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Deletion of the Alternative Sigma Factor σ^B in *Staphylococcus aureus* Reveals Its Function as a Global Regulator of Virulence Genes

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A deletion of the *sigB* operon was constructed in three genetically distinct *Staphylococcus aureus* strains, and the phenotypes of the resulting mutants were analyzed. Compared to the corresponding wild-type strains, the Δ*sigB* mutants showed reduced pigmentation, accelerated sedimentation, and increased sensitivity to hydrogen peroxide during the stationary growth phase. A cytoplasmic protein missing in the Δ*sigB* mutants was identified as alkaline shock protein 23, and an extracellular protein excreted at higher levels in one of the Δ*sigB* mutants was identified as staphylococcal thermonuclease. Interestingly, most *sigB* deletion phenotypes were only seen in *S. aureus* COL and Newman and not in 8325, which was found to contain an 11-bp deletion in the regulator gene *rsbU*. Taken together, our results show that σ^B is a global regulator which modulates the expression of several virulence factors in *S. aureus* and that laboratory strain 8325 is a σ^B-defective mutant.

The initial event during infection by the human pathogen *Staphylococcus aureus* is the expression of certain virulence genes. Virulence factors are required for colonization of host tissue and for protection against the host defense. Timely correct expression of the virulence factors is essential for the establishment and maintenance of an infection and represents a highly regulated process (29). As a prerequisite, the microorganism has to recognize and respond to certain signals provided by the host. Such signals could be temperature (increases upon infection), peroxide (released by macrophages), pH shifts, or the presence or absence of specific carbon or energy sources (24).

In bacteria, alternative sigma factors of RNA polymerase are known to play a crucial role in regulating gene expression upon major changes in the environment. We recently identified the alternative sigma factor σ^B in *S. aureus* 8325 and showed that σ^B is induced during stationary phase and upon heat shock (20). The corresponding sigma factor in *B. subtilis* is known to be itself target of a complex regulatory network, which controls gene expression in response to certain stress and stationary-phase-specific signals (14). It has recently been shown that *S. aureus* σ^B also has sigma factor activity in vitro and that transcription of the global regulator Sar in *S. aureus* is at least partially controlled by σ^B (8). Since the Sar protein represents a global regulator involved in the expression of virulence genes (2, 4), it is tempting to speculate that σ^B is directly or indirectly involved in the regulation of virulence genes. To test this hypothesis, we constructed a *sigB* deletion in several staphylococcal backgrounds and analyzed the phenotype of these mutants. Here we report that deletion of *sigB* caused a drastic phenotype in two of the three backgrounds tested and revealed a natural σ^B defect in strain 8325. Compared to strain COL, strain 8325 has an 11-bp deletion in the gene encoding the σ^B regulator RsbU (20, 36), which we suggest is the reason for the σ^B defect.

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MATERIALS AND METHODS

Bacterial strains, plasmids, culture conditions, and general methods. The bacterial strains and plasmids used in this study are listed in Table 1. *S. aureus* cells were routinely grown in Luria-Bertani (LB) medium at 37°C. Antibiotics were used at the following concentrations: for *Escherichia coli*, ampicillin at 100 μg ml^{-1}; for *S. aureus*, erythromycin at 10 μg ml^{-1} and tetracycline at 10 μg ml^{-1}. All DNA manipulations and handling of *E. coli* were performed in accordance with standard protocols (31). Manipulations with *S. aureus* were done as described earlier (20).

Construction of plasmid pIK58, used to create a *sigB* deletion mutant. In a first step, we constructed the suicide vector pIKET by introducing appropriate antibiotic resistance cassettes into pBLSK(+), namely, the blunted 1.75-kb *Ava*I fragment with the *ermB* gene from transposon Tn*551* in the *Eco*RV site of pBLSK(+), as well as the blunted 2.3-kb *Hin*dIII fragment with the *tetK* gene from pT181 in the *Sma*I site. This plasmid cannot replicate in *S. aureus*, and erythromycin resistance can only be rescued by integration of this plasmid into the chromosome. To provide sites for homologous recombination, we cloned the *S. aureus* 1.1-kb *Nsi*I fragment (downstream region of *sigB*) in the *Pst*I site of the vector and the 1.7-kb *rsbU′*-containing *Hin*dIII fragment in the *Hin*dIII site, leading to plasmid pIK58 (Fig. 1). Fifteen micrograms of this plasmid was used to transform *S. aureus* RN4220 by electroporation with erythromycin for selection.

Lipase assay. To assay lipase activity, strains were grown for 12 h at 37°C in LB medium. Different dilutions of the culture supernatants were tested for lipase activity by monitoring hydrolysis of *p*-nitrophenyl-caprylate at 405 nm as described elsewhere (27). Calculation of lipase activity was adjusted for cell density and expressed as a percentage of that of the respective wild-type parent. Since the levels of lipase production differed substantially among the three backgrounds tested, dilutions of the supernatants were used to achieve a linear reaction in each case. Supernatants of strain 8325 were diluted 20-fold, those of strain Newman were diluted 4-fold, and those of strain COL were used without dilution; the wild type and mutants were always diluted equally. Lipase activity was also monitored on LB agar plates containing 1% Tween 20 and 1% xylose.

Peroxide susceptibility testing. For disk assays, bacteria were plated on LB agar and a disk soaked with 10 μl of a 3% H_2O_2 solution was placed on the surface. Plates were incubated at 37°C for 48 h, and inhibition zones were compared. MICs and MBCs of H_2O_2 were determined by broth microdilution by using the National Committee for Clinical Laboratory Standards protocol (26) with serial dilutions of H_2O_2 (2.2 M to 0.125 mM). Microtiter plates were incubated for 48 h at 37°C.

SDS-gel electrophoresis of protein, protein blotting, and N-terminal protein sequencing. Cellular or excreted proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis by using standard protocols (31). For analysis of excreted proteins, culture supernatants were concentrated about 10- to 15-fold by using Microcon 10 spin columns (Amicon Inc., Beverly, Mass.). Blotting of the proteins onto polyvinylidene difluoride membranes was done as previously described (1), and N-terminal sequencing of the respective proteins was done by P. Hunziker in the Laboratory of Biochemistry, University of Zürich.

Construction of pIK64 for complementation of Δ*sigB* strains. For complementation assays, we used vector pTX15, which contains the xylose-inducible

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype[a]	Reference or source
Strains		
E. coli DH10B	F⁻ ϕ80d*lacZ*ΔM15 *recA1*	Gibco/BRL, Gaithersburg, Md.
S. carnosus TM300		13
S. aureus		
RN4220	NCTC 8325-4-r (restriction mutant)	19
8325	NCTC 8325	
COL	High-Mc^r clinical isolate	
Newman	High level of clumping factor	9
8325 Δ*sigB*	Deletion of *sigB* operon, Em^r	This study
COL Δ*sigB*	Deletion of *sigB* operon, Em^r	This study
Newman Δ*sigB*	Deletion of *sigB* operon, Em^r	This study
Plasmids		
pBLSK(+)	Ap^r	Stratagene, La Jolla, Calif.
pIKET	*tetK* and *ermB* in pBLSK+; Ap^r in *E. coli*; Tc^r Em^r in *S. aureus*	This study
pTX15	Tc^r P_{Xyl}; staphylococcal origin of replication	28
pIK58	1.1-kb *Nsi*I and 1.7-kb *Hin*dIII *S. aureus* fragments in pIKET	This study
pIK64	800-bp *sigB* fragment in pTX15	This study

[a] Abbreviations are as follows: Ap^r, ampicillin resistant; Em^r, erythromycin resistant; Mc^r, methicillin resistant; Tc^r, tetracyclin resistant.

promoter P_{Xyl} and a tetracycline resistance determinant (28). We first amplified the entire *sigB* gene by PCR and subcloned it into pBLSK(+). The correct sequence of this fragment was confirmed by plasmid sequencing. We then subcloned the *sigB*-containing *Bam*HI/*Eco*RI fragment into the *Bam*HI/*Eco*RI sites of pTX15 downstream of P_{Xyl}, leading to plasmid pIK64. Since pTX15 only contains a staphylococcal origin of replication, we directly transformed pIK64 into *S. carnosus* TM300 by using protoplast transformation as previously described (13). pIK64 isolated from *S. carnosus* was then used to transform *S. aureus* RN4220. From there, the plasmid was moved into wild-type and Δ*sigB* mutant *S. aureus* by phage transduction as previously described (20). In those strains, P_{Xyl} and, therefore, expression of *sigB* were induced by adding 1% xylose to the medium. pIK64 could not be used for complementation of strain COL, since this strain naturally contains a tetracycline resistance-encoding plasmid.

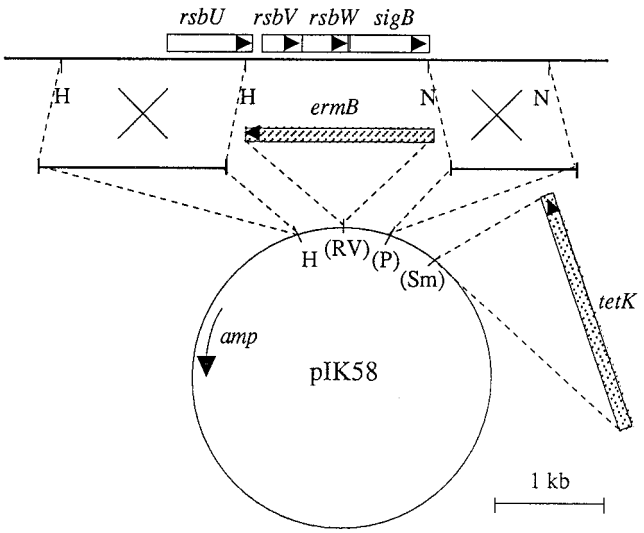

FIG. 1. Physical map of the *sigB* operon of *S. aureus* and the construction of a *sigB* deletion using suicide plasmid pIK58. Open reading frames are depicted as rectangles with arrows indicating their orientation. The genes for ampicillin resistance (*amp*), erythromycin resistance (*ermB*), and tetracycline resistance (*tetK*) are indicated. Recognition sites for restriction enzymes are designated as follows: H, *Hin*dIII; N, *Nsi*I; P, *Pst*I; RV, *Eco*RV; Sm, *Sma*I. Sites in parentheses were destroyed during cloning. The bold line corresponds to *S. aureus* chromosomal DNA, the thin line corresponds to pBLSK(+) DNA, and the dashed line shows the cloning procedures. The crosses indicate sites of homologous recombination.

RESULTS AND DISCUSSION

Construction of different *sigB* deletion mutants. To create a *sigB* deletion mutant by homologous recombination, we constructed pBLSK(+)-based suicide plasmid pIK58. This plasmid contained two *S. aureus* DNA fragments flanking the region to be deleted, as well as appropriate antibiotic resistance markers for selection (Fig. 1). We transformed pIK58 into *S. aureus* RN4220 and obtained approximately 100 erythromycin-resistant colonies. Since no plasmid could be isolated from those transformants, pIK58 must have integrated into the chromosome through at least a single crossover in one of the two homologous regions. The colonies were replica plated onto tetracycline, and three colonies were tetracycline sensitive. This suggested that in these clones a double crossover and, therefore, deletion of the *sigB* region had occurred (Fig. 1). By using phage transduction and selection for erythromycin resistance, the deletion was transferred to three genetically different backgrounds: *S. aureus* 8325, COL, and Newman. *S. aureus* 8325 represents a standard laboratory strain, whereas strain COL is a highly methicillin-resistant clinical isolate. Strain Newman is characterized by a high level of clumping factor and is therefore often used in clumping or adhesion assays (9). After transduction, we isolated erythromycin-resistant and tetracycline-sensitive clones in all three backgrounds. Southern blot hybridization using a *sigB*-specific probe confirmed that *sigB* was deleted in all three strains (data not shown). We characterized the phenotype of those mutants as follows.

Reduced pigmentation. The first obvious phenotype of the *sigB* deletion mutants was their color on agar plates. Whereas colonies of the wild-type parents of strains COL and Newman produced an orange pigment after 24 h of incubation, the colonies of the respective *sigB* mutants were unpigmented. In contrast, both wild-type strain 8325 and the Δ*sigB* mutant were unpigmented. Two major pigments are produced in *S. aureus* during stationary phase; the yellow carotenoid 4,4′-diaponeurosporene is converted to the orange pigment staphyloxanthin after prolonged cultivation (34). The genes responsible for the biosynthesis of these pigments have been characterized (34, 35). By homology, we were able to identify a σ^B-dependent promoter consensus sequence upstream of the staphyloxanthin biosynthesis operon and thus propose that the conversion of

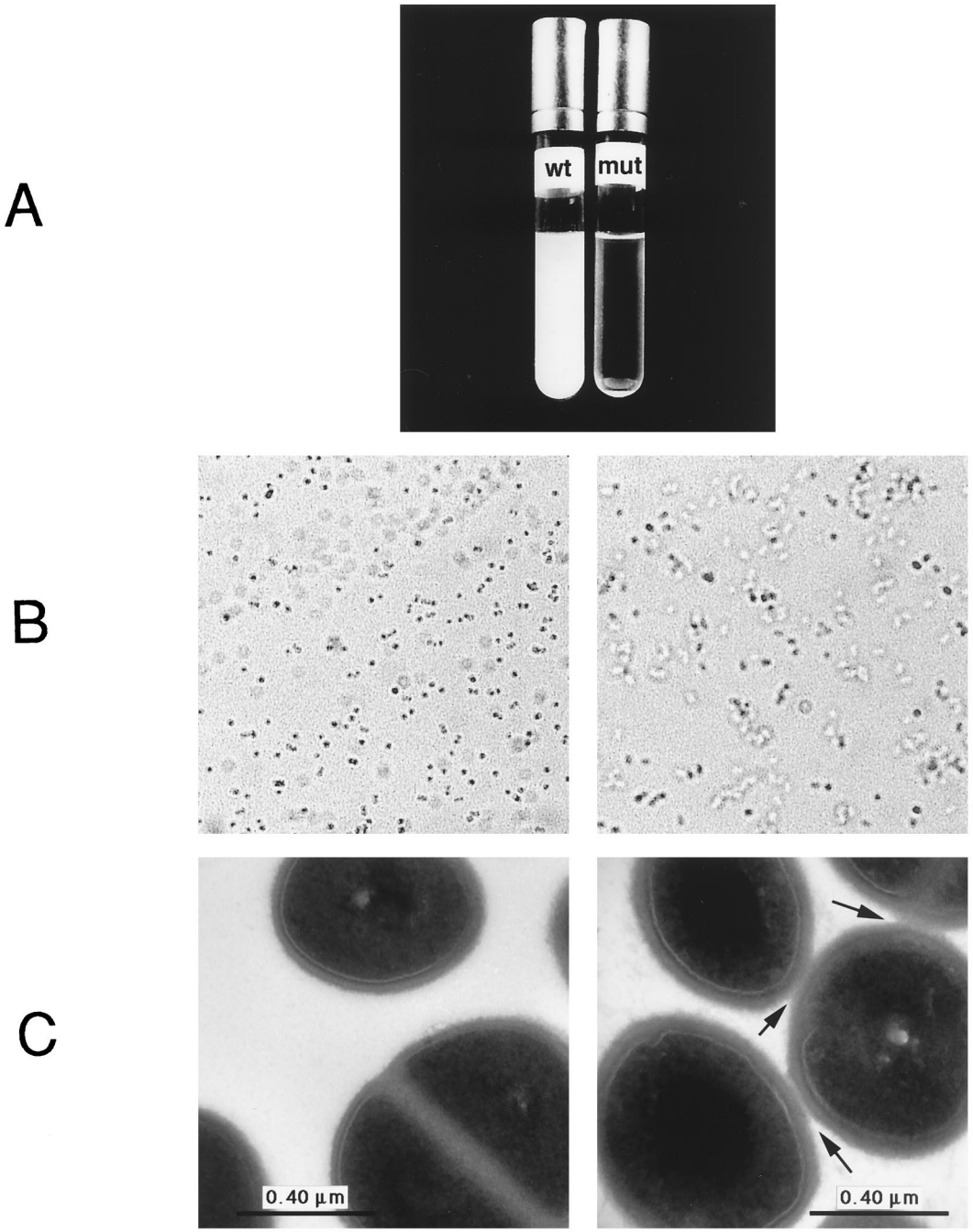

FIG. 2. Comparison of liquid cultures of wild-type (wt) and Δ*sigB* mutant (mut) forms of *S. aureus* Newman. (A) Sedimentation of a culture grown overnight is shown after 1 h without shaking. Overnight cultures were also analyzed by light microscope (×1,000 magnification) (B) or by electron microscope (C). In both cases, aggregation of the cells was visible. The arrows indicate areas of close contact between cells.

4,4′-diaponeurosporene to staphyloxanthin during late stationary phase is σ^B dependent in *S. aureus*. Interestingly, carotenoid synthesis in *Streptomyces setonii* is dependent on the σ^B homologue CrtS (17).

Increased sedimentation and aggregation. The growth rates of all of the strains tested were unaffected by the *sigB* deletion, but strain Newman Δ*sigB* exhibited accelerated sedimentation when a culture grown overnight was left without agitation. In contrast to the wild-type parent, almost all cells in the mutant culture were completely sedimented after less than 1 h (Fig. 2A). Light microscopic analysis revealed that Newman Δ*sigB* cells were clustered while wild-type cells were mostly separated (Fig. 2B). Electron microscopy confirmed close cell contact in strain Newman Δ*sigB* (Fig. 2C). Close cell contact can be due to either incomplete separation after cell division or secondary interaction of cell surface proteins, and the correct expression and cellular localization of cell surface proteins is essential for successful colonization of surfaces by *S. aureus* (11, 12). However, the increased cell aggregation of the Δ*sigB* mutant was observed only in strain Newman and not in strain COL or 8325.

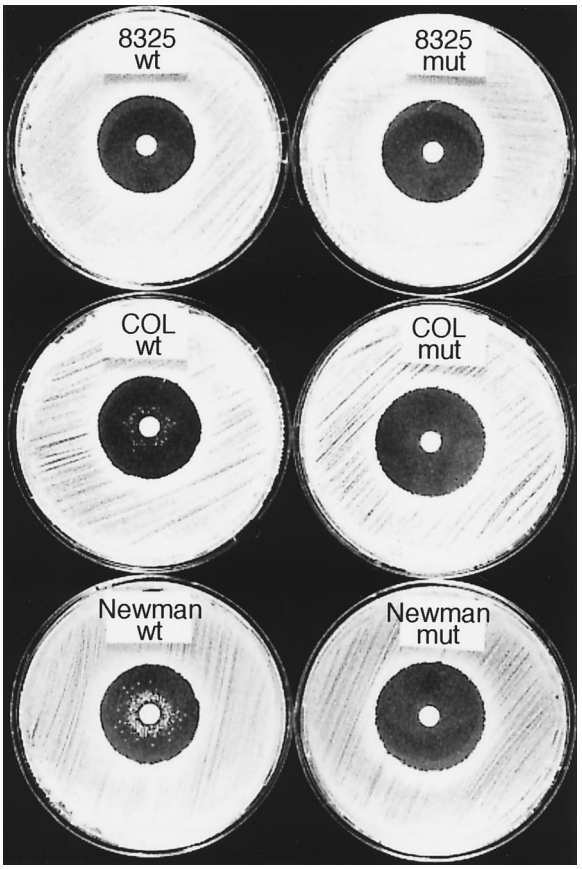

FIG. 3. Comparison of the H_2O_2 susceptibilities of wild-type (wt) and Δ*sigB* mutant (mut) strains of *S. aureus* in a disk diffusion assay after 48 h. The primary inhibition zones of all strains were comparable, but wild-type strain COL and Newman colonies growing close to the central H_2O_2 disk were visible after 48 h of incubation.

Strain Newman is known to produce high levels of clumping factor, a cell surface-associated fibrinogen receptor (23). It is possible that σ^B modulates expression of the clumping factor or of other adhesins, which might lead to the observed cell aggregation also in cultures.

Decreased susceptibility to H_2O_2 during stationary phase. Since H_2O_2 represents an important stress factor for *S. aureus* during infection, we tested the H_2O_2 susceptibility of the Δ*sigB* mutants in a disk diffusion assay. The diameters of the inhibition zones were the same in the wild type and Δ*sigB* mutants, yet interestingly, after 48 h of incubation, we repeatedly observed wild-type colonies of strains COL and Newman close to the disk (Fig. 3). Those colonies were never observed with the *sigB* deletion mutants or with wild-type strain 8325. It was possible that those colonies represented highly H_2O_2-resistant second-site mutants. However, when they were reassayed for H_2O_2 susceptibility, they were not highly resistant to H_2O_2, but the same growth pattern as in the original assay was observed. This implies that the appearance of such highly H_2O_2-resistant colonies in late stationary phase (48 h) is due to a transient effect, possibly an adaptive response to H_2O_2. We also examined the MICs and MBCs of H_2O_2 (Table 2). For the wild-type COL and Newman strains, the MBC was higher than the MIC, whereas for COL Δ*sigB*, Newman Δ*sigB*, and wild-type and Δ*sigB* 8325, the MICs and MBCs were identical. Thus, higher concentrations of H_2O_2 are required to kill wild-type COL and Newman cells than to inhibit growth, whereas in the respective *sigB* deletion mutants, as well as in strain 8325, the same concentration of H_2O_2 which inhibits growth also kills the cells. This is in accordance with the results seen in the zone assay, where after 48 h only wild-type COL and Newman cells could again grow after being initially inhibited. Taken together, these data suggest that in *S. aureus* growth at high concentrations of peroxide during late stationary phase requires σ^B.

TABLE 2. Susceptibilities of *S. aureus sigB* mutant strains and their wild-type parents to H_2O_2

Strain	MIC (mM)	MBC (mM)
8325		
Wild type	0.5	0.5
Δ*sigB* mutant	0.5	0.5
COL		
Wild type	1	4
Δ*sigB* mutant	1	1
Newman		
Wild type	0.25	4
Δ*sigB* mutant	0.25	0.25

H_2O_2 is detoxified by the enzyme catalase, which is considered to be an important virulence factor in *S. aureus*. Catalase protects the cells from the oxidative burst released from host macrophages upon infection, and in *S. aureus*, a correlation between catalase activity and virulence has been observed (16, 22). In *E. coli*, expression of the two catalase genes is regulated by the stationary-phase sigma factor σ^S (15, 25), and in *B. subtilis*, one catalase gene has been shown to be regulated by σ^B (10). *S. aureus* also appears to have multiple catalase activities (7), and since the growth of *S. aureus* at high concentrations of H_2O_2 in late stationary phase was σ^B dependent, we suggest that expression of at least one of the catalases is regulated by σ^B. Preliminary data from catalase activity stains of native protein gels with total protein from *S. aureus* wild-type and Δ*sigB* strains revealed the existence of at least two bands with catalase activity, of which one was less abundant in the Δ*sigB* strains (data not shown). However, further biochemical analysis is required to define those activities.

Expression of Asp23 and thermonuclease is affected by the *sigB* deletion. By using SDS-polyacrylamide gel electrophoresis, we compared the total protein expression patterns and the levels of excreted protein of the wild-type and Δ*sigB* strains (Fig. 4). The patterns of total protein expression of the wild-type and mutant strains were very similar. However, one very abundant protein of about 23 kDa was clearly missing in the Δ*sigB* mutants of strains COL and Newman and also in wild-type 8325 (arrow in Fig. 4A). N-terminal sequencing of this protein revealed the amino acid sequence MTVDNNKAKQ AYDNQ, which shows 100% identity with the first 15 amino acids of alkaline shock protein 23 (Asp23) of *S. aureus*. The *asp23* gene was previously found to encode a 169-amino-acid protein with an unknown function and a molecular mass of 19.2 kDa (21). Although the molecular mass of Asp23 is predicted to be 19.2 kDa, it reveals an apparent molecular mass of 23 kDa on a protein gel. It was furthermore demonstrated that expression of Asp23 is strongly induced upon a pH upshift to 10 (21). The transcriptional start site of *asp23* has been mapped (21), and upon examination of the sequence, we found a perfect σ^B-dependent promoter consensus sequence, instead of the proposed σ^A promoter, at the correct distance from the

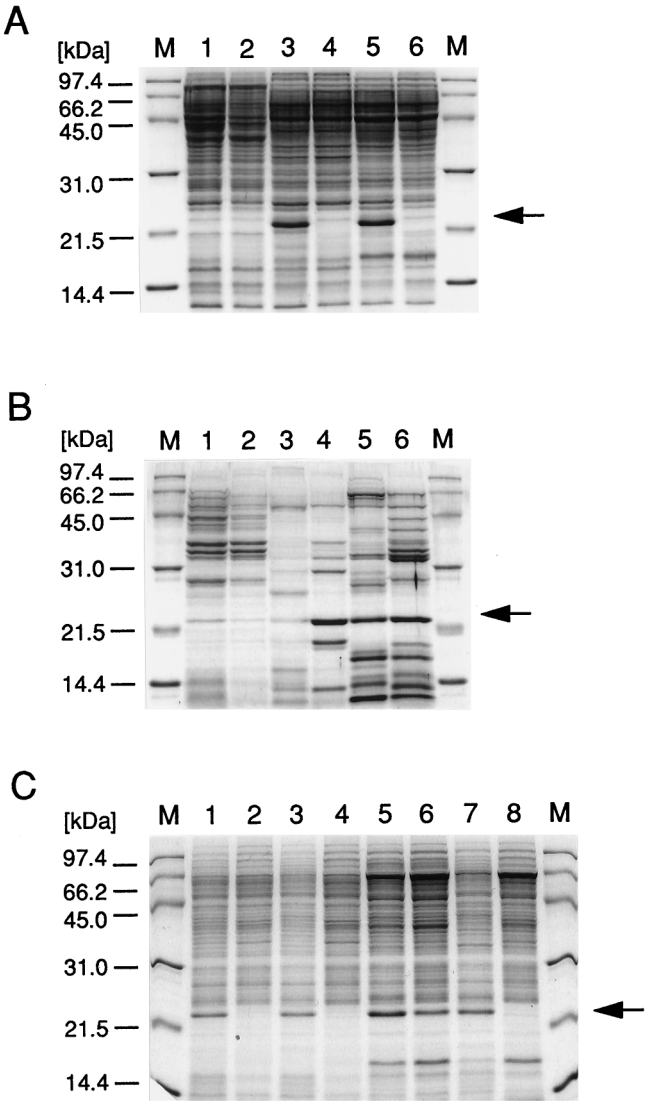

FIG. 4. SDS-polyacrylamide gel electrophoresis analysis of wild-type and Δ*sigB* mutant *S. aureus* strains. In A, about 20 μg of total cellular protein per lane was analyzed; in B, about 5 μg of excreted protein per lane was analyzed. Samples were analyzed as follows: lanes 1 and 2, strain 8325; lanes 3 and 4, strain COL; lanes 5 and 6, strain Newman. Extracts in lanes 1, 3, and 5 were from the wild-type parent, and those in lanes 2, 4, and 6 were from the corresponding Δ*sigB* mutant. In C, 10 μg of total cellular protein per lane from the following strains was analyzed: lanes 1 and 2, strain 8325 wild type; lanes 3 and 4, strain 8325 Δ*sigB*; lanes 5 and 6, strain Newman wild type; lanes 7 and 8, strain Newman Δ*sigB*. Lanes 1, 3, 5, and 7 were complemented with plasmid pIK64. A protein standard was loaded in lanes M. The arrows in A and C indicate the protein identified as Asp23. The arrow in B indicates the protein identified as staphylococcal thermonuclease.

transcriptional start of the gene. These observations strongly suggest that *asp23* is a σ^B target in *S. aureus*. In growth assays, the Δ*sigB* mutant strains were slightly more sensitive to a pH upshift from 7 to 10 during exponential growth phase (data not shown). However, the function of Asp23 in *S. aureus* and its possible role in permitting *S. aureus* to grow at high pHs remain to be determined.

On gels of the excreted proteins of *S. aureus*, the amount and pattern of excreted proteins varied significantly among wild-type strains 8325, COL, and Newman (Fig. 4B). Comparison of each wild-type parent with the respective Δ*sigB* mutant revealed three classes of proteins: (i) those which were identical in the wild type and the mutant, (ii) those which were less abundant in the mutant, and (iii) those which were enhanced in the mutant. We determined the N-terminal sequence of a 23-kDa protein from Δ*sigB* mutant strains COL and Newman (arrow in Fig. 4B) and in both cases obtained the amino acid sequence SQTDNGVNR, which showed 100% identity with amino acids 64 to 72 of the staphylococcal thermonuclease (5, 6, 18). Staphylococcal thermonuclease is an excreted toxin of 231 amino acids with a molecular mass of 25.5 kDa. Amino acids 1 to 63 serve as a signal peptide which is cleaved off after protein export. Therefore, the mature excreted protein begins with amino acid 64, which is exactly where the homology to our protein sequence starts. Thermonuclease was hardly detectable in wild-type and mutant 8325 but highly abundant in both wild-type and Δ*sigB* mutant strain Newman. Interestingly, in strain COL, thermonuclease was more prominent in the Δ*sigB* mutant than in the wild type, indicating that in this strain σ^B has a negative effect either on protein expression or on export of this protein into the medium. Taken together, our data indicate that deletion of σ^B can have both positive and negative effects on certain proteins. It also shows that additional strain-specific factors may exist which modulate σ^B-dependent regulation in the respective background.

TABLE 3. Lipase activities of *S. aureus sigB* mutant strains and their wild-type parents

Strain	Avg % activity (SD)[a]
8325	
Wild type	100
Δ*sigB* mutant	162 (8)
COL	
Wild type	100
Δ*sigB* mutant	153 (16)
Newman	
Wild type	100
Δ*sigB* mutant	373 (3)

[a] The values shown are results of four independent assays.

Increased lipase activity. Lipase represents an important virulence factor that is excreted mainly during stationary growth phase and can easily be assayed either on plates or in liquid culture supernatants. On plates, we observed that all of the *sigB* deletion strains studied produced more lipase than did their respective wild-type parents (data not shown). This effect was confirmed by a quantitative assay of culture supernatants. Interestingly, the levels of lipase expression varied significantly among the three wild-type strains, with strain 8325 showing the highest activity and strain COL showing the lowest activity. Since we focused on the effect of the *sigB* deletion in each case, the lipase activity of each wild-type strain was set as 100% and the Δ*sigB* mutant levels were compared with those of the corresponding parents (Table 3). For strains 8325 and COL, lipase production was about 1.5 times as high in the Δ*sigB* mutant as in the parent, and for strain Newman, mutant lipase activity was increased three- to fourfold. Those values were highly reproducible and were confirmed in four independent assays. Hence, we speculate that lipase production or excretion during stationary growth phase is negatively regulated by σ^B or by a σ^B-dependent factor.

Complementation of Δ*sigB* mutants restores pigmentation and Asp23 production. To prove that the observed phenotypes

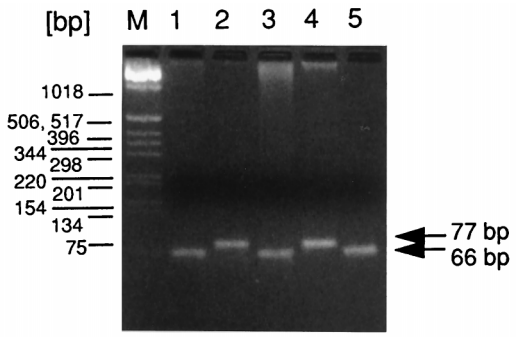

FIG. 5. PCR of different *S. aureus* strains using primers flanking the 11-bp deletion in the *rsbU* gene. Amplification products were analyzed on a 4% agarose gel as follows: lane 1, strain 8325; lane 2, strain COL; lane 3, strain RN4220, lane 4, strain Newman; lane 5 independent source of strain 8325. A 100-bp DNA ladder was loaded in lane M as a molecular size marker.

of the mutants are due to the deletion of *sigB* itself, we complemented the mutants with plasmid pIK64, carrying the *sigB* gene under control of the xylose-inducible promoter P_{Xyl}. pIK64 was introduced into the wild-type and Δ*sigB* mutant forms of strains 8325 and Newman but could not be used in strain COL because of the overlapping tetracycline resistance encoded by plasmid pIK64 and a plasmid naturally present in this background. When Δ*sigB* strains 8325 and Newman containing pIK64 were grown in medium with xylose, the formerly pigmentless strains produced a very intense orange pigment, even during exponential growth phase and not only in late stationary phase. In contrast, without xylose in the medium, no such pigment was produced. These results show that (i) *sigB* alone restores pigmentation in the pigmentless Δ*sigB* mutants and (ii) overexpression of plasmid-encoded *sigB* causes overexpression of the orange pigment staphyloxanthin even much earlier than in the wild-type situation. Therefore, we conclude that the operon for staphyloxanthin biosynthesis is a direct target of σ^B. pIK64 even caused orange pigmentation in otherwise pigmentless wild-type 8325, meaning that the missing pigmentation in this strain is not due to a loss or defect of the pigmentation genes but rather to a nonfunctional σ^B protein, most likely due to a defective RsbU protein, as described later in the report.

We also analyzed the expression of the protein Asp23 in the complemented mutants (Fig. 4C). Addition of pIK64 and xylose restored Asp23 expression in wild-type 8325, Δ*sigB* 8325, and Δ*sigB* Newman (lanes 1, 3, and 7). In wild-type strain Newman, the presence of pIK64 even slightly increased the amount of Asp23 (lane 5). These results support our assumption that *asp23* is also a direct target of σ^B. Taken together, the complementation data prove that deletion of σ^B alone leads to the observed phenotypes in the mutants described.

Deletion of 11 bp of *rsbU* in strain 8325 and derivatives. We previously found that strain 8325 contains an 11-bp deletion in the *rsbU* gene in comparison to the *B. subtilis* sequence and also in comparison to the *sigB* sequence in *S. aureus* COL (20). To test whether this deletion was present in other 8325 derivatives as well, we performed PCR with oligonucleotides flanking the deletion site. PCR should give rise to a 77-bp fragment in the wild type and a 66-bp fragment in a mutant with an 11-bp deletion. Two independent 8325 strains (lanes 1 and 5) and strain RN4220 (an 8325 derivative) (lane 3) had an 11-bp deletion, whereas strains COL and Newman (lanes 2 and 4) had no deletion (Fig. 5).

In *B. subtilis*, an intact RsbU phosphatase is required for full σ^B activity under stress conditions (32, 33). Therefore, it is to be expected that *S. aureus* σ^B can only be activated under the respective stress conditions in strains with functional RsbU. Consequently, it is plausible that deletion of σ^B results in a mutant phenotype only in strains with intact RsbU.

Wild-type strain 8325 resembles the COL and Newman Δ*sigB* mutants with respect to Asp23 expression, H_2O_2 susceptibility, and pigmentation. Therefore, we suggest that strain 8325 represents a natural σ^B-defective mutant most probably due to the 11-bp deletion in the *rsbU* gene. In contrast, Δ*sigB* strain 8325 does show a mutant phenotype with respect to lipase production during stationary growth phase, where σ^B might activate a negative effector. From work with *B. subtilis*, it is known that σ^B can be activated via two pathways: during exponential phase by an RsbU-dependent pathway and by an RsbU-independent pathway during stationary phase (33). We propose that to control lipase production during stationary phase, σ^B does not require stress induction via RsbU but can still be activated during stationary phase in an RsbU-independent way.

In summary, we have demonstrated that a *sigB* deletion in *S. aureus* produces a pleiotropic phenotype. Our results suggest that *asp23* and the operon for staphyloxanthin biosynthesis are direct targets of σ^B in *S. aureus*. In *B. subtilis*, σ^B is a stationary-phase- and stress-specific sigma factor and several target genes are known to be σ^B dependent. However, in many cases, the function of the target genes is unknown, and a *sigB* mutation has no clear phenotype. Stress survival does also not appear to be impaired in the mutants (14). σ^B may not be essential for survival but might give a competitive advantage under specific environmental conditions. In a pathogen like *S. aureus*, expression of virulence genes is not essential but enables the cells to colonize and survive in human hosts, who serve as a specialized ecological niche. Several of the functions which we have demonstrated to be modulated by σ^B in *S. aureus*, such as peroxide resistance, possibly alkali stress response, cell aggregation, or lipase and thermonuclease production, may play an important role during infection. In addition, expression of the global regulator Sar is σ^B dependent (8). The Sar protein is required for expression of the regulator Agr (3), which, in turn, affects the expression of a variety of virulence factors (30). Thus, we suggest that *S. aureus* σ^B is a stress- and stationary-phase-specific global regulator which is directly and indirectly involved in the expression of virulence genes. We predict that the identification of additional σ^B-dependent target genes will provide insight into the regulatory pathways controlling the process of infection.

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