Vaginal Microbe Medium

A novel medium to culture fastidious vaginal bacteria

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Background

Research into the vaginal microbiome has increased in recent years and has shown the integral role that it plays in women's health. Studies have demonstrated a link between the vaginal microbiome and many negative sequalae, including Pre-Term Birth, acquisition of STIs, development of UTIs, and Bacterial Vaginosis.

While molecular methods have generated a large amount of information, characterisation and contextualisation of many fastidious species found in this niche is still lacking. Additionally, there has also been a limited number of mechanistic studies. This can be attributed in part to the difficulty of culturing these bacteria, especially in liquid media.

This difficulty is particularly true for *Lactobacillus iners,* which is one of the most commonly found bacteria in this niche yet compared to other less-fastidious species (e.g. streptococci and other lactobacilli) relatively little is known.

A medium named Vaginal Microbe Medium (VMM) has been developed and patented which enables the growth of *L. iners* and many other bacterial species.

Methods

Development of Vaginal Microbe Medium (VMM)

A media that best supported the growth of *L. iners* was developed and named Vaginal Microbe Medium (VMM).

Testing of VMM compared to other available media

VMM's ability to support the growth of *L. iners* in comparison to other media (Columbia, supplemented MRS (L-cys, L-glut)[i] and NYCIII), was tested. *L. iners* strains from culture collections (DSM13335 and DSM13336) and patient samples (21N1 and 24C1) were used to inoculate the broths to an optical density (OD_{600}) =0.01. The OD_{600} was measured over 36 hours. All media was pre-reduced and cultures were incubated anaerobically using the Don Whitley A20.

Microscopy

L. iners cultures were prepared and incubated as above for 36 hours. Cultures were concentrated to OD_{600} = 4 and stained with 2 µM of SYTO59 for 30 minutes. Culture (2 µl) was placed onto an agar pad[ii] to reduce Brownian motion. Samples were visualised using a Leica SP8 inverted confocal microscope with a 63x/1.4 oil objective, and images were processed with Icy.

Investigating which bacterial species can be cultured using VMM

Pure cultures of 57 species (Table 1) were used to inoculate VMM agar and broth. The growth of the bacteria was confirmed by visually observing the agar for colonies and the broth for turbidity after 48 hours of anaerobic incubation.

i. Bloom SM, *et al.*. Nat Microbiol. 2022.

ii. Skinner SO , *et al.* Nat Protoc. 2013.

Results

Testing of VMM compared to other available media

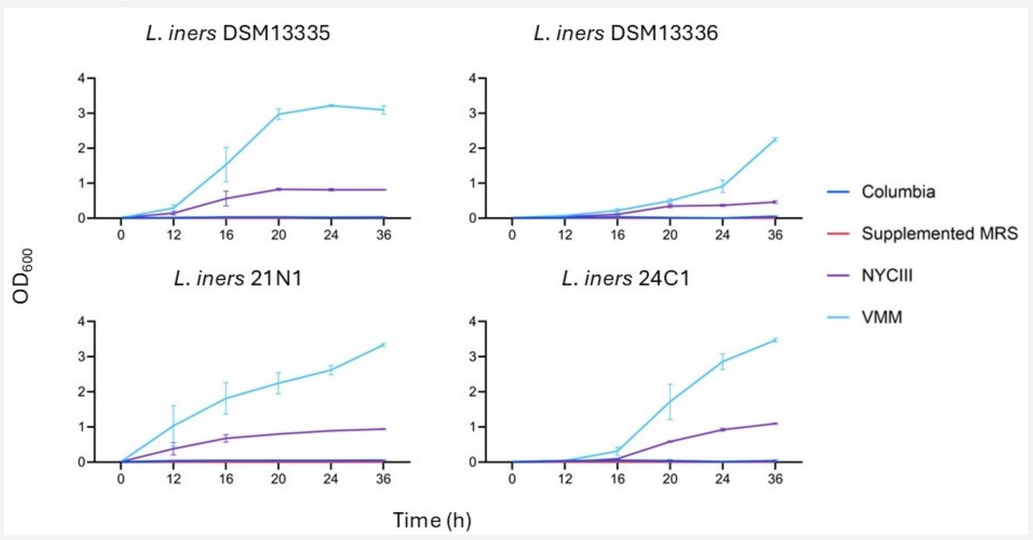


Fig 1: Growth curves of *L. iners* strains in various culture media over 36 hours.

Investigating which bacterial species can be cultured using VMM

Table 1: Bacterial species that can be cultured using VMM

- *Bifidobacterium* spp.
- *Dialister microaerophilus*
- *Fannyhessea (Atopobium) vaginae*
- *Fingolida magna*
- *Gardnerella vaginalis*
- *Lactobacillus* spp. (*crispatus, jensenii, gasseri* etc.)
- *Limosilactobacillus vaginalis*
- *Megasphaera lornae*
- *Mobiluncus mulieris*
- *Prevotella* spp.
- *Sneathia vaginalis*
- *Streptococcus agalactiae* (GBS)
- *Actinomyces neuii*
- *Alistipes* spp.
- *Alloscardovia omnicolens*
- *Bacteroides* spp.
- *Barnesiella intestinihominis*
- *Bifidobacterium spp.*
- *Citrobacter freundii*
- *Clostridium* spp.
- *Corynebacterium aurimucosum*
- *Cutibacterium acnes*
- *Enterobacter cloacae*
- *Enterococcus faecalis*
- *Escherichia coli*
- *Fusobacterium nucleatum*
- *Gemella morbillorum*
- *Granulicatella adiacens*
- *Klebsiella pneumoniae*
- *Lacticaseibacillus rhamnosus*
- *Odoribacter splanchnicus*
- *Parabacteroides merdae*
- *Parvimonas micra*
- *Peptostreptococcus anaerobius*
- *Peptoniphilus asaccharolyticus*
- *Porphyromonas gingivalis*
- *Pseudomonas aeruginosa*
- *Roseburia hominis*
- *Ruminococcus gnavus*
- *Schaalia odontolytica*
- *Staphylococcus* spp.
- *Streptococcus*
- *Tissierellia bacterium*
- *Ureaplasma parvum*
- *Veillonella atypica*
- *Winkia neuii*

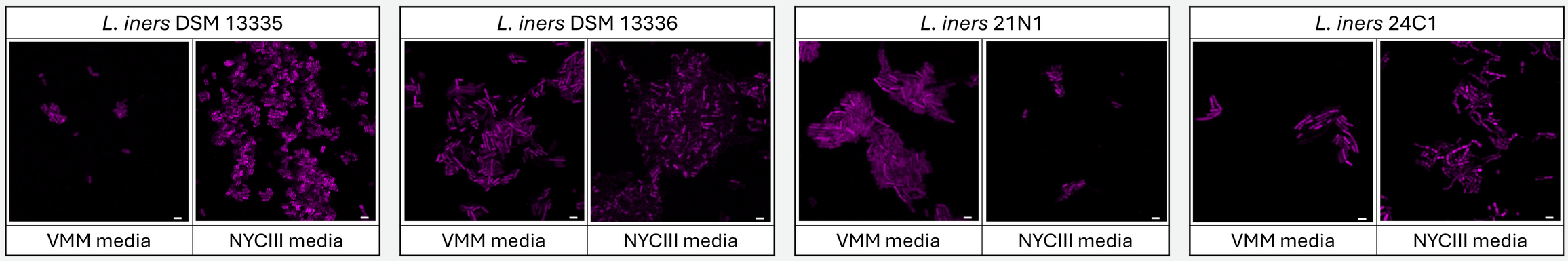


Fig 2: Microscopy of *L. iners* strains cultured in VMM and NYCIII media.

Discussion

L. iners isolates grew most rapidly in VMM, while little to no growth was observed in Columbia and Supplemented MRS. Cultures in NYCIII showed aggregation, and microscopy imaging revealed that bacteria cultured in VMM were longer and more bacillus-shaped compared to those cultured in NYCIII. These results indicate that VMM provides more favourable nutritional conditions for *L. iners* growth compared to NYCIII. VMM supports the growth of a number of bacterial isolates commonly found in the vagina, gut, and mouth and on the skin.

The ability to reliably grow *L. iners* in broth enables various experiments that will enhance our understanding of this bacterium. The ability to culture a variety of bacteria in one medium is advantageous as it removes a confounding variable when analysing results and allows co-incubation of bacteria to create defined consortia of bacteria.

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