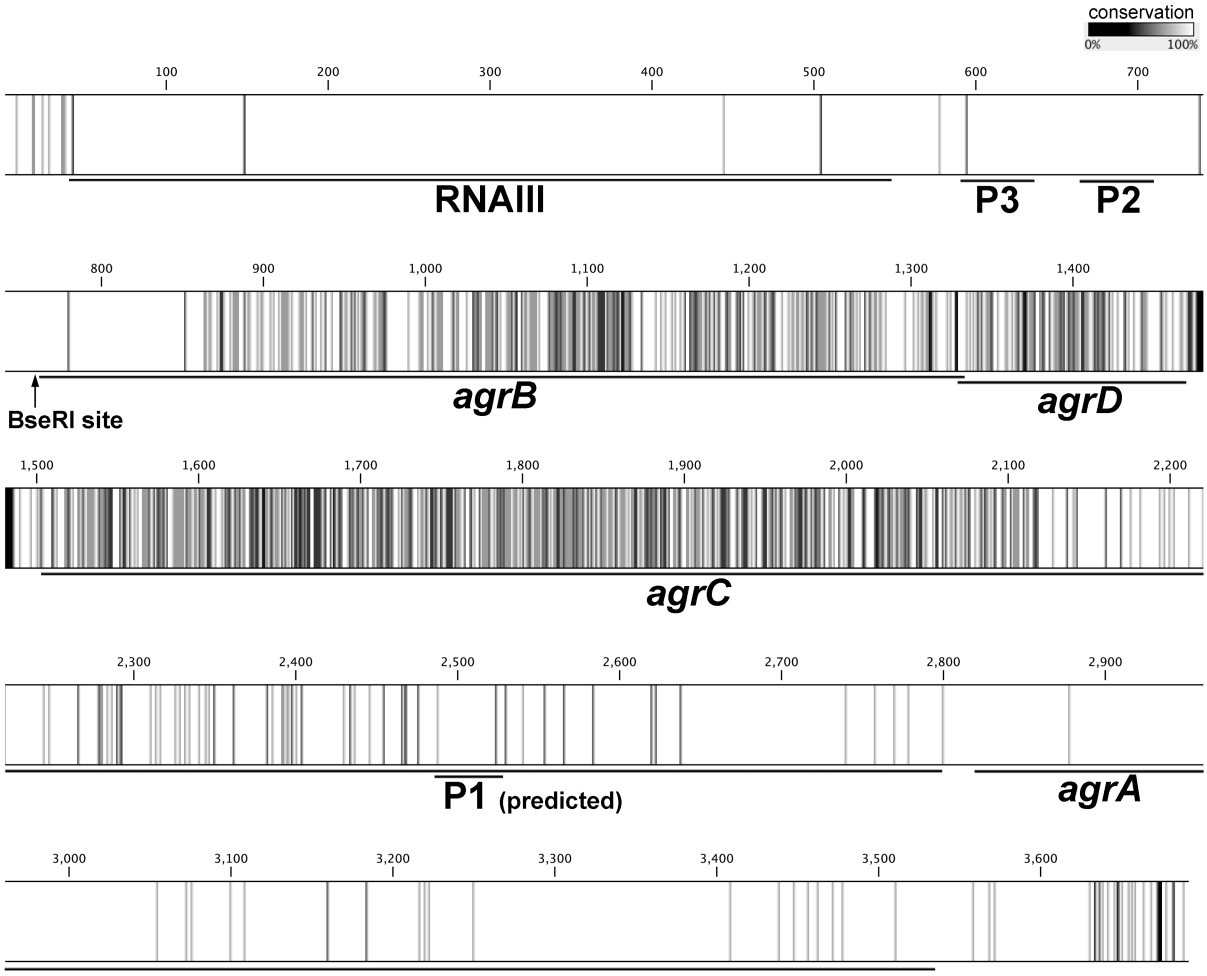

Figure S1. Sequence conservation among the four *S. aureus agr* alleles. Nucleotide sequences of the four *agr* alleles cloned in this study (*S. aureus agr*-I, -II, -III, and -IV) were aligned with CLC Sequence Viewer and conservation score was plotted via grayscale bars. Genes, promoters, and the BseRI restriction site referred to in the text are indicated; the *agrC* and *agrA* coding regions extend onto two lines. A segment known as the hypervariable region encompasses the 3' variable part of *agrB*, *agrD*, and the 5' variable region of *agrC*. The location of the *agr* P1 promoter was predicted with BPROM (Softberry) using the DNA sequence of the RsaI-PvuII fragment of the *agr* locus, also known as "A8," which was shown to contain weak, constitutive promoter activity in previous gene fusion experiments (12); predicted -10 box, *tggtattat*; predicted -35 box, *tcgatt*.

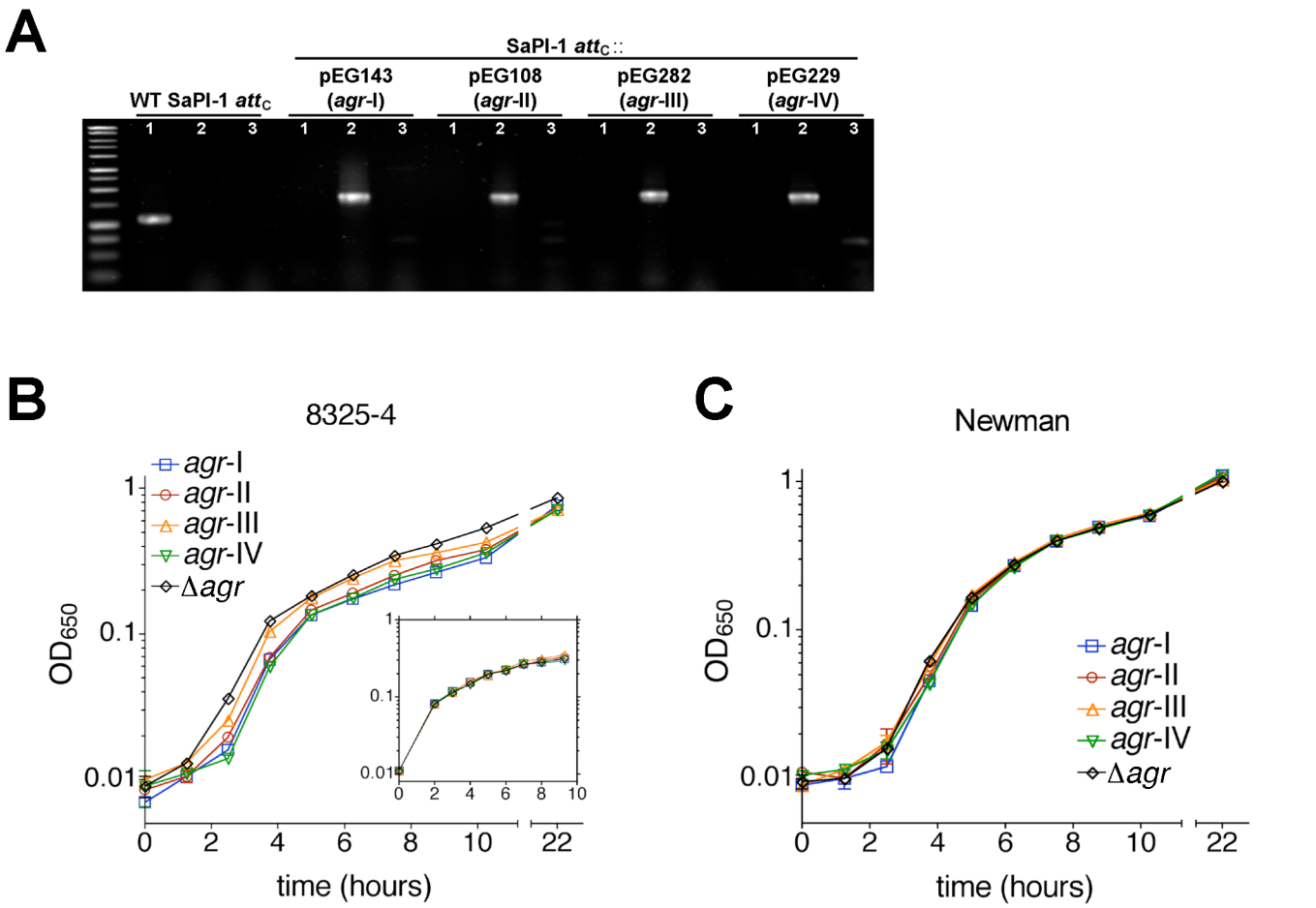

Figure S2. PCR analysis and growth of congenic *S. aureus* strains. A. PCR determination of locus orientation. DNA was extracted from 8325-4 derivatives containing the indicated *agr* construct, and amplified using the indicated primers flanking the SaPI-1 att_C site or extending out from the *agr* locus. Primer combinations: 1, att_C-F + att_C-R; 2, att_C-F + *agr*-out; 3, att_C-R + *agr*-out. B, C. Growth in liquid culture. Cells containing the indicated *agr* allele were grown in CYGP media without glucose overnight and subcultured at a 1:200 dilution into fresh media. Growth was monitored via optical density as described in the text. B, Inset. After subculture cells were grown to OD_{650} 0.05 (corresponding to approximately 2 hours), then diluted down 5-fold in fresh media, representing time point zero.

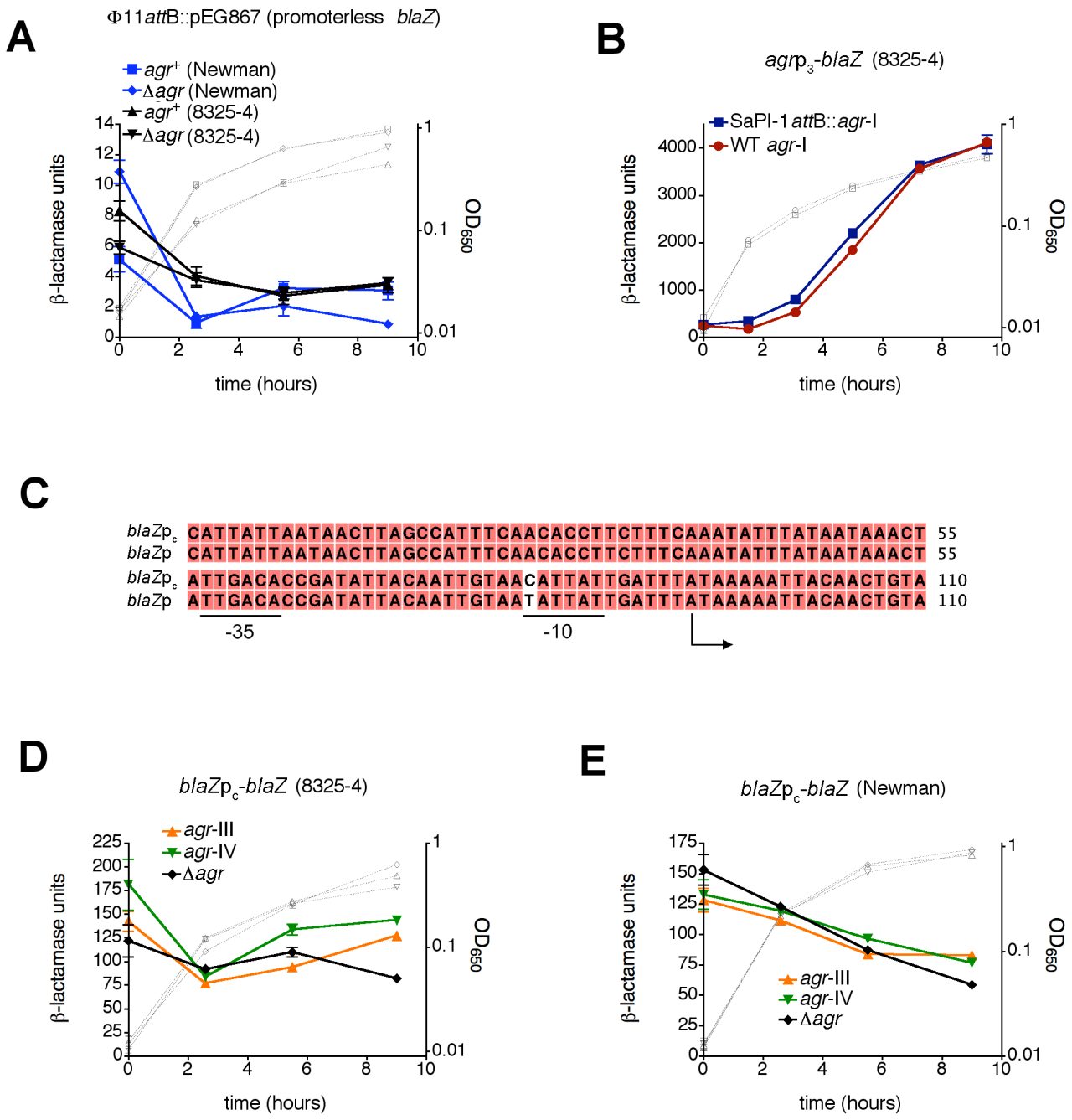

Figure S3. Tests of transcription from the ϕ11 att_B site and from the *blaZ*p$_c$ promoter. A. Congenic *agr*$^+$ or Δ*agr* strains of the indicated background containing a promoterless *blaZ* gene within the ϕ11att_B site were assayed for β-lactamase activity as described in the text. B. *S. aureus* cells containing *agr*-I at its native site (WT) or expressed from pEG143 within the SaPI-1 att_C site were assayed for β-lactamase activity as described. C. Alignment of the promoter regions from *blaZ*p (14) and a stable constitutive mutant, *blaZ*p$_c$, derived from this promoter. -35, -10, and start sites (14) are indicated. D, E. Congenic strains of the 8325-4 (D) or Newman (E) background containing the indicated *agr* allele and a plasmid-carried *blaZ*p$_c$-*blaZ* reporter (pEG832) were assayed for β-lactamase activity as described in the text. Assay data are presented as β-lactamase units ± SEM (closed symbols, left axis), and growth as optical density measurements (grey open symbols, right axis).

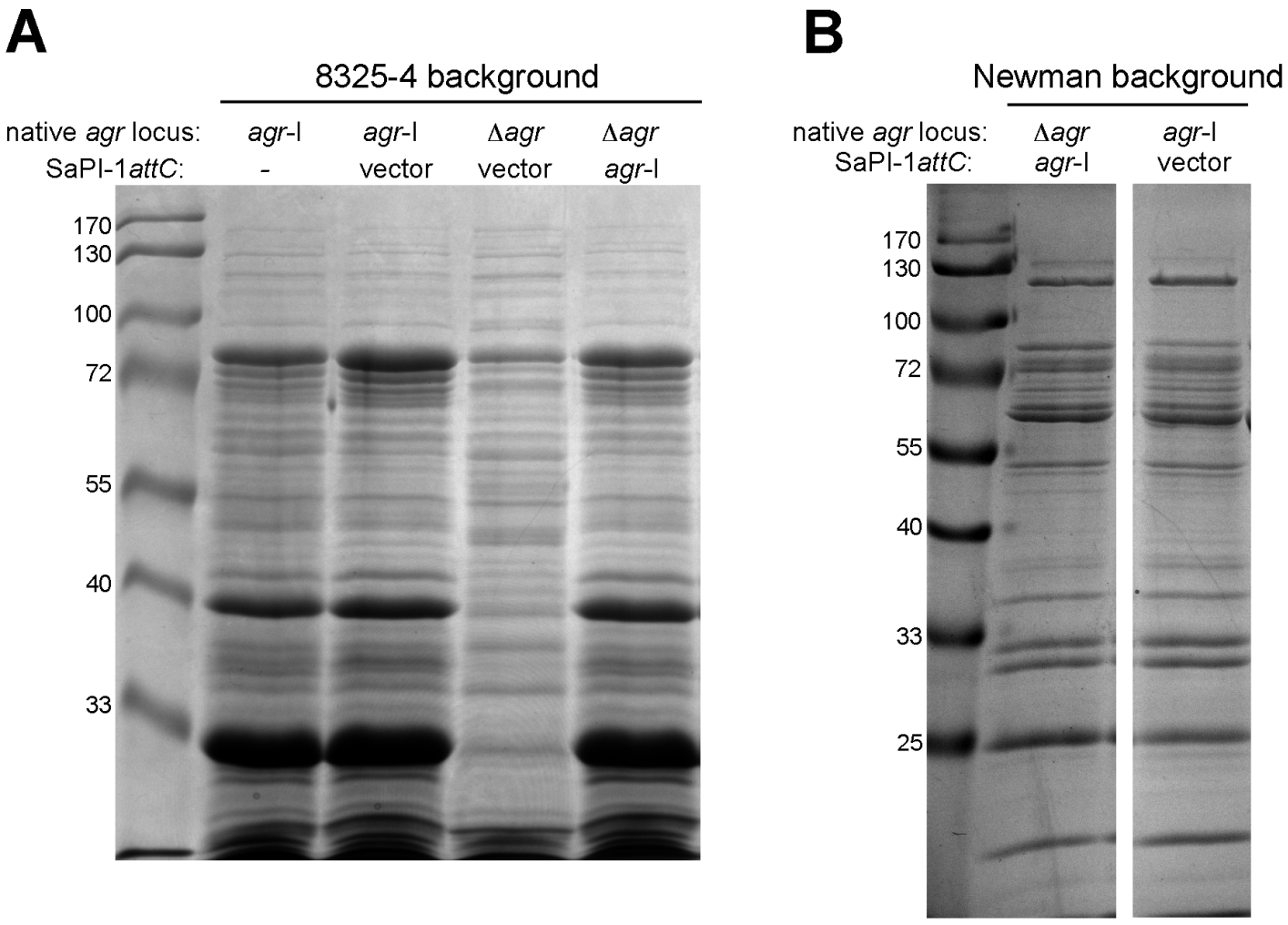

Figure S4. Comparison of exoproteins isolated from *S. aureus* strains with the *agr* operon in its native or heterologous location. Culture supernatants were collected after growth to post-exponential phase and exoproteins were isolated, separated via SDS-PAGE, and stained with Coomassie dye. A, 8325-4 strains. Lane 1, RN6734 (native *agr*-I); lane 2, EG180 (native *agr*-I, SaPI-1 att_C::vector alone); lane 3, EG248 (native *agr*$^-$, SaPI-1 att_C::vector alone); lane 4, EG170 (native *agr*$^-$, SaPI-1 att_C::*agr*-I). B, Newman strains. Lane 1, EG802 (native *agr*$^-$, SaPI-1 att_C::*agr*-I); lane 2 (same gel), EG806 (native *agr*-I, SaPI-1 att_C::vector alone).

Table S1. Strains and plasmids.

Strain or plasmid	Genotype or description	Reference
***S. aureus* strains**		
RN6734	*agr* group I prototype, derivative of NTCT8325-4	(11)
RN7206	*agr* group I prototype, derivative of NTCT8325-4 Δ*agr::tetM*	(11)
RN4220	Restriction-deficient mutant of NTCT8325-4	(2)
RN10149	Strain Newman, *agr* group I	(3)
HF6122	RN10149 Δ*agr::tetM*	This work
RN6607	*agr* group II prototype	(8)
RN3984	*agr* group III prototype	(9, 11)
RN4850	*agr* group IV prototype	(7)
RN9011	RN4220 with pRN7023	(5)
RN11679	RN4220 with pTL2787	(10); This work
EG170	RN7206 SaPI-1*att*$_c$::pEG143 (congenic *agr*-I, 8325-4)	This work
EG133	RN7206 SaPI-1*att*$_c$::pEG108 (congenic *agr*-II, 8325-4)	This work
EG292	RN7206 SaPI-1*att*$_c$::pEG282 (congenic *agr*-III, 8325-4)	This work
EG248	RN7206 SaPI-1*att*$_c$::pEG229 (congenic *agr*-IV, 8325-4)	This work
EG248	RN7206 SaPI-1*att*$_c$::pJC1111 (congenic *agr*$^-$, 8325-4)	This work
EG180	RN6734 SaPI-1*att*$_c$::pJC1111 (vector alone, 8325-4)	This work
EG802	HF6122 SaPI-1*att*$_c$::pEG143 (congenic *agr*-I, Newman)	This work
EG803	HF6122 SaPI-1*att*$_c$::pEG108 (congenic *agr*-II, Newman)	This work
EG804	HF6122 SaPI-1*att*$_c$::pEG282 (congenic *agr*-III, Newman)	This work
EG805	HF6122 SaPI-1*att*$_c$::pEG229 (congenic *agr*-IV, Newman)	This work
EG801	HF6122 SaPI-1*att*$_c$::pJC1111 (congenic *agr*$^-$, Newman)	This work
EG806	RN10149 SaPI-1*att*$_c$::pJC1111 (vector alone, Newman)	This work
***E. coli* strains**		
DH5α	Standard recipient for plasmid cloning	Promega
Plasmids		
pJC1111	Shuttle/suicide vector (Cd^R) containing SaPI-1 *att*$_S$	(5)
pEG143	pJC1111 containing *agr*-I	This work
pEG108	pJC1111 containing *agr*-II	This work
pEG282	pJC1111 containing *agr*-III	This work
pEG229	pJC1111 containing *agr*-IV	This work
pEG858	pJC1111 containing *agr*-III-IV chimera	This work
pEG859	pJC1111 containing *agr*-III-I chimera	This work
pEG860	pJC1111 containing *agr*-IV-III chimera	This work
pEG861	pJC1111 containing *agr*-I-III chimera	This work
pEG924	pJC1111 containing *agr*-II-IV chimera	This work
pEG925	pJC1111 containing *agr*-IV-II chimera	This work
pRN7023	Shuttle vector (Cm^R) containing SaPI-1 *int*	(13)
pEG813	Shuttle/suicide vector (Em^R) containing ϕ11 *att*$_P$	This work
pCN41	Shuttle vector (Em^R) containing promoterless *blaZ*	(1)
pEG867	pEG813 containing promoterless *blaZ*	This work
pEG835	pEG813 containing *agr*p3-*blaZ* fusion	This work
pTL2787	Shuttle vector (Cm^R) containing ϕ11 *int*	(10)
pEG832	pCN41 containing *blaZ*p$_C$-*blaZ*	This work
pRN9162	Shuttle vector (Em^R) containing *rot-blaZ* translational fusion	(4)
pJC1122	pUC18 containing *blaZ*p$_C$	This work
pJC1280	pCN41(Cm^R)	This work
pJC1435	pJC1280 containing *tst*p-*blaZ* transcriptional fusion	This work
pJC1287	pJC1280 containing *lukSF-PV*p-*blaZ* transcriptional fusion	This work
pJC1295	pJC1280 containing *hla*p-*blaZ* transcriptional fusion	This work
pRN6788	Shuttle vector (Cm^R) containing *spa*p-*blaZ* transcriptional fusion	(6)

Table S2. Oligonucleotide primers.

Primer name	Sequence (5' – 3'; restriction site underlined)	RE site
agrII-F	GATAACTCAGTAAGAATCCATTTCGCCCTTTGC	
agrII-R	TTGCGCCATAGGATTGTAGAGTGAAATAG	
agrIII-F	AGATAACTCAGTAAGAACCAGTTTCGCCCTTAGC	
agrIII-R	GCGAGCAAAATAAGATTTATTGGGTAGGATATTGTAGC	
agrIV-F1	CATTAGACTTATTCATAAATTTTAACGGTGGATCTC	
agrIV-R	GTTAAAAATTGCGCCATAGGATTGTAGAGTGAAATAG	
SaPI-1 att_C-F	GCTGCGGTAATGGAAAC	
SaPI-1 att_C-R	CCATAATAAATGCCTCCTCG	
agr-out	GCAAATGCACTGTATAGCTGGC	
p11att-F	TTTTGGGCCCTCTGGAATTAATGAGGCATTCTAAC	ApaI
p11att-R	ATCCCCTAGGATCGATCCCCACACAACCAAC	AvrII
Pbla-F	ATATAAGCATGCAGCTTACTATGCCATTATT	SphI
blaZtt-R	AACAGTGGCGCCTGTCACTTTGCTTGATATATGAG	NarI
Phla-F	GGTACCAGTCAGTGTATGGATG	KpnI
Phla-R	GAATTCATTTTCATCATCCTTC	EcoRI
Ppvl-F	GGTACCGCGACAGGAGAGGTAG	KpnI
Ppvl-R	GAATTCATAAAATCATTTCCTTTCTTTATAAATTTTATTAC	EcoRI
Ptst-F	GGTACCACCACTAGAAAGTGAC	KpnI
Ptst-R	GAATTCATTTTTAATTCTCCTTACTTCAAATGTGTAAACG	EcoRI

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