

SUPPLEMENTARY INFORMATION

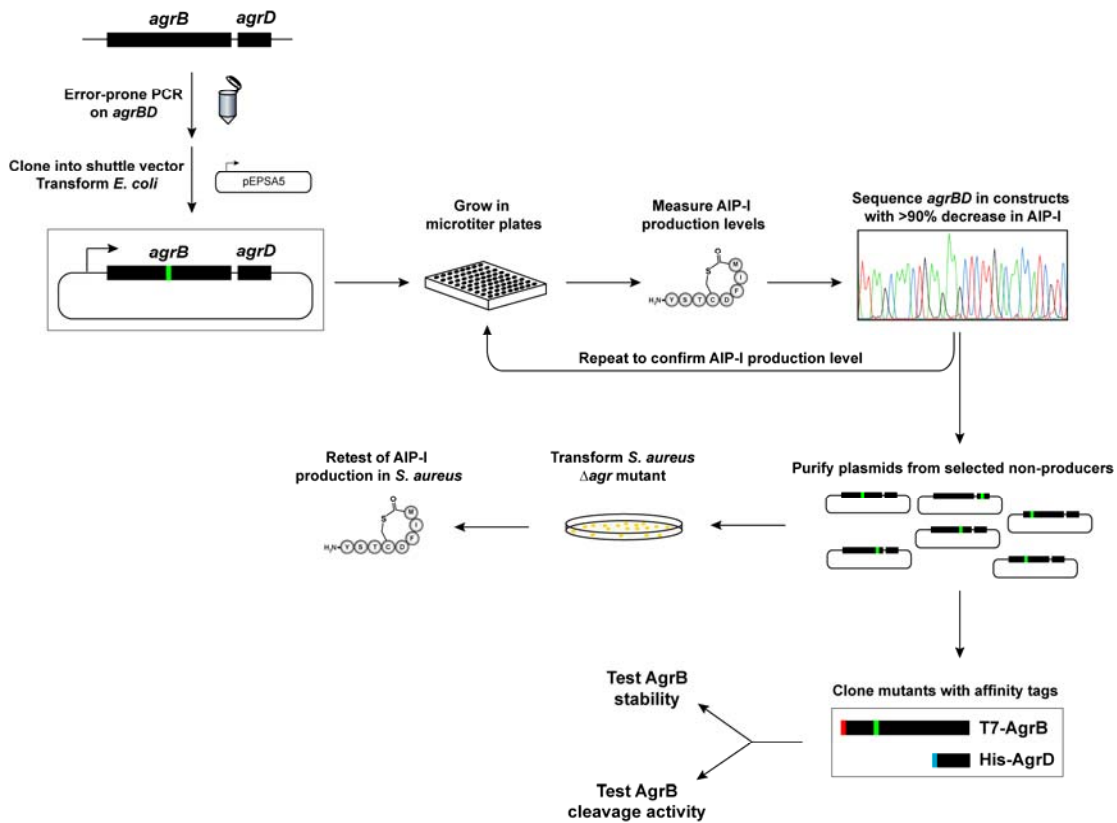


Figure 1. Schematic of the screen for identifying *agrBD* mutants. As outlined in the text, the *agrBD* genes were mutagenized by error-prone PCR and cloned onto *E. coli* – *S. aureus* shuttle vector pEPSA5. Spent media from the *E. coli* transformants was tested for AIP-I production using a *lux*-based *agr* activation bioassay with an *S. aureus* reporter strain. Transformants producing less than 10% bioluminescence were retested in the bioassay, and the mutagenized *agrBD* genes that confirmed in the retest were sequenced. After removing candidates with multiple or nonsense mutations, the pEPSA5-*agrBD* mutant plasmids were transformed into *S. aureus* Δagr strain (AH1292) for testing AIP-I production in the native host. In parallel, T7-AgrB and His6-AgrD constructs were built for testing AgrB cleavage activity.

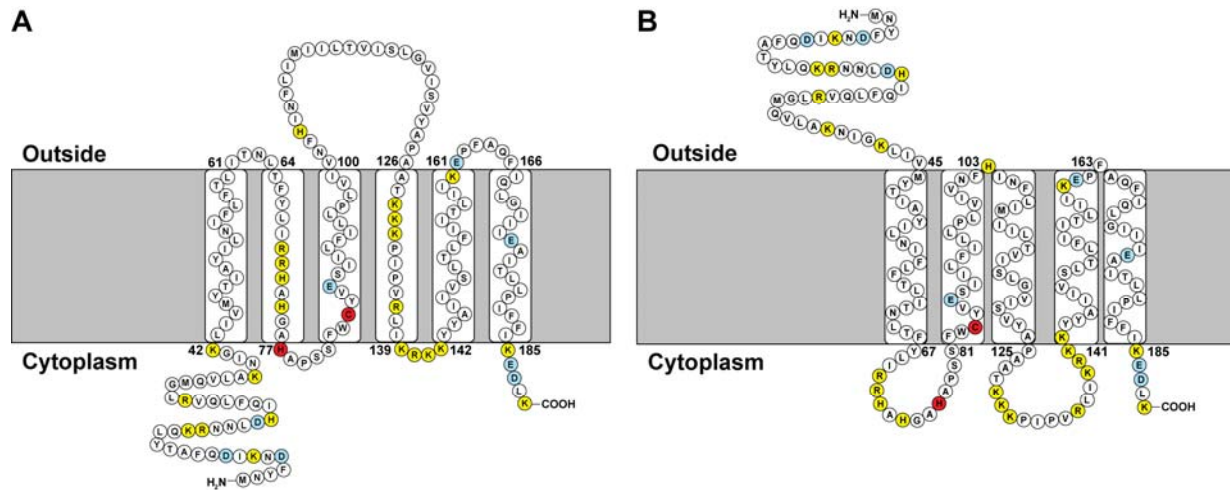


Figure S2. Topology maps of AgrB. **A**, The topology map reported by Zhang, et al. (Zhang et al., 2002) based on alkaline phosphatase fusions. **B**, AgrB topology as predicted by the topology analysis program TOPCONS (Bernsel et al., 2009). Positively charged residues are shaded yellow, negatively charged ones are blue. Red indicates the catalytic histidine and cysteine residues.

			1	10	20	30	40	50	60	70			
<i>S. aureus</i> type 1	(1)	----	LNYFDN	KIQDFATY	LQKRNLDHIQ	-FLQVR	LGQVLA	KNI	IGKLI	VMTIAYILNI	FLFLITNLT	FYILRRH	
<i>S. aureus</i> type 2	(1)	----	LNYFDN	KIQDFATY	LQKRNLDHIQ	-FLQVR	LGQVLA	KNI	IGKLI	VMTIAYILNI	FLFLITNLT	FYILRRH	
<i>S. aureus</i> type 3	(1)	----	LNYFDN	KIQDFATY	LQKRNLDHIQ	-FLQVR	LGQVLA	KNI	IGKLI	VMTIAYILNI	FLFLITNLT	FYILRRH	
<i>S. aureus</i> type 4	(1)	----	MNYFDN	KIQDFATY	LQKRNLDHIQ	-FLQVR	LGQVLA	KNI	IGKLI	VMTIAYILNI	FLFLITNLT	FYILRRH	
<i>S. capitis</i>	(1)	----	MNIIDR	KIDEFANY	LQKRNLDHIQ	-FLKVR	LGQVLA	KNI	IGKLI	VMTIAYILNI	FLFLITNLT	FYILRRH	
<i>S. carnosus</i>	(1)	----	MQVLER	KIDAWAQA	LQKRNLDRIA	-YLIK	IGLGV	FNNL	FRTI	VVYGL	LALFHV	FLYTLTVHLSYFAIRHY	
<i>S. epidermidis</i>	(1)	----	MKIIDK	KIEQFAQY	LQKRNLDHIQ	-FLKIR	LGQVLA	KNI	IGKLI	VMTIAYILNI	FLFLITNLT	FYILRRH	
<i>S. haemolyticus</i>	(1)	----	MKAIDN	KIEQFALY	LQKRNLDHIQ	-FLKVR	LGQVVA	SNLAKT	IVTV	GVALIF	HFTFLY	TLTHTNISFYILRRY	
<i>S. intermedius</i>	(1)	----	MLLDNG	IEKMLK	LQQRNLSHIE	-FLKVR	LGQVVA	SNLAKT	IVTV	GVALIF	HFTFLY	TLTHTNISFYILRRY	
<i>S. lugdunensis</i>	(1)	----	LKAI	DKKIERFARY	LQRNNLDHIQ	-FLKIR	IGQVAL	CNPF	FKTI	VTVGV	ALLFHT	FLYTLTHTLVTVFVRRF	
<i>S. warneri</i>	(1)	----	MKIIDT	KIQDFANY	LQKRNLDRIQ	-FLKVR	LGQVVA	SNLAKT	IVTV	GVALIF	HFTFLY	TLTHTNISFYILRRY	
<i>C. acetobutylicum</i>	(1)	---	MKGKSS	VMEKLA	EVVSKLNKHL	KMEGIE	-LIR	LKLG	VEI	I	FINIS	KLAILFLVSYFFGLIKETI	
<i>C. beijerinckii</i>	(1)	---	MEVDL	SKKAL	SEI	IAKL	VAKIN	RDN	QLD	NVN	-YK	MMYGL	VEI
<i>C. botulinum</i>	(1)	---	MKLSE	KFSIK	VNTY	IKK	TL	PNK	TE	ED	LQI	IKY	GV
<i>C. butyricum</i>	(1)	---	MKNKI	QISF	MER	LAETI	VNN	MNS	YLH	KE	GLE	-IQ	KL
<i>C. cellulolyticum</i>	(1)	---	MLEVI	TKK	IT	NE	IV	LV	NP	G	ITE	KA	E
<i>C. difficile</i> 1	(1)	---	MFKRY	AEK	M	T	S	V	L	C	N	M	D
<i>C. difficile</i> 2	(1)	---	MFKRL	S	Y	K	F	A	N	I	V	N	E
<i>C. novyi</i>	(1)	---	MKLSE	KFSIK	VNTY	IKK	TL	PNK	TE	ED	LQI	IKY	GV
<i>C. thermocellum</i>	(1)	---	MPLL	K	K	C	E	N	T	N	V	K	E
<i>L. grayii</i>	(1)	---	MSNYS	PNV	PL	SAR	LA	E	K	I	T	K	R
<i>L. innocua</i>	(1)	---	MSNFT	A	K	V	L	S	E	R	M	A	D
<i>L. monocytogenes</i>	(1)	---	LSNFT	A	K	V	L	S	E	R	M	A	D
<i>L. seeligeri</i>	(1)	---	MSNFT	A	K	V	L	S	E	R	M	A	D
<i>L. welshimeri</i>	(1)	---	MSNFT	V	K	V	L	S	E	R	M	A	D

			80	90	100	110	120	130	140
<i>S. aureus</i> type 1	(73)	AG	GAH	APSS	FWCY	VESI	ILE	ILL	PLV
<i>S. aureus</i> type 2	(73)	AG	GAH	AKSS	ILCY	IQSIL	TE	VFV	FL
<i>S. aureus</i> type 3	(73)	AG	GAH	APSS	FWCY	IESIT	LE	IVL	LLV
<i>S. aureus</i> type 4	(73)	AG	GAH	APSS	FWCY	IESIF	LE	IVL	LLV
<i>S. capitis</i>	(73)	AG	GAH	AKSS	LLCH	IQNI	ILE	IFL	PP
<i>S. carnosus</i>	(74)	AG	GAH	AKST	FA	CY	IESI	LE	IVL
<i>S. epidermidis</i>	(73)	AG	HT	ANSS	LLCH	IQNI	IFL	IF	PP
<i>S. haemolyticus</i>	(73)	AG	GAH	AKSS	LLCH	VQNL	AL	VAL	P
<i>S. intermedius</i>	(73)	SH	GAH	AKT	SM	LCH	VQNL	IV	F
<i>S. lugdunensis</i>	(73)	AG	GAH	AKSS	LLCH	IQNL	V	L	V
<i>S. warneri</i>	(73)	AG	GAH	AKSS	LLCH	IQNI	IFL	IF	PP
<i>C. acetobutylicum</i>	(77)	AF	GL	HAK	NS	I	V	C	T
<i>C. beijerinckii</i>	(80)	AF	GI	HAK	NS	I	S	C	T
<i>C. botulinum</i>	(74)	AAG	I	HAK	S	Y	T	C	L
<i>C. butyricum</i>	(79)	T	F	GL	HAK	S	F	I	C
<i>C. cellulolyticum</i>	(72)	L	G	V	HAK	T	Q	I	G
<i>C. difficile</i> 1	(71)	S	G	V	HAK	N	Y	K	E
<i>C. difficile</i> 2	(71)	T	G	V	HAK	N	Y	K	E
<i>C. novyi</i>	(74)	AAG	I	HAK	S	Y	T	C	L
<i>C. thermocellum</i>	(74)	AG	G	S	HAK	T	F	W	G
<i>L. grayii</i>	(78)	S	F	L	HAK	T	K	S	W
<i>L. innocua</i>	(78)	S	F	L	HAK	T	K	S	W
<i>L. monocytogenes</i>	(78)	S	F	L	HAK	T	K	S	W
<i>L. seeligeri</i>	(78)	S	F	L	HAK	T	K	S	W
<i>L. welshimeri</i>	(78)	S	F	L	HAK	T	K	S	W

			150	160	170	180
<i>S. aureus</i> type 1	(149)	SL	TL	FI	IT	LI
<i>S. aureus</i> type 2	(149)	YLL	V	L	S	L
<i>S. aureus</i> type 3	(149)	ST	L	F	I	T
<i>S. aureus</i> type 4	(149)	SL	I	F	I	T
<i>S. capitis</i>	(149)	Y	C	F	I	V
<i>S. carnosus</i>	(150)	A	G	I	L	I
<i>S. epidermidis</i>	(149)	Y	C	T	I	V
<i>S. haemolyticus</i>	(149)	T	M	V	L	I
<i>S. intermedius</i>	(149)	F	V	L	M	T
<i>S. lugdunensis</i>	(149)	S	I	C	F	L
<i>S. warneri</i>	(149)	Y	M	L	V	I
<i>C. acetobutylicum</i>	(155)	M	L	L	M	A
<i>C. beijerinckii</i>	(158)	S	I	M	T	L
<i>C. botulinum</i>	(152)	T	L	F	L	S
<i>C. butyricum</i>	(157)	L	I	L	M	I
<i>C. cellulolyticum</i>	(148)	L	V	I	C	F
<i>C. difficile</i> 1	(151)	V	L	L	T	I
<i>C. difficile</i> 2	(151)	V	I	C	L	I
<i>C. novyi</i>	(152)	I	L	F	L	S
<i>C. thermocellum</i>	(154)	I	A	E	L	S
<i>L. grayii</i>	(156)	L	L	T	G	I
<i>L. innocua</i>	(156)	L	L	T	G	I
<i>L. monocytogenes</i>	(156)	L	L	T	G	I
<i>L. seeligeri</i>	(156)	L	L	T	G	I
<i>L. welshimeri</i>	(156)	L	L	T	G	I

Figure S3. Sequence alignments of AgrB. All known AgrB sequences from *Staphylococcus*, *Clostridium* and *Listeria* species were aligned using Vector NTI Advance 11.0 AlignX software (Invitrogen). Residues that are completely conserved in all species are boxed in gray and residues conserved in *Staphylococcus* species are boxed in yellow. Residues where mutations were found to decrease AIP production are bolded in red.