

Microarray-Based Analysis of the *Staphylococcus aureus* σ^B Regulon

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Microarray-based analysis of the transcriptional profiles of the genetically distinct *Staphylococcus aureus* strains COL, GP268, and Newman indicate that a total of 251 open reading frames (ORFs) are influenced by σ^B activity. While σ^B was found to positively control 198 genes by a factor of ≥ 2 in at least two of the three genetic lineages analyzed, 53 ORFs were repressed in the presence of σ^B . Gene products that were found to be influenced by σ^B are putatively involved in all manner of cellular processes, including cell envelope biosynthesis and turnover, intermediary metabolism, and signaling pathways. Most of the genes and/or operons identified as upregulated by σ^B were preceded by a nucleotide sequence that resembled the σ^B consensus promoter sequence of *Bacillus subtilis*. A conspicuous number of virulence-associated genes were identified as regulated by σ^B activity, with many adhesins upregulated and prominently represented in this group, while transcription of various exoproteins and toxins were repressed. The data presented here suggest that the σ^B of *S. aureus* controls a large regulon and is an important modulator of virulence gene expression that is likely to act conversely to RNAPIII, the effector molecule of the *agr* locus. We propose that this alternative transcription factor may be of importance for the invading pathogen to fine-tune its virulence factor production in response to changing host environments.

Transcription of DNA into RNA is catalyzed by RNA polymerase. In bacteria, one RNA polymerase generates nearly all cellular RNAs, including ribosomal, transfer, and mRNA. This enzyme consists of six subunits, $\alpha_2\beta\beta'\omega\sigma$, with $\alpha_2\beta\beta'\omega$ forming the catalytically competent RNA polymerase core enzyme (E). The core is capable of elongation and termination of transcription, but it is unable to initiate transcription at specific promoter sequences. The σ subunit, which when bound to E forms the holoenzyme (E- σ), directs the multisubunit complex to specific promoter elements and allows efficient initiation of transcription (reviewed in references 5 and 6). Therefore, σ factors provide an elegant mechanism in eubacteria to allow simultaneous transcription of a variety of genetically unlinked genes, provided all of these genes share the same promoter specificities.

In addition to the housekeeping sigma subunit, σ^{70} or σ^A , most bacteria produce one or more additional σ subunits, termed alternative σ factors, which direct the respective E- σ complex to distinct classes of promoters that contain alternative σ factor-specific sequences. Alternative σ factors have been shown in various bacteria to be of importance for survival under extreme conditions (7, 14, 23, 31, 38, 44, 49, 60, 68, 73, 78, 79, 80) and to influence virulence and pathogenicity (8, 13, 32, 35, 37, 42, 51, 57, 61, 71, 75, 78, 81).

At least six alternative σ factors are produced by the enteric bacterium *Escherichia coli* (reviewed in reference 6). Genomic sequence analysis suggests that many alternative σ factors also

exist in a number of other pathogenic species such as *Treponema pallidum* (4 alternative σ factors) (21), *Vibrio cholerae* (7 alternative σ factors) (29), *Mycobacterium tuberculosis* (12 alternative σ factors) (12), and *Pseudomonas aeruginosa* (23 alternative σ factors) (76). Two alternative σ factors, σ^B and σ^H , have been identified in *Staphylococcus aureus* (43, 82). σ^H has only recently been characterized as a bona fide *S. aureus* sigma factor, which is involved in the transcriptional regulation of DNA competence factors (56).

In contrast to σ^H , the *S. aureus* alternative transcription factor σ^B has been studied intensively. It has been shown to be involved in the general stress response (7, 24, 26, 34, 43, 44). σ^B also directly or indirectly influences the expression of a variety of genes (25, 44, 84), including many associated with virulence, such as α -hemolysin (26, 34, 84), clumping factor (58, 60), coagulase (55, 60) fibronectin-binding protein A (58), lipases (44, 84), proteases (34, 36, 84), and thermonuclease (44, 84). In addition, σ^B has been shown to influence the expression of several global virulence factor regulators, including SarA (4, 15, 25, 52), SarS (also known as SarH1) (76), and RNAPIII (4, 34). However, no effect of σ^B on *S. aureus* pathogenicity has been demonstrated in any in vivo model analyzed to date (7, 34, 60).

Besides its function in regulating virulence determinants, σ^B is likely to play a role in mediating antibiotic resistance. Inactivation of the gene coding for σ^B , *sigB*, in the homogeneously methicillin-resistant strain COL increases its susceptibility to methicillin (82) while mutations within the *rsbU*-defective strain BB255, leading to σ^B hyperproduction, are associated with an increase in glycopeptide resistance (3). Moreover, σ^B was shown to affect pigmentation (26, 44), to increase resistance to hydrogen peroxide (26, 44) and UV (26), and to

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TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype and phenotype ^a	Reference or source
Strains		
<i>E. coli</i> XL1-Blue	<i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac</i> [F' <i>proAB lacI^q</i> ZΔM15 Tn10(Tc ^r)]	Stratagene
<i>S. aureus</i>		
BB255	<i>rsbU</i> , low σ^B activity	2
COL	<i>mec</i> , highly Mc ^r clinical isolate, Mc ^r Tc ^r	41
Newman	Clinical isolate, high level of clumping factor (ATCC 25904)	17
IK181	BB255 Δ <i>rsbUVW-sigB</i> , Em ^r	44
IK183	COL Δ <i>rsbUVW-sigB</i> , Em ^r Mc ^r Tc ^r	44
IK184	Newman Δ <i>rsbUVW-sigB</i> , Em ^r	44
GP268	BB255 <i>rsbU</i> ⁺ , Tc ^r	26
Plasmids		
pAC7	Cm ^r , expression plasmid containing the P _{BAD} promoter and the <i>araC</i> gene	70
pAC7-sigB	Cm ^r , 767-bp PCR fragment of the <i>sigB</i> ORF from strain COL into pAC7	This study
pSB40N	Ap ^r , promoter probe plasmid	39
pSA0455p	Ap ^r , 360-bp PCR fragment covering the promoter region of the COL homologue of ORF N315-SA0455 into pSB40N	This study

^a Abbreviations are as follows: Ap^r, ampicillin resistant; Cm^r, chloramphenicol resistant; Em^r, erythromycin resistant; Mc^r, methicillin resistant; Tc^r, tetracycline resistant.

promote microcolony formation (1) and biofilm production (67).

The genetic organization of the *S. aureus sigB* operon (43, 82) closely resembles that of the distal part of the well-characterized homologous operon of the soil-borne gram-positive bacterium *Bacillus subtilis* (reviewed in references 28 and 65). DNA microarray technology-based analysis of the general stress response in *B. subtilis* identified 127 genes controlled by σ^B (66), and heat shock studies suggest that the σ^B regulon of this organism comprises up to 200 genes (reviewed in references 27 and 30). Because *S. aureus* σ^B seems to be a pleiotropic regulator that plays a role in a number of clinically relevant processes, a number of investigators have begun characterizing the σ^B regulon. Proteomic approaches have identified 27 *S. aureus* cytoplasmic proteins and one extracellular protein to be under the positive control of σ^B , and 11 proteins were found to be repressed by the factor (25, 84), indicating that the σ^B regulon of this pathogen is likely to comprise a much higher number of genes than known to date.

In this study, we present DNA microarray-based data from three distinct genetic backgrounds that suggest that the *S. aureus* σ^B influences the expression of at least 251 genes. Of these, 198 genes are positively controlled by σ^B while 53 genes are repressed in the presence of the alternative σ factor.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. Strains and plasmids used in this study are listed in Table 1. *S. aureus* was routinely cultured on sheep blood agar or Luria-Bertani (LB) medium with rotary agitation at 200 rpm at 35°C. Exogenous glucose was not added to the growth medium. When included, antibiotics were used at the following concentrations: ampicillin, 50 mg liter⁻¹; chloramphenicol, 40 mg liter⁻¹.

Sampling, RNA isolation, and transcriptional profiling. Overnight cultures of *S. aureus* were diluted 1:100 into fresh prewarmed LB medium and grown as described above. For experiment 1, cultures were grown to an optical density at 600 nm (OD₆₀₀) of 2, at which time RNA samples were prepared as described below. For experiment 2, cultures were grown for 9 h and sample volumes corresponding to 10¹⁰ cells were removed after 1, 3, 5, and 8 h of growth. For

RNA isolation, samples were centrifuged at 7,000 × *g* at 4°C for 5 min, the culture supernatants were removed, and the cell sediments were snap-frozen in a dry ice-alcohol mixture. Frozen cells were resuspended in 5 ml of ice-cold acetone-alcohol (1:1) and incubated for 5 min on ice. After centrifugation at 7,000 × *g* and 4°C for 5 min, cells were washed with 5 ml of TE buffer (10 mM Tris, 1 mM EDTA [pH 8]) and resuspended on ice in 900 μl of TE. The cell suspensions were transferred to 2-ml lysing matrix B tubes (Bio 101, Vista, Calif.), and the tubes were shaken in an FP120 reciprocating shaker (Bio 101) two times at 6,000 rpm for 20 s. After centrifugation at 14,000 × *g* at 4°C for 5 min, the supernatants were used for RNA isolation with the RNeasy Midi system (QIAGEN, Inc., Valencia, Calif.) according to the manufacturer's recommendations. To remove any contaminating genomic DNA, approximately 125 μg of total RNA was treated with 20 U of DNase I (Amersham Biosciences, Piscataway, N.J.) at 37°C for 30 min. The RNA was then purified with an RNeasy mini column (QIAGEN) by following the manufacturer's cleanup protocol. The integrity of the RNA preparations was analyzed by electrophoresis in 1.2% agarose-0.66 M formaldehyde gels. Reverse transcription-PCR, cDNA fragmentation, cDNA terminal labeling, and hybridization of approximately 1.5 μg of labeled cDNA to custom-made Wyeth *S. aureus* GeneChips were carried out in accordance with the manufacturer's (Affymetrix Inc., Santa Clara, Calif.) instructions for antisense prokaryotic arrays. The GeneChip contains 7,723 qualifiers representing the consensus open reading frame (ORF) sequences of the genomes of N315, Mu50, COL, 8325, 252, and 476 as well as those of N315 intergenic regions greater than 50 bp in length (P. Dunman, E. Murphy, and S. Projan, unpublished data). GeneChip arrays were scanned with the GeneArray laser scanner (Agilent Technologies, Palo Alto, Calif.). Data for biological duplicates were normalized and analyzed by using the GeneSpring, version 5.1, gene expression software package (Silicon Genetics, Redwood City, Calif.). Genes that were considered upregulated in a σ^B -dependent manner were found to demonstrate a >2-fold increase in RNA titers under σ^B -producing conditions in comparison to isogenic non- σ^B -producing cells. In addition, these genes were considered present by Affymetrix algorithms in the σ^B -producing strains and demonstrated a significant difference in expression (*t* test, with a *P* cutoff of at least 0.05). Genes considered downregulated in a σ^B -dependent manner demonstrated at least a twofold reduction in RNA transcript titers in the wild-type as opposed to their isogenic σ^B mutant background and were both considered present by the Affymetrix criteria in mutant cells and where characterized as having significantly differing amounts of transcripts based on *t* tests with a *P* cutoff of at least 0.05.

Construction of plasmids pAC7-sigB and pSA0455p. A DNA fragment constituting the *sigB* ORF of *S. aureus* COL was amplified by PCR with an upstream primer (5'-GATCATATGGCGAAGAGTTCGAAATCAGC-3') containing an NdeI site (underlined) and a downstream primer (5'-GCGAAGCTTCAAATTCATTGATGTGCTGC-3') containing a HindIII site (underlined), with italic

nucleotides corresponding to positions 2687 to 2709 and 3443 to 3463 of the sequence found under GenBank accession no. Y09929, respectively. The resulting PCR product was digested with NdeI and HindIII and cloned into plasmid pAC7 (70) to obtain pAC7-*sigB*, which was subsequently transformed by electroporation into *E. coli* XL1-Blue (Stratagene, La Jolla, Calif.). Sequence analysis and comparison confirmed the identity of the construct. For pSA0455p, a DNA fragment representing 360 bp of the N315-SA0455 promoter region of COL was generated by PCR with an upstream primer (5'-CGGATCCAGTAG TAGTGATTAGAAAAGAC-3') containing a BamHI site (underlined) and a downstream primer (5'-CGGCTCGAGATAAACTGTTGCCAGGTTCTACG-3) containing an XhoI site (underlined), with italic nucleotides corresponding to positions 227569 to 227592 and 227895 to 227919, respectively, of the sequence found under GenBank accession no. AP003130. The PCR product was digested with BamHI and XhoI and cloned into promoter probe plasmid pSB40N (39) to obtain pSA0455p. Sequence analysis confirmed the identity of the insert. Plasmid pSA0455p was transformed into *E. coli* XL1-Blue containing either compatible plasmid, pAC7-*sigB* or pAC7.

High-resolution S1 nuclease mapping. For RNA isolation from recombinant *E. coli* cultures, strains were grown at 37°C in LB supplemented with ampicillin and chloramphenicol to an OD₆₀₀ of 0.3. At this growth stage, expression of *S. aureus sigB* was induced by adding 0.0002% (wt/vol) arabinose, and cultivation was continued for an additional 3 h. Isolation of total RNA and high-resolution S1 nuclease mapping were performed as described by Kormanec (40). A 450-bp DNA fragment covering the SA0455 promoter region was amplified by PCR from pSA0455p, with universal oligonucleotide primer -47 (5'-CGCCAGGGT TTTCCAGTCCAGAC-3'), labeled at the 5' end with [γ -³²P]ATP, and mut80 primer (5'-GAGGTTCCGCGCACATTTCCTCCG-3'). Forty micrograms of RNA was hybridized to 0.02 pmol of the 5' end-labeled DNA fragment (approximately 3×10^6 cpm/pmol of probe) and treated with 100 U of S1 nuclease. The protected DNA fragment was analyzed on a DNA sequencing gel together with G+A and T+C sequencing ladders derived from the end-labeled probe (54).

RESULTS AND DISCUSSION

Identification of σ^B -regulated genes. Proteomic approaches and computational analyses, based on the method described by Petersohn and colleagues (64), indicate that the σ^B regulon of *S. aureus* comprises many more genes than described to date, suggesting that the regulon might be as large as that of the well-characterized homologous regulon of *B. subtilis* (reviewed in references 27 and 30). In an effort to better define the *S. aureus* σ^B regulon, DNA microarray studies were performed in three genetically distinct backgrounds. DNA microarray technology is a powerful tool to analyze the transcription profiles of the whole genome, provided that all genes are represented on the respective GeneChip. There is increasing evidence that extensive variation in gene content exists among strains of many pathogenic bacterial species. A genomic comparison of 36 *S. aureus* strains of divergent clonal lineage identified a very large genetic variation to be present in this pathogen, with approximately 22% of the genome being dispensable (18). The custom-made Affymetrix *S. aureus* GeneChip used in this study includes probes that monitor the expression of virtually all ORFs from six *S. aureus* genomes, making it an ideal tool for the identification of almost all transcriptional changes that are caused by the alternative transcription factor σ^B .

Two different approaches were chosen to identify σ^B -dependent genes. In experiment 1, the transcriptional profiles of three genetically distinct *S. aureus* strains harboring an intact *sigB* operon (COL, Newman, and GP268) and their isogenic Δ *rsbUVW-sigB* mutants were analyzed. For this purpose, total bacterial RNA was obtained from cells that were grown to the late-exponential growth phase (OD₆₀₀ = 2), a time point at which σ^B has been shown to be active (26). Comparison of the transcriptional profiles of the *sigB*⁺ strains to their respective

isogenic *sigB* mutants identified 229 ORFs to be influenced by σ^B by a factor of more than 2 in at least two of the three genetic backgrounds analyzed (Tables 2 and 3). While the majority of ORFs were positively influenced by σ^B (Table 2), as expected for a σ factor, a number of ORFs that were repressed in the presence of σ^B were also identified (Table 3). Forty-six of the genes identified were previously shown to be influenced by σ^B in *S. aureus*. Additionally, 23 genes were previously identified to be regulated by σ^B in *B. subtilis* (30, 66). This high correlation indicates that the GeneChip method used accurately identified the genes belonging to the σ^B regulon of the strains analyzed.

Transcriptional start point (tsp) determinations of σ^B -driven transcripts (15, 33), coupled with σ^B -dependent in vitro transcription analyses of the *asp23* P1 and *coa* promoters (55), suggest that the promoter region of *S. aureus* σ^B -regulated genes contains a consensus sequence that is highly similar to that of *B. subtilis* σ^B -regulated genes (GttTww₁₂₋₁₅gGgwAw) (64). The similarity of the σ^B promoter consensus sequences of both species is further corroborated by the findings of Gertz et al. (24, 25), who demonstrated that the *S. aureus asp23* P1 promoter is recognized by E- σ^B in *B. subtilis* and that all proteins that were identified to be influenced by σ^B in *S. aureus* by a proteomic approach are encoded by genes harboring a nucleotide sequence resembling the *B. subtilis* σ^B promoter consensus. Most of the genes identified as upregulated by σ^B in this study were also preceded by nucleotide sequences resembling the σ^B promoter consensus of *B. subtilis*, either directly or as part of a putative operon. None of the genes identified as downregulated in a σ^B -specific manner contained this sequence within their promoter regions.

Genes influenced by σ^B during early growth stages. The approach used in experiment 1 proved useful for the identification of a large number of σ^B -regulated genes (Tables 2 and 3). However, this strategy was likely to miss σ^B -dependent genes that were expressed only during the early growth stages. In a second approach, the transcriptional profiles of strain Newman and its Δ *rsbUVW-sigB* mutant, IK184, were analyzed during several growth stages, e.g., 1, 3, 5, and 8 h after inoculation (Fig. 1A). Monitoring of the transcriptional profiles during different growth stages confirmed almost all genes identified by experiment 1 as σ^B dependent. The experiment also enabled us to identify 23 additional ORFs as positively regulated by σ^B (Table 4). The majority of these ORFs, represented by transcriptional profile type 1 (Fig. 1B), were expressed primarily during the early growth stages (1 and 3 h after inoculation) while no transcripts were detectable during later growth (5 and 8 h after inoculation). Members of this group include several putative virulence factors such as *coa*, encoding staphylococcal coagulase, and *fnb*, encoding fibronectin binding protein A, which have previously been demonstrated to be influenced by σ^B and confirmed in this study (55, 58, 60). In addition, ORFs N315-SA0620, N315-SA2093, and N315-SA2332, which are all homologues of *ssaA* of *Staphylococcus epidermidis*, encoding the highly antigenic staphylococcal secretory antigen A (48), were found to be influenced by σ^B . Most of the ORFs listed in Table 4 lacked a significant σ^B consensus promoter in their upstream regions, suggesting that σ^B indirectly regulates their transcript titers.

TABLE 2. Genes upregulated by σ^B

N315 ORF no. ^a	N315 gene ^a	N315 description ^a	Fold change ^b in strain:			σ^B consensus ^{c,d}	Reference(s) with reported σ^B dependence ^e for:	
			COL	Newman	GP268		<i>S. aureus</i>	<i>B. subtilis</i> ^f
N315-SA1984	<i>asp23</i>	Alkaline shock protein 23	Up	Up	Up	Yes	24, 26, 44, 55	—
CAB75732.1	<i>bbp</i>	Bone sialoprotein-binding protein Bbp	3.2	4.5	4.8	?	—	—
N315-SA2008	<i>budB</i>	α -acetolactate synthase	Up	Up	Up	Yes ^d	—	—
N315-SA0144	<i>cap5A</i>	Capsular polysaccharide synthesis enzyme Cap5A	Up	Up	12.8	?	—	—
N315-SA0145	<i>cap5B</i>	Capsular polysaccharide synthesis enzyme Cap5B	Up	Up	10.8	?	—	—
N315-SA0146	<i>cap5C</i>	Capsular polysaccharide synthesis enzyme Cap5C	Up	Up	8.6	?	—	—
N315-SA0147	<i>cap5D</i>	Capsular polysaccharide synthesis enzyme Cap5D	Up	Up	7.3	?	—	—
N315-SA0148	<i>cap5E</i>	Capsular polysaccharide synthesis enzyme Cap5E	Up	Up	7.5	?	—	—
N315-SA0149	<i>cap5F</i>	Capsular polysaccharide synthesis enzyme Cap5F	Up	Up	7.5	?	—	—
N315-SA0150	<i>cap5G</i>	Capsular polysaccharide synthesis enzyme Cap5G	Up	Up	6.8	?	—	—
N315-SA0151	<i>cap5H</i>	Capsular polysaccharide synthesis enzyme Cap5H	Up	Up	5.1	?	—	—
N315-SA0152	<i>cap5I</i>	Capsular polysaccharide synthesis enzyme Cap5I	Up	Up	5.7	?	—	—
N315-SA0153	<i>cap5J</i>	Capsular polysaccharide synthesis enzyme Cap5J	Up	Up	3.5	?	—	—
N315-SA0155	<i>cap5L</i>	Capsular polysaccharide synthesis enzyme Cap5L	Up	Up	5.1	?	—	—
N315-SA0156	<i>cap5M</i>	Capsular polysaccharide synthesis enzyme Cap5M	Up	Up	4.5	?	—	—
N315-SA0157	<i>cap5N</i>	Capsular polysaccharide synthesis enzyme Cap5N	2.7	Up	5.2	?	—	—
N315-SA0158	<i>cap5O</i>	Capsular polysaccharide synthesis enzyme Cap8O	2.6	Up	4.2	?	—	—
CAA79304	<i>clfA</i>	Clumping factor A	35.7	Up	7.8	Yes	33, 60	—
N315-SA2336	<i>clpL</i>	ATP-dependent Clp proteinase chain ClpL	17.3	13.2	Up	Yes	25, 33	—
N315-SA2349	<i>crtM</i>	Squalene desaturase	Up	Up	Up	Yes ^d	26	66 (<i>yisP</i>)
N315-SA2348	<i>crtN</i>	Squalene synthase	Up	Up	Up	Yes ^d	26	—
N315-SA1452	<i>csbD</i>	HP, σ^B -controlled gene product CsbD (Csb8)	37.0	Up	Up	Yes	25, 33	30, 66 (<i>ywmG</i>)
COL-SA1872	<i>epiE</i>	Epidermin immunity protein EpiE	Up	Up	Up	Yes ^d	—	—
COL-SA1873	<i>epiF</i>	Epidermin immunity protein EpiF	Up	Up	Up	Yes	—	—
N315-SA1634	<i>epiG</i>	Epidermin immunity protein EpiG	Up	Up	Up	Yes ^d	—	—
N315-SA2260	<i>fabG</i>	HP, similar to glucose 1-dehydrogenase	Up	Up	Up	Yes	—	30 (<i>yxbG</i>)
N315-SA1901	<i>fabZ</i>	(3R)-hydroxymyristoyl-[acyl carrier protein] dehydratase	2.2	5.1	2.0	Yes ^d	—	—
N315-SA2125	<i>hutG</i>	HP, similar to formiminoglutamase	3.7	14.6	2.9	Yes	—	—
N315-SA1505	<i>lysP</i>	Lysine-specific permease	2.4	7.9	2.0	?	—	—
N315-SA1962	<i>mtlA</i>	PTS system, mannitol-specific IIA component	8.5	17.2	Up	Yes ^d	—	—
N315-SA1963	<i>mtlD</i>	Mannitol-1-phosphate 5-dehydrogenase	8.2	Up	Up	Yes ^d	—	—
N315-SA1902	<i>murA</i>	UDP-N-acetylglucosamine 1-carboxyvinyl transferase 1	2.2	5.1	2.0	Yes ^d	—	—
N315-SA0547	<i>mvaK1</i>	Mevalonate kinase	2.4	4.5	1.3	Yes	—	—
N315-SA0548	<i>mvaD</i>	Mevalonate diphosphate decarboxylase	3.3	7.3	1.8	Yes ^d	—	—
N315-SA0549	<i>mvaK2</i>	Phosphomevalonate kinase	3.7	10.6	2.2	Yes ^d	—	—
N315-SA1987	<i>opuD</i>	Glycine betaine transporter <i>opuD</i> homologue	Up	Up	Up	Yes	26, 33	—
N315-SA1871	<i>rsbV</i>	Anti- σ^B factor antagonist	Up	Up	Up	Yes	26, 43, 82	30, 66
N315-SA1870	<i>rsbW</i>	Anti- σ^B factor	Up	Up	Up	Yes ^d	26, 43, 82	30, 66
N315-SA0573	<i>sarA</i>	Staphylococcal accessory regulator A (Csb35)	2.9	3.8	2.0	Yes	4, 15, 25, 55	—
N315-SA0108	<i>sarS</i>	Staphylococcal accessory regulator A homologue S	2.6	1.1	2.1	Yes	77	—
N315-SA0099	<i>sbtA</i>	HP, similar to transmembrane efflux pump protein	Up	Up	Up	?	—	66 (<i>yusP</i>)
N315-SA1869	<i>sigB</i>	Alternative transcription factor σ^B	Up	Up	Up	Yes ^d	26, 43, 82	30, 66
N315-SA0456	<i>spoVG</i>	Stage V sporulation protein G homologue	4.3	9.8	3.0	Yes ^d	—	—
N315-SA1114	<i>truB</i>	tRNA pseudouridine 5S synthase	2.1	Up	2.3	Yes	—	—
N315-SA2119	<i>ydaD</i>	HP, similar to dehydrogenase (Csb28)	4.8	33.1	16.9	Yes	25	30 (<i>yhxD</i>)
N315-SA0084		HP, similar to <i>Homo sapiens</i> CG1-44 protein	Up	Up	3.0	Yes	—	—
N315-SA0098		HP, similar to aminoacylase	Up	Up	Up	?	—	—
N315-SA0102		67 kDa myosin-crossreactive streptococcal antigen homologue	Up	Up	Up	Yes	—	—
N315-SA0105		HP	Up	Up	Up	?	—	—
N315-SA0163		HP, similar to cation efflux system membrane protein CzcD	Up	Up	Up	?	—	—
N315-SA0164		HP	Up	Up	Up	Yes	—	—
N315-SA0261		HP, similar to <i>rbs</i> operon repressor RbsR	2.5	Up	Up	Yes	—	—
N315-SA0296		Conserved HP	7.6	20.5	3.9	Yes	—	—
N315-SA0297		HP, similar to ABC transporter ATP-binding protein	6.3	13.1	2.8	Yes ^d	—	—
N315-SA0317		HP, similar to dihydroflavonol-4-reductase	11.6	20.7	3.9	Yes	—	—
N315-SA0326		Conserved HP	2.5	2.1	2.0	Yes	—	—
N315-SA0327		Conserved HP	2.2	2.1	2.0	Yes ^d	—	—
N315-SA0359		Conserved HP	Up	Up	Up	Yes	33	—
N315-SA0360		Conserved HP	Up	Up	77.7	Yes	—	30, 66 (<i>ydaS</i>)

Continued on following page

TABLE 2—Continued

N315 ORF no. ^a	N315 gene ^a	N315 description ^a	Fold change ^b in strain:			σ^B consensus ^{c,d}	Reference(s) with reported σ^B dependence ^e for:	
			COL	Newman	GP268		<i>S. aureus</i>	<i>B. subtilis</i> ^f
N315-SA0372		HP (Csb12)	1.6	3.3	2.0	Yes	25	—
N315-SA0455		Translation initiation inhibitor homologue	3.2	6.2	2.3	Yes	33	—
N315-SA0509		Conserved HP	2.0	12.1	2.0	?	—	—
N315-SA0528		HP, similar to hexulose-6-phosphate synthase (Csb4)	1.8	6.8	2.0	Yes	25	—
N315-SA0529		Conserved HP (Csb4-1)	1.9	8.7	2.0	Yes ^d	25	—
N315-SA0541		HP, similar to cationic amino acid transporter	11.3	14.4	7.7	Yes	—	30 (<i>yhdG</i>)
N315-SA0572		HP, similar to esterase/lipase	Up	Up	Up	Yes	—	—
N315-SA0577		HP, similar to FimE recombinase	Up	Up	Up	?	—	—
N315-SA0578		HP, similar to NADH dehydrogenase	Up	Up	Up	Yes	—	—
N315-SA0579		HP, similar to Na ⁺ H ⁺ antiporter	Up	Up	4.0	Yes ^d	—	—
N315-SA0580		HP, similar to Na ⁺ H ⁺ antiporter	Up	Up	Up	Yes ^d	—	—
N315-SA0581		MnhD homologue, similar to Na ⁺ H ⁺ antiporter subunit	Up	Up	6.0	Yes ^d	—	—
N315-SA0582		HP, similar to Na ⁺ H ⁺ antiporter	Up	Up	4.0	Yes ^d	—	—
N315-SA0583		HP, similar to Na ⁺ H ⁺ antiporter	Up	Up	4.7	Yes ^d	—	—
N315-SA0584		Conserved HP	Up	Up	5.3	Yes ^d	—	—
N315-SA0633		HP	2.0	8.7	2.9	Yes ^d	33	—
N315-SA0634		Conserved HP	1.9	6.6	2.3	Yes ^d	—	—
N315-SA0635		Conserved HP	5.1	14.8	2.8	Yes ^d	—	—
N315-SA0636		Conserved HP	5.5	22.9	2.2	Yes ^d	—	—
N315-SA0637		Conserved HP	5.3	24.3	3.5	Yes	—	—
N315-SA0658		HP, similar to plant metabolite dehydrogenases	3.0	10.5	2.5	Yes	—	—
N315-SA0659		HP, similar to CsbB stress response protein	3.3	10.4	2.5	Yes ^d	—	30, 66 (<i>csbB</i>)
N315-SA0665		Coenzyme PQQ synthesis homologue	2.1	4.5	1.8	?	—	—
N315-SA0666		6-Pyruvoyl tetrahydrobiopterin synthase homologue	2.3	5.7	2.1	?	—	—
N315-SA0681		HP, similar to multidrug resistance protein (Csb29)	2.4	Up	Up	Yes	25	30, 66 (<i>bmrU</i>)
N315-SA0721		Conserved HP	4.2	10.3	2.4	Yes	—	—
N315-SA0722		Conserved HP	3.4	9.4	1.5	Yes ^d	—	—
N315-SA0724		HP, similar to cell division inhibitor	2.5	3.8	2.5	Yes	—	30, 66 (<i>yfhF</i>)
N315-SA0725		Conserved HP	Up	Up	Up	?	—	—
N315-SA0740		HP	Up	Up	Up	Yes	—	—
N315-SA0741		Conserved HP	Up	Up	Up	Yes ^d	—	—
N315-SA0748		HP	3.0	Up	4.8	Yes ^d	—	—
N315-SA0749		HP	2.5	Up	6.6	Yes	—	—
N315-SA0751		HP	4.3	5.7	4.1	?	—	—
N315-SA0752		HP	Up	Up	Up	Yes	33	—
N315-SA0755		HP, similar to general stress protein 170	Up	Up	Up	Yes	—	30, 66 (<i>ykzA</i>)
N315-SA0768		Conserved HP	2.0	5.6	4.5	?	—	—
N315-SA0772		Conserved HP	Up	Up	Up	Yes	33	30, 66 (<i>csbD</i>)
N315-SA0774		HP, similar to ABC transporter ATP-binding protein homologue (Csb10)	2.1	2.0	1.4	Yes	25	—
N315-SA0780		HP, similar to hemolysin	3.3	Up	2.2	Yes	—	30, 66 (<i>yqhB</i>)
N315-SA0781		HP, similar to 2-nitropropane dioxygenase	2.2	Up	2.0	Yes ^d	—	—
N315-SA0933		HP	13.1	26.9	7.1	Yes	—	—
N315-SA1014		Conserved HP	Up	Up	Up	Yes	—	—
N315-SA1057		Conserved HP	2.4	3.9	3.1	Yes	—	—
N315-SA1559		HP, similar to smooth muscle caldesmon	3.6	12.1	2.1	Yes ^d	—	—
N315-SA1560		HP, similar to general stress protein homolog	2.8	8.2	2.2	Yes	—	30, 66 (<i>ytxG</i>)
N315-SA1573		HP	5.9	21.0	3.0	Yes	—	—
N315-SA1590		HP	2.0	4.3	2.1	Yes	—	—
N315-SA1657		Conserved HP	2.0	4.5	2.4	Yes	—	—
N315-SA1671		HP (Csb33)	3.0	9.4	2.1	Yes	25	—
N315-SA1692		Conserved HP (Csb3)	1.8	5.6	4.0	?	25	—
N315-SA1697		HP, similar to protein-tyrosine phosphatase	2.3	5.0	3.7	Yes	—	30 (<i>yfkJ</i>)
N315-SA1698		HP	1.3	2.9	2.0	Yes ^d	—	—
N315-SA1699		HP, similar to transporter	5.0	23.1	6.1	Yes ^d	—	30, 66 (<i>yfkH</i>)
N315-SA1814		HP, similar to succinyl-diaminopimelate desuccinylase	Up	Up	Up	?	—	—
N315-SA1903		Conserved HP	3.7	10.9	3.7	Yes ^d	—	—
N315-SA1924		HP, similar to aldehyde dehydrogenase (Csb24)	3.7	26.1	3.2	Yes	25	—
N315-SA1942		Conserved HP	2.3	7.9	3.6	?	—	—
N315-SA1946		Conserved HP (Csb9)	Up	Up	Up	Yes	25, 33	—
N315-SA1961		HP, similar to transcription AT BglG family	9.7	8.2	Up	Yes ^d	—	—
N315-SA1980		Conserved HP	3.4	4.7	1.1	Yes ^d	—	—
N315-SA1981		Conserved HP	5.7	7.7	1.6	Yes	—	—
N315-SA1985		HP	Up	Up	Up	Yes ^d	26	—
N315-SA1986		HP	Up	Up	Up	Yes	26	—

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TABLE 2—Continued

N315 ORF no. ^a	N315 gene ^a	N315 description ^a	Fold change ^b in strain:			σ^B consensus ^{c,d}	Reference(s) with reported σ^B dependence ^e for:	
			COL	Newman	GP268		<i>S. aureus</i>	<i>B. subtilis</i> ^f
N315-SA2006		HP, similar to MHC class II analog	Up	Up	Up	?	—	—
N315-SA2101		Conserved HP	2.2	3.3	1.5	Yes ^d	66	(<i>yrhD</i>)
N315-SA2102		Conserved HP	2.2	3.3	1.7	Yes	—	—
N315-SA2104		HP, similar to suppressor protein SuhB	2.1	2.2	1.8	Yes	—	—
N315-SA2158		HP, similar to TpgX protein	2.2	3.5	2.5	Yes	—	—
N315-SA2203		HP, similar to multidrug resistance protein	2.1	3.9	2.2	Yes	—	—
N315-SA2219		Conserved HP	Up	Up	3.0	Yes	33	—
N315-SA2240		HP, similar to <i>para</i> -nitrobenzyl esterase chain A	1.9	2.0	2.0	?	—	—
N315-SA2242		Conserved HP	Up	Up	Up	?	—	—
N315-SA2243		HP, similar to ABC transporter (ATP-binding protein)	Up	Up	Up	?	—	—
N315-SA2262		Conserved HP (Csb7)	Up	Up	Up	Yes	25	—
N315-SA2267		HP	3.0	Up	3.9	Yes	—	—
N315-SA2298		Conserved HP	3.4	30.9	6.1	?	33	30, 66 (<i>ydhT</i>)
N315-SA2309		Conserved HP	2.0	2.5	2.9	?	—	—
N315-SA2327		HP, similar to pyruvate oxidase	51.1	Up	17.9	?	30, 66	(<i>ydhP</i>)
N315-SA2328		Conserved HP	Up	Up	Up	?	66	(<i>yxaC</i>)
N315-SA2350		Conserved HP	Up	Up	Up	Yes ^d	—	—
N315-SA2351		HP, similar to phytoene dehydrogenase	Up	Up	Up	Yes ^d	—	—
N315-SA2352		HP	Up	Up	Up	Yes	—	—
N315-SA2366		Conserved HP	7.3	Up	4.5	Yes	—	—
N315-SA2367		Conserved HP	10.4	Up	8.9	Yes	—	—
N315-SA2374		Conserved HP	Up	Up	Up	?	—	—
N315-SA2398		HP	Up	Up	Up	Yes	—	—
N315-SA2403		Conserved HP	10.3	Up	8.7	Yes	—	—
N315-SA2440		HP	2.3	5.9	1.7	?	—	—
N315-SA2441		HP, similar to lipopolysaccharide biosynthesis protein	2.5	6.6	2.0	?	—	—
N315-SA2442		Preprotein translocase SecA homologue	3.5	8.5	2.0	?	—	—
N315-SA2451		HP	Up	Up	Up	Yes	33	—
N315-SA2452		Conserved HP	Up	Up	3.5	?	—	—
N315-SA2479		Conserved HP	Up	4.3	4.6	Yes	—	—
N315-SA2485		HP	Up	Up	Up	Yes	—	—
N315-SA2488		HP	Up	Up	Up	Yes	—	—
N315-SA2489		HP, similar to high-affinity nickel-transport protein	Up	Up	Up	Yes ^d	—	—
N315-SA2491		Conserved HP	Up	Up	Up	Yes	—	—
N315-SAS023		HP, similar to thioredoxin	2.1	4.6	3.2	?	—	—
N315-SAS049		HP	Up	Up	Up	Yes ^d	—	—
N315-SAS053		HP	4.0	12.8	2.1	Yes ^d	—	—
N315-SAS056		HP	2.0	5.7	1.9	Yes	—	—
N315-SAS068		HP	5.2	5.7	3.3	Yes	—	—
N315-SAS082		HP	Up	Up	Up	?	—	—
N315-SAS083		HP	Up	Up	Up	?	—	—
N315-SAS089		HP	2.6	5.7	2.3	?	—	—
COL-SA0866		HP	Up	Up	Up	?	—	—
COL-SA1046		HP	6.6	12.0	9.0	Yes	—	—
COL-SA2012		HP, acetyltransferase (GNAT) family	5.8	2.9	2.0	?	—	—
COL-SA2013		HP	Up	Up	Up	?	—	—
COL-SA2379		Conserved HP	2.2	17.1	3.0	?	—	—
COL-SA2433		HP	2.6	3.6	2.1	Yes ^d	—	—
COL-SA2481		HP	Up	Up	Up	Yes ^d	—	—
COL-SA2595		HP	2.3	4.1	2.1	?	—	—
COL-SA2631		Conserved HP	Up	Up	3.8	Yes	—	—
AAB05395		HP, ORF 3 of the <i>sarA</i> locus	11.8	46.6	6.8	Yes	4, 15, 52, 55	—
CAB60754		HP	32.1	Up	13.9	Yes	—	—

^a Based on the published sequence of strain N315 (accession no. NC_002745). For genes not present in N315, the gene name and description given are from the COL genome, available from The Institute for Genomic Research Comprehensive Microbial Resource website (<http://www.tigr.org>), or the respective accession number. ABC, ATP binding cassette; GNAT, GCN5-related N-acetyltransferases; HP, hypothetical protein; MHC, major histocompatibility complex; PTS, phosphotransferase system.

^b Normalized values in the *rsbU*⁺*V*⁺*W*⁺ *sigB*⁺ strain over values in the Δ *rsbUVW*-*sigB* mutant. "Up" denotes genes highly downregulated in the Δ *rsbUVW*-*sigB* mutant, such that the transcripts were below detectable levels and the change could not be accurately calculated.

^c ORFs preceded by a consensus sequence that resembles the σ^B consensus sequence for *B. subtilis* as described by *Petersohn et al.* (64). Only sequences deviating not more than three nucleotides from the consensus GttTww₁₂₋₁₅gGgwAw (w = a, t) sequence and lying within 500 bp upstream of predicted ORFs were considered σ^B -dependent promoters. ?, genes or operons are not preceded by a σ^B consensus promoter that matches the criteria given above.

^d ORFs likely to form an operon.

^e References reporting an influence of σ^B on the respective gene or its gene product in *S. aureus* or the homologous gene in *B. subtilis*.

^f *B. subtilis* gene names are given in parentheses, if different from those of *S. aureus*. The absence of a homologous ORF in the *B. subtilis* genome is indicated by a dash.

TABLE 3. Genes downregulated by σ^B

N315 ORF no. ^a	N315 gene ^a	N315 description ^a	Fold change ^b in strain:			Reference(s) with reported σ^B dependence ^c	Regulation by SarA ^d (reference[s])
			COL	Newman	GP268		
N315-SA2430	<i>aur</i>	Zinc metalloprotease aureolysin	7.4	6.1	9.1	36, 84	Down (16, 37, 84)
N315-SA2411	<i>citM</i>	HP, similar to magnesium citrate secondary transporter	Down	Down	4.3		
N315-SA0820	<i>glpQ</i>	Glycerophosphoryl diester phosphodiesterase	3.6	2.6	1.9	4	Down (84)
N315-SA1007	<i>hla</i>	α -Hemolysin precursor	2.1	2.8	4.1	26, 84	Up (16)
N315-SA2207	<i>hlgA</i>	γ -Hemolysin component A	1.7	2.0	2.1		
N315-SA2209	<i>hlgB</i>	γ -Hemolysin component B	2.2	4.2	Down		Up (16)
N315-SA2208	<i>hlgC</i>	γ -Hemolysin component C	2.0	4.7	4.1		Up (16)
N315-SA2463	<i>lip</i>	Triacylglycerol lipase precursor	2.0	6.2	2.0	44, 84	Up (84)/Down (16)
N315-SA0252	<i>lrgA</i>	Holin-like protein LrgA		5.8	9.4		Up (22)
N315-SA0253	<i>lrgB</i>	Holin-like protein LrgB		6.2	6.5		Up (22)/Down (16)
N315-SA1812	<i>lukF</i>	HP, similar to synergohymenotropic toxin precursor	2.7	3.9	Down		
N315-SA1813	<i>lukM</i>	HP, similar to leukocidin chain <i>lukM</i> precursor	3.8	4.8	Down		
N315-SA0746	<i>nuc</i>	Staphylococcal nuclease	29.7	5.1	Down	44, 84	Down (16, 84)
N315-SA0091	<i>plc</i>	l-Phosphatidylinositol phosphodiesterase precursor	Down	3.9	Down		Down (84)
N315-SA0963	<i>pycA</i>	Pyruvate carboxylase	2.3	1.9	2.3		
N315-SA0259	<i>rbsD</i>	Ribose permease	2.9	2.8	1.5		
N315-SA0258	<i>rbsK</i>	Probable ribokinase	2.8	2.3	1.3		
N315-SA1758	<i>sak</i>	Staphylokinase precursor (protease III)		2.7	7.0		
N315-SA0128	<i>sodM</i>	Superoxide dismutase	4.6	2.0	2.8		
N315-SA1631	<i>splA</i>	Serine protease SplA	Down	9.9	Down	84	Up (16)
N315-SA1630	<i>splB</i>	Serine protease SplB	Down	7.9	Down		Up (16)
N315-SA1629	<i>splC</i>	Serine protease SplC	Down	Down	Down	84	
N315-SA1628	<i>splD</i>	Serine protease SplD	Down	Down	Down		Up (16)
COL-SA1865	<i>splE</i>	Serine protease SplE	Down	Down	Down		
BAB95617_1	<i>splF</i>	Serine protease SplF		Down	Down		
N315-SA0901	<i>sspA</i>	Staphylococcal serine protease (V8 protease)	3.8	2.1	3.3	36, 84	Down (36)
N315-SA0900	<i>sspB</i>	Cysteine protease	3.2	2.2	4.3	36	Down (16, 36)
N315-SA0899	<i>sspC</i>	Cysteine protease	3.0	1.9	3.0		Down (16)
N315-SA2302	<i>stpC</i>	HP, similar to ABC transporter	6.3	2.3	4.0		
N315-SA0022		HP, similar to 5' nucleotidase	2.6	1.8	3.3		
N315-SA0089		HP, similar to DNA helicase	2.4	Down	2.1		
N315-SA0260		HP, similar to ribose transporter RbsU	3.0	2.6	2.3		
N315-SA0270		HP, similar to secretory antigen precursor SsaA	4.6	Down	Down		
N315-SA0272		HP, similar to transmembrane protein Tmp7	4.4	Down	Down		
N315-SA0276		Conserved HP, similar to diarrheal toxin-like protein	3.7	Up			
N315-SA0285		HP	2.6	Down	3.4		
N315-SA0291		HP	3.1		3.3		
N315-SA0295		HP, similar to outer membrane protein precursor	4.9	3.6	10.4		
N315-SA0368		HP, similar to proton/sodium glutamate symport protein	2.7	3.1	1.4		
N315-SA0841		HP, similar to cell surface protein Map-w	5.7	3.4	2.2		
N315-SA0977		29-kDa cell surface protein	2.5	2.1	1.8		
N315-SA1725		Staphopain, cysteine protease	5.9	4.2	10.6	36, 84	Down (36, 84)
N315-SA1726		HP	3.8	3.4	6.5		
N315-SA1815		HP, similar to Na ⁺ -transporting ATP synthase	Down	Down	Down		
N315-SA1853		HP, similar to DNA mismatch repair protein MutS	2.1	Down	4.0		
N315-SA2132		HP, similar to ABC transporter (ATP-binding protein)	2.7	Down	2.3		
N315-SA2133		Conserved HP	3.1	Down	3.2		
N315-SA2303		HP, similar to membrane-spanning protein	Down	1.8	Down		
N315-SAS020		HP, similar to phosphoglycerate mutase	2.1	2.4	2.2		
COL-SA0450		HP	2.2	2.2	3.1		
COL-SA1884		HP	3.3	Down	Down		
COL-SA2693		HP	2.2	7.1	2.2		

^a Based on the published sequence of strain N315 (accession no. NC_002745). For genes not present in N315, the gene name and description given are from the COL genome, available from The Institute for Genomic Research Comprehensive Microbial Resource website (<http://www.tigr.org>) or the respective accession number. HP, hypothetical protein.

^b Normalized values in the $\Delta rsbUVW$ -*sigB* mutant over values in the *rsbU⁺V⁺W⁺ sigB⁺* strain. "Down" denotes genes highly downregulated in the *rsbU⁺V⁺W⁺ sigB⁺* strain, such that the transcripts were below detectable levels and the change could not be accurately calculated.

^c References reporting an influence of σ^B on the respective gene or its gene product.

^d References reporting an influence of SarA on the respective gene or its gene product.

The transcript titers of a number of ORFs were not only increased in the wild-type strain during early growth (1 h after inoculation) but were found to be further enhanced during late growth (8 h after inoculation), as represented by transcription profile type 2 (Fig. 1B). It is conceivable that

the expression of these ORFs is again influenced indirectly by σ^B , most likely via regulator(s) which are mainly active during the late growth phase. The increase in expression observed for these ORFs during the early growth phase may be due to a carryover of the regulators that were produced

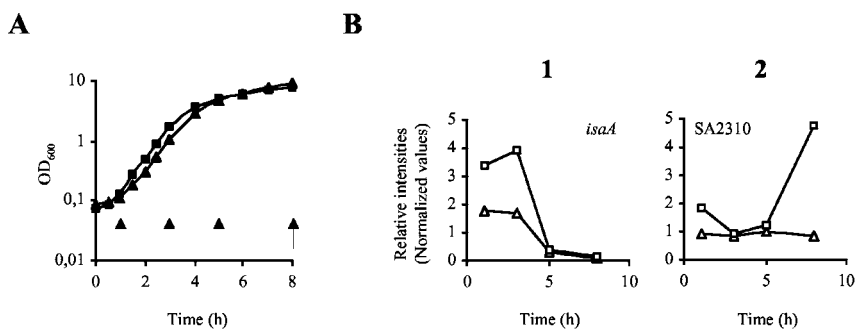


FIG. 1. Expression pattern variation of ORFs influenced positively by σ^B . (A) Growth curves of *S. aureus* Newman (■) and its mutant IK184 (▲). Time points of sampling are indicated by arrows. (B) Examples of expression pattern types of ORFs found to be influenced positively by σ^B in experiment 2. Transcript levels for Newman (□) and IK184 (△) cells sampled at different time points of growth (x axis) are shown. Data points were plotted as relative intensity values (y axis).

during late growth in the preculture and may be still active even 1 h after inoculation.

Functional classification of ORFs influenced by σ^B . The ORFs influenced by σ^B represent all functional categories that have been proposed by Kunst et al. (45), e.g., (i) cell envelope and cellular processes, including cell wall production, trans-

port, signal transduction, membrane bioenergetics, and protein secretion; (ii) intermediary metabolism, including carbohydrate metabolism, glycolytic pathways, tricarboxylic acid cycle, and amino acid and lipid metabolism; (iii) information pathways, including DNA modification and repair, RNA synthesis, and regulation; (iv) other functions, such as adaptation to

TABLE 4. Genes upregulated by σ^B in strain Newman during early growth phase

N315 ORF no. ^a	N315 gene ^a	N315 description ^a	Fold change ^b	σ^B consensus ^{c,d}	Expression profile	References with reported σ^B dependence ^e for:	
						<i>S. aureus</i>	<i>B. subtilis</i> ^f
N315-SA0222	<i>coa</i>	Staphylocoagulase precursor	2.4	Yes	1	55, 60	—
N315-SA2291	<i>fnb</i>	Fibronectin binding protein A	2.5	?	1	58	—
N315-SA2356	<i>isuA</i>	Immunodominant antigen A	2.4	?	1	—	—
N315-SA0265	<i>lytM</i>	Peptidoglycan hydrolase	3.4	Yes	1	—	—
N315-SA2093	<i>ssaA</i>	Secretory antigen precursor SsaA homolog	2.4	?	1	—	—
COL-SA0857	<i>vwb</i>	Secreted von Willebrand factor-binding protein	2.6	?	1	—	—
N315-SA0336		HP	2.1	?	1	—	—
N315-SA0612		Conserved HP	3.1	?	2	—	—
N315-SA0620		Secretory antigen SsaA homologue	2.7	?	1	—	—
N315-SA0903		Conserved HP	2.5	?	1	—	—
N315-SA0937		Cytochrome <i>d</i> ubiquinol oxidase subunit I homolog	2.2	?	1	—	—
N315-SA0938		Cytochrome <i>d</i> ubiquinol oxidase subunit II homolog	2.0	?	1	—	—
N315-SA1275		Conserved HP	2.6	?	1	—	—
N315-SA1898		HP, similar to SceD precursor	Up	Yes	1	—	—
N315-SA2301		HP, similar to alkaline phosphatase	2.2	?	2	—	—
N315-SA2310		Conserved HP	2.0	?	2	—	—
N315-SA2321		HP	2.3	Yes	2	—	—
N315-SA2332		HP, similar to secretory antigen precursor SsaA	2.8	?	1	—	—
N315-SA2355		Conserved HP	2.3	Yes	1	—	—
N315-SA2378		Conserved HP	2.5	?	1	—	—
N315-SA2447		HP, similar to streptococcal hemagglutinin protein	Up	Yes	2	—	—
N315-SAS051		HP	2.1	?	2	—	—
COL-SA0210		HP	Up	?	1	—	—

^a Based on the published sequence of strain N315 (accession no. NC_002745). For genes not present in N315, the gene name and description given are from the COL genome, available from The Institute for Genomic Research Comprehensive Microbial Resource website (<http://www.tigr.org>) or the respective accession number. ABC, ATP binding cassette; GNAT, GCN5-related N-acetyltransferases; HP, hypothetical protein; MHC, major histocompatibility complex; PTS, phosphotransferase system.

^b Normalized values in the *rsbU⁺V⁺W⁺sigB⁺* strain over values in the Δ *rsbUVW-sigB* mutant. "Up" denotes genes highly downregulated in the Δ *rsbUVWsigB* mutant, such that the transcripts were below detectable levels and the change could not be accurately calculated.

^c ORF preceded by a consensus sequence that resembles the σ^B consensus sequence for *B. subtilis* as described by Petersohn et al. (64). Only sequences deviating not more than three nucleotides from the consensus GttTww₁₂₋₁₅gGgwAw (w = a, t) and lying within 500 bp upstream of predicted ORFs were considered σ^B -dependent promoters. ?, genes or operons are not preceded by a σ^B consensus promoter that matches the criteria given above.

^d ORF likely to form an operon.

^e References reporting an influence of σ^B on the respective gene or its gene product in *S. aureus* or the homologous gene in *B. subtilis*.

^f *B. subtilis* gene names are given in parentheses if different from that of *S. aureus*. The absence of a homologous ORF in the *B. subtilis* genome is indicated by a dash.

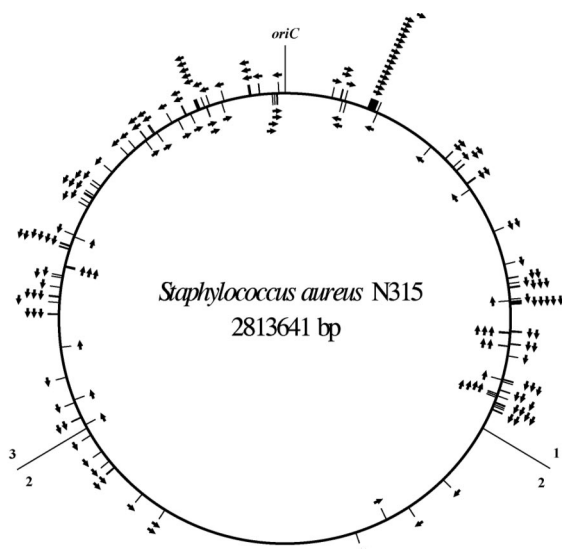


FIG. 2. Chromosomal distribution and orientation of ORFs up-regulated by σ^B . ORFs and their respective orientations are represented by arrows. The origin of replication (*oriC*) and the borders of genome fragments 1 to 3 are indicated.

atypical conditions or detoxification; and (v) ORFs similar to proteins with unknown function. The latter group alone comprises 100 of the 251 ORFs regulated by σ^B , representing a large reservoir of potential factors that may be responsible for phenotypic properties of *S. aureus* associated with σ^B activity, such as the development of resistance to methicillin, glycopeptides, and hydrogen peroxide (3, 26, 44, 82) that have not been associated with specific genes.

Chromosomal distribution of σ^B -regulated genes. The ORFs that are positively controlled by σ^B are not evenly distributed over the *S. aureus* chromosome (Fig. 2) but rather are overabundant in the genomic regions that are close to the origin of replication (*oriC*). While 77 of 828 ORFs (9.3%) or 69 of 861 ORFs (8%) encoded by the genome fragments 1 and 3, corresponding to positions 1 to 937880 and 1875761 to 2813641, respectively, are influenced by σ^B , only 12 of 816 (1.5%) of the ORFs encoded by genomic region 2 (positions 937880 to 1875760), which is most distal to *oriC*, are controlled by σ^B . The majority of genes and/or operons in these segments are oriented with respect to *oriC* in a manner that minimizes collisions between the transcribing RNA polymerase and the replication apparatus. Thus, 71.5% of all genes and 77% of the σ^B -regulated ORFs located on genome fragment 1 are encoded by the clockwise replicating strand, and 72.8% of all genes and 72.5% of the σ^B -regulated ORFs located on genome fragment 3 are encoded by the counterclockwise strand. It has been suggested by Neidhardt and colleagues (59) that the location of a gene relative to *oriC* can affect its level of expression. Genes located near the point of origin of replication are present in higher numbers in a rapidly growing cell than those near the terminus, which may be of importance, especially for those genes that are controlled by promoters operating near the maximum possible frequency.

Putative regulators acting downstream of σ^B . A significant number of ORFs (50 of 176 from experiment 1 and 17 of 23

from experiment 2) found to be upregulated by σ^B were not preceded by nucleotide sequences resembling the σ^B promoter consensus. Some of these genes were expressed only in *sigB*⁺ strains. It is possible that these ORFs were transcribed by the direct action of E- σ^B , despite the lack of an obvious σ^B promoter consensus. Alternatively, it is possible that σ^B controls the expression of a regulator(s), which would subsequently promote the expression of these genes. Promising candidates for such a scenario are the putative regulator homologues YabJ and SpoVG (N315-SA0455/6), which are likely to be cotranscribed, and were found to be controlled by σ^B (Fig. 3). The use of a recently described two-plasmid system (33) allowed us to confirm that *yabJ* expression is driven by σ^B (Fig. 3C). *tsp* determination by S1 mapping identified *yabJ*-specific RNA-protected fragments only in the presence of σ^B but not in the absence of the alternate transcription factor. A perfectly conserved σ^B consensus promoter sequence is present upstream of the *yabJ* *tsp*, indicating a direct influence of σ^B on the expression of this gene or operon. YabJ belongs to the highly conserved family of YigF proteins, which have been suggested to influence a variety of biological processes (69). YabJ of *B. subtilis* was found to have a role in the repression of *purA* by adenine (69). *spoVG*, encoding the stage V sporulation protein G, was the first developmentally regulated gene that was cloned from *B. subtilis* (74), and its regulation has been investigated intensively. However, little is known about the function of this protein. A mutation in *spoVG* was shown to impair the sporulation of *B. subtilis*, apparently as a result of disintegration of an immature spore cortex (72). More recent results suggest that SpoVG interferes with or is a negative regulator of the pathway leading to asymmetric septation (53). In addition to *S. aureus*, *spoVG* homologues have been found in the genomes of several bacteria, such as *Archaeoglobus fulgidus*, *Borrelia burgdorferi*, *Listeria monocytogenes*, and *S. epidermidis*, none of which produce spores. Thus, the SpoVG homologues of these organisms are likely to mediate functions other than sporulation. Inactivation of *spoVG* in a methicillin-resistant *S. epidermidis* drastically decreased methicillin resistance and the formation of a biofilm (D. Mack, personal communication). Interestingly, both attributes have also been linked positively to σ^B activity in *S. aureus* (67, 82). Attempts to inactivate the *S. aureus yabJ* and *spoVG* homologues are currently ongoing in our laboratory to elucidate their roles in this organism.

Another potential regulator acting downstream of σ^B is the gene product of ORF N315-SA1961, a homologue of the BglG/SacY family of transcriptional antiterminators (ATs). ATs are regulatory protein factors that bind to specific sites in the nascent mRNA to prevent premature termination of gene transcription and to stimulate elongation by RNA polymerase (83). Expression of N315-SA1961 was found to be highly up-regulated in strains harboring an intact *sigB* operon (Table 2), and the ORF is preceded by a nucleotide sequence (GTTAT T₋₁₄-GGGTAT) that matches the proposed σ^B promoter consensus, indicating that the BglG/SacY homologue is controlled directly by σ^B .

Influence of σ^B on known regulatory elements. *S. aureus* possesses an array of virulence factor regulatory elements, such as two-component signal transduction systems and winged-helix transcription regulatory proteins. Presumably, these elements interact to influence different networks of virulence fac-

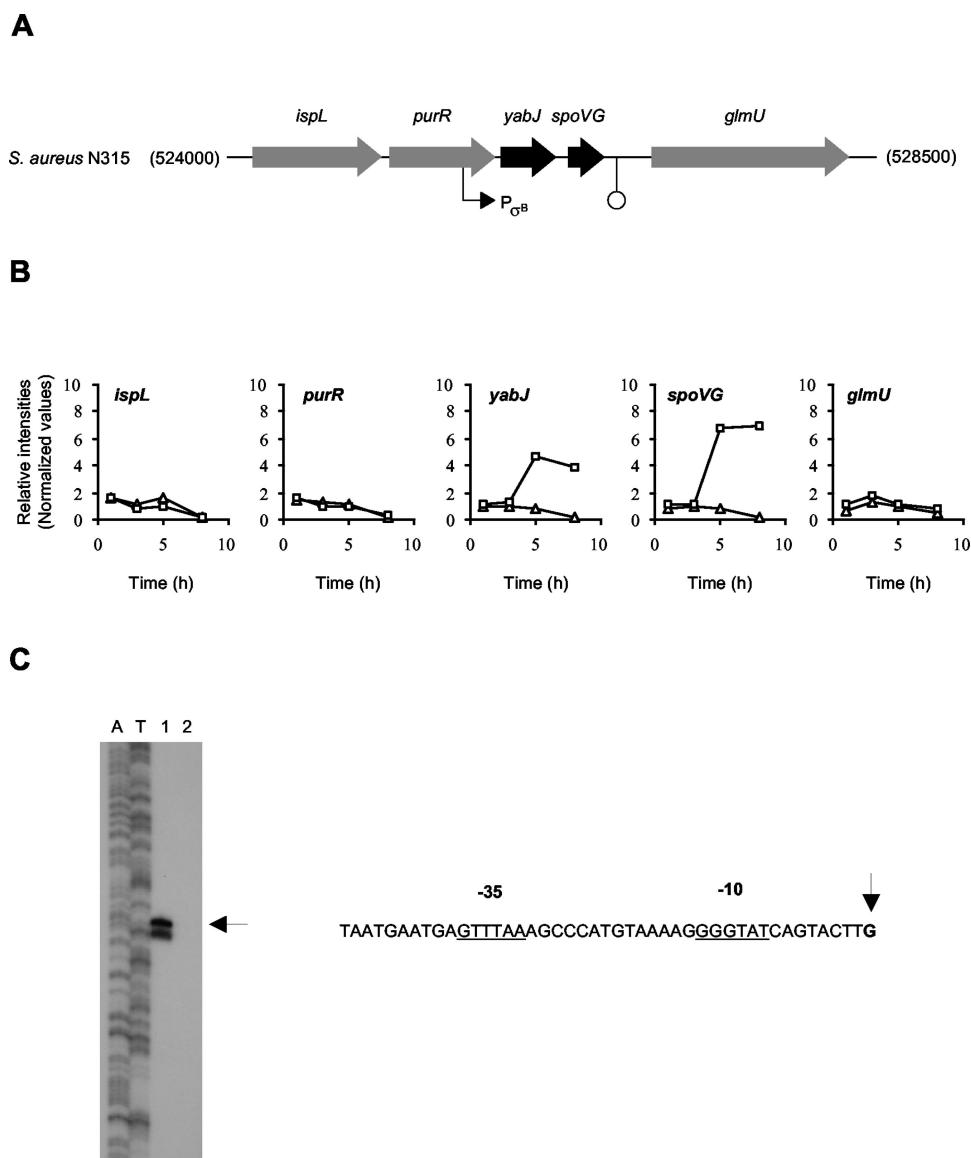


FIG. 3. The *yabJ-spoVG* locus of *S. aureus*. (A) Schematic representation of the *yabJ-spoVG* operon of *S. aureus* N315 (GenBank accession no. AP003130). Proposed ORFs and promoter and terminator sequences are indicated. (B) Transcript levels for Newman (\square) and IK184 (\triangle) cells sampled at different time points of growth (x axes). Data points were plotted as relative intensity values (y axes). (C) High-resolution S1 nuclease mapping of the transcriptional start point for *yabJ* in the *E. coli* two-plasmid system. The 5' end-labeled DNA fragment was hybridized with 40 μ g of RNA and treated with 100 U of S1 nuclease (as described in Materials and Methods). RNA was isolated from exponentially grown *E. coli* containing pSA3C and pAC7-sigB (lane 1) and pSA3C and pAC7 (lane 2). The RNA-protected DNA fragments were analyzed on DNA sequencing gels together with G+A (lane A) and T+C (lane T) sequencing ladders derived from the end-labeled fragments. The horizontal arrow indicates the position of the RNA-protected fragment, and the vertical arrow indicates the nucleotide corresponding to *tsp*. Before assigning the *tsp*, 1.5 nucleotides were subtracted from the length of the protected fragment to account for the difference in the 3' ends resulting from S1 nuclease digestion and the chemical sequencing reactions. The predicted -35 and -10 boxes are indicated.

tors on an as-needed basis, thereby providing cells with the necessary arsenal of virulence determinates to respond to environmental changes or stimuli (reviewed in reference 10). The data presented here indicate that three of these virulence regulators, *sarA*, *sarS*, and *arlRS*, are upregulated by σ^B (Fig. 4A). Transcription of other well-studied virulence regulators, such as Sae and Rot, were not significantly influenced by σ^B in these studies.

The staphylococcal accessory regulator A, SarA, a member of the winged-helix transcription proteins is encoded by the *sar*

locus. Although it is well established that expression of the *sar* locus is in part controlled by the action of σ^B (4, 15, 52), it is still a matter of debate whether σ^B has a positive or negative effect on the overall level of SarA production. Much of what is published regarding the influence of σ^B on SarA expression is difficult to interpret because most of these studies were done in strains, such as RN6390 and 8325-4, that harbor mutations in *rsbU*, the positive activator of σ^B , rendering them *sigB* deficient (26). The discrepancies between the positive influence of σ^B on SarA production observed by Gertz et al. (25) in a proteomic

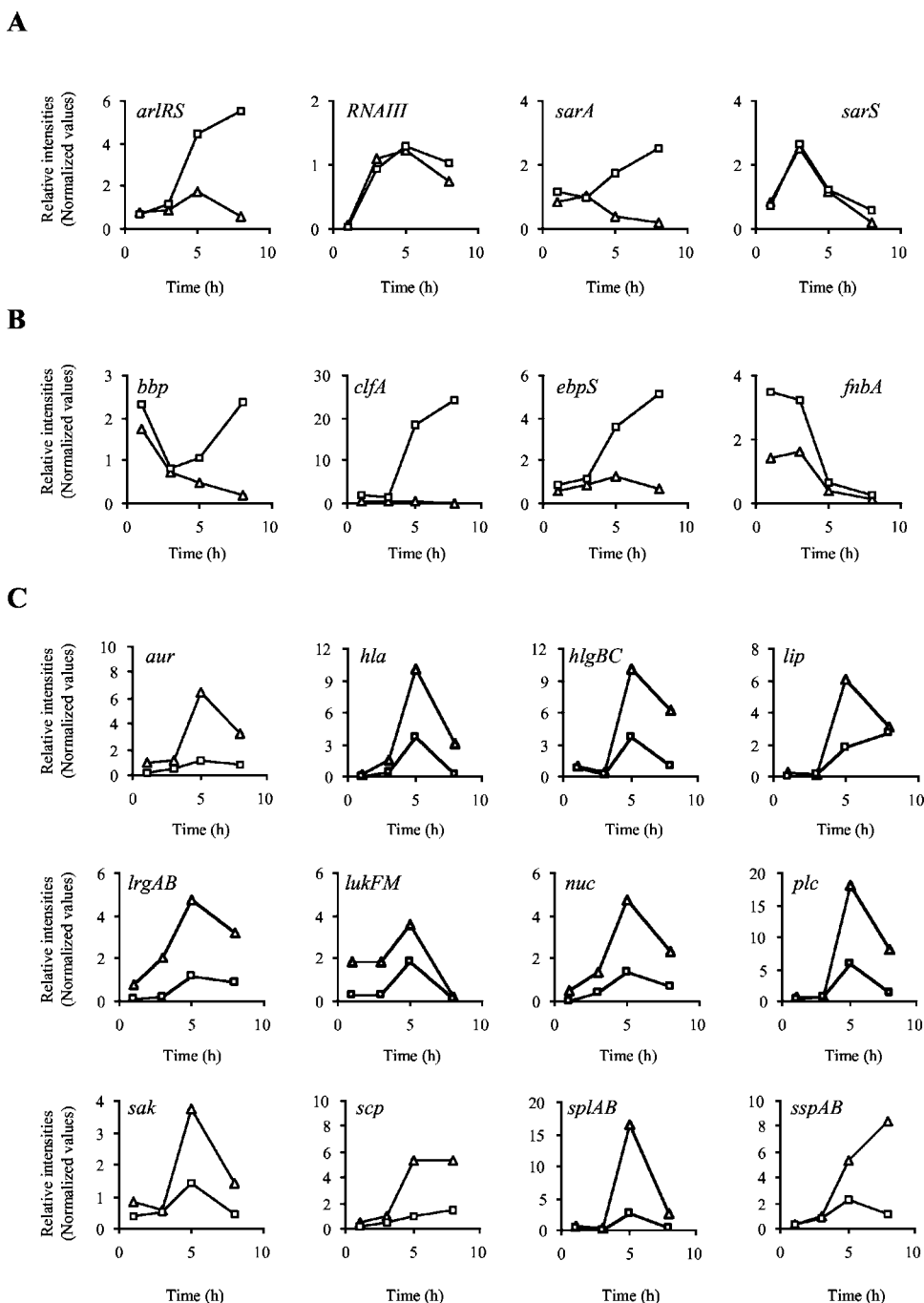


FIG. 4. Transcription profiles of ORFs influenced by σ^B . Transcript levels for Newman (\square) and IK184 (\triangle) cells sampled at different time points of growth (x axes). Data points were plotted as relative intensity values (y axes). (A) Influence of σ^B on known regulatory elements. *arlR*, autolysis-related locus regulator protein (response regulator); *arlS*, autolysis-related locus sensor protein (histidine kinase); RNAlII, effector molecule of the *agr* locus; *sarA*, staphylococcal accessory regulator A; *sarS*, staphylococcal accessory regulator S. (B) Adhesions factors upregulated by σ^B . *bbp*, bone sialoprotein-binding protein; *clfA*, clumping factor A; *ebpS*, elastin binding protein S; *fnbA*, fibronectin binding protein A. (C) Exoproteins and toxins downregulated by σ^B . *aur*, zinc metalloprotease aureolysin; *hla*, α -hemolysin; *hlgBC*, γ -hemolysin components B and C; *lip*, lipase; *lrgAB*, holin-like proteins LrgA and LrgB; *lukFM*, synergohymenotropic toxin precursor; *lukM*, leukocidin chain precursor; *nuc*, staphylococcal nuclease; *plc*, 1-phosphatidylinositol phosphodiesterase precursor; *sak*, staphylokinase precursor (protease III); *scp*, staphopain; *splAB*, serine proteases SplA and SplB; *sspA*, staphylococcal serine protease (V8 protease); *sspB*, cysteine protease SspB.

approach and by Bischoff et al. (4) via reporter gene fusion experiments versus the observed downregulatory effect of σ^B on SarA production reported by Manna et al. (52) and Cheung et al. (9) may be explained by the fact that, in the latter studies,

an *rsbU* mutant was used as parental strain to compare it with its respective *sigB* mutant. However, this explanation cannot account for the findings of Horsburgh et al. (34), who did not observe any influence of σ^B on SarA production either at the

transcriptional or at the protein level. The transcriptional profiling data presented here strongly suggest that σ^B increases the expression of the *sar* locus (Table 2; Fig. 4A), especially during the later growth stages (5 and 8 h after inoculation). Moreover, a direct correlation between the increase in SarA transcript levels and an increase in SarA protein is indirectly suggested by the findings that the expression of four major extracellular proteases of *S. aureus* (staphylococcal serine protease V8 [SspA], cysteine protease [SspB], metalloprotease aureolysin [Aur], and staphopain [Scp]) is significantly decreased in *sigB*⁺ strains (Table 3; Fig. 4). It was recently demonstrated by Karlsson and Arvidson (36) that transcription of these protease genes was suppressed due to increased σ^B -dependent expression of SarA. This is further supported by the findings that several of the ORFs found to be downregulated by σ^B , such as *glpQ*, encoding glycerophosphoryl diester phosphodiesterase, *nuc*, encoding staphylococcal thermonuclease, and *plc*, encoding a 1-phosphatidylinositol phosphodiesterase precursor, have previously been demonstrated to be downregulated by SarA (16, 84). It is likely that the increase in expression of these genes found in the Δ *rsbUVW-sigB* mutants is due to decreased production of SarA. Although appealing, this assumption remains speculative, as both Dunman et al. (16) and Ziebandt et al. (84) used the *rsbU*-defective RN6390 lineage as the genetic background for their analyses, leaving it open to question what might happen with respect to the *sarA* regulon in strains carrying an intact *sigB* operon. The genetic background chosen may also explain the observed discrepancy that several of the genes listed in Table 3 were found to be downregulated by σ^B but upregulated by SarA. Support for such a process is conferred by the observations that RNAIII expression of the *agr* locus is known to be promoted by SarA (11) but decreased by σ^B (4, 34) in an unidentified way that is, however, supposed to be independent from SarA (34).

The expression of a second winged-helix transcription protein, SarS (also known as SarH1), belonging to the family of SarA homologues was previously shown to be influenced by σ^B (77). This was confirmed in two of the three backgrounds analyzed in this study (Table 2). Interestingly, no difference in *sarS* expression was observed when comparing strain Newman and its Δ *rsbUVW-sigB* mutant either in the microarray experiments (Table 2; Fig. 4A) or by Northern blot analysis (data not shown), further demonstrating that strain-to-strain differences influence regulon constituents. Sequencing of the σ^B promoter regions of *sarS* of strains Newman and GP268 did not reveal any differences between the respective regions (which were identical with the N315 region corresponding to nucleotides 125868 to 126073 of GenBank accession no. AP003129), leaving the question open as to why expression of *sarS* in strain Newman is not affected by σ^B .

The third known virulence regulatory element observed to be influenced by σ^B was *arlRS*, encoding a two-component signal transduction system that influences adhesion, autolysis, and extracellular proteolytic activity of *S. aureus* (19). More recently, it was also demonstrated to decrease expression of the *agr* locus while increasing the expression of SarA (20). The data obtained from experiment 2 suggest that *arlRS* of strain Newman is upregulated by σ^B (Fig. 4A). However, *arlRS* did not show up in experiment 1 as influenced by σ^B either in strain

COL or strain GP268 and is not preceded by a σ^B consensus promoter.

Recent results suggest that expression of RNAIII, the effector molecule of the *agr* locus, is negatively influenced by σ^B (4, 34). However, results of the two experiments presented here did not effectively corroborate these observations, as although slight differences in RNAIII transcription were detectable between wild-type strains and their respective Δ *rsbUVW-sigB* mutants (Fig. 4A), changes in expression were not determined to be significant. RNAIII is by far the most prominent RNA molecule produced by *S. aureus* during the later growth stages. As a result, the RNAIII transcript levels of the wild-type strains already reached amounts that saturated the RNAIII-specific target oligonucleotides represented on the GeneChip, thus impeding the detection of differences in RNAIII transcript levels that might be present between the strain pairs analyzed.

Influence of σ^B on expression of virulence determinants. Previous studies demonstrated that σ^B influences the expression of various factors associated with virulence and pathogenicity of *S. aureus* (4, 15, 25, 34, 44, 58, 60, 84), which led to the assumption that σ^B may be important for virulence of this organism (4, 44). However, in vivo studies have failed to demonstrate an effect of σ^B on the virulence of *S. aureus* (34, 60), implying that such an assumption is no longer tenable. Alternatively, σ^B may play a role in pathogenesis; however, the effects of σ^B -mediated virulence mechanisms do not play a role in the models chosen in those experiments.

Analysis of the GeneChip data suggests that σ^B influences the expression of a large number of virulence genes in *S. aureus* (reviewed in references 10 and 47). Many of these are reported here for the first time as genes that are altered transcriptionally by σ^B . By comparing the expression profiles of these virulence genes, a pattern has emerged; most of the exoenzymes and toxins produced by *S. aureus* were negatively influenced by σ^B (Fig. 4C) while the expression of several adhesins was found to be clearly increased by σ^B (Fig. 4B). The function of σ^B in virulence factor production is therefore exactly the opposite of that of RNAIII, which is known to act as a negative regulator of cell wall proteins and a positive regulator of exoenzymes and toxins in a growth phase-dependent manner (Table 5) (10, 62). The decreased amounts of exoprotein and toxin transcripts observed in wild-type strains compared to their respective mutants may in part be a consequence of lower RNAIII transcript levels that are present in strains harboring an intact *sigB* operon (4, 34). Expression of the *cap* gene cluster is influenced by a variety of environmental stimuli and affected by several global regulators, such as RNAIII, SarA, and MgrA, (reviewed in reference 63). The microarray data presented here add a further regulator, σ^B , to this list and suggest that the alternate transcription factor influences *cap* expression in a growth phase-dependent manner (Fig. 5). While virtually no *cap* transcripts were detectable during the early growth stages (1 and 3 h), expression of the *cap* genes increased with ongoing growth (5 and 8 h), being highest at the latest time point analyzed. After 8 h of growth, a >50-fold increase in *cap*-specific transcripts was observable in strain Newman that was totally missing in its Δ *rsbUVW-sigB* mutant (Fig. 5). However, the effect of σ^B on *cap* expression is likely to be indirect, as the

TABLE 5. Influence of σ^B on virulence determinants regulated by the *agr* locus

Virulence determinant	Gene ^a	Result for ^b :	
		<i>agr</i>	σ^B
Aureolysin	<i>aur</i>	+	-
Capsular polysaccharide synthesis enzyme 5J	<i>cap5J</i>	+	+
Clumping factor B	<i>clfB</i>	+	∅
Coagulase	<i>coa</i>	-	+
Cystein protease	<i>sspC</i>	+	-
Enterotoxin A	<i>sea</i>	+	Unknown
Enterotoxin B	<i>seb</i>	+	- ^c
Exotoxin 2	<i>set8</i>	+	Unknown
Factor effecting methicillin resistance B	<i>femB</i>	+	∅
Fibronectin-binding protein A	<i>fnbA</i>	-	+
Fibronectin-binding protein B	<i>fnbB</i>	-	∅
Glycerol ester hydrolyase	<i>geh</i>	+	-
α -Hemolysin	<i>hla</i>	+	-
β -Hemolysin	<i>hlb</i>	+	- ^c
γ -Hemolysin	<i>hlgBC</i>	+	-
δ -Hemolysin	<i>hld</i>	+	∅
Hyaluronate lyase	<i>hysA</i>	+	∅
Lipase	<i>lip</i>	+	-
LrgAB (holin-like proteins)	<i>lrgAB</i>	+	-
Myosin-cross-reactive antigen	N315-SA0102	-	+
Phosphatidylinositol-specific phospholipase C	<i>plc</i>	+	-
Protein A	<i>spa</i>	-	∅
Secretory antigen A	<i>ssaA</i>	-	+
Serine protease A, B, D, and F	<i>spLA,B,D,F</i>	+	-
Staphylokinase	<i>spc</i>	+	-
Toxic shock syndrome toxin 1	<i>tst</i>	+	Unknown
V8 protease	<i>sspA</i>	+	-

^a Genes that are regulated conversely by *agr* and σ^B are shown in boldface type.

^b Influence of *agr* and σ^B on transcription of the respective gene. ∅, not influenced; +, increased; -, decreased.

^c Based on transcript levels detected in strains COL and IK183.

promoter region of the *cap* operon lacks an obvious σ^B consensus promoter sequence.

The finding that expression of so many virulence genes is significantly altered by σ^B warrants further investigation to elucidate its role in infectivity of *S. aureus* in additional models of infection. To date, nothing is known about the expression or activity of σ^B during the course of infection. *S. aureus* is known for its ability to cause a variety of unrelated infections (reviewed in reference 50). It is feasible that the σ^B -dependent downregulation of toxins and exoenzymes, combined with the simultaneous upregulation of adhesins, may enable *S. aureus* to cause very specific host-pathogen interactions that have not been investigated to date. Recent results indicate that σ^B is

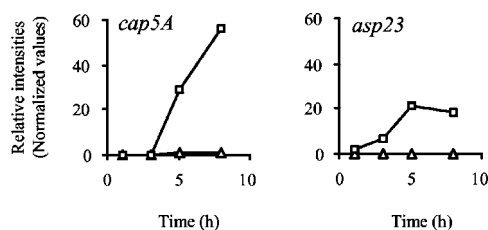


FIG. 5. Transcription profiles of *capA* and *asp23*. Transcript levels for Newman (□) and IK184 (△) cells sampled at different time points of growth (*x* axes). Data points were plotted as relative intensity values (*y* axes). The influence of σ^B on the expression of *capA*, encoding capsular polysaccharide synthesis enzyme A, and *asp23*, encoding alkaline shock protein 23, is shown.

involved in processes that are important for biofilm formation (1, 67); therefore, a comparison of the transcription profile of biofilm cells to the results we have obtained may identify genes that are essential for biofilm formation. Additionally, based on the virulence factor pattern caused by σ^B , it is tempting to speculate that this alternative transcription factor may also be an important player during nasal colonization, thereby promoting adherence to the host cell matrix without evoking an inflammatory response. Investigations in our laboratories are ongoing to address these questions. It is also quite possible that in vivo conditions leading to *S. aureus* stress, including those of high temperature at the site of infection, may induce the stress responsive σ^B factor. Under such conditions, when the host is trying to mount an immune response at the site of infection, it may be more beneficial for the bacterium to produce cell surface components that are involved in camouflaging the organism from the host's defense than to produce exoproteins.

The present study was designed to extensively characterize the genes that are regulated by the alternative sigma factor σ^B during standard laboratory growth conditions. Under these conditions, a >20-fold increase in the σ^B -regulated gene *asp23* was observed (Fig. 5). In addition, very stringent criteria were used for the identification of σ^B -regulated genes: (i) transcripts demonstrated the same σ^B -dependent phenotype in at least two of the three genetic backgrounds tested and (ii) transcripts passed strict statistical cutoff values. Based on these criteria, there was an extremely high correlation between the genes that we identified to be regulated in a σ^B -dependent manner and previously recorded results. As a result, it is likely that the GeneChip method used accurately identified the genes belonging to the σ^B regulon of the strains analyzed. While defining the σ^B regulon, we observed a distinguishable pattern among virulence factors. Subsequent studies that have focused on two *S. aureus* adhesions (*clfA* and *fnbA*) have confirmed that each gene is indeed regulated in a σ^B -dependent manner and further validated the method used (unpublished data).

The finding that σ^B downregulates the transcription of secreted virulence factors but upregulates cell surface virulence factors is in direct contrast to the observations of Kupferwasser et al. (46). In that study it was found that salicylic acid mildly induces *asp23* (1.9-fold) and corresponds to both the downregulation of certain cell surface adhesions and the upregulation of secreted proteases. Based on the low induction rate of *asp23*, it is difficult to reconcile whether the virulence factor effects seen in that study are directly mediated by σ^B versus another salicylic acid-responsive process or a combination of the two. It also raises the question of whether low to moderate levels of σ^B produce a much different physiological phenotype than the levels tested here. It is also possible that salicylic acid and other stresses that have previously been shown to modulate σ^B activity direct the expression of portions of the σ^B regulon. More completely characterizing the σ^B regulon will allow subsequent experiments to fully address these questions and further understand the role, if any, that the σ^B regulon plays in pathogenesis.

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