

Systematic mutational analysis of the LytTR DNA binding domain of *Staphylococcus aureus* virulence gene transcription factor AgrA.

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SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Multiple sequence alignment of the AgrA LytTR domain of representative staphylococci strains. Conserved residues are represented by a dot. The aa residues displaying similar colours have similar properties. The conserved histidine at position 174 is highlighted in yellow.

Fig. S2. Image of a Western blot showing accumulation of AgrA in whole-cell lysates of SH1000^{-agr} IR P3-GFP + pSN-*tet-agrA* over time grown in rich media. The intensity of the band corresponding to AgrA is quantified and expressed in arbitrary units.

SUPPLEMENTARY TABLES

Table S1. Bacterial strains and plasmids used in this study.

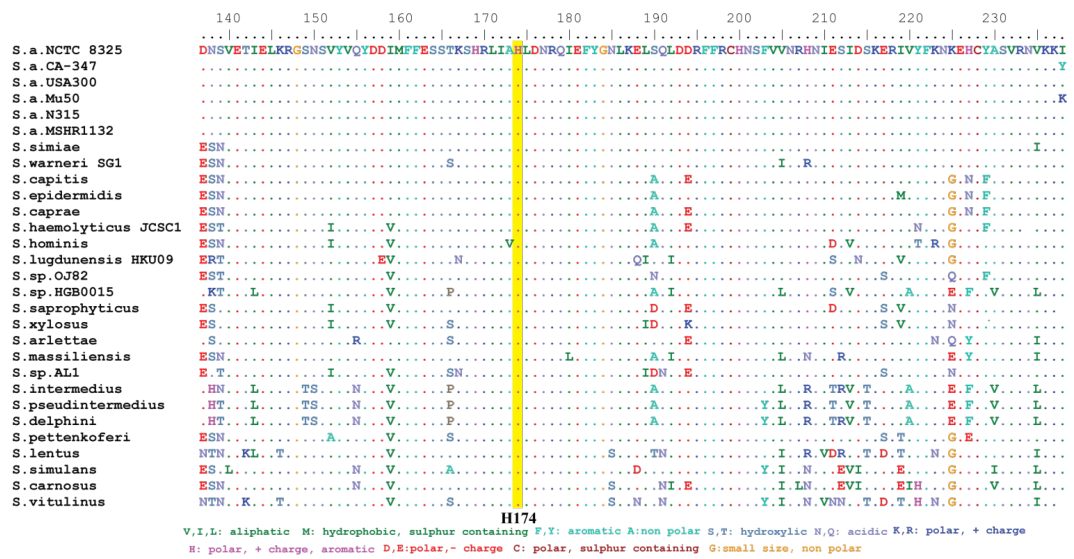
Table S2. Primers used for cloning and EMSA.

SUPPLEMENTARY MOVIES

Supplementary movie 1. aMD simulations showing the consequences of the *in silico* Y229-A mutation on AgrA LytTR domain conformation (see text for details). The Y229-A, I219, C199 and H200 aa residues are shown as van der Waals spheres in purple, orange, yellow and blue, respectively.

Supplementary movie 2. aMD simulations showing the consequences of the *in silico* I229-A

mutation on AgrA LytTR domain conformation (see text for details). The Y229, I219-A, E217 and R218 aa residues are shown as van der Waals spheres in dark green, purple, red and blue, respectively.



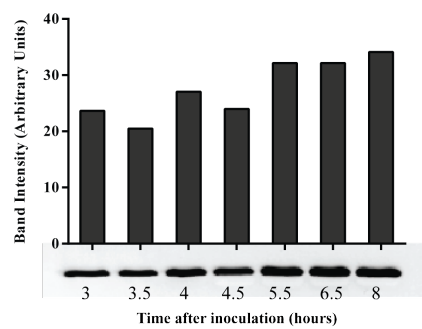


Table S1. Bacterial strains and plasmid used in this study

	Strain/plasmid	Genotype/characteristics*	Source/reference
Strains	<i>E. coli</i>		
	XL1-blue	Efficient cloning strain	Stratagene
	DC10B	Δdcm in the DH10B background; Dam methylation only	Monk et al. (2012)
	ER2566	Expression strain, IPTG inducible T7 RNA polymerase	New England Biolabs
	BL21(DE3)	Deficient in <i>lon</i> and <i>ompT</i> proteases	
		Expression strain, IPTG inducible T7 RNA polymerase	Stratagene
<i>S. aureus</i>	SH1000	Functional <i>rbsU</i> derivative of NCTC8325-4 <i>rbsU</i>	Horsburgh et al. (2002)
	SH1000-	<i>agr</i> -deficient SH1000 because of H174L mutation in <i>agrA</i>	Tsompanidou et al. (2010), this study
	SH1001	<i>agr::tet</i>	Horsburgh et al. (2002)
	SH1000- <i>agr</i> IR P3-GFP	pCL55 _{<i>agr</i> IR P3-GFP} integrated into SH1000-	this study
	SH1000 <i>agr</i> IR P3-GFP	pCL55 _{<i>agr</i> IR P3-GFP} integrated into SH1000	James et al. (2013)
	SH1001 <i>agr</i> IR P3-GFP	pCL55 _{<i>agr</i> IR P3-GFP} integrated into SH1001	James et al. (2013)
Plasmids	pCN34	Shuttle vector for Gram-positive bacteria. Amp ^R in <i>E. coli</i> , Kan ^R in <i>S. aureus</i>	Chapentier et al. (2004)
	pCN34 <i>itet</i>	pCN34 carrying a tetracycline inducible promoter Amp ^R in <i>E. coli</i> , Kan ^R in <i>S. aureus</i>	Corrigan et al. (2011)
	pCN44	Shuttle vector for Gram-positive bacteria. Amp ^R in <i>E. coli</i> , Erm ^R in <i>S. aureus</i>	Chapentier et al. (2004)
	pCL55 <i>agr</i> IR P3-GFP	pCL55 carrying P2GFP	James et al. (2013)
	pTYB2	Expression vector carrying a self-cleavable affinity tag Amp ^R	New England Biolabs
	pJR _{P2+P3}	Promoter template for <i>in vitro</i> transcription Amp ^R	Reynolds and Wigneshweraraj (2011)
	pJR28-[6His] <i>rpoD</i>	Expression vector carrying <i>rpoD</i> Kan ^R	Reynolds and Wigneshweraraj (2011)
	pAM1847	DNA bending vector, pCY7 containing the AgrA P2 tandem site Amp ^R	Reyes et al. (2011)
	pSN-P2- <i>agrA</i>	pCN34 carrying <i>agr</i> IR P2- <i>agrA</i>	this study
	pSN- <i>itet-agrA</i>	pCN34 <i>itet</i> carrying <i>agrA</i>	this study
	pSN- <i>agrA</i>	pTYB2 carrying <i>agrA</i>	this study

* Amp, ampicillin; Cm, chloramphenicol; Kan, kanamycin; Erm, erythromycin.

Table S2. Primers used for cloning and EMSA

Primer	Use	Sequence (5'-3')
P2-RBS F (for P2-AgrA)	cloning P2-RBS-AgrA-TT into pCN34; fuse to AgrA. <i>Bam</i> HI site.	CGCGGATCCCGGAATTCCTTTTCTTAAGTAGTCG
P2-RBS R (for P2-AgrA)	cloning P2-RBS-AgrA-TT into pCN34; fuse to AgrA. Contains RBS.	CGCAAAATGAAATTTTCATTTTTTCTCCTTAGGATCACC
P2-AgrA F (for P2-AgrA)	cloning P2-RBS-AgrA-TT into pCN34; fuse to P2-RBS	ATGAAATTTTCATTTGCGAAGACGATCCAAAAC
P2-AgrA R (for P2-AgrA)	cloning P2-RBS-AgrA-TT into pCN34; fuse to P2-RBS	GCATTTAGAATATTATTTTTTAACGTTTCTCACC
P2-TT F (for P2-AgrA)	cloning P2-RBS-AgrA-TT into pCN34; fuse to P2-RBS-AgrA.	CGTTAAAAAATATAATATCTAAATGCATAATAATACTG
P2-TT R (for P2-AgrA)	cloning P2-RBS-AgrA-TT into pCN34tet; <i>Kpn</i> I site.	CGGGGTACCTGTCACTTTGCTTGATATATGAGAATTATTTAACC
AgrA pCN34tet F	cloning RBS-AgrA-TT into pCN34. Contains RBS. <i>Kp</i> MI site.	CGGGGTACCGATCCTAAGGAGGAAA
AgrA pCN34tet R	cloning RBS-AgrA-TT into pCN34. <i>Eco</i> RI site.	CCGGAATTCGTCACTTTGCTTGATATATGAGAATTATTTAACC
AgrA pTYB2 F	cloning AgrA into pTYB2. <i>Nde</i> I site.	GAAGCATATGAAATTTTCATTTGCGAAGACG
AgrA pTYB2 R	cloning AgrA into pTYB2. <i>Sma</i> I site.	GAAGCCCGGTATTTTTTAACGTTTCTCACCAGTGC
P2-IR-P3 F	EMSA probe	ACCACTCTCCTCACTGTCATTATACG
P2-IR-P3 R	EMSA probe	ACATCTCTGTGATCTAGTTATATTAACATGC