDVVPQYLNDYILSSYESINWQ
HLHNLEVPKLGDVLVLPVRSFSADEKKNLSFVKHYGDKIYKQV

>gi|50312081|ref|XP_456072.1| hypothetical protein [Kluyveromyces lactis NRRL Y-1140]
MLGLPKISRTYTVLVIYLLYLSVQWNTAVNKHYNFSNGTRVLSFLSTARDVHLNLKNLTDAGTSNNGD
LMLDRVQLASQFPYSDRVPQKVWQTWDPSKSQVSSSISKCIDWNKHFSASEEPPYQPLYITDO
MLPLEQLYGGVQPIFAQMLPLPKLAIKDFDRLILYARGIYISIMDFTFPLKPSWSTFSQYFSSL
QPQYRNSLDNLETLESEPGFVGIEADPDRSDWAEMYARRIQFCQWTTIQSKSHPLRELITNITATTL
ESVANVKSIPPLDAEVLDIADDYNVNRMDKFKFKNYKIQKKTAKNTGDTDDNMTGWTPGIFSDFI
FYQKNVIQKNNLDLIFNDNLNYISGKSHDTTMRFYKDIVKMLQNDPFLFWGFSFSLMETPILVDIML
PITSFSGPIRTGAKEDNNEMAFVHKIFEGSWKD

>gi|50287115|ref|XP_445987.1| hypothetical protein [Candida glabrata CBS 138]
MTMARKRSNRIFIIIIFLQFLAILLALCKLSNARASDIQKLQNLKPEITQINSVANSNQKADADV
MFAKAKLADIKLKQEEQSKQFERQKRKLRLNMKQ1STDATRIKLYNFEYDGSRSIPALFWQTWW
SQTPEEVHLVHKATQWKEQPMQFVHELITPEMMALVHHYSSIVPEVEAYKLLPNDLIRIDFVKLYL
GGTYADIDTSPLQIPNWFETVEPDSIDGLTGIEHDAQAVDRWSHYRVRQLFGTWIQAKGPQHPVLRV
VAQIVHEVLNKKDSTVNVRLNL5VMKFTGSAFTDAINTMLNDFKSLRDKTVRDFTKITSPKLVS
DILVFPEFSNFVENFDKEDLRAQFFISHKGEKFVKAPKVAS

>gi|6321400|ref|NP_011477.1| Och1p [Saccharomyces cerevisiae S288c]
MSRKLHSLATRKSATIVTUVTTILYSSLTTFHLSNKRLLSQFYPKDDFQTLLPTTTHSQRDINLKKQITV
NKKKNQLHNLQDLQFADFYDSQPQIPFQVWQTWKGADDKNFPSFTFTQKTWGSGSYSDPSDYQSLSSDD
SIIPFRLENYAPVPIVIQAFKLMPGNLKADFLRLYLFFARGGIYSDMDDMLKPDWSQNKSNNLNI
IDLNMPIFYNYSNKPSLSSDEISHEQGPLLVIGIEADPDRDDSEWYARRIQFCQWTIQAKGPILRELIL
NITATTASVQNPQVSEMIDTFREEDYNVSHYARDEQDVASKALVSSFGDSYSVQIAMMPLPV
LADFFRFLVLLAKGGIYSDIATPLKHHNNWIPREYKRNNLIVGIEADPDRDDWNYARRVQFCWQ
ITAAAAGPHILMEVRLIVDTEKWLDHSDKLKLSKNGESVMETGPGIWTDAMIDYLNWQYGPSVENITNL
EPYLVGLVILILPITAFSPGVGHMSKPSNPDMPAYQHVFFAGSWKDD

>gi|19115764|ref|NP_594852.1| alpha-1,6-mannosyltransferase Och1
[Schizosaccharomyces pombe 972h]
MLRLRLRSIVIGAASISIIILHNHSIEGMEDLTEISMLEDYTPEAAKNDYVQGQQESEELYDDPSYEIE
EEDDPDEAFLSLERELHLESLELDEENNYYKLHLRSFQSLOQMFDINEAVHMIIVKDTYFEEVYHA
DIDKLMQSDKDFDRESDKYMRVNHPSYSHDLEDDQESLKLVSSFGDSYSVQIAMMPLPV
KADFFRFLVLLAKGGIYSDIATPLKHHNNWIPREYKRNNLIVGIEADPDRDDWNYARRVQFCWQ
ITAAAAGPHILMEVRLIVDTEKWLDHSDKLKLSKNGESVMETGPGIWTDAMIDYLNWQYGPSVENITNL
EPYLVGLVILILPITAFSPGVGHMSKPSNPDMPAYQHVFFAGSWKDD

>gi|156841296|ref|XP_001644022.1| hypothetical protein Kpol_1026p11
[Vanderwaltozyma polyspora DSM 70294]
MVKLVPFLRNFLASKRGGILVIISLNLISFFSIQIQSNSSKQQFDKDKDLPLTTSHSDSINLKKDVLNNS
NDIDDLRLKLSFAPFYPPKPIFPRRIWTQKVTDSSEKFSDKFRTYQKEWTSKSDYSLIPPDKLVPFL
LNYAEVPTQVQAEAFKAMPNMLIKADFFRFLYLLFFARGGIYSMDTIPMLDLEWPSVDLNNKIKIKALNSNP
QVYKNLKMSPFYEPVQLLVIYIEADPDRDDWNDYARRVQFCWQITAAAGPHILMVRLIVEKDPWEKDL
IVTDAMIDYLNWQYGPSVENITNL
EPYLVGLDILILPITAFSPGVGHMSKPSNPDMPAYQHVFFAGSWKDD

>gi|6322535|ref|NP_012609.1| Hoc1p [Saccharomyces cerevisiae S288c]
MAKTTKRSFSSRFLMAIFIALISLFRFYLHNSNATDLQKILQLNPKEISINSANIQSSSDLVL
QHFSLAQEIRHQQEQAQFQDKQRLKIKQDQLKTQPPATLRERIAMTFYDHSKVPAFWQITWSN
DEGPERVQDIKGMESEKNPGFAHEVLNVHQLSVAILVHHYFYSISPEILEYAEPSILKIDEFKYLILLVLV
GGVYADIDTFVQVIPNPWIEELSPSDEGGLVGEDEAQARDWRTKYIRLQFTIQAGPKHPVLRLEI
ISRIETTLQRRKDDQLNVNLRNLNSMWTGSGLWTDTIFTYFNDFMSRGVEKVTWKLHNQLQPKKLL
SDLVVFPLKFSFNPQNIDNDDPHKKKFYFITHLASQFWKNTPKVEQK

>gi|259147537|emb|CAY80788.1| Hoc1p [Saccharomyces cerevisiae EC1118]
MAKTTKRSFSSRFLMAIFIALISLFRFYLHNSNATDLQKILQLNPKEISINSANIQSSSDLVL
QHFSLAQEIRHQQEQAQFQDKQRLKIKQDQLKTQPPATLRERIAMTFYDHSKVPAFWQITWSN
DEGPERVQDIKGMESEKNPGFAHEVLNVHQLSVAILVHHYFYSISPEILEYAEPSILKIDEFKYLILLVLV
>gi|255719976|ref|XP_002556268.1| KLTH0H09064p [Lachancea thermotolerans]  
MLKFNKERTILSVVFVLSQSSLQWLEKQFFRGKTLIPTATATEGQTINLKTFKPMNGCAPIAELRSGQLQFYPDQSPFPVRPIRWTQKWTPFNSMPQSMGQKDFSGRWASAASEPQLYDLYTLVPHDMLPLHHNLYGAVFPQIIQAFFMDSLNMLKADDFRYLVLYARGGIYSDMTDFPLKALDEWPSIKTSSLKPEVPIKYGLDASSPPTQEPFGIAGIAEADPRDDMDWNYARRVQFCWQTIOQSKFQHLLRELINITIALTASTNVTNIKAPFIDDEGKHDDYFVNAIGINRAKRLDFSKPPFTQKKTAKNTDGTDMTNWGTGPGVSFAIFNYLNNIQTNPDIMIINNNLKANMEDGSGTGRKGYRKLITTGEMSSATFVEFSSLIEEPFIVDDVMILPITSFSPGVMNMGSKPEDDAMAVKHMFGTWWKNPFPSSQGQRQE

>gi|45198983|ref|NP_986012.1| AFR465Cp [Ashbya gossypii ATCC 10985]  
MFKVTRRVARAVLAVAILGLAVELSTWNRRRLHVFWRQAEGFLEVPSSYHVQELNMRERFGAWERRDRLRSQLLAQFPRDPAPPIRPRWQTVKVRHPRHSQAQFEFHRSLSDAWEASKDAEGYEYFLVGEDMLPLRNLYGGPVQVLQAFAPLIMRADDREYLLYARGGIYSDIDTEPLVQITAQFDVSQAALQKFKNKHYHYGTLSVFGESSLTPLGAIEADORPDPMGWAYKALQAKQHTLRINLTGTLTSHVARRTGYARLPFVTFDTDELEDVINNYRHRKHHDAAYPTKEKTAKNTDTEMNWTGPGEFSDVVDYNYLNNLITNDDEVYIVDLLEKNPEGTGRCIPAIATSTKRFAAIETALSKSKPKFLWDSFSMLMQTPLALIDDVVPLPTSFSPGVHMQAGEPDPHMAVFVHHHEGWSKQGGFKPQDDAGEGDKSEHAEK

>gi|15685958|ref|XP_001645868.1| hypothetical protein Kpol_1054p58 [Vanderwaltozyma polyspora DSM 70294]  
MAKTRRADPKTLISVGVITVISVIHKFISSSHSHIDENIIENLNKEINSNSVSRFKSGSDDKVLSRLEELISDVLTEQGRADELVKQRKLEKVLKAEQMPESGLRKLAYLFYENTARKFPAFYQWTVKFPNSKAPQRANKARNGWNEKNSFVHELFDMDVISAMVQHYSSIPDVVEAYSMKSDLKIDFFKYLILLARGGVSYSDYDTDIQIPINWEPEFINPKDVGIIVGYEVDLSSGSLPKNYERLQFATWQIQCKPGHIQEREIQAQETELLQFKRKECSLEVNYKEIINWTGSGVWTDIIFSFYNNLYQSLGDKVVWKFHNLLVPKLMVDSLVLPLPFSPFNAPONKLDGHFDRTYHYCTHDKESWKKVPTL

>gi|254571473|ref|XP_002492846.1| Alpha-1,6-mannosyltransferase involved in cell wall mannans biosynthesis [Pichia pastoris GS115]  
MQQQLFRKVWLTGLVLTI6VILIISSKSTATLQKLKVNANIPQDVINYNRSKVRTDELAISKDEIQTKYLSKQDDRISKLAEARADLLEQVRFLNRPAGSSLREKLAYLFYENFKFPAFYIQWTQKWYGDLNDDRFEKFKGGETQWASKNPGFVHELINDDTSQVFIHHLYINVFEPKVAYELLPNIILKDMFFRYLVYAKGGVADVDTMPQLQFPVWPVENPSKSIQIGQNDANPNWKKITELIAKTEDTLQRAESNSLEADISEEGGLSDKLSIMQWGTGTFDAIFTYFDNYIQSSITYTKVTKEFSKLKRPKLVSVDLVPLPIISFSGAGSGKSTELDNLAPFQVHYFUELHRNFLNH

>gi|254585655|ref|XP_002498395.1| ZYRO0G09284p [Zygosaccharomyces rouxii]  
MIKPLNKFRELKDRKTQIQFPGILLLYSLVAFHSLSKKYAYIKATFAPISNALVPTLTHVQSLNLKERVDRKLDLREQLMVAPFYEPEKPIRPIRQWTQKWKEKDSLFPSRFYQDTEAATLQGFEYMLVPDDNI  
EAFLQNYGEPLVLKAFKEMPNKILKADFLRLYLLYARGGIYSDMTDFPLKPLKNKPSVDREKLFKTRKFPYKGFKSEEVAQNEREPGVIGVEADPDRTDSDWFVARRIQFCWQITIONSKPGHPVLRLELINITATTLYSVVVSSVKKTAELIDKTHKNDYNVNYRDKRARDSHYDHEETFDTKDNVGDSSIMWTGPGEFSDMVM
QYNLNNLIQNNNDVLLLNGHHTPVDDPAAEAAQDENVSTKFFYRKIESSLLYLNHRHIPPEFFLITEPI
TYDLMILPITSFSDPVQMMNAKGMDDEMELVHDFRFGSWKGDAGHD

>gi|213410162|ref|XP_002175851.1| initiation-specific alpha-1,6-
mannosyltransferase [Schizosaccharomyces japonicus yFS275]
MIKRGRYLLVVAIILYVSSTISDFDGLNEPFAEKNYADSIERDQLKQSRVVQTATATSASATAP
TASYFTPEKNNGFDETDAADVENDDEGGYKDFPENFDVYKDFIAEMLQMLPSLKKNQAIIFKTI
WQLTKSDFSEDYGLYLWIKANPQHVHLUTDDLAEELRHGFDESKSLRMYEFFKLPEVMKADFFR
YLSSLLAEGGYTIDDIPTSPIKIQDKWPAKFKDSIGMMGIEADFOPDQNDYWYARRVQFCQWMTIAAVPN
HPILWMDAQRTIKETIKNKLDTRYGNMVETGPGVWTDADMVLWNYGFPWSKVNVTNQLQPMVLVG
DVLVPITAFSPVGHMNSKFDHPSALVRHFSWSKATTHENKGH

>gi|323336961|gb|EGA78218.1| Hoclp [Saccharomyces cerevisiae Vin13]
MTFYDHPKFAIWQTWTSNDEGPEQVDIKGMWSKNPGFAEHVLNHDIVNALVHNYFYISEPEITLEY
EALPSLLKIDFFKYLIIIWVGHGYADTDFVQFIPNPEWPEELSDGLGIVGVEEADQRADWTRYIR
RLQFGTWIIQAKGPHELQILNVLRNLDNISWGSTGLWTITFYNDFFMR
SGVREKVTWKLHFLNQFPKLSSDLVLPFKFSNCNPQIDNDDPHKKFYFITHLASQFWKNTFKVEQK

>gi|50284827|ref|XP_444841.1| hypothetical protein [Candida glabrata CBS 138]
MSKWQKRHLAVLAVLVLVFLRVHGNRLGLGQFRAKPNYLVNGDTSLGLNLKFLNSDLRSDLASKA
FPYDPSPKPIPPRQWQTVKVPIDSPEFPGDLKQYVRWSENSEYVEDEIYSEQENQNYLPDDQIESI
LSEFYGEVIPIVAQYKLPSNLKIDFLRLLLARYGQYSDIDTFPKLDFRDWFMSQOQDMKKLLKAKKII
PYKNFDTMINGMNFPIDGPLDGIEAPDLRDPNWWDYRRQPPQFCQTQSKPHGVPNLRELINIATTY
SSAKTPAHLKSLIDNSADEKDFINIYRHRFFDKEYDNSQHKRKNVGGTDITMNWTGPGIFSDSILNYLT
NIRKRNLLILNSNLDSQKKEIPSSAASSQEEEEEEDLYSTSKKFYNEISTSSLSSAKMPEWFFSLITE
PILLDDDVMILPITSFSDPVMQMQAESQHELALVKHMFGSWKWEADGH

>gi|323309089|gb|EGA62317.1| Ochlp [Saccharomyces cerevisiae FostersO]
MPGNLKDACLFRYLLLFARGGYSMDTMLLKPIDSWPSQNKSWSNLNNIDLNPKLYSNKPSLSSLSDI
EHSQPGVGIEAADPDRDDWSEYAWRIQQFCQWTQIAKPGHPIRELINLINITATLASSQNNPVPSEMID
PREFEDYNVNYRNKRHIDETYKSLKNNKNDGDSMNWTGPGIFSDIIEFMYNNVLRNLDIINLPN
LNKNDDEGESASATTPAKDVNDSTSKSTRFYKKEISELQSSNSMPFEWFFSLKEPVIVDDVMVLPITSF
SPDVGQMQGAQSSDDKMFAVKHMFGSWKBEADNAGKH

>gi|33321202|gb|AAQ06408.1| Ocr1p [Pichia angusta]
MSKASYPFGINSSTSSTKPFKKLSIFVGLDLLLGLFLKFSTSWINTEDKIVSEYLNIFNKLPKFRGAND
YDAAVTAERALKFYPFDYNSARRIEKSITQWQMVKVPSTDPPFHELNQWKNNPETYKYNLTDDEILEIL
IRKRFKDTPVEFLAVEFMLEPK进行了不正确的翻译
IVENVANYHMTIRQQFQWTFPKAKAPHLKILAKIVETTTQFQQKNDLQAYFKDFKVGCAVSDIMV
WTGPFVWVTDTIAYLHLSIPSPITVIDHDRQDIAGEYLPETGEGRIDVSWRFFAHLRAPVIMDDVYIPRA
SFRREDCKENCGKYCVHHHFGGSWKNNGKIEKFGMEYEGEDPNEEELRKNDSKRVDVIPGEKSDVAD
VKKLAKRCAAYTPY
>gi|310801436|gb|EFQ36329.1| glycosyltransferase sugar-binding region containing DXD domain-containing protein [Glomerella graminicola M1.001] MSSPIMSPLSRSPIGSTKANLFRNRVPTQLRRCIALYACICIIILVANLDLFGVSPKPVHLSKRNRH SSISNRAAFFKKIQWSKVFAPFAPAEEQVLTARTWTDKNGYRVEVLTDNNMDNEYVVEEYHPGDFDRPDI VDYMNVNTIIAKDLLRMYMIMYAEYGVYADVIDVDELPRVSPRIPQYAEADIDLIVIGVEIDQPFKNHP ILGKKSMSFCQWTFSRPGPHVMKLMKVENIAIWLSDVAKSOGVTISEVKLDFEVISGTGSPSAFTAKAVLDMVSARSHSGPITWDHTFHDIDESKVSVNILVRDVESFAAGQGHSDSGNHSRGALVKHHYHASNWFPSRHRFRHPIFGEVERCNWNIECMWQDQDIAAFNSLPPEEQQEIEKHDNLQRLQTQAEQKAEEQKDAANMAL HIPPQQPLQALAQQPALQELRLK

>gi|302891711|ref|XP_003044737.1| glycosyltransferase family 32 [Nectria haematococca mpVI 77-13-4] MASGPRRLPSLRIIPSTVEWTYIVDSINTREIEKMGSLGSPTLAASSATKFRNKVPAMQCRPLPILY IGCVCLLLLILNFDVFYAIPLSLDWERGDHPTPPRSFDFQKIQWTKLDPLNFEDRDVTSRTWVA KNFGPRYVLTDANEMAFEEHFGPGLNRDPVFKYRSLNHAIKADLLRMYMQVYAGVYADIVEAL RPNFPEPERYYDKIDMIVGEVIDQPFIDHPILGKKSKSCFQWTIIISKPHQVPMLKLIENMAWLLNV TKEQKVPLGEVQLDFQVISGTGSPAFTKALEEQMNQRSSGFPKVTDWDMFHMDESKIVGRVVLVDEAF CAGGGHDSGHNDSRGALVKHHYHASNWFSKHFRYSHPVYQVEDCWNNAECVRLWENDVAEYNKLEE KKTKLDEAKKGGLE
> Sclerotinia sclerotiorum 1980
MVFLTRTHNPSTNDLLQDSIPKSEEKDVGSKSASTNAVPPAPKRQASQALLQDSLRAPLREKLAYQFFYDEKFPAIYIWQTWKYPASGDFGEAFPAEASEWSEKHFGVHEVITDSVAVHLLKLYASLPVELDAYNSSLPLVKADFFRLILLARGGIGSIDIDTHALKPAEFLPESVPREAIGLVIIGEADPD
RFPDAEWYRSRIRQFCQWTIQSKPHGHPVLREIVANITEQTLSMKESGTLKHFNDKVNVFETGPAWLWTDTIFDFLNDERYFDMTTSKGKNITWRFQGQSAKVGDVVLVLPITSFSHPGQMQSKYDPPMAFVKHEFGETW

> Botryotinia fuckeliana B05.10
MLTFRRALIAAFFTLTMFLVTSSHQPTNDLLQDTPAPSKSJEADLTKPFGSSASTNAVPSTPPKNTQVSTQLLDQSLRAPHREKLAYQFFYDVFPAIYIWQTWKYPASGDFGEAFPAEASEWSEKHFGVHEVITDSVAVHLLKLYASLPVELDAYNSSLPLVKADFFRLILLARGGIGSIDIDTHALKPAEFLPESVPREAIGLVIIGEADPD
RFPDAEWYRSRIRQFCQWTIQSKPHGHPVLREIVANITEQTLSMKESGTLKHFNDKVNVFETGPAWLWTDTIFDFLNDERYFDMTTSKGKNITWRFQGQSAKVGDVVLVLPITSFSHPGQMQSKYDPPMAFVKHEFGETW

> Neurospora crassa OR74A
MLTFRRALVAAAFLLTFLYLISSSSSNNPSTTTTVPDKTADVETAKHASTTAVDKSSSAGANAAQNPQGQRPIQDMSTLYEKLAQFFYDESKFFPAYIWQTWKWTAPAEENFREQEASWSEKHFGVHEVITDKVAVHLRLLYAASVEPVEAYNALSPLPLVKADFFRLILLARGGIGSIDIDTHALKPAEFLPESVPREAIGLVIIGEADPD
RFPDAEWYRSRIRQFCQWTIQSKPHGHPVLREIVANITEQTLSMKESGTLKHFNDKVNVFETGPAWLWTDTIFDFLNDERYFDMTTSKGKNITWRFQGQSAKVGDVVLVLPITSFSHPGQMQSKYDPPMAFVKHEFGETW

> Glomerella graminicola 1_001
MLTRRAIAAFFTTILVFYLISSSSSNNPSTTTTVPDKTADVETAKHASTTAVDKSSSAGANAAQNPQGQRPIQDMSTLYEKLAQFFYDESKFFPAYIWQTWKWTAPAEENFREQEASWSEKHFGVHEVITDKVAVHLRLLYAASVEPVEAYNALSPLPLVKADFFRLILLARGGIGSIDIDTHALKPAEFLPESVPREAIGLVIIGEADPD
RFPDAEWYRSRIRQFCQWTIQSKPHGHPVLREIVANITEQTLSMKESGTLKHFNDKVNVFETGPAWLWTDTIFDFLNDERYFDMTTSKGKNITWRFQGQSAKVGDVVLVLPITSFSHPGQMQSKYDPPMAFVKHEFGETW

> Sordaria macrospora
MLTFRRALVAAAFLLTLYLISSSSSNNPSTTTTVPDKTADVETAKHASTTAVDKSSSAGANAAQNPQGQRPIQDMSTLYEKLAQFFYDESKFFPAYIWQTWKWTAPAEENFREQEASWSEKHFGVHEVITDKVAVHLRLLYAASVEPVEAYNALSPLPLVKADFFRLILLARGGIGSIDIDTHALKPAEFLPESVPREAIGLVIIGEADPD
RFPDAEWYRSRIRQFCQWTIQSKPHGHPVLREIVANITEQTLSMKESGTLKHFNDKVNVFETGPAWLWTDTIFDFLNDERYFDMTTSKGKNITWRFQGQSAKVGDVVLVLPITSFSHPGQMQSKYDPPMAFVKHEFGETW

1_Glomerella graminicola M1.001
MLTFRRAIAAFFTLVFYLISSSSHHTSPADEVKVPVAVSNHDTAGSKKSLSTFADTDEIKPSAPKAAQKPMMDMSKMSLYLDKALAYQFYDYDFPRTFRAYIPAYIWQTWKWTAPAEENFREQEASWSEKHFGVHEVITDKVAVHLRLYASVEPVEAYNALSPLPLVKADFFRLILLARGGIGSIDIDTHALKPAEFLPESVPREAIGLVIIGEADPD
RFPDAEWYRSRIRQFCQWTIQSKPHGHPVLREIVANITEQTLSMKESGTLKHFNDKVNVFETGPAWLWTDTIFDFLNDERYFDMTTSKGKNITWRFQGQSAKVGDVVLVLPITSFSHPGQMQSKYDPPMAFVKHEFGETWKPESERHIGENK

> Neurospora crassa OR74A
MLTFRRAIAAFFTLVFYLISSSSHHTSPADEVKVPVAVSNHDTAGSKKSLSTFADTDEIKPSAPKAAQKPMMDMSKMSLYLDKALAYQFYDYDFPRTFRAYIPAYIWQTWKWTAPAEENFREQEASWSEKHFGVHEVITDKVAVHLRLYASVEPVEAYNALSPLPLVKADFFRLILLARGGIGSIDIDTHALKPAEFLPESVPREAIGLVIIGEADPD
RFPDAEWYRSRIRQFCQWTIQSKPHGHPVLREIVANITEQTLSMKESGTLKHFNDKVNVFETGPAWLWTDTIFDFLNDERYFDMTTSKGKNITWRFQGQSAKVGDVVLVLPITSFSHPGQMQSKYDPPMAFVKHEFGETW

> Sordaria macrospora
MLTFRRAIAAFFTLVFYLISSSSHHTSPADEVKVPVAVSNHDTAGSKKSLSTFADTDEIKPSAPKAAQKPMMDMSKMSLYLDKALAYQFYDYDFPRTFRAYIPAYIWQTWKWTAPAEENFREQEASWSEKHFGVHEVITDKVAVHLRLYASVEPVEAYNALSPLPLVKADFFRLILLARGGIGSIDIDTHALKPAEFLPESVPREAIGLVIIGEADPD
RFPDAEWYRSRIRQFCQWTIQSKPHGHPVLREIVANITEQTLSMKESGTLKHFNDKVNVFETGPAWLWTDTIFDFLNDERYFDMTTSKGKNITWRFQGQSAKVGDVVLVLPITSFSHPGQMQSKYDPPMAFVKHEFGETW
Chaetomium globosum CBS 148.51
MLSFRRALVAAAFITLFFTRSTPSASSLEAYEPVKQTQSDAGSHPKENSQTTKTPPSDEIPHAGVNRPRPFPVEQOGQIFQDSKMTNLKDQAYQFPFYDVEKFPAYIQWTKWTPHAEFQPQFRDPFQREATWQFGHPEHVITDQVAVHLLRLLYGSAVPEVLAEAYALPLPVKADFFRYLILLARGGIYSDTDFAIRSAYDWDIPAVEPPHTVLVIGAEAPDPRDPKWQDSRMISRIQFCWQIASKGHPVLRVETVQTVNRTRKRDGDLKELNNSVIEFTGPALWTDKTFYFNDARFDFMSKTFIDWKNFTGMECSSRVGDVIVLPITSFSPGVPQVMGAKDYDDPMAFVKHDYEMGSMQFPAIS

Podospora anserina S mat+
MLTFRRALVAAVFFIAVFLFTRITTSSSPAASAAAAYEPVKQPGAEAEARQKSQTASSRQKADQDVQVQNGHGQGPKQPKQMDPMNLVDKGYQYPDYDTEKFPAYIQWTKWTPAPDGEAEFREQTAEWTEQHPFGHIHEVITDQVAVHLLRLLYGSAVPEVLAEAYALPLPVKADFFRYLILLARGGIYSDTDFAIRSALEVWPESVPRDQIGLVLVIGAEAPDPRDPKWQDSRMISRIQFCWQIASKGHPVLRVETVQTVNRTRKRDGDLKELNNSVIEFTGPALWTDKTFYFNDARFDFMSKTFIDWKNFTGMECSSRVGDVIVLPITSFSPGVPQVMGAKDYDDPMAFVKHDYEMGSMQFPAIS

Magnaporthe oryzae 70-15
MLTFKRALIAAAAFFLTYLTTTPAAPATSTTPPLAKGAQDGTQTEKVKANASSAKISNTASSASTSSQDGKSNRLAPEGKKPMDPMNLVDKGYQYPDYDTEKFPAYIQWTKWTPAPDGEAEFREQTAEWTEQHPFGHIHEVITDQVAVHLLRLLYGSAVPEVLAEAYALPLPVKADFFRYLILLARGGIYSDTDFAIRSALEVWPESVPRDQIGLVLVIGAEAPDPRDPKWQDSRMISRIQFCWQIASKGHPVLRVETVQTVNRTRKRDGDLKELNNSVIEFTGPALWTDKTFYFNDARFDFMSKTFIDWKNFTGMECSSRVGDVIVLPITSFSPGVPQVMGAKDYDDPMAFVKHDYEMGSMQFPAIS

Phaeosphaeria nodorum SN15
MLTFKRALIAAAAFFLTYLTTTPAAPATSTTPPLAKGAQDGTQTEKVKANASSAKISNTASSASTSSQDGKSNRLAPEGKKPMDPMNLVDKGYQYPDYDTEKFPAYIQWTKWTPAPDGEAEFREQTAEWTEQHPFGHIHEVITDQVAVHLLRLLYGSAVPEVLAEAYALPLPVKADFFRYLILLARGGIYSDTDFAIRSALEVWPESVPRDQIGLVLVIGAEAPDPRDPKWQDSRMISRIQFCWQIASKGHPVLRVETVQTVNRTRKRDGDLKELNNSVIEFTGPALWTDKTFYFNDARFDFMSKTFIDWKNFTGMECSSRVGDVIVLPITSFSPGVPQVMGAKDYDDPMAFVKHDYEMGSMQFPAIS

Pyrenophora tritici-repentis Pt-1C-BFP
MLTFKRALVSLACLTLFFLKFHKHQQSSAPNFRANENEMPRPTAWKPEFEKQEEAQAKAKTAKAEKAENFTEpeePPEPUPKPPKQTPQISMDVLTLQKPLREQLEYPHYDPYDSTKCPAYIQWTKWTPADGEAEFREQTAEWTEQHPFGHIHEVITDQVAVHLLRLLYGSAVPEVLAEAYALPLPVKADFFRYLILLARGGIYSDTDFAIRSALEVWPESVPRDQIGLVLVIGAEAPDPRDPKWQDSRMISRIQFCWQIASKGHPVLRVETVQTVNRTRKRDGDLKELNNSVIEFTGPALWTDKTFYFNDARFDFMSKTFIDWKNFTGMECSSRVGDVIVLPITSFSPGVPQVMGAKDYDDPMAFVKHDYEMGSMQFPAIS

Leptosphaeria maculans
MLTFKRALVSLACLTLFFLKFHKHQQSSAPNFRANENEMPRPTAWKPEFEKQEEAQAKAKTAKAEKAENFTEpeePPEPUPKPPKQTPQISMDVLTLQKPLREQLEYPHYDPYDSTKCPAYIQWTKWTPADGEAEFREQTAEWTEQHPFGHIHEVITDQVAVHLLRLLYGSAVPEVLAEAYALPLPVKADFFRYLILLARGGIYSDTDFAIRSALEVWPESVPRDQIGLVLVIGAEAPDPRDPKWQDSRMISRIQFCWQIASKGHPVLRVETVQTVNRTRKRDGDLKELNNSVIEFTGPALWTDKTFYFNDARFDFMSKTFIDWKNFTGMECSSRVGDVIVLPITSFSPGVPQVMGAKDYDDPMAFVKHDYEMGSMQFPAIS
> Nectria haematococca mpVI 77-13

MINSRRAIVAAVFVLTVLRLSHTQPSVPAKDAVADTDDKAAAPASNFDFVQFQPPPEPKEMEVEKRRTRPRIDMSGMSTYKELAYAYPYDVFKFAVIPAYQWTQKPSQDPQDKQHQASWTVEHPGFHVHITDDVAVNLRLPILYATVEPIDYASRLPLVIRAFFYRNLILYARGGTYTIDTTAQISSSSVKLPEKIPREITGLVIGIEADPRFDFWQAWSRIRIQFCWQTIQSKPGHVPLRDIIAIIKNSTLALKRDGKLSSFQGKHVVDLTGPAVWTDTIMDYLNDGRFFDMRKSQGKIDWHNFTGMETSKRVGDVIVLPTSFSPGVQMGAEKPDPMAFVKHDFEGTWKFESEPHERHMGEOQKEDDGEQPOQQQQQQAPAQ

> Metarhizium acridum CQMq 102

MANSSRALAAAVLFLPLFLRLRSHSSHPSAQSSSDTARAHAAASPNNAVDSTRAVPFQPKMDMRSMTDYDKLAYAYPYDSEKFAVIPAYQWTQKPSQDPQDKQHQASWTVEHPGFHVHITDDVAVNLRLPILYATVEPIDYASRLPLVIRAFFYRNLILYARGGTYTIDTTAQISSSSVKLPEKIPREITGLVIGIEADPRFDFWQAWSRIRIQFCWQTIQSKPGHVPLRDIIAIIKNSTLALKRDGKLSSFQGKHVVDLTGPAVWTDTIMDYLNDGRFFDMKKSQGKIDWHNFTGMEMSKRVGDVIVLPTSFSPGVQMGAEKPDPMAFVKHDFEGMW

> Coccidioides posadasii str. Silveira

MISFRRCLIIAAATFLAPYLHLSGSQGSGGRQPEEESNAGPESDKLSSDGLQFQPQQPQIPLEDLARKPLRELRYQPQFYDQKFPAYQWTQKTYPASGKEELPEASWEHHPGFHVQVITDDGLIYVVYLYAAMEIEFESMPLVRLKADYFRLILLARQYGDIYIDTFFALKPATEWVPAVDRTTIIIGLIGIIEADEPRDKWQTWYSSRIQFCWQTIQSKPGHPIRLRADVANITEEALRMSEGLKKNKMDKITEVEFTGFAWTSDFIFRYFNNPYNFDMGRRDDGNTSEISYKYHTGFTGMVAQKAVGDVIVLPTSFSPGVQMGAAEEPHEMPAFVKHNFQAKWTKFA

> Coccidioides immittis

MISFRRCLIIAAATFLAPYLHLSGSQGSGGRQPEEESNAGPESDKLSSDGLQFQPQQPQIPLEDLARKPLRELRYQPQFYDQKFPAYQWTQKTYPASGKEELPEASWEHHPGFHVQVITDDGLIYVVYLYAAMEIEFESMPLVRLKADYFRLILLARQYGDIYIDTFFALKPATEWVPAVDRTTIIIGLIGIIEADEPRDKWQTWYSSRIQFCWQTIQSKPGHPIRLRADVANITEEALRMSEGLKKNKMDKITEVEFTGFAWTSDFIFRYFNNPYNFDMGRRDDGNTSEISYKYHTGFTGMVAQKAVGDVIVLPTSFSPGVQMGAAEEPHEMPAFVKHNFQAKWTKFA

> Ajellomyces dermatitidis SLH14081

MITFRSSSAAATIFLLRTSFFFLSSHSNNSTRPAITSTVRAEHSTTPPPDPAPQTPTATNQQAHTQSGSSSGISKPAPQHVHPNNPPDNLRRYLRPRLYQPYQYPDPQSKFAPAYQWTQKTYPASGEFEKELRPPLESWTWEHPTVHIVTDDEAAPLRRYLAAPEEILDAYRSLPLVLRADFYYRLLILLARQYGDIYIDTALQPÆEDWLFYVNRKSTGLIGIIEALDREDWYQWSRQVQFCWQTIQSKPGHPIRLADVATITEETLRMQRAGILTPKSMDSIVETFTGPAWTVDAPFNPNEEFARPHGRNTAMDGFSTQTVQRLGDVDVIPITSFSPGVQMGAAKPDPMAFVKHEFEGTWKPEQERFIGHGIAVTTTTTATPEIQN

> Ajellomyces capsulatus H88

MITFRSSSAAATLLLASSYFLLSSRSSCHRCLCHPHPLPIRKAQQSKVAASSESPSTHSNELPPTSA
>Uncinocarpus reesii 1704
MISFRRCVILAVSSLSTPFILHSLSTGSHNHQQPKSDLLEEKEPSSGLKSRQRLQFQIPELDLQRKLRERLRLRYQFYPFLNKKFPAYIWQTWKYAPSSMFFSESLSDFESSWSHELHPGHEVVFDPDTQRHLLKLFGVAPFVEAFDALLPVLKADFDFRYYLLILLARGGIYSDDTTLAPASEWPLSTSLDSSTIVVGGIADDFWDIPHDWSRIRRIOFCQWIFIQKPGHIPIFRDIVAYITEELMRRKAKILKGGKMDKITEVEFTGPAAWTDVFYRYPNFPYINFNSDNSKEYETFDNQEEYRKVGDDVIVLPITSFSPGVQMGAKDVPDAFAVHKHFDFGSGKDDPSL

>Aspergillus terreus NIH2624
MITFRKSLIAAFVYTFIFIPFLRSLSSHSSPSYELSFLYTTPEENVQGDADGTEERSFGKQKDAQQPLQPSASAFLERLRYYFYPFLNKKFPAYIWQTWKYAPSSMFFSESLSDFESSWSHELHPGHEVVFDPDTQRHLLKLFGVAPFVEAFDALLPVLKADFDFRYYLLILLARGGIYSDDTTLAPASEWPLSTSLDSSTIVVGGIADDFWDIPHDWSRIRRIOFCQWIFIQKPGHIPIFRDIVAYITEELMRRKAKILKGGKMDKITEVEFTGPAAWTDVFYRYPNFPYINFNSDNSKEYETFDNQEEYRKVGDDVIVLPITSFSPGVQMGAKDVPDAFAVHKHFDFGSGKDDPSL

>Aspergillus niger CBS 513.88
MLTFRKSLAAALITFIVYLYRSLTASSLPSPDSTSSAHGLYNQSYGHDGANDERKGGTRDTVQQLPLTPPSAPLQDRLRLYHFPYLDEAKFFAFIWIQWTWKYAPSSMFFSESLSDFESSWSHELHPGHEVVFDPDTQRHLLKLFGVAPFVEAFDALLPVLKADFDFRYYLLILLARGGIYSDDTTLAPASEWPLSTSLDSSTIVVGGIADDFWDIPHDWSRIRRIOFCQWIFIQKPGHIPIFRDIVAYITEELMRRKAKILKGGKMDKITEVEFTGPAAWTDVFYRYPNFPYINFNSDNSKEYETFDNQEEYRKVGDDVIVLPITSFSPGVQMGAGDVDPRPAFAVHKHFDFGSGKDDPSL

>2_Ajellomyces capsulatus G186AR
MITFRRSSIAATLLLALASFYFLISRSRSICFRISPRLQPLPIRKAQQKSQAASSSESEPSTHENVESLPPPSAPA
SLSPEDLARKPLRLRRLYQFYPDVAFHAKFPAYIWQTWKYTPASGKFGDKFRPMEASWTELHSFHPHEVIDTD
DDADPLLLRYAFFPEELDAYSLRLPVLKADFDFRYYLLILLARGGIYSDDTTLAPASEWPLSTSLDSSTIVVGGIADDFWDIPHDWSRIRRIOFCQWIFIQKPGHIPIFRDIVAYITEELMRRKAKILKGGKMDKITEVEFTGPAAWTDVFYRYPNFPYINFNSDNSKEYETFDNQEEYRKVGDDVIVLPITSFSPGVQMGAGDVDPRPAFAVHKHFDFGSGKDDPSL

>1_Paracoccidioides brasiliensis Pb18
MITFRRSSLVALRSLSSCIVLLHSHSSPKLLNPSHTSSKPQPNQKIQEQKSFSSAEINNQPPTPPPLHSTDPA
ASKPLRLRLYQFYPDVAFHAKFPAYIWQTWKNTPASPDFHELRAEASEWTTTHPSFIHQVITDDMDFLPLRLYAFFPEELDAYSLRLPVLKADFDFRYYLLILLARGGIYSDDTTLAPASEWPLSTSLDSSTHVGGIADDFWDIPHDWSRIRRIOFCQWIFIQKPGHIPIFRDIVAYITEELMRRKAGILRPGIMDKSIV
EFTGPAWVTDCVFLRYLNPDEYFEEARPPHPHGNLNTAKDFAGITVQRKVGDVVVLPLITSFSPGVQMGAGDVDPRPAFAVHKHFDFGSGKDDPSL

>2_2_Ajellomyces capsulatus G186AR
MITFRRSSIAATLLLALASFYFLISRSRSICFRISPRLQPLPIRKAQQKSQAASSSESEPSTHENVESLPPPSAPA
SLSPEDLARKPLRLRRLYQFYPDVAFHAKFPAYIWQTWKYTPASGKFGDKFRPMEASWTELHSFHPHEVIDTD
DDADPLLLRYAFFPEELDAYSLRLPVLKADFDFRYYLLILLARGGIYSDDTTLAPASEWPLSTSLDSSTIVVGGIADDFWDIPHDWSRIRRIOFCQWIFIQKPGHIPIFRDIVAYITEELMRRKAKILKGGKMDKITEVEFTGPAAWTDVFYRYPNFPYINFNSDNSKEYETFDNQEEYRKVGDDVIVLPITSFSPGVQMGAGDVDPRPAFAVHKHFDFGSGKDDPSL

>1_Paracoccidioides brasiliensis Pb18
MITFRRSSLVALRSLSSCIVLLHSHSSPKLLNPSHTSSKPQPNQKIQEQKSFSSAEINNQPPTPPPLHSTDPA
ASKPLRLRLYQFYPDVAFHAKFPAYIWQTWKNTPASPDFHELRAEASEWTTTHPSFIHQVITDDMDFLPLRLYAFFPEELDAYSLRLPVLKADFDFRYYLLILLARGGIYSDDTTLAPASEWPLSTSLDSSTHVGGIADDFWDIPHDWSRIRRIOFCQWIFIQKPGHIPIFRDIVAYITEELMRRKAGILRPGIMDKSIV
EFTGPAWVTDCVFLRYLNPDEYFEEARPPHPHGNLNTAKDFAGITVQRKVGDVVVLPLITSFSPGVQMGAGDVDPRPAFAVHKHFDFGSGKDDPSL
1. Aspergillus fumigatus A1163
MLTFRKSLIAAVVLITFVVLLRSAHSSPSAEAPVLNTETTAHDTSQAAEHLTDQKQDIQQQPLKPPPTA
PLRERLYQFPYDLENRFAYIQYWTWKFYPQSFHMFASDLRAEPASEWTHELPGFHEVIPDTRAQRLVYKYL
YGVSPEVFEAYDMLPVLKADFQYRLLLARLGGYISDITYALKPAVWLPGLDLATVGFVGIEADFP
DRPDWDHYSRQIYFCQWTIQAPGHPIRLRDIVAYITEEALRMKGGKILKEGMKDTIVEFTGPAWTDARVFYFNNPEYSIEFGSTHNVYTEDFNQRYYKGKVGQVQMGAGDLDDPMAFVKHDFS
GTIMEDEGSLNVDCAISSHLQLQPLPETCAGCQADLDPSLARANLALKRQDFYPFGVSFTTVSIPKDQYV

2. Aspergillus fumigatus Af293
MLTFRKSLIAAVVLITFVVLLRSAHSSPSAEAPVLNTETTAHDTSQAAEHLTDQKQDIQQQPLKPPPTA
PLRERLYQFPYDLENRFAYIQYWTWKFYPQSFHMFASDLRAEPASEWTHELPGFHEVIPDTRAQRLVYKYL
YGVSPEVFEAYDMLPVLKADFQYRLLLARLGGYISDITYALKPAVWLPGLDLATVGFVGIEADFP
DRPDWDHYSRQIYFCQWTIQAPGHPIRLRDIVAYITEEALRMKGGKILKEGMKDTIVEFTGPAWTDARVFYFNNPEYSIEFGSTHNVYTEDFNQRYYKGKVGQVQMGAGDLDDPMAFVKHDFS
GTIMEDEGSLNVDCAISSHLQLQPLPETCAGCQADLDPSLARANLALKRQDFYPFGVSFTTVSIPKDQYV

2. Neosartorya fischeri NRRL 181
MLTFRKSLIAAVVLITFVVLLRSAHSSPSAEAPVLNTETTTHTDSSQAAEHLTNQKQDIQQQPLKPPPTA
PLRERLYQFPYDLESRFAYIQYWTWKFYPQSFHMFASDLRAEPASEWTHELPGFHEVIPDTRAQRLVYKYL
YGVSPEVFEAYDMLPVLKADFQYRLLLARLGGYISDITYALKPAVWLPGLDLATVGFVGIEADFP
DRPDWDHYSRQIYFCQWTIQAPGHPIRLRDIVAYITEEALRMKGGKILKEGMKDTIVEFTGPAWTDARVFYFNNPEYSIEFGSTHNVYTEDFNQRYYKGKVGQVQMGAGDLDDPMAFVKHDFS
GTIMEDEGSLNVDCAISSHLQLQPLPETCAGCQADLDPSLARANLALKRQDFYPFGVSFTTVSIPKDQYV

2. Paracoccidioides brasiliensis Pb03
MITFRRSLVSSFLSCLIVLHSSHPKLLNPSHTSTSKIPNQKIQQIEKSPASSAEINQPPTTPLHSTDPA
ASKPRRDLRDLRQFPPFDPKPFAIYQWTWKFYPQSFHMFASDLRAEPASEWTHELPGFHEVIPDTRAQRLVYKYL
YGVSPEVFEAYDMLPVLKADFQYRLLLARLGGYISDITYALKPAVWLPGLDLATVGFVGIEADFP
DRPDWDHYSRQIYFCQWTIQAPGHPIRLRDIVAYITEEALRMKGGKILKEGMKDTIVEFTGPAWTDARVFYFNNPEYSIEFGSTHNVYTEDFNQRYYKGKVGQVQMGAGDLDDPMAFVKHDFS
GTIMEDEGSLNVDCAISSHLQLQPLPETCAGCQADLDPSLARANLALKRQDFYPFGVSFTTVSIPKDQYV

2. Arthroderma otae CBS 113480
MISFRKCVFIAFLILSSGFLHLAHIKPVSVANEVETHHQRSEQQPLRTSTAKELSEPEPEPKVEDPE
EEPEPKMTLRRLKHLYHPFYGATKFGGYIQYWTWKFYPQSFHMFASDLRAEPASEWTHELPGFHEVIPDTRAQRLVYKYL
YGVSPEVFEAYDMLPVLKADFQYRLLLARLGGYISDITYALKPAVWLPGLDLATVGFVGIEADFP
DRPDWDHYSRQIYFCQWTIQAPGHPIRLRDIVAYITEEALRMKGGKILKEGMKDTIVEFTGPAWTDARVFYFNNPEYSIEFGSTHNVYTEDFNQRYYKGKVGQVQMGAGDLDDPMAFVKHDFS
GTIMEDEGSLNVDCAISSHLQLQPLPETCAGCQADLDPSLARANLALKRQDFYPFGVSFTTVSIPKDQYV

HQFSGSWKAEEAGRPAPVILEE
>Aspergillus clavatus NRRL 1
MLTFRKSLIAAVILITFVILRSLRSAHSGPSSTHSIVPKPDEAVFDVNQAVSNESHESTQEKQAQAIPPLPKPT
APLRDRRLRYQFPYDLENKFPAYIQWTKYTPSSWVFGEDLRPAEASWTLHPGFVHEVVTDETQRLIKY
LGYGLPEVFEAYSMLPLVKADFFRLYLILLARGGYSDIDTFALKPASDNLFLSELDLSKIGFVVGIEAD
PDRPDWDHWSRRIQFCQWQTIAQKAGHPSLISDIVAYITEEALRMKKGAVLKEGKMDKTIVEFTGPAAWTD
AVFRYFNDEPFFEISIPDSTHNVTYEDFTNQQQFGRMVGVVVLPITSFSFGVNMQAGYDDPMAFVHDF
SGSWKTDPSL

>Penicillium marneffei ATCC 18224
MKDLESRYYPARTSTMARRAIADFALLVSIILLFRSFSASTIYDSPRPQPQARQGRAFESQLTIGD
TTYPLTEKLHRQYPYDLDKFPAAIYWQTWKSYSVSSSPQHPFHYLESTWDTNPQVFVEHVISDEAVHLL
QVQLYGSMPVEVEAYNSLFAPVLKADFFRLYLILLARGGYSDIDTEALKPVVDWLPEDMDPS8VGLVIGI
EADPRPDWDHWSRRIQFCWQTIAQKAPGHVLRDVVATVEDALRMKKGAVLKEGKMDKTIVEFTGPAAWTD
AVFRYFNDEPFFEISIPDSTHNVTYEDFTNQQQFGRMVGVVVLPITSFSFGVNMQAGYDDPMAFVHDF
SGSWKTDPSL

>Aspergillus oryzae RIB40
MLTYRKSLIAALFLIFVVLRLRSHSSASSSSFSPFAPAPHLDEVAYNTNEVTEEEHLSQGKKEAIMQQPLKP
SPSAPLRERLRHYFPYDLDKFPAYIQWTKYTPSDSWFQPQHELRAEASWTELHPGFVHQVVPDOTTGQL
IKLYLSLIDPFAEAYNSLFPVLKADFFFLYLLILLARGGYSIDTEALKPVVDWLPEDMDPS8VGLVIGI
EADPRPDWDHWSRRIQFCWQTIAQKAPGHVLRDVVATVEDALRMKKGAVLKEGKMDKTIVEFTGPAAWTD
AVFRYFNDEPFFEISIPDSTHNVTYEDFTNQQQFGRMVGVVVLPITSFSFGVNMQAGYDDPMAFVHDF
SGSWKTDPSL

>Talaromyces stipitatus ATCC 10500
MKELIERSQYPAKNPTMAPARRAIADFALLVSIILLFRSFSASTIYDSPRPQPQARQGRAFESQLTIGD
QSSLQVGTEAYTPLEKLHRQYPYDLDKFPAYIQWTWKSYSVSSSPQHPFHYLESTWDTNPQVFVEHVISDEAVHLL
SDEAAADLVILQYLSGMPVEVEAYNSLFAPVLKADFFRLYLILLARGGYSIDTEALKPVVDWLPEDMDPS
S8VGLVIGIADPRPDWDHWSRRIQFCWQTIAQKAPGHVLRDVVATVEDALRMKKGAVLKEGKMDKTIVEFTGPAAWTD
AVFRYFNDEPFFEISIPDSTHNVTYEDFTNQQQFGRMVGVVVLPITSFSFGVNMQAGYDDPMAFVHDF
SGSWKTDPSL

>Aspergillus nidulans FGSC A4
MLTFRKALIVAIFITLIRSLRSHSGPSGPEAAASAPDIAIEYISQETKGTSSSESSDSTQQQPLKPQPPP
ATAPLRERLRYHPYDFNLEAKFPAYIQWTKYTPASWFSQDLRPEASWTEMPSFHVQVIPDOTTLLHLY
KLYLSLIDPFAEAYNSLFPVLKADFFFLYLLILLARGGYSIDTEALKPVVDWLPEDMDPS8VGLVIGI
EADPRPDWDHWSRRIQFCWQTIAQKAPGHVLRDVVATVEDALRMKKGAVLKEGKMDKTIVEFTGPAAWTD
AVFRYFNDEPFFEISIPDSTHNVTYEDFTNQQQFGRMVGVVVLPITSFSFGVNMQAGYDDPMAFVHDF
NFGGTWKTDPASL

>Aspergillus flavus NRRL3357
MLTYRKSLIAALFLIFVVLRLRSHSSASSSSFSPFAPAPHLDEVAYNTNEVTEEEHLSQGKKEAIMQQPLKP
SPSAPLRERLRYHPYDLDKFPAYIQWTKYTPSDSWFQPQHELRAEASWTELHPGFVHQVVPDOTTGQL
IKLYLSLIDPFAEAYNSLFPVLKADFFFLYLLILLARGGYSIDTEALKPVVDWLPEDMDPS8VGLVIGI
EADPRPDWDHWSRRIQFCWQTIAQKAPGHVLRDVVATVEDALRMKKGAVLKEGKMDKTIVEFTGPAAWTD
AVFRYFNDEPFFEISIPDSTHNVTYEDFTNQQQFGRMVGVVVLPITSFSFGVNMQAGYDDPMAFVHDF
NFGGTWKTDPASL
EADPDRPDWHEWYSRRLQFCQWTIQSKPGHPILRDIVAYITEETLRMKKAGILKVGKMDKTIVEFTGPGA
WTDAIFRYFNDFDYFNIPEDSNHNITYEDFNSQNKRKDVGDVVLPLITSFSGVGMQMGAGDPPDMAFVKB
HDFEGT

>3_Paracoccidioides brasiliensis Pb01
MITFRRLVAILFLLSICILLHHSKAKLPKPSHTSSKIPRpNRKQNQQEKSFSSAELNPKSLPHSPDL
ARKTLRDLRLRQYFPFYYLDPSKFPAYIQWTKNTAPSDFYESHTREASWTIHAFTPQHVQMDMVJDDPL
RLYLYAPFPEIETYRSLPLPLKADFFRLYLLLARGGIYSDITYVLKPADEDLPRHNIRSTVGVLVGIE
ADTREDWAQFSRRLOFCQWTRIQAQPGHPVLRDVVATITEALRMKEGI11LGMMKSCMEFTEFGPAWV
TDAIFRYBNLPYFETAQPNNRNLTADHDFSVGKEHRQ1GDVVLPLITSFSGVGGMAGADDPMASHVKB
HHFSGWKSEAGRPAPVPVLEE

>Penicillium chrysogenum Wisconsin 54-1255
MITPRKVVLLSLLCVTVFLILSRFRDPEVFPAGARPYVRKKISQEEDDEVTSNLARKPPQGRALPLGASP
TAPLRERLRFQFYQENKFPAVAYWTKYDPGSDFWFDEEDRLGEASWEMHGFHEVIFDEQTQQLIK
LYLSFGVEPVEFAYDSRMVLQVLEAGGYSIDDDITALRPWTLPEELDRSTIGLVIGEA
DPFDREDWHDWSRLRFQFWTLVSDKPGHPILRDMVAYTEHALRMKARGLIKVGKMDKTIMEFTGPAGW
DSVFRVFNPPAYFNNQPDGKNITYEDFQHTHTRKVDVVLPLITSFSGVQGMADPDDMPAFVKB
HNFSGKWKSAAAGRPAPVPVLEE

>Arthroderma benhamiae CBS 112371
MASLLNLLEGGVRNEAGPPEPEDOOGSSLLLTLLHNSPGHISAGCSCSSGSEGW5LRSVAVLLSSIFDI
AGDEVVAGLAAGV-NTRSPFLPPFHPKTDPPPSAPSHQPPAYIKPNPALSFLFHFHVIPFLRL
LFLFAAFSFLAVGPANMISFRKVCPVIAFFLIGFLILHTAHIQPALAKAEQTQHRQSEQNARLT
TQLGSKADSPSEQEQVEQVSEQEPQKEPEKEPEETTLRGRKLHYHPQKFGPYIQWTKYTFADGEFDPM
LRPLEASWETHELHFQVQVVDJAIYFLKLYSSPEIIEAYESMLPVLKADFFRILYLLHARGGIYSD
IDTTALSATDDWPFTDRSTFGLVVGIEADPDRAWAKWYRRIQFQWTLQSKPGHPVLVRDVIASITE
DALRMKEEGI11TKKMDKSIVEFTGPAWTVTAIFRFNDFPLAFFSEGDHRKHNISAAHFTGMKTQKQVGDV
VVLPLITSFSGVQGMGSEEADSDMAFQHEFSGYTV

>Trichophyton verrucosum HKI 0517
MASLLNLLEGGVRNEAGPPEPEDOOGSSLLLTLLHNSPGHISAGCSCSSGSEGW5LRSVAVLLSSIFDIAG
DEVDVAGLAAGV-NTRSPFLPPKHFADDFPFAAPSFHPQPPAYITFPPAFSLFPLFHFHVIPFRL
LLLFAAFSFLAVGPANMISFRKVCPVIAFFLIGFLILHTAHIQPALAKAEQTQHRQSEQNARLT
TQLGSKADSPSEQEQVEQVSEQEPQKEPEKEPEETTLRGRKLHYHPQKFGPYIQWTKYTFADGEFDPMVL
RPLEASWETHELHFQVQVVDJAIYFLKLYSSPEIIEAYESMLPVLKADFFRILYLLHARGGIYSD
IDTTALSATDDWPFTDRSTFGLVVGIEADPDRAWAKWYRRIQFQWTLQSKPGHPVLVRDVIASITE
DALRMKEEGI11TKKMDKSIVEFTGPAWTVTAIFRFNDFPLAFFSEGDHRKHNISAAHFTGMKTQKQVGDV
VVLPLITSFSGVQGMGSEEADSDMAFQHEFSGYTV

>3_Ajellomyces capsulatus H143
MITFRRLVAILFLLSICILLHHSKAKLPKPSHTSSKIPRPNRQNNQEKSFSSAELNPKSLPHSPDL
ARKTLRDLRLRQYFPFYYLDPSKFPAYIQWTKNTAPSDFYESHTREASWTIHAFTPQHVQMDMVJDDPL
RLYLYAPFPEIETYRSLPLPLKADFFRLYLLLARGGIYSDITYVLKPADEDLPRHNIRSTVGVLVGIE
ADTREDWAQFSRRLOFCQWTRIQAQPGHPVLRDVVATITEALRMKEGI11LGMMKSCMEFTEFGPAWV
TDAIFRYBNLPYFETAQPNNRNLTADHDFSVGKEHRQ1GDVVLPLITSFSGVGGMAGADDPMASHVKB
HHFSGWKSEAGRPAPVPVLEE

>3_Ajellomyces capsulatus H143
GLVGIEADAPRFGLAKPGHPVLRDIVATITEDTLRMKRAGILRPGIMDRSIVEFTGPAVWTDCVFRLY
NDFEYFEEARPPHGRNLTKADFAGITVQKRGVDVVLPITSFSFPGVGQMGAGQDAGDPFMAFVHLQFEGSWK
GEAEEDEDEKHELQNAADIIASALDIIIRTNGQKTE

>Arthroderma gypseum CBS 118893
MISFRKCVCVIAFFILSFGLFFHLTAHARPALAKTGFGTHQRSEPLGTQITAALKGSVDSTPEQEQEPEQEAE
PEPEPEPEPEPEPFEPTTLRLKLYHFYPVYKAAKFGFYIQWGTYKTFQAQGFDPLRPLEASWTEHLPGFV
HQQVVDSSAVYFLKLYASFPEVIEAYDAPLPLLKLADFFRYLILTARGDIIDTDLARAATEWIPT
FDRSTIGLVHIEAPDRDARWARIWSYRIQFCQWTQISPKGPVLRDVGATSWETERMKFGEKLNQGVHRP
AVWTDFAIFKHFNDPVNPSEFGKHQNITALHFTGMPKQGVVDVVLPITSFSFPGVQQMGSEEIDSMAPT
VQHEFSADGMLAGCFGRICFINAGRDNGVADGVKLCA

>Yarrowia lipolytica
MALITARRVILLGLAVATVCFLFFGSSEKIAPTLLLPGDMSAFENKIYKMENITEKALKEQEEAAAKRFD
SFNKKLESELENRVEKIQRLTRFPGASLEKLAYLFPYEYTYYKFFAIQWTKDEITDDTDPTIRQ
PRTWTEKNSFVHEVLTDDAADDEAAFAQVHHFAIPPEVEAYAKMPKINLRADFFRYVLLARGGVYSDVDT
EDLPKIPNPWEPSSTVGLVGIEADPDPRDFWKEWYARRIQLCQWTQIAKGPHVLRDIVARIKETK
AKKRTGGTLEMAGKTDGSEMDTQPVGWTSVFDYFNDKKSGLKSQVGKDFZNNFLKTPHVDVLLP
VTSFSFPGVDQGAHKDTPDIFAVKHLFSGSWKPEDERMNQNPQVKNQ

>Verticillium albo-atrum VaMs.102
MLTPRRALGRGGLCSHPYNAAALPVLKADFFRYLILLARGGVYSDIDTVSALWVPDRIPTTSIGLV
IGIEADPDRPWHDWYSRIQFCQWTQIAKGPHVLRDIVARIETEELRRLVKAQGLSLKMDDNVEFTG
AVWTDVFYNDLARYFDMAEKSGPITDNSFTGMEQSKKGVDVVLPITSFSFPGVQQMGAKDDDPMAFV
KHEFEGTWKPEALRHIENND

>1_Meyerozyma guilliermondii ATCC 6260
MKQKQVKIAVVVLAVFHYLYTFHTSFPPHSAAEFHSNTAKKLREHERHTNWKLYGFIQFSPNNRAQLP
KITTVRQQLAQFPFYPEPERPKNHqryqWkDLESENFRPSYRKFQOTDSWNPKYHYHIDEACEELV
NQLFSVPPDVARAINMPKSLKADFFRYLILFARGGVYSDITVSLKIPDVMSANETYLQGPPNPLG
VGIEADPDRPDWADWYARRAQFCQWTQIAKGKHMLREIVALITEELERKKQNHLKVGLDGQDDMVW
WGTGPIWTDVEFAYMNNAALQSPFMAAKKHEDEVWKTQFTGKMMPAIADVLVLPITSFSFPGVQQMGAK
SKDPMAYAHKFMAGSWKPEDER

>1_Pichia angusta
MKINTVRKLLLVSISVIAVVLRLSSDSTPDLPQVLSPOGPSMVPSDSSSQQDHQGRGRRSGGSLGLSGK
NSEEELARKLNEQVDRQLQERQLSKEQLSPLNNPEATRLRLAMYMPYDQANKFFA
YIWSQKYGFLNDANFPFRQKEEEQWALKLNPFGVHELLENTSNARVHYLYHMHEVKAYELLTVILK
MDFFRILFLFAGGGYADWTLPLQIPNWPNEVPDSEPSEAGIIGIQSDPTDFRWKRFARRRLQFANWVI
QAKPGPHPIREVIAITETLEKRAEGTTLFDAASSDMIEWTGAVWVDVFYFNVDVLSGFISKVTN
KDFTKLDVPKLVSDLVLPIFTCPFSPGISGKMAGADADHPFAFVHYSEHLFQ

>1_Debaromyces hansenii CBS767
MIKKRIEV1KVVAILYLYLFREVFSFVYKSKTEFHNTLDSIKELEHHTNWLKYGFKFHSNANCLP
>1 Scheffersomyces stipitis CBS 6054
MINKRVTGLAVLVLVLYLGYFFRTFSVPVGHSHRRREESSSKQLKRELEIHSNWAKTLNFQPNNKARLP1
IETTVRQQLSFQFYPPESSRFKFPQKNIQWTQKVWALDDSFSSLKYRTYOQSTWDDKNGYHYVAADVCEELI
SQLYSTVDPVARAYNIMPKSILKADFFRYLILYARGGTVYTDITVGLKLPICWSSNTTYLDKPINFGLV
VGIEADPDRPDWAEMYARYRIIQFCQWTIQAKKGFMLRELIAEITEKTLRARKQOLKVLGKDDEGDIMN
WTGPGLWTVDVVFYMNNILQFDPFDKNTKYDDIIINVSVTGMTPVIAIDDVVLVPITSPDVGQMAGK
MDPAMYAKHMFSGSKWKPEDEKINT

>2 Candida albicans SC5314
MLQLREPQMVKHLKLALVGIVVFTYFIIISSLSSTERTKTEYNSPKQLIAKELELNSWKEGLNFLQP
PNKKSILPDDELRLQQLSYQFPYDESKPFKPNIQWTQKVIDEKSFKRKLKYQQTWDKNPDYKHYVVF
DKQCDLIEQLYSQVFDVAKAYKIMPKSILKADFFRYLILFARGGVYTDITVGLKLPICWDEISNSEMILE
KHNRSGLVVGIEADPDRPDWAEMYARYRIIQFCQWTIQSKRGHFMLRELIAEITEKTLRARKQOLKVLGK
NEGGDIMNWTPGIFTDDTVFVEYMNILQPSFEVKKNTKWWI1DWWKFLTGGMEQPIAIDDVVLVPITSP
DVNQMAGAKDHSFMAKHMFSGSKWDGGMEPEMQ

>1 Candida dubliniensis CD36
MLQLREPVMHRHKLALIGIVVFTYFIIISSLSSTERTKTEYNSPKQLIAKELELNSWKEGLNFLQP
PNKKSILPDDELRLQQLSYQFPYDESKPFKPNIQWTQKVIDEKSFKRKLKYQQTWDKNPDYKHYVVF
DKQCDLIEQLYSQVFDVAKAYKIMPKSILKADFFRYLILFARGGVYTDITVGLKLPICWDEISNSEMILE
KHNRSGLVVGIEADPDRPDWAEMYARYRIIQFCQWTIQSKRGHFMLRELIAEITEKTLRARKQOLKVLGK
NEGGDIMNWTPGIFTDDTVFVEYMNILQPSFEVKKNTKWWI1DWWKFLTGGMEQPIAIDDVVLVPITSP
DVNQMAGAKDHSFMAKHMFSGSKWDGGMEPEMQ

>1 Candida tropicalis MYA-3404
MRSKDIRLVLVGLFTISVTYFLISFFGNSNRTSINISKLQFERELSNPWNQKIGLEFLKPTQKLQIDE
SSIIQQQLSKRFYDLNSFPPKNIQWTQKVGLNDEFTFSPYSFIFKSWDKKNFHYIIPDCENELIE
QLYEIEPDVSKYAMPKSIKLFADFFRYLILFARGGVYTDITVGLKLPICWDEISNSEMILE
KHNRSGLVVGIEADPDRPDWAEMYARYRIIQFCQWTIQSKRGHFMLRELIAEITEKTLRARKQOLKVLGK
NEGGDIMNWTPGIFTDDTVFVEYMNILQPSFEVKKNTKWWI1DWWKFLTGGMEQPIAIDDVVLVPITSP
DVNQMAGAKDHSFMAKHMFSGSKWDGGMEPEMQ

>1 Clavispora lusitaniae ATCC 42720
MVGPRKRLIGIISIVLVEMLFLVTYFTSVSPHRVHTKELSTNKLKARELEKNSWKKTGLNFTKNAAN
LPALSTRQLQFLSFQFPYEPSSKFKPNIQWTQKVGLNDEFTFPTTTYRQETWEALNSGYHYVIPDECDDT
MVKQLFASVPDQAANSMPKKNLADFFRYLILYARGVYTDITKLCKFIDTWSSKNQTYIGDNHAG
LVGIEADPDRPDWAEMYARYRIIQFCQWTIQAKGHFMRELIAEITEKTLRWNKELKKIKGKDSGDDI
MNWTGPGLWDMVFYMNNILQPSFEVKKNYDEITWVKFLTGGMEQPIAIDDVVLVPITSPDVGQMAGK

>1 Clavispora lusitaniae ATCC 42720
MVGPRKRLIGIISIVLVEMLFLVTYFTSVSPHRVHTKELSTNKLKARELEKNSWKKTGLNFTKNAAN
LPALSTRQLQFLSFQFPYEPSSKFKPNIQWTQKVGLNDEFTFPTTTYRQETWEALNSGYHYVIPDECDDT
MVKQLFASVPDQAANSMPKKNLADFFRYLILYARGVYTDITKLCKFIDTWSSKNQTYIGDNHAG
LVGIEADPDRPDWAEMYARYRIIQFCQWTIQAKGHFMRELIAEITEKTLRWNKELKKIKGKDSGDDI
MNWTGPGLWDMVFYMNNILQPSFEVKKNYDEITWVKFLTGGMEQPIAIDDVVLVPITSPDVGQMAGK
Pichia pastoris GS115

>1_Pichia pastoris GS115
MAKADGSLLYYNPHNPPRRYYFYMAIFAVSVICVLYGPSQQLSSPKIDYDPLTLRSLDLKTLTEAPSQSF
GTVEDNLRLQLEPAASATLREKLSFQFYPDFEKPVPQNIQWTKVWDINDKSFRFRKQETWQQLNSGYTY
HLIQPSIVDFMRSLFANVEPIVAYANLMPLKNIKADFRRYLIVARGGTYSDITCILKVPVENWATFN
EQTVSHYTKNQTLQPEVDPSTRKPIGMGHEQPLMKVPGYKQAQQVLWEQFTNLRSPLDLIILPITSFSPF

>2_Pichia minuta var. minuta

>1_Pichia minuta var. minuta

>2_Pichia angusta

>1_Ashbya gossypii ATCC 10895

>1_Lachancea thermotolerans

>1_Lachancea thermotolerans
>2_Clavispora lusitaniae ATCC 42720
MNQTSMGRPKKPKVIIIVLVAILLIVLVRSTAQSRKDAILDNGIFSSVFSENDKKQDELFKKEKMQR
EFTPSEQELRQLARERQVEQLYFVQRQLIFIPQSMQSMQEMRKLQFMYDVTAKKFGYIQWSKWHGIDNFED
STFKEGGQAVQAVPNLRPPLPLIDTAKKAFPLSYKLYARGYISMETDLPLKLSSWPSSTQYSYFSSLKNEP
QYRNNSLDNLETASEPGVIEATPDRSDVDAEWYARRIQFCQWITQSISGHPLLRERLNTTATE
SVANVSSSIPLDDAVLKDADDYVNMRMRDFFPKNKNQKQQKTTKNTGDTMDINMTPGPISDFSIVQY
LNNVIQKNDLNLNDFNLSKVNHGKSFHSDTMYKDFIDKVNQKNSKPSLFWGFFSMLNTEPILVDDIMVLP
ITSFSFGRTMGAKEDNDEAMFKHHIFEGSWKD

>2_Kluyveromyces lactis NRRL Y-1140
MGLPKISRRTRYIIIVLILYLLFSQVNTKAVSNHFYNSIGTVLTPSTARVDHNLNKLNLGTSNNGDHI
LMDLRVQLASQFYDSRVPKPKVQWTKIDPSKSVQSVSSIKCQNDWHFSASEFFPYQYLITDDQMI
PLEQQLYGGVPVQIFKESLPILPDLIDTAAYKFRLYLIARQGYISMDTFLPPLSSWPSSTQYSYFSSLKNEP
QYRNNSLDNLETASEPGVIEATPDRSDVDAEWYARRIQFCQWITQSISGHPLLRERLNTTATE
SVANVSSSIPLDDAVLKDADDYVNMRMRDFFPKNKNQKQQKTTKNTGDTMDINMTPGPISDFSIVQY
LNNVIQKNDLNLNDFNLSKVNHGKSFHSDTMYKDFIDKVNQKNSKPSLFWGFFSMLNTEPILVDDIMVLP
ITSFSFGRTMGAKEDNDEAMFKHHIFEGSWKD

>1_Candida glabrata CBS 138
MTMARKRNSFILRRIFFFIAGLAGAIIALLKLSSNNARASDIQKILQNLKPeITQSinVANSNQKADVAD
AKFKALEDKLRKQEQSKQFERQKVRKLRLNNMQISMTDATLREKLAYNFEYDGKRSFPAFLWQTMWSP
SQTPEEVLHVATWQKNGPFCVHEITPMMNALVHHDYSIVPEVEAYKLLPDNLRDFKYYLVLLAR
GGTYADĐTSPQIPNPWPETVESDGGILTVGIEHDAQAVDWSHRHYYRLQFSTVIQAKPGHPVLREV
VAQIJEVHVINNKLDDSTVSNRLVSMKGFSTDSALTAIMTYLNIKLKVSKDQLGDRKTVRDFKITSPLKVS
DILVFPEFSFNFVPEDKEDPLRAQFFISHKGEKFKWAAPKVAS

>1_Saccharomyces cerevisiae S288c
MSRKLSHLATRSKTSTIVTVLYLISLTLTTTLFHSNKLRLLGSFQFSPKDDFKQTLPTTSHSQRDINLKKQITV
NKKKNQHLRNRLQDQLSFAPYDQSAPIPQQRVQWTKVKGADDNKFPSRFQTKWQSYSYPSQYLSILSDD
SIIPFLENLYAPVIPVIAFKLMPGNFLKADFLRYLLFARGYISMDMTPKIPDSWPSQNKSLLNIN
IDLNKPFPYNKRSFKLSSDEISHQPGVLIGEIAEDPRDDWSEYWARRIQFCQWITQQAKPGHHPRLLIF
NITATLASSQNQFPVSEMIDRFSEEDNYNVRHKHHKETYKSCSLLKNKVVSMGIDTSWMTGPGIFS
IIFEYMNNVNLINPDLNKLNDSEGSESATEMACDMLDLSKSTRKFKYYKIIESLQSSNSMPWE
FFSFLKEVDPVDDVLMVLPITSESPDFPVGMPAGQASSDDKMAFVKHMGWSKEDADNAGHK

>1_Schizosaccharomyces pombe 972h
MLRRLRSVIGAASGILLLFNHNSIEGMEDLTEISMLEDYTPEAANKDYVGQEESEELLYDQFSYIE
EEDPDEAELNSLLEELEEEDEENNDKLYHLRSFSQLQDFDEENEAVHIMVPKDYTEFEFVYHA
DIPIKLWQTSKDDFDREVMKTRFWHRINSHPHYSHALPDESEQKALVISISSIFGDSSVSKISQAYAMMPPLV
KLADFDFLYLVLAALKGISDIDFREEDNYNVRHKHHKETYKSFLLKLNNKVVSMGIDTSWMTGPGIFS
IIAAPPGLHPILWELVRRIETDWTKLHDSKLSKNGESVMETGPGTMDIAIMDYLNWQYPFSGVENITNLE
EPYLVGDVLPILPITAFSPGTVGHMGSKSPNDEMAYQQHFFAGSWSKD

>2_Vanderwaltozyma polyspora DSM 70294
> 2. **Saccharomyces cerevisiae** S288c
MAKTTKRASSFRRLMIFAIIALISLAFGVRYLFHNSNATDLQKILQNLPKEIQSINSANNIQSSDSDLV
QHFESLAQEIRHQEQVAKQFDKQKILEKKIQDNLQKTPPEATLRRIAMTFYPDHVFKPAFIWQTWS
DEGPERVQDIKMWEKSNPGFAEHLVNHVDNALVHHYFYSIFEILEETYALPSILKIDFFKYLILLVH
GVYADITFVQHIPWIEPEELSIPILGIVGEADQQRADWRKTYIRRLQQTGWIKAPGHVP
ISRIIETLQRKRDQDLVNVLNRNDLNMSTGSGLWDTDITYFYNDFMRSVGREKVTKLFHN
SDVLFVFKFSNCPQIDNDDPHKFFYFITHLASQFWKNTPKVEQK

> 3. **Saccharomyces cerevisiae** EC1118
MAKTTKRASSFRRLMIFAIIALISLAFGVRYLFHNSNATDLQKILQNLPKEIQSINSANNIQSSDSDLV
QHFESLAQEIRHQEQVAKQFDKQKILEKKIQDNLQKTPPEATLRRIAMTFYPDHVFKPAFIWQTWS
DEGPERVQDIKMWEKSNPGFAEHLVNHVDNALVHHYFYSIFEILEETYALPSILKIDFFKYLILLVH
GVYADITFVQHIPWIEPEELSIPILGIVGEADQQRADWRKTYIRRLQQTGWIKAPGHVP
ISRIIETLQRKRDQDLVNVLNRNDLNMSTGSGLWDTDITYFYNDFMRSVGREKVTKLFHN
SDVLFVFKFSNCPQIDNDDPHKFFYFITHLASQFWKNTPKVEQK

> 4. **Saccharomyces cerevisiae** AWR1796
MIFAIIALISLAFGVRYLFHNSNATDLQKILQNLPKEIQSINSANNIQSSDSDLVQHFESLAQEIRHQ
EVQAKQFDKQKILEKKIQDNLQKTPPEATLRRIAMTFYPDHVFKPAFIWQTWS
DEGPERVQDIKMWEKSNPGFAEHLVNHVDNALVHHYFYSIFEILEETYALPSILKIDFFKYLILLVH
GVYADITFVQHIPWIEPEELSIPILGIVGEADQQRADWRKTYIRRLQQTGWIKAPGHVP
ISRIIETLQRKRDQDLVNVLNRNDLNMSTGSGLWDTDITYFYNDFMRSVGREKVTKLFHN
SDVLFVFKFSNCPQIDNDDPHKFFYFITHLASQFWKNTPKVEQK

> 5. **Saccharomyces cerevisiae** Lalvin QA23
MIFAIIALISLAFGVRYLFHNSNATDLQKILQNLPKEIQSINSANNIQSSDSDLVQHFESLAQEIRHQ
EVQAKQFDKQKILEKKIQDNLQKTPPEATLRRIAMTFYPDHVFKPAFIWQTWS
DEGPERVQDIKMWEKSNPGFAEHLVNHVDNALVHHYFYSIFEILEETYALPSILKIDFFKYLILLVH
GVYADITFVQHIPWIEPEELSIPILGIVGEADQQRADWRKTYIRRLQQTGWIKAPGHVP
ISRIIETLQRKRDQDLVNVLNRNDLNMSTGSGLWDTDITYFYNDFMRSVGREKVTKLFHN
SDVLFVFKFSNCPQIDNDDPHKFFYFITHLASQFWKNTPKVEQK

> 6. **Saccharomyces cerevisiae** YJM789
MAKTTKRASSFRRLMIFAIIALISLAFGVRYLFHNSNATDLQKILQNLPKEIQSINSANNIQSSDSDLV
QHFESLAQEIRHQEQVAKQFDKQKILEKKIQDNLQKTPPEATLRRIAMTFYPDHVFKPAFIWQTWS
DEGPERVQDIKMWEKSNPGFAEHLVNHVDNALVHHYFYSIFEILEETYALPSILKIDFFKYLILLVH
GGVYADIDTFPVQPIPNWEELPSDLIQGIVEEDEAQRADWRTKYIRRLQFAQFVMQSKP
GHPILREIIETTLQRKRDDQLNVNLRNDINIMSWTGSLWTDITFTYFNDFMRSGVREKVTWKL
FHNLPQKLL
SDVLVFPKFSNPCNPQIDNDDPHKQFYFITHLASQFKNTPKVEQK

>2 Candida tropicalis MYA-3404
MTKFKTSSRVRGKRYVPIIILTILTGLITIFTFSSPSLNGGKDIQEVISLPNDKMLGLSNNLTSTENKNQ
GELLAIKIEKLHAFEEQNERNLVRIEXQNNKLLKLEIKLLKAPPATASIRDKTTLFYADARVFAPAYWQ
TKWHGLNDERDFDREGERQWAYKPNPGFVHELFDNTDAMVHFKQVPEIQAEEKPLEVVLKMDFF
RYLILFARKGGVYADIDTFPVQPIPNWEIPENSPSPLDGLISVSVESDNSNPWRQDSVSRLQFAQFVMQSKP
GHPILREIIETTLQRKRDDQLNVNLRNDINIMSWTGSLWTDITFTYFNDFMRSGVREKVTWKL
GHPILREIIETTLQRKRDDQLNVNLRNDINIMSWTGSLWTDITFTYFNDFMRSGVREKVTWKL
SDVLVFPKFSNPCNPQIDNDDPHKQFYFITHLASQFKNTPKVEQK

>2 Saccharomyces cerevisiae
MACKRASSRRLMIAIAILISLAFGVRYLHFNANSDLQKILQNLPEISQINSANNIQSSDSDDL
QHFESELAQEIRIQGVQVMQSKDKRQLIELKIDQLKQTFPEATLRERIAMTFYDHSVFKPFAIWTQNS
DEGEERVQDIKWGMWLNDVHNLVWAYGYSMDTFLPLKALDEWSPSITLSPKPEVPIKYKG
GTVYADIDTFPVQPIPNWEELPSDLIQGIVEEDEAQRADWRTKYIRRLQFAQFVMQSKP
GHPILREIIETTLQRKRDDQLNVNLRNDINIMSWTGSLWTDITFTYFNDFMRSGVREKVTWKL
SDVLVFPKFSNPCNPQIDNDDPHKQFYFITHLASQFKNTPKVEQK

>2 Lachancea thermotolerans
MLKFNKERTILTSVFLVYIASQFQLSSWKLKEQDFGRFGKTLIPPTATEQTQINTLTKFPPANMGAPIAEL
RSQILQQFFYDFSQPIPIRRIQWWTNTFNSPKMGQGQDFSGRASASAASEAPLYDLVPDSPHMLPNN
LYGAVPQIQADSMSNLNKLADFFFLYLVLYARQYGYSMDTFLPLKALDEWSPSITLSPKPEVPIKYKG
GTVYADIDTFPVQPIPNWEELPSDLIQGIVEEDEAQRADWRTKYIRRLQFAQFVMQSKP
GHPILREIIETTLQRKRDDQLNVNLRNDINIMSWTGSLWTDITFTYFNDFMRSGVREKVTWKL
SDVLVFPKFSNPCNPQIDNDDPHKQFYFITHLASQFKNTPKVEQK

>2 Ashbya gossypii ATCC 10895
MKFVTRRARVAVLAAVIVLSTEWNSRRHLHRVFWRQAEQGLEVFSSHYVGELNMRFRGAWERRDRLS
QLIAAQFPFDTPAGIFIQVVQWTVVHFSAQQFEHFLSDLAWSENKADEGEVYFLVMEFLDMLPNLY
GGVQVQLQAFESLPLAIMRADFFRYLILYARGGIYSDIDTEQLPLITAWSDLQAQLKFKMKRYHGDT
ELSVGFESSFPLGAIQPACDPFRDSEYYARIQFCQWTIQCAKAGHPLREILNITGTLHVASRT
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|MQQQLFRKVIWTVGLTIVLVIKKISSSKSTADLQKLKPKNAMNPQDVINVNSRKVTDNKLDEIQKKLSQKQDDSKSAEAREADLQVRLPMFNSSDAALTRLLFYNFENKFPAYILQMTKVLGLNNDREFGEKFKEGETQWASKNPFGVHETFNDTSGVFIHHLYNVPEVIKAYELLPNILKMDFFRYLVLYAKGGVYADVDTMLQPVFPNIPENPSKPSIIMGIQNDANNPNDWKKTEIATLDQRAESNLSLELADSEEGGLSDKNSMNPWQTGFTIDAFIYFMDFNQIYQSIYKTVKWEFSKLKPKLVSDLVLPILISFSAGAGSGKSTELNDPLAFVQHYFERLHNDNH

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|WQTLKSFSEGYLGTVINWTKAANFPGYVHAVLTDAAEFLREHFGDESKRELMEYFKKLPEEMKADFR YLSLLAEGGYYTDIDSTPIKPDKWVPAKFKDSMGIVGEDPEODPWNDDYARRQFQWCTIAAVPNGYQHLDMAQIRTKETIKRAETNKLDTRYGNMEWTGPGTVTDVAVMDNWYNPGFWSKNTLQLPEMLGV DVRLLVLPITAFSPGVGHNMSFDFHSALVRHHFSGWKTATENGH

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CLUSTAL W (1.81) multiple sequence alignment

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Penicillium chrysogenum Wison
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Chaeotomium globosum CBS 148.51
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VARP
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1 Pichia angusta
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| Aspergillus_nidulans_FGSC_A4 | L |
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2 Zygosaccharomyces rouxii
2 Candida glabrata_CBS_138
2 Lachancea_thermoTolerans
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Coccioides_immitis_RS
Uncinocarpus reesii_1704
Arthroderma_benhamiae_CBS_1123
Trichophyton_verrucosum_NHI_05
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3 Ajellomyces_capsulatus_H143
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1 Ajellomyces_dermatitidis_SLH
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Talaromyces_stipitatus_ATCC_10
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Pyrenophora_teres_f_teres_0-1
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Phaeosphaeria_nodorum_SN15
Tuber_melanoporum_Mel28
Sclerotinia_sclerotiorum_1980
Botryotinia_fuckelliana_H05.10
bgp1704
1 Glomerella_graminicola_M1.00
Magnaporthe_oryzae_70-15
Verticillium_albo-atrum_VaMa.1
Grosmannia_clavigera_kw1407
Neurospora_crassa_CD74A
Sordaria_macrospora
Chaetomium_globosum_CBS_148.51
Podospora_anserina_Smat+
Gibberella_zeae_PH-1
1 Nectria_haematococca_mpVI_77
2 Nectria_haematococca_mpVI_77
3 Pichia_angusta
Schizosaccharomyces_pombe_972h
Schizosaccharomyces_japonicus
2 Glomerella_graminicola_M1.00
2 Nectria_haematococca_mpVI_77
3 Pichia_angusta
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  . Pezizomycotina [ascomycetes]
  . leotiomycota [ascomycetes]
  . sordariomycota [ascomycetes]
    . Sclerotiniaceae [ascomycetes]
      . Botrytis tuckelliana B05.10 493 2 hits [ascomycetes] hypothetical protein BC1G_07558 [Botrytis tuckelliana B05.10]
      . Neurospora crassa OR74A 472 2 hits [ascomycetes] hypothetical protein NCU00609 [Neurospora crassa OR74A]
      . Glomerella graminicola M1.001 466 2 hits [ascomycetes] glycosyltransferase sugar-binding region containing DXD dom
    . Sordaria macrospora 464 1 hit [ascomycetes] unnamed protein product [Sordaria macrospora]
    . Gibberella zeae PH-1 428 1 hit [ascomycetes] hypothetical protein FG00425.1 [Gibberella zeae PH-1]
    . Metarhizium anisopliae ARSEF 23 425 1 hit [ascomycetes] initiation-specific alpha-1,6-mannosyltransferase [Metarhizium anisopliae ARSEF 23]
    . Verticillium albo-atrum VaMs.102 415 1 hit [ascomycetes] initiation-specific alpha-1,6-mannosyltransferase [Verticillium albo-atrum VaMs.102]
    . Phaeosphaeria nodorum SN15 410 2 hits [ascomycetes] hypothetical protein SN05_02920 [Phaeosphaeria nodorum SN15]
    . Pyrenophora tritici-repentis Pt1C-FBP 447 2 hits [ascomycetes] initiation-specific alpha-1,6-mannosyltransferase [Pyrenophora tritici-repentis Pt1C-FBP]
    . Leptosphaeria maculans (blackleg of crucifers ... 447 1 hit [ascomycetes] hypothetical protein [Leptosphaeria maculans]
    . Pyrenophora teres f. teres 0-1 444 1 hit [ascomycetes] hypothetical protein PTT_09202 [Pyrenophora teres f. teres 0-1]
    . Coccidioides posadasii str. Silveira 404 1 hit [ascomycetes] hypothetical protein CPSG_07345 [Coccidioides posadasii]
    . Coccidioides posadasii C735 delta SOWGp 401 2 hits [ascomycetes] initiation-specific alpha-1,6-mannosyltransferase, putative [Coccidioides posadasii C735 delta SOWGp]
    . Coccidioides immitis RS 400 1 hit [ascomycetes] hypothetical protein CIMG_06774 [Coccidioides immitis RS]
    . Ajellomyces dermatitidis SLH14081 399 2 hits [ascomycetes] alpha-1,6-mannosyltransferase subunit [Ajellomyces dermatitidis SLH14081]
    . Ajellomyces dermatitidis ER-3 399 1 hit [ascomycetes] alpha-1,6-mannosyltransferase [Ajellomyces dermatitidis ER-3]
    . Ajellomyces capsulatus H88 398 1 hit [ascomycetes] alpha-1,6-mannosyltransferase-like protein [Ajellomyces capsulatus H88]
    . Coccidioides posadasii 387 1 hit [ascomycetes] mannosyltransferase-like protein [Coccidioides posadasii]
    . Uncinocarpus reesi 386 2 hits [ascomycetes] conserved hypothetical protein [Uncinocarpus reesi 1704]
    . Aspergillus niger CBS 531.88 384 1 hit [ascomycetes] alpha-1,6-mannosyltransferase subunit (Och1) [Aspergillus niger]
    . Aspergillus niger 384 1 hit [ascomycetes] alpha-1,6-mannosyltransferase subunit [Aspergillus niger]
    . Ajellomyces capsulatus G186AR 381 1 hit [ascomycetes] alpha-1,6-mannosyltransferase [Ajellomyces capsulatus G186AR]
    . Paracoccidioides brasiliensis Ph18 380 1 hit [ascomycetes] initiation-specific alpha-1,6-mannosyltransferase [Paracoccidioides brasiliensis Ph18]
    . Aspergillus fumigatus A1653 380 1 hit [ascomycetes] alpha-1,6-mannosyltransferase subunit (Och1), putative [Aspergillus fumigatus A1653]
    . Nectria haematococca mpVII 181 380 2 hits [ascomycetes] alpha-1,6-mannosyltransferase subunit (Och1), putative [Nectria haematococca mpVII 181]
    . Aspergillus fumigatus AF293 380 1 hit [ascomycetes] alpha-1,6-mannosyltransferase [Aspergillus fumigatus AF293]
    . Paracoccidioides brasiliensis Ph03 380 1 hit [ascomycetes] initiation-specific alpha-1,6-mannosyltransferase [Paracoccidioides brasiliensis Ph03]
    . Arthrobotrys otiae CBS 113480 379 2 hits [ascomycetes] alpha-1,6-mannosyltransferase [Arthrobotrys otiae CBS 113480]
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Sclerotinia sclerotiorum 1980 UF.70 [ascomycetes] taxid 665079
ref|XP_001595070.1| hypothetical protein SS1G_03158 [Sclerotinia sclerotiorum] 241 9 hits [ascomycetes] Och1p [Saccharomyces cerevisiae S288c] >gi|400663|sp|P31755
ref|XP_001222309.1| hypothetical protein SS1G_03158 [Sclerotinia sclerotiorum] 241 2 hits [ascomycetes] Ochlp [Saccharomyces cerevisiae S288c] >gi|400663|sp|P31755

Botryotinia fuckeliana B05.10 [ascomycetes] taxid 332648
ref|XP_001553998.1| hypothetical protein BC1G_07558 [Botryotinia fuckeliana] 238 1 hit [ascomycetes] Ochlp [Saccharomyces cerevisiae AWRI796] >gi|323354952|gb|EFQ36329.1
ref|XP_001553998.1| hypothetical protein BC1G_07558 [Botryotinia fuckeliana] 238 2 hits [ascomycetes] alpha-1,6-mannosyltransferase Ochlp [Schizosaccharomyces pombe] >gi|323354952|gb|EFQ36329.1

Neurospora crassa OR74A [ascomycetes] taxid 367110
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Glomerella graminicola M1.001 [ascomycetes] taxid 645133
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ref|XP_001967497.1| hypothetical protein NCU00609 [Neurospora crassa] 471 5e-131

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ref|XP_001967497.1| hypothetical protein NCU00609 [Neurospora crassa] 464 9e-129
ref|XP_001967497.1| hypothetical protein NCU00609 [Neurospora crassa] 464 9e-129

Chaetomium globosum CBS 148.51 [ascomycetes] taxid 306901
ref|XP_001967497.1| hypothetical protein CHGG_06214 [Chaetomium globosum] 1e-127
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Podospora anserina S mat+ [ascomycetes] taxid 515849
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**Magnaporthe oryzae 70-15** [ascomycetes] taxid 242507
ref|XP_366783.1| hypothetical protein MGG_02859 [Magnaporthe... 460 2e-127
ref|XP_001522514.1| hypothetical protein MGCH7_ch7g617 [Ma... 460 2e-127
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gb|EDK01686.1| hypothetical protein MGG_02859 [Magnaporthe... 460 2e-127

**Phaeosphaeria nodorum SN15** [ascomycetes] taxid 321614
ref|XP_001793513.1| hypothetical protein SNOG_02920 [Phaeo... 457 1e-126
gb|EAT89651.1| hypothetical protein SNOG_02920 [Phaeosphae... 457 1e-126

**Pyrenophora tritici-repentis Pt-1C-BFP** [ascomycetes] taxid 426418
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gb|EDU41602.1| initiation-specific alpha-1,6-mannosyltrans... 447 1e-123

**Leptosphaeria maculans** (blackleg of crucifers fungus, ...) [ascomycetes] taxid 5022
emb|CBY02465.1| hypothetical protein [Leptosphaeria maculans] 447 1e-123

**Pyrenophora teres f. teres 0-1** [ascomycetes] taxid 861557
gb|EFQ93438.1| hypothetical protein PTT_09202 [Pyrenophora... 444 8e-123

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gb|EFX02215.1| alpha-mannosyltransferase [Grosmannia clav... 441 8e-122

**Tuber melanosporum Mel28** [ascomycetes] taxid 656061
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ref|XP_389601.1| hypothetical protein FG09425.1 [Gibberell... 428 7e-118

**Metarhizium anisopliae ARSEF 23** [ascomycetes] taxid 655844
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gb|EU410172.1| hypothetical protein NECHADRAFT_105919 [Nec... 422 5e-116
ref|XP_003047377.1| glycosyltransferase family 32 [Nectria... 157 3e-36
gb|EU39024.1| glycosyltransferase family 32 [Nectria haem... 157 3e-36

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Aspergillus terreus NIH2624 [ascomycetes] taxid 341663
hypothetical protein ATEG_05779 [Aspergill... 386 3e-105

Aspergillus niger CBS 513.88 [ascomycetes] taxid 425011
alpha-1,6-mannosyltransferase subunit ... 384 1e-104

Aspergillus niger [ascomycetes] taxid 5061
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Ajellomyces capsulatus G186AR [ascomycetes] taxid 447093
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Paracoccidioides brasiliensis Pb18 [ascomycetes] taxid 502780
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Neosartorya fischeri NRRL 181 [ascomycetes] taxid 331117
ref|XP_001275987.1| alpha-1,6-mannosyltransferase subunit (Och1...
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380 2e-103

Paracoccidioides brasilensis Pb03 [ascomycetes] taxid 482561
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380 2e-103

Arthroderma otae CBS 113480 [ascomycetes] taxid 554155
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379 5e-103

Aspergillus clavatus NRRL 1 [ascomycetes] taxid 344612
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378 8e-103

Penicillium marneffei ATCC 18224 [ascomycetes] taxid 441960
ref|XP_002147474.1| alpha-1,6-mannosyltransferase subunit (Och1...
376 4e-102

Aspergillus oryzae RIB40 [ascomycetes] taxid 510516
ref|XP_001823952.2| alpha-1,6-mannosyltransferase subunit (Och1...
375 8e-102

Talaromyces stipitatus ATCC 10500 [ascomycetes] taxid 441959
ref|XP_002481608.1| alpha-1,6-mannosyltransferase subunit (Och1...
372 5e-101

Aspergillus nidulans FGSC A4 [ascomycetes] taxid 227321
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371 9e-101

Aspergillus flavus NRRL 3357 [ascomycetes] taxid 332952
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- gb|AAQ11191.1| putative mannosyltransferase [Pichia angusta] 294 2e-77
- gb|AAS77488.1| Och1p [Pichia angusta] 266 4e-69
- gb|AAQ06408.1| Ocr1p [Pichia angusta] 171 1e-40

**Pichia angusta DL-1** [ascomycetes] taxid 871575
- gb|EFW95452.1| putative mannosyltransferase [Pichia angusta] 294 2e-77
- gb|EFW97215.1| chromatin structure remodeling complex subunit [Pichia angusta] 266 4e-69
- gb|EFW96809.1| putative mannosyltransferase [Pichia angusta] 171 1e-40

**Debaryomyces hansenii CBS767** [ascomycetes] taxid 284592
- ref|XP_002770210.1| DEHA2C13508p [Debaryomyces hansenii CBS767] 291 7e-77
- ref|XP_461124.2| DEHA2F17534p [Debaryomyces hansenii CBS767] 252 5e-65

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- emb|CAR65573.1| DEHA2C13508p [Debaryomyces hansenii] 291 7e-77
- emb|CAG89506.2| DEHA2F17534p [Debaryomyces hansenii] 252 5e-65

**Scheffersomyces stipitis CBS 6054** [ascomycetes] taxid 322104
- ref|XP_001383155.2| membrane-bound alpha-1,6-mannosyltransferase [Scheffersomyces stipitis CBS 6054] 288 6e-76
- gb|ABN65126.2| membrane-bound alpha-1,6-mannosyltransferase [Scheffersomyces stipitis CBS 6054] 288 6e-76
- ref|XP_001384720.2| hypothetical protein PICST_72561 [Scheffersomyces stipitis CBS 6054] 260 3e-67
- gb|ABN66691.2| predicted protein [Scheffersomyces stipitis CBS 6054] 260 3e-67

**Candida albicans** [ascomycetes] taxid 5476
- gb|AAL49987.1| putative mannosyltransferase [Candida albicans] 284 1e-74

**Candida albicans SC5314** [ascomycetes] taxid 237561
- ref|XP_716632.1| mannosyltransferase Och1p [Candida albicans SC5314] 284 1e-74
- gb|EAK97637.1| mannosyltransferase Och1p [Candida albicans SC5314] 284 1e-74
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- ref|XP_716693.1| potential mannosyltransferase Hoc1p [Candida albicans SC5314] 248 9e-64
- gb|EAK97701.1| potential mannosyltransferase Hoc1p [Candida albicans SC5314] 248 9e-64
- gb|EAK97764.1| potential mannosyltransferase Hoc1p [Candida albicans SC5314] 248 9e-64

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- gb|EEQ44644.1| hypothetical protein CAWG_02918 [Candida albicans WO-1] 284 1e-74

**Candida dubliniensis CD36** [ascomycetes] taxid 573826
- ref|XP_002419506.1| initiation-specific alpha-1,6-mannosyltransferase [Candida dubliniensis CD36] 283 4e-74
- emb|CAX43101.1| initiation-specific alpha-1,6-mannosyltransferase [Candida dubliniensis CD36] 283 4e-74
- ref|XP_002420925.1| alpha-1,6-mannosyltransferase, putative [Candida dubliniensis CD36] 248 1e-63
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gb|EER33436.1| hypothetical protein CTRG_02254 [Candida tropicalis MYA-3404] 281 1e-73
ref|XP_002548355.1| hypothetical protein CTRG_02832 [Candida tropicalis MYA-3404] 237 2e-60
gb|EER34014.1| hypothetical protein CTRG_02832 [Candida tropicalis MYA-3404] 237 2e-60

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**Pichia pastoris GS115** [ascomycetes] taxid 644223
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**Pichia minuta var. minuta** [ascomycetes] taxid 53936
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gb|AAS53836.1| AFR465Cp [Ashbya gossypii ATCC 10895] 233 3e-59

**Lachancea thermotolerans CBS 6340** [ascomycetes] taxid 559295
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gb|CAR30406.1| KLTH0H09064p [Lachancea thermotolerans] 236 4e-60

**Lodderomyces elongisporus NRRL YB-4239** [ascomycetes] taxid 379508
ref|XP_0015236176.1| hypothetical protein LELG_02734 [Lodderomyces elongisporus NRRL YB-4239] 258 9e-67
gb|EDK44555.1| hypothetical protein LELG_02734 [Lodderomyces elongisporus NRRL YB-4239] 258 9e-67
ref|XP_001523919.1| conserved hypothetical protein [Lodderomyces elongisporus NRRL YB-4239] 252 6e-65
gb|EDK46551.1| conserved hypothetical protein [Lodderomyces elongisporus NRRL YB-4239] 252 6e-65
Zygosaccharomyces rouxii CBS 732 [ascomycetes] taxid 559307
ref|XP_002498104.1| ZYRO0G02310p [Zygosaccharomyces rouxii] 258 1e-66
ref|XP_002498395.1| ZYRO0G09284p [Zygosaccharomyces rouxii] 230 3e-58

Zygosaccharomyces rouxii [ascomycetes] taxid 4956
emb|CAR29171.1| ZYRO0G02310p [Zygosaccharomyces rouxii] 258 1e-66
emb|CAR29462.1| ZYRO0G09284p [Zygosaccharomyces rouxii] 230 3e-58

Vanderwaltozyma polyspora DSM 70294 [ascomycetes] taxid 436907
ref|XP_001647504.1| hypothetical protein Kpol_1018p186 [Vand... 257 2e-66
gb|EDO19646.1| hypothetical protein Kpol_1018p186 [Vanderwa... 257 2e-66
ref|XP_001644022.1| hypothetical protein Kpol_1026p11 [Van... 240 2e-61
gb|EDO16164.1| hypothetical protein Kpol_1026p11 [Vanderwa... 240 2e-61
ref|XP_001645868.1| hypothetical protein Kpol_1054p58 [Van... 233 5e-59
gb|EDO18010.1| hypothetical protein Kpol_1054p58 [Vanderwa... 233 5e-59

Kluyveromyces lactis NRRL Y-1140 [ascomycetes] taxid 284590
ref|XP_452168.1| hypothetical protein [Kluyveromyces lacti... 248 9e-64
ref|XP_456072.1| hypothetical protein [Kluyveromyces lacti... 243 3e-62

Kluyveromyces lactis [ascomycetes] taxid 28985
emb|CAH02561.1| KLLA0B14322p [Kluyveromyces lactis] 248 9e-64
emb|CAD21465.1| mannosyltransferase [Kluyveromyces lactis] 243 3e-62
emb|CAG98780.1| KLLA0F22220p [Kluyveromyces lactis] 243 3e-62

Candida glabrata CBS 138 [ascomycetes] taxid 284593
ref|XP_445987.1| hypothetical protein [Candida glabrata CB... 243 3e-62
ref|XP_444841.1| hypothetical protein [Candida glabrata CB... 202 7e-50

Candida glabrata [ascomycetes] taxid 5478
emb|CAG58911.1| unnamed protein product [Candida glabrata] 243 3e-62
emb|CAG57734.1| unnamed protein product [Candida glabrata] 202 7e-50

Saccharomyces cerevisiae S288c [ascomycetes] taxid 559292
ref|NP_011477.1| Och1p [Saccharomyces cerevisiae S288c] 241 1e-61
tpg|DA0A08062.1| TPA: Mannosyltransferase of the cis-Golgi ... 241 1e-61
ref|NP_012609.1| Hoc1p [Saccharomyces cerevisiae S288c] 239 6e-61
tpg|DA0A08861.1| TPA: Alpha-1,6-mannosyltransferase involve... 239 6e-61

Saccharomyces cerevisiae (yeast, ...) [ascomycetes] taxid 4932
sp|P31755|OCH1_YEAST RecName: Full=Initiation-specific a... 241 1e-61
dbj|BA01869.1| mannosyltransferase [Saccharomyces cerevisi... 241 1e-61
emb|CAA96740.1| OCH1 [Saccharomyces cerevisiae] 241 1e-61
Saccharomyces cerevisiae YJM789 [ascomycetes] taxid 307796

Saccharomyces cerevisiae RM11-1a [ascomycetes] taxid 285006

Saccharomyces cerevisiae AWRI1631 [ascomycetes] taxid 545124

Saccharomyces cerevisiae JAY291 [ascomycetes] taxid 574961

Saccharomyces cerevisiae EC1118 [ascomycetes] taxid 643680

Saccharomyces cerevisiae Vin13 [ascomycetes] taxid 764099

Schizosaccharomyces pombe 972b- [ascomycetes] taxid 284812

Schizosaccharomyces pombe [ascomycetes] taxid 4896

Saccharomyces cerevisiae FostersB [ascomycetes] taxid 764102

Saccharomyces cerevisiae AWRI796 [ascomycetes] taxid 764097

Saccharomyces cerevisiae JAY291 [ascomycetes] taxid 574961

Saccharomyces cerevisiae EC1118 [ascomycetes] taxid 643680

Saccharomyces cerevisiae Vin13 [ascomycetes] taxid 764099

Schizosaccharomyces pombe [ascomycetes] taxid 4896
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<td>. . . . . . . . . . . . . Verticillium albo-atrum VaMs.102 2 hits 1 orgs [Verticillium; Verticillium albo-atrum]</td>
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<td>. . . . . . . . . . . . . . Pleosporaceae]</td>
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<td>. . . . . . . . . . . . . Pyrenophora teres f. teres 0-1 1 hits 1 orgs [Pyrenophora teres; Pyrenophora teres f. teres]</td>
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<td>. . . . . . . . . . . . . . . . . Coccidioides posadasii C735 delta SOWgp . 2 hits 1 orgs</td>
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Paracoccidioides brasiliensis ............... 4 hits    3 orgs [Paracoccidioides]
Paracoccidioides brasiliensis Pb18 ....... 1 hits    1 orgs
Paracoccidioides brasiliensis Pb03 ....... 1 hits    1 orgs
Paracoccidioides brasiliensis Pb01 ........ 2 hits    1 orgs
Ajellomyces .....................................  6 hits    5 orgs [Ajellomycesaceae]
Ajellomyces dermatitidis ....................  3 hits    2 orgs
Ajellomyces dermatitidis SLH14081 .........  2 hits    1 orgs
Ajellomyces dermatitidis ER-3 .............  1 hits    1 orgs
Ajellomyces capsulatus ......................  3 hits    3 orgs
Ajellomyces capsulatus H88 ...............  1 hits    1 orgs
Ajellomyces capsulatus G186AR .............  1 hits    1 orgs
Ajellomyces capsulatus H143 ...............  1 hits    1 orgs
Uncinocarpus reesii 1704 .....................  2 hits    1 orgs [Onygenaceae; Uncinocarpus; Uncinocarpus reesii]
Arthrodermataceae ..............................  8 hits    4 orgs
Arthroderma ......................................  6 hits    3 orgs
Arthroderma otae CBS 113480 ...............  2 hits    1 orgs [Arthroderma otae]
Arthroderma benhamiae CBS 112371 ..........  2 hits    1 orgs [Arthroderma benhamiae]
Arthroderma gypseum CBS 118893 ............  2 hits    1 orgs [Arthroderma gypseum]
Trichophyton verrucosum HKI 0517 ..........  2 hits    1 orgs [mitosporic Arthrodermataceae; Trichophyton; Trichophyton verrucosum]
Trichocomaceae .................................. 24 hits    14 orgs [Eurotiales]
mitosporic Trichocomaceae ..................... 14 hits    9 orgs
Aspergillus ...................................... 10 hits    7 orgs
Aspergillus terreus NIH2624 ...............  2 hits    1 orgs [Aspergillus terreus]
Aspergillus niger ................................  2 hits    2 orgs
Aspergillus niger CBS 513.88 ...............  1 hits    1 orgs
Aspergillus clavatus NRRL 1 ...............  2 hits    1 orgs [Aspergillus clavatus]
Aspergillus oryzae .........................  2 hits    2 orgs
Aspergillus oryzae RIB40 ....................  1 hits    1 orgs
Aspergillus flavus NRRL3357 ...............  2 hits    1 orgs [Aspergillus flavus]
Penicillium ......................................  4 hits    2 orgs
Penicillium marneffei ATCC 18224 ..........  2 hits    1 orgs [Penicillium marneffei]
Penicillium chrysogenum Wisconsin 54-1255  2 hits    1 orgs [Penicillium chrysogenum complex; Penicillium chrysogenum]
Neosartorya .................................  5 hits    3 orgs
Aspergillus fumigatus ......................  3 hits    2 orgs [Neosartorya fumigata]
Aspergillus fumigatus A1163 ...............  1 hits    1 orgs
Aspergillus fumigatus Af293 ...............  2 hits    1 orgs
Neosartorya fischeri NRRL 181 ............  2 hits    1 orgs [Neosartorya fischeri group; Neosartorya fischeri]
Talaromyces stipitatus ATCC 10500 ..........  2 hits    1 orgs [Talaromyces; Talaromyces stipitatus]
Aspergillus nidulans FGSC A4 .............  3 hits    1 orgs [Emericella; Emericella nidulans; mitosporic Emericella nidulans]
Tuber ............................................  2 hits    2 orgs [Pezizomycetes; Pezizales; Tuberaceae]
Tuber melanosporum ..........................  2 hits    2 orgs
Tuber melanosporum Mel28 ..................  1 hits    1 orgs
Saccharomycetales ............................ 106 hits   40 orgs [Saccharomycotina; Saccharomycetes]
Yarrowia ........................................ 3 hits 2 orgs [Dipodascaceae]
Yarrowia lipolytica .............................. 3 hits 2 orgs
Yarrowia lipolytica CLIB122 ..................... 1 hits 1 orgs
Debaryomycetaceae ................................ 16 hits 5 orgs
Meyerozyma guilliermondii ATCC 6260 ............. 4 hits 1 orgs [Meyerozyma; Meyerozyma guilliermondii]
Debaryomyces ....................................... 4 hits 2 orgs
Debaryomyces hansenii ......................... 4 hits 2 orgs
Debaryomyces hansenii CBS767 .................... 2 hits 1 orgs [Debaryomyces hansenii var. hansenii]
Scheffersomyces stipitis CBS 6054 ................. 4 hits 1 orgs [Scheffersomyces; Scheffersomyces stipitis]
Lodderomyces elongisporus NRRL YB-4239 ........... 4 hits 1 orgs [Lodderomyces; Lodderomyces elongisporus]
Saccharomycetaceae ................................ 67 hits 27 orgs
Pichia ............................................. 11 hits 4 orgs
Pichia angusta .................................... 6 hits 2 orgs
Pichia angusta DL-1 ............................ 3 hits 1 orgs
Pichia pastoris GS115 ........................... 4 hits 1 orgs [Pichia pastoris]
Pichia minuta var. minuta ...................... 1 hits 1 orgs [Pichia minuta]
Ashbya gossypii ATCC 10895 ..................... 4 hits 1 orgs [Eremothecium; Eremothecium gossypii]
Lachancea .......................................... 4 hits 2 orgs
Lachancea thermotolerans .......................... 4 hits 2 orgs
Lachancea thermotolerans CBS 6340 .............. 2 hits 1 orgs
Zygosaccharomyces ................................ 4 hits 2 orgs
Zygosaccharomyces rouxii ........................ 4 hits 2 orgs
Zygosaccharomyces rouxii CBS 732 ............... 2 hits 1 orgs
Vanderwaltozyma polyspora DSM 70294 ............ 6 hits 1 orgs [Vanderwaltozyma; Vanderwaltozyma polyspora]
Kluiveromyces ..................................... 5 hits 2 orgs
Kluiveromyces lactis ............................. 5 hits 2 orgs
Kluiveromyces lactis NRRL Y-1140 .............. 2 hits 1 orgs
mitosporic Nakaseomyces ......................... 4 hits 2 orgs [Nakaseomyces]
Candida glabrata ................................... 4 hits 2 orgs
Candida glabrata CBS 138 ........................ 2 hits 1 orgs
Saccharomyces .................................... 29 hits 13 orgs
Saccharomyces cerevisiae ........................ 29 hits 13 orgs
Saccharomyces cerevisiae S288c .................... 4 hits 1 orgs
Saccharomyces cerevisiae YJM789 .................. 2 hits 1 orgs
Saccharomyces cerevisiae RM11-1a .................. 2 hits 1 orgs
Saccharomyces cerevisiae AWRI1631 ............... 1 hits 1 orgs
Saccharomyces cerevisiae JAY291 ................... 2 hits 1 orgs
Saccharomyces cerevisiae EC1118 ................... 2 hits 1 orgs
Saccharomyces cerevisiae Vin13 ..................... 2 hits 1 orgs
Saccharomyces cerevisiae FostersB ................ 1 hits 1 orgs
Saccharomyces cerevisiae AWRI796 ............... 1 hits 1 orgs
Saccharomyces cerevisiae Lalvin QA23 ............. 1 hits 1 orgs
Saccharomyces cerevisiae FostersO ................ 1 hits 1 orgs
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[mitosporic Saccharomycetales]

[Candida dublinensis]

[Candida tropicalis]

[Metschnikowia; Clavispora; Clavispora lusitaniae]

[Taphrinomycotina; Schizosaccharomycetes; Schizosaccharomycetales; Schizosaccharomycetaceae]

[Schizosaccharomyces japonicus]