JOURNAL OF BACTERIOLOGY, Sept. 2011, p. 4954–4962 Vol. 193, No. 18
0021-9193/11/$12.00 doi:10.1128/JB.05362-11
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The σ^B-Dependent *yabJ-spoVG* Operon Is Involved in the Regulation of Extracellular Nuclease, Lipase, and Protease Expression in *Staphylococcus aureus*▽†

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Received 22 May 2011/Accepted 15 June 2011

The alternative sigma factor σ^B of *Staphylococcus aureus* is involved in the coordination of the general stress response, expression of virulence determinants, and modulation of antibiotic resistance levels. It controls a large regulon, either directly by recognizing conserved σ^B promoter sequences or indirectly via σ^B-dependent elements. The σ^B-controlled *yabJ-spoVG* operon encodes two such putative downstream elements. We report here transcriptome analysis in *S. aureus* Newman, showing that inactivation of the *yabJ-spoVG* operon had primarily a repressing effect on a small subregulon encoding mainly virulence factors, including a nuclease (*nuc*), a protease (*splE*) and a lipase (*lip*). As a consequence, extracellular nuclease, protease, and lipase activities were reduced in a Δ*yabJ-spoVG* mutant. *trans*-complementation by SpoVG was sufficient to restore their reduced phenotypic expression and lowered transcription due to the *yabJ-spoVG* deletion. It did not restore, however, the changes triggered by σ^B inactivation, indicating that both regulons only partially overlap, despite the σ^B dependency of the *yabJ-spoVG* expression. Thus, σ^B is likely to control additional, SpoVG-independent factors affecting the expression of numerous hydrolytic enzymes. SpoVG, on the other hand, seems to fine-tune the σ^B-dependent regulation of a subset of virulence factors by antagonizing the σ^B effect.

Staphylococcus aureus secretes a wide array of extracellular virulence factors that enable it to cause superficial skin infections or severe invasive infections, such as bacteremia, endocarditis, and osteomyelitis (4, 9, 18). The growth- and environment-dependent expression of virulence factors is a prerequisite for the survival of *S. aureus* in the host and the pathogenesis of staphylococcal infections. During initial colonization steps, the expression of cell surface adhesins promotes the binding to human extracellular matrix components (16). Extracellular enzymes, such as nucleases, lipases, and proteases, are, on the other hand, produced mainly in later infection stages. They destroy the local host tissue to access nutrients required for growth and spreading within the host (18) and facilitate immune evasion by interfering with the host's innate immune system (5, 41).

The coordinated expression of virulence determinants in *S. aureus* is controlled by a complex network of regulatory elements, including two-component regulatory systems, DNA-binding proteins, and alternative sigma factors (8, 13, 32, 53). σ^B is the best characterized alternative sigma factor of *S. aureus*. It is involved in general stress responses (11, 22, 28, 32, 38) and part of the regulatory network controlling the expression of virulence determinants (25, 28, 31, 54) and modulates antibiotic resistance levels (6, 37, 46, 48, 53). σ^B activity is growth phase regulated, peaking toward early stationary phase (47), and controls a large regulon of genes involved in many different cellular processes by recognizing and binding to a conserved nucleotide consensus sequence (GTTTAA-12-15-G GGTAT) in the upstream region of the respective genes/operons (7, 27, 38). However, many of the genes that are regulated by σ^B are not preceded by such a σ^B promoter. It is therefore thought that σ^B-dependent regulation of those genes occurs by downstream regulatory elements (7, 38).

The bicistronic *yabJ-spoVG* operon, whose transcription depends heavily on σ^B activity, was hypothesized to play a role in the indirect σ^B-dependent regulation of genes lacking an obvious σ^B promoter (7, 36, 46). The molecular mechanisms of YabJ, which belongs to the YjgF protein family of unknown biochemical function (49), and of the stage V sporulation protein G SpoVG (30), originally identified in *Bacillus subtilis* as being involved in the formation of the spore cortex, are yet unknown for the nonsporulating *S. aureus*. However, the finding that inactivation of the staphylococcal *yabJ-spoVG* operon, like inactivation of σ^B, reduces the transcription of the *capA* gene, which is not preceded by a σ^B promoter (36), and reduces the resistance level of methicillin-resistant *S. aureus* (MRSA) and glycopeptide intermediate-resistant *S. aureus* (GISA) (46) support the hypothesis that the *yabJ-spoVG* operon may encode a σ^B downstream regulatory element.

Early work on σ^B activity in *S. aureus* showed that extracellular nuclease and lipase activities are negatively regulated by the alternative sigma factor σ^B via a yet unknown indirect

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† Supplemental material for this article may be found at http://jb.asm.org/.

▽ Published ahead of print on 1 July 2011.

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype[a]	Reference or source
S. aureus strains		
Newman	Clinical isolate, ATCC 25904, *saeS* constitutively active	1, 20
SM148	Newman Δ*yabJ-spoVG::erm*(B); Em^r	46
MS64	Newman *sigB1*(Am); Tc^r	47
IK184	Newman Δ*rsbUVW-sigB::erm*(B); Em^r	32
Coln	Tc^s derivative of *S. aureus* Col; Mc^r	29
SM165	Coln Δ*yabJ-spoVG::erm*(B); Em^r	46
BS128	Coln Δ*rsbUVW-sigB::erm*(B); Em^r	46
Plasmids		
pMGS100	Overexpression plasmid containing the *bacA* promoter of *E. faecalis*; Cm^r	21
pBus1	*E. coli-S. aureus* shuttle plasmid with multicloning site from pBlue-script II SK (Stratagene) and the rrnT14 terminator sequence from pLL2443; Tc^r	43
pSTM08	pBus1 with a 2.1-kb PCR fragment covering *yabJ*, *spoVG*, and the preceding σ^B-dependent promoter P_{yabJ}; Tc^r	46
p08-*yabJ*-His	pSTM08 with a 6-histidine tag added to *yabJ*; Tc^r	This study
p08-*spoVG*-His	pSTM08 with a 6-histidine tag added to *spoVG*; Tc^r	This study
p*yabJ*	pBus1 containing a *bacA* promoter-*yabJ* ORF fusion construct; Tc^r	This study
p*yabJ*-His	p*yabJ* with a 6-histidine tag added to *yabJ*; Tc^r	This study
p*spoVG*	pBus1 containing a *bacA* promoter-*spoVG* ORF fusion construct; Tc^r	This study
p*spoVG*-His	p*spoVG* with a 6-histidine tag added to *spoVG*; Tc^r	This study
p*spoVG-Bsu*	pBus1 containing the *bacA* promoter fused to the *spoVG* ORF of *B. subtilis*; Tc^r	This study
p*yabJspoVG*	pBus1 containing a *bacA* promoter-*yabJ-spoVG* operon fusion construct; Tc^r	This study

[a] Abbreviations are as follows: Cm^r, chloramphenicol resistant; Em^r, erythromycin resistant; Mc^r, methicillin resistant; Tc^r, tetracycline resistant; Tc^s, tetracycline sensitive.

mechanism (31). In this study, we show that deletion of the σ^B-dependent *yabJ-spoVG* operon reduces nuclease, lipase, and protease activities in *S. aureus* Newman. We present transcriptomic data allowing the comparison of the *yabJ-spoVG* regulon with the σ^B regulon, and we identify a set of genes that is influenced by σ^B as well as by *yabJ-spoVG*. We demonstrate that SpoVG, and not YabJ, is the major regulator of the *yabJ-spoVG* operon.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The bacterial strains and plasmids used in this study are listed in Table 1. Bacteria were grown on Luria Bertani (LB) agar (Becton Dickinson, Allschwil, Switzerland) or in LB broth with shaking (180 rpm) at 37°C. Where required, media were supplemented with either 20 μg chloramphenicol or 10 μg tetracycline per ml.

Molecular biological methods. General molecular biology techniques were performed according to standard protocols described by Sambrook et al. (44) and Ausubel et al. (2). Sequencing was done using the BigDye Terminator cycle sequencing ready reaction kit and an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA). Sequences were analyzed with the Lasergene software package (DNASTAR, Inc., Madison, WI).

Plasmid construction. For the construction of the complementing plasmids p*yabJ*, p*spoVG*, and p*yabJspoVG*, DNA fragments covering *yabJ*, *spoVG*, or the whole *yabJ-spoVG* operon were amplified from *S. aureus* Newman genomic DNA using primer pairs oSTM51/oBS09, oBS11/oBS10, and oSTM51/oBS10, respectively. These fragments were digested with NruI and EclXI and inserted into plasmid pMGS100 downstream of the *bacA* promoter. The promoter-gene fusions were amplified from the resulting plasmids with primer pair oDB2/oBS30 or oBS28/oBS30, digested with PstI and XbaI, and cloned into *Escherichia coli-S. aureus* shuttle vector pBus1 to give p*yabJ*, p*spoVG*, and p*yabJspoVG*.

p*spoVG-Bsu* was constructed by amplifying *spoVG* with primers Bsu-spoVG-f and Bsu-spoVG-r from *Bacillus subtilis* ATCC 6633 genomic DNA, digesting the resulting fragment with Eco521 and SacI and cloning it into Eco521/SacI-digested p*yabJ*.

A 6-histidine tag was added to the 3′ end of *yabJ* and *spoVG* in pSTM08 by amplifying it with primer pairs YabJ-his/oBS33 and SpoVG-his/oBS34, respectively, and ligating it to give plasmids p08-*yabJ*-His and p08-*spoVG*-His. Similarly, a 6-histidine tag was added to p*yabJ* and p*spoVG* by amplifying the respective plasmid with primer pair YabJ-his/oBS33 for p*yabJ* and SpoVG-his/oBS34 for p*spoVG* and ligating it to give plasmids p*yabJ*-His and p*spoVG*-His.

All plasmid constructs were confirmed by sequence analyses.

Northern blot analysis. Overnight cultures were diluted 1:100 into LB broth, grown for 2 h, and then used to inoculate 100 ml prewarmed LB to an optical density at 600 nm (OD_{600}) of 0.05. Cell samples were taken at the time points indicated and centrifuged at 9,000 × *g* and 4°C for 2 min, the culture supernatants discarded, and the cell sediments snap-frozen in liquid nitrogen. Total RNA was isolated according to the method of Cheung et al. (14). RNA samples (8 μg) were separated in a 1.5% agarose gel containing 20 mM guanidine thiocyanate in 1× Tris-borate-EDTA buffer (24). RNA transfer and detection were performed as previously described (35). Digoxigenin (DIG)-labeled probes were amplified using the PCR DIG probe synthesis kit (Roche, Basel, Switzerland). The primer pairs used for amplification of the hybridization probes are listed in Table 2. All Northern blots were repeated at least twice, using independently isolated RNA samples.

Microarray design and manufacturing. The microarray was manufactured by *in situ* synthesis of 60-base-long oligonucleotide probes (Agilent, Palo Alto, CA), selected as previously described (12). It covers approximately >98% of all open reading frames (ORFs) annotated for strains N315 and Mu50 (33), MW2 (3), COL (23), NCTC8325 (accession no. NC_007795), USA300 (17), and MRSA252 and MSSA476 (26), including their respective plasmids.

Expression microarrays. Total RNA was purified from bacteria grown in LB broth for 5 h as described for the Northern blot analysis. DNA-free total RNA was obtained after DNase treatment on RNeasy columns (Qiagen). For each strain, RNA of three independently grown cultures was analyzed. The absence of remaining DNA traces was confirmed by quantitative PCR (SDS 7700; Applied Biosystems, Framingham, MA) with assays specific for 16S rRNA genes (40, 45). Batches of 5 μg of total *S. aureus* RNA were labeled by Cy-3-dCTP using the SuperScript II (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Labeled products were purified onto QiaQuick columns (Qiagen).

Purified genomic DNA from the different sequenced strains used for the design of the microarray was extracted (DNeasy, Qiagen), labeled with Cy5-dCTP using the Klenow fragment of DNA polymerase I (BioPrime; Invitrogen) (12), and used for the normalization process (50). Cy5-labeled DNA (500 ng) and Cy3-labeled cDNA mixture were diluted in 50 μl Agilent hybridization buffer and then hybridized at a temperature of 60°C for 17 h in a dedicated hybridization oven (Robbins Scientific, Sunnyvale, CA). Slides were washed with Agilent proprietary buffers, dried under nitrogen flow, and scanned (Agilent) using 100% photon multiplier tube power for both wavelengths.

TABLE 2. Oligonucleotide primers used in this study

Primer name	Sequence (5′–3′)[a]	Source or reference
DIG probes		
NWMN_0095-DIG f	CAGTTTATGGCGCAAGAGT	This study
NWMN_0095-DIG r	GAACCCAATACAGGCAATCC	This study
NWMN_0218-DIG f	CCTGCCCAGTTTTTAGCATC	This study
NWMN_0218-DIG r	AACAACACGCCAAACAACAA	This study
NWMN_0219-DIG f	TCCAGAGGAAATCAGAGCAAA	This study
NWMN_0219-DIG r	CTTGTTCTTGAACGGCATCA	This study
NWMN_0394-DIG f	ACATCAGAAGGCCAAGCAGT	This study
NWMN_0394-DIG r	TCTTGCTTTTCTCCATCTTTCA	This study
NWMN_0655-DIG f	TTTCATCAAATGCATGAATGACT	This study
NWMN_0655-DIG r	CAATGCTCAAAGACAAGTTAATCG	This study
NWMN_0760-DIG f	GAAAGGGCAATACGCAAAGA	This study
NWMN_0760-DIG r	TAGCCAAGCCTTGACGAACT	This study
NWMN_1560-DIG f	GATTAACCCCTCCTGGTGCT	This study
NWMN_1560-DIG r	AGCTTAACGCAAACATCACG	This study
NWMN_1702-DIG f	TTGCCTGGTTCTACGAAAGC	This study
NWMN_1702-DIG r	GGCACAACAGTGGTTGAGG	This study
NWMN_1718-DIG f	AGTTACCACGCGCCAATAA	This study
NWMN_1718-DIG r	GCAGCCGGAAACATTAATTC	This study
NWMN_2569-DIG f	AAATTTCGCCACCATGTTTC	This study
NWMN_2569-DIG r	CCAAACAAAGGGACAAAGGA	This study
oSTM16 (*glmU* f)	GAAGACACGCGATAATTTTGG	This study
oSTM17 (*glmU* r)	CAGTTGCTTGAGCATTAGCATC	This study
SA*asp23*+	ATGACTGTAGATAACAATAAAGC	22
SA*asp23*−	TTGTAAACCTTGTCTTTCTTGG	22
Plasmid constructions		
oDB02	CGCTCTAGATTATAATTCCTTAATTTTACTTAC	This study
oBS09	TTTTTCGCGATAATTCCTTAATTTTACTTACTAATTC	This study
oBS10	TTTTTCGCGAAGCTTCTTCTGAATCTTCTGATGTAG	This study
oBS11	TTTTTCGGCCGGCATGAAAGTGACAGATGTAAGAC	This study
oBS28	CGTTCTAGATTAAGCTTCTTCTGAATCTTCTG	This study
oBS30	GGGCTGCAGTGATTGAAACTCAAGATAGATATG	This study
oBS33	TTTTCGATTAATATGTTTAATC	This study
oBS34	TCAATTTTATATTTAGCGATG	This study
oSTM51	TTTTTCGGCCGGCATGAAAATGATTAACACAACAAG	This study
SpoVG-his	GCGGTAGGTACCTTAATGATGATGATGATGATGAGCTTCTTCTGAATCTTCTG	This study
YabJ-his	GTAGGTACCTTAATGATGATGATGATGATGTAATTCCTTAATTTTACTTAC	This study
Bsu-spoVG-f	GCACGGCCGGCATGGAAGTTACTGACGTAAG	This study
Bsu-spoVG-r	GCAGAGCTCTTAAGAAGCTCCAGCTTCTTC	This study

[a] Restriction sites are underlined.

Microarray analysis. Fluorescence intensities were extracted using the Feature extraction software (Agilent, version 8). Local background-subtracted signals were corrected for unequal dye incorporation or unequal loading of the labeled product. The algorithm consisted of a rank consistency filter and a curve fit using the default Lowess (locally weighted linear regression) method. Data consisting of three independent biological experiments were analyzed using GeneSpring 8.0 (Silicon Genetics, Redwood City, CA). A filter was applied to select oligonucleotides mapping ORFs in the Newman genome, yielding approximately 92% coverage. Statistical significance of differentially expressed genes was identified by analysis of variance (ANOVA) (15, 45), using GeneSpring, including the Benjamini and Hochberg false discovery rate correction of 5% (*P* value cutoff of 0.05) and an arbitrary cutoff of 2-fold for expression ratios. Microarray data have been posted on the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) under accession numbers GPL7137 for the platform and GSE30115 for the complete experimental data set.

Enzyme activity assays. (i) Protease. The proteolytic activity of *S. aureus* was determined on skim milk agar plates (75 g liter^{-1} skim milk [Becton Dickinson]) as clear zones surrounding the colonies. The strains were grown on skim milk plates for 24 h at 37°C and incubated for 72 h at room temperature.

(ii) Nuclease. The nuclease activity was measured as described by Berends et al. (5). Supernatants were harvested from early stationary-phase cultures (OD_{600} = 4) after centrifugation for 5 min at 2,000 × *g*.

(iii) Lipase. Lipase activity was tested by monitoring hydrolysis of 4-nitrophenyl octanoate by supernatants of cultures grown to an OD_{600} of 7 (31). A volume of 50 μl of the supernatant was incubated with 1.2 ml of 4-nitrophenyl octanoate (Sigma) solution (1 mg ml^{-1} in 20 mM Tris-HCl [pH 9.0], 10 mM $CaCl_2$, and 0.1% [vol/vol] Triton X-100) for 15 min at 37°C. The absorption at 405 nm was measured, and the lipase activity was expressed as a percentage of that of the wild-type strain. Values of four independent assay were statistically evaluated using a paired, two-tailed Student *t* test.

Western blot analysis. Cytoplasmic protein extracts were obtained by lysing *S. aureus*, grown in LB broth at 37°C for 5 h, in phosphate-buffered saline (pH 7.4) containing 2 mM phenylmethylsulfonyl fluoride and 40 μg each of lysostaphin, lysozyme, DNase, and RNase per ml. Protein fractions (10 to 15 μg/lane) were separated using SDS-PAGE, blotted onto a nitrocellulose membrane, and subjected to Western blot analysis using rabbit anti-6×His tag antibodies (Abcam, Cambridge, MA).

Population analysis profiles. Oxacillin resistance profiles were determined by plating aliquots of overnight cultures on LB agar plates containing increasing concentrations of oxacillin and determining the number of CFU after 48 h of incubation at 37°C.

RESULTS AND DISCUSSION

Transcriptional profiling of genes depending on the *yabJ-spoVG* operon. The σ^B-controlled *yabJ-spoVG* operon has pleiotropic effects in *S. aureus*, stimulating *cap* operon transcription and capsule formation, and supporting resistance to

TABLE 3. Genes with ≥2 times higher expression in wild-type *S. aureus* Newman than in the Δ*yabJ-spoVG* mutant SM148

Newman TIGR locus name[a]	Gene symbol[b]	Description[b]	Newman/ SM148 fold change[c]	Newman/ MS64 fold change[c]	Reference(s) with reported σ^B dependence	σ^B promoter[d]	Transcription unit 5′–3′[e]	Pattern[f]
NWMN_0095*	*capA*	Capsular polysaccharide biosynthesis protein Cap5A	9.3	2.8	7, 25, 36, 38	No	*nwmn_0095-0110*	i
NWMN_0096	*capB*	Capsular polysaccharide biosynthesis protein Cap5B	11.0	2.8	7, 38	No	*nwmn_0095-0110*	
NWMN_0097	*capC*	Capsular polysaccharide biosynthesis protein Cap5C	9.5	2.5	7, 38	No	*nwmn_0095-0110*	
NWMN_0098	*capD*	Capsular polysaccharide biosynthesis protein Cap5D	12.1	2.8	7, 38	No	*nwmn_0095-0110*	
NWMN_0099	*capE*	Capsular polysaccharide biosynthesis protein Cap5E	7.2	2.4	7, 38	No	*nwmn_0095-0110*	
NWMN_0100	*capF*	Capsular polysaccharide biosynthesis protein Cap5F	7.5		7, 38	No	*nwmn_0095-0110*	
NWMN_0101	*capG*	Capsular polysaccharide biosynthesis protein Cap5G	6.0		7, 38	No	*nwmn_0095-0110*	
NWMN_0102	*capH*	Capsular polysaccharide biosynthesis protein Cap5H	4.4	2.1	7, 38	No	*nwmn_0095-0110*	
NWMN_0103	*capI*	Capsular polysaccharide biosynthesis protein Cap5I	4.9	2.4	7, 38	No	*nwmn_0095-0110*	
NWMN_0104	*capJ*	Capsular polysaccharide biosynthesis protein Cap5J	3.1	2.1	7, 38	No	*nwmn_0095-0110*	
NWMN_0105	*capK*	Capsular polysaccharide biosynthesis protein Cap5K	2.7	2.1	7, 38	No	*nwmn_0095-0110*	
NWMN_0106	*capL*	Capsular polysaccharide biosynthesis protein Cap5L	2.1		7, 38	No	*nwmn_0095-0110*	
NWMN_0107	*capM*	Capsular polysaccharide biosynthesis protein Cap5M	2.2	2.1	7, 38	No	*nwmn_0095-0110*	
NWMN_0108	*capN*	Capsular polysaccharide biosynthesis protein Cap5N	2.0		7, 38	No	*nwmn_0095-0110*	
NWMN_0152		Maltose ABC transporter substrate-binding protein, putative	2.8		25	No	*nwmn_0151-0156*	iii
NWMN_0218*		Staphyloxanthin biosynthesis protein, putative	2.9	0.5	7, 25	No	*nwmn_0218*	ii
NWMN_0219*	*esxA*	Virulence factor EsxA	2.7			Yes	*nwmn_0219*	iii (ii)
NWMN_0220	*esaA*	Membrane protein EsaA	2.2		7, 25	No	*nwmn_0220-0227*	iii
NWMN_0451		Conserved hypothetical protein	2.1			No	*nwmn_0451-0452*	iii
NWMN_0460	*yabJ*	YabJ protein	254.0	3.6	7, 36, 46	Yes	*nwmn_0460-0461*	−
NWMN_0461	*spoVG*	Regulatory protein SpoVG	77.6	4.1	7, 36, 46	No	*nwmn_0460-0461*	−
NWMN_0525	*sdrE*	Ser-Asp rich fibrinogen/bone sialoprotein-binding protein SdrE	2.2	0.4		No	*nwmn_0525*	ii
NWMN_0559		Conserved hypothetical protein	2.3			No	*nwmn_0559-0560*	iii
NWMN_0753		Lipoprotein, putative	2.1			No	*nwmn_0753*	iii
NWMN_0760*	*nuc*	Thermonuclease precursor	3.0		7, 25, 38	No	*nwmn_0760*	iii (ii)
NWMN_1279	*trpE*	Anthranilate synthase component I	2.1			No	*nwmn_1279-1285*	iii
NWMN_1560*	*abrB*	Ammonia monooxygenase	2.4	3.3		No	*nwmn_1560*	i
NWMN_1702*	*splE*	Serine protease SplE	2.1	0.1	7, 54	No	*nwmn_1702*	ii
NWMN_1708		Conserved hypothetical protein	2.0			No	*nwmn_1708*	iii
NWMN_1718*	*lukD*	Leucotoxin LukD	2.9	0.1		No	*nwmn_1719-1718*	ii
NWMN_1719	*lukE*	Leucotoxin LukE	2.7	0.1		No	*nwmn_1719-1718*	
NWMN_2061	*fmtB*	Methicillin resistance determinant FmtB protein	2.5			No	*nwmn_2061*	iii
NWMN_2365		Conserved hypothetical protein	2.2		38	No	*nwmn_2367-2365*	iii
NWMN_2553	*hsa*	LPXTG cell wall surface anchor family protein	2.1		7	Yes	*nwmn_2553*	iii
NWMN_2569*	*lip*	Lipase	2.3	0.3	7, 54	No	*nwmn_2569*	ii

[a] Locus number for *S. aureus* Newman (http://www.tigr.org). *, genes selected for Northern blot analysis.
[b] Gene symbol and description of the gene based on the TIGR annotation (http://www.tigr.org).
[c] Fold change of gene transcription comparing *S. aureus* Newman with either SM148 or MS64.
[d] ORFs preceded by a promoter sequence that resembles the σ^B consensus sequence of *S. aureus* described by Homerova et al. (27). Sequences matching the GNNTNN-12-15-NGGTAN consensus, with no more than three mismatches of the degenerated bases from the GTTTAA-12-15-GGGTAT sequence and lying within a range of 500 bp upstream of the ORF were accepted.
[e] Transcription units were deduced from Northern blot analyses done in this study or published by Pané-Farré et al. (38) or Meier et al. (36). Where no experimental data were available, the operons are indicated as predicted by Wang et al. (52).
[f] Assignment of transcription units into regulatory patterns: i, upregulated by YabJ/SpoVG and σ^B; ii, upregulated by YabJ/SpoVG, downregulated by σ^B; iii, upregulated by YabJ/SpoVG; −, deleted *yabJ-spoVG* operon, upregulated by σ^B; in parentheses, assignment to class ii according to Northern blot analyses.

cell wall antibiotics (36, 46). To explore the extent of the *yabJ-spoVG* regulon, we used full-genome DNA microarrays and compared the transcriptional patterns of *S. aureus* Newman and its isogenic Δ*yabJ-spoVG* mutant, SM148, in the early stationary growth phase, when σ^B activity and *spoVG* transcription are known to be maximal (7, 36, 47). Forty-three genes belonging to 27 predicted transcriptional units were found to be differently regulated. Nineteen operons were downregulated, and 8 were upregulated 2-fold or more in SM148 (Tables 3 and 4), suggesting that the *yabJ-spoVG* operon had primarily activating functions. The majority of the differentially expressed genes belong to the group of virulence factors, including those that encode members of the capsular polysaccharide synthesis (*cap*) operon, members of the staphylococcal ESX-1 secretion system (*esxA* and *esaA*), leukotoxins (*lukED*), exotoxins (*set1*/*6*/*7nm*), a nuclease (*nuc*), a serine protease (*splE*), a lipase (*lip*) and an adhesin (*sdrE*).

Overlap of the *yabJ-spoVG* and the σ^B regulons. To identify the overlap of the σ^B and *yabJ-spoVG* regulons, we performed in parallel differential transcriptome microarrays of *S. aureus* Newman and its isogenic Δ*sigB* mutant, MS64. This analysis identified 191 genes to be differentially influenced by σ^B 2-fold or more (see Table S1 in the supplemental material). The intersection of the *yabJ-spoVG* and the σ^B regulons covered 10 transcriptional units summarized in Tables 3 and 4. The microarrays revealed five different regulatory patterns (i to v) of the YabJ/SpoVG subregulon: pattern i contained genes upregulated by both YabJ/SpoVG and σ^B; pattern ii contained genes upregulated by YabJ/SpoVG but downregulated by σ^B; pattern iii contained genes upregulated by YabJ/SpoVG; pattern iv contained genes that were downregulated by YabJ/SpoVG but upregulated by σ^B; and pattern v contained genes that were downregulated by YabJ/SpoVG. Interestingly, 8 operons affected by σ^B activity were regulated by YabJ/SpoVG

TABLE 4. Genes with ≥2 times lower expression in wild-type *S. aureus* Newman than in the Δ*yabJ-spoVG* mutant SM148

Newman TIGR locus name[a]	Gene symbol[b]	Description[b]	Newman/ SM148 fold change[c]	Newman/ MS64 fold change[c]	Reference(s) with reported σ^B dependence	σ^B promoter[d]	transcription unit 5′–3′[e]	Pattern[f]
NWMN_0388	*set1nm*	Superantigen-like protein, exotoxin NM1	0.4			No	*nwmn_0388*	v
NWMN_0393	*set6nm*	Superantigen-like protein, exotoxin NM6	0.5	2.6		No	*nwmn_0393*	iv
NWMN_0394*	*set7nm*	Superantigen-like protein, exotoxin NM7	0.3	2.2		No	*nwmn_0394*	iv
NWMN_0462*	*glmU*	UDP-*N*-acetylglucosamine pyrophosphorylase	0.2			No	*nwmn_0462-0464*	v
NWMN_0463	*prsA*	Ribose-phosphate pyrophosphokinase	0.3			No	*nwmn_0462-0464*	
NWMN_0464	*rplY*	50S ribosomal protein L25, Ctc form	0.4			No	*nwmn_0462-0464*	
NWMN_0655*	*norR*	HTH-type transcriptional regulator MgrA	0.4			No	*nwmn_0655*	v (iv)
NWMN_2074		Conserved hypothetical protein	0.5			No	*nwmn_2074*	v
NWMN_2086*	*asp23*	Alkaline shock protein 23	0.3	33.9	7, 22, 38	Yes	*nwmn_2088-2086*	iv
NWMN_2187	*yut*	Urea transporter	0.4			No	*nwmn_2187*	v

[a] Locus number for *S. aureus* Newman (http://www.tigr.org). *, genes selected for Northern blot analysis.
[b] Gene symbol and description of the gene based on the TIGR annotation (http://www.tigr.org).
[c] Fold change of gene transcription comparing *S. aureus* Newman with either SM148 or MS64.
[d] ORFs preceded by a promoter sequence that resembles the σ^B consensus sequence of *S. aureus* described by Homerova et al. (27). Sequences matching the GNNTNN-12-15-NGGTAN consensus, with no more than three mismatches of the degenerated bases from the GTTTAA-12-15-GGGTAT sequence and lying within a range of 500 bp upstream of the ORF were accepted.
[e] Transcription units were deduced from Northern blot analyses done in this study or published by Pané-Farré et al. (38) or Meier et al. (36). Where no experimental data were available, the operons are indicated as predicted by Wang et al. (52).
[f] Assignment of transcription units into regulatory pattern: iv, downregulated by YabJ/SpoVG, upregulated by σ^B; v, downregulated by YabJ/SpoVG; in parentheses, assignment to class iv according to Northern blot analyses.

in an opposite way, indicating that YabJ/SpoVG might antagonize the σ^B activity to fine-tune the σ^B-driven expression of a subset of σ^B-dependent genes/operons.

Computer-based searches for putative σ^B-binding sites in the region upstream of the translational start site of the ORFs of the *yabJ-spoVG* regulon were done using the σ^B promoter consensus sequence determined by Homerova et al. (27) (Tables 3 and 4). Most genes were not preceded by a σ^B consensus sequence, suggesting an indirect σ^B-dependent regulation for the majority of the genes of the *yabJ-spoVG* regulon. The only exceptions were the genes *asp23*, which is known to be σ^B dependent (7, 22, 38), and, *esxA* and *hsa*. The latter are preceded by putative σ^B promoter sequences, but their functionality remains to be confirmed.

Confirmation of microarray results. To confirm the microarray results, the transcriptional profiles of a selection of ORFs found to be regulated by the *yabJ-spoVG* operon were verified by Northern blot analyses. The transcription levels of eight down- and four upregulated ORFs were compared for the wild-type strain Newman, the Δ*yabJ-spoVG* mutant, SM148, and the Δ*sigB* mutant, MS64, selecting those genes with the highest differential expression upon *yabJ-spoVG* inactivation and one representative per predicted operon. The Northern blots of the selected ORFs as well as their genomic context are shown in Fig. 1. All downregulated ORFs tested, including *capA*, *nuc*, *nwmn_0218*, which encodes a putative staphyloxanthin biosynthesis protein, *lukD*, *esxA*, *abrB*, which encodes an ammonia monooxygenase, *lip*, and *splE*, produced weaker transcripts in the Δ*yabJ-spoVG* mutant, SM148, compared to the wild type. In the Δ*sigB* mutant, MS64, which consequently does not express the σ^B-dependent *yabJ-spoVG* operon, two different responses were observed for these eight genes: (i) *capA* and *abrB* transcripts were reduced, while (ii) the remaining six ORFs were overexpressed, confirming the microarray data for *splE*, *lukD*, and *lip.* The Northern blot analyses also suggested that σ^B downregulated *esxA*, *nwmn_0218*, and *nuc*, despite the fact that the σ^B microarray data did not reach the threshold of 2 for these last genes, but downregulation of *nwmn_0218* and *nuc* by σ^B was reported previously (7). Taking into account these results, the predominant regulatory pattern for genes influenced by YabJ/SpoVG and σ^B was pattern ii, in which YabJ/SpoVG acts as activator and counteracts the repressing effect of σ^B.

Only two of the four genes predicted by the microarray data to be upregulated in the Δ*yabJ-spoVG* mutant, SM148, could be confirmed by Northern blot analyses, namely, *glmU*, encoding a UDP-*N*-acetylglucosamine pyrophosphorylase, and *set7nm*, encoding an exotoxin. Transcription of the genes encoding the alkaline shock protein 23 (*asp23*) and the transcriptional regulator NorR (*norR*) apparently did not respond to *yabJ-spoVG* inactivation. The reasons for this discrepancy need further exploration.

Complementation analyses. Since the *yabJ-spoVG* operon is under σ^B control and therefore not transcribed in Δ*sigB* mutants (36, 46), we fused *yabJ* and *spoVG* to the *bacA* promoter of *Enterococcus faecalis*, which is expressed constitutively in *S. aureus* (21). The resulting plasmids p*yabJ*, p*spoVG*, and p*yabJspoVG* allowed a σ^B activity-independent complementation of both the Δ*yabJ-spoVG* and Δ*sigB* mutants.

The typical and most common regulatory pattern, ii, namely, upregulation by YabJ/SpoVG and downregulation by σ^B as exemplified by *nuc* (Fig. 1) was used to analyze if YabJ and/or SpoVG was required for restoring *nuc* expression in the Δ*yabJ-spoVG* mutant SM148 and to assess their effect on *nuc* regulation in the absence of σ^B. For this purpose we complemented SM148 and the σ^B mutant IK184 with either gene separately or both genes together, expressed from the σ^B-independent constitutive promoter. In analogy to the complementation of *capA* transcription in a Δ*yabJ-spoVG* background, where SpoVG was shown to be the effector of the *yabJ-spoVG* operon (36, 46), the plasmids p*spoVG* and p*yabJspoVG* did largely restore *nuc* expression

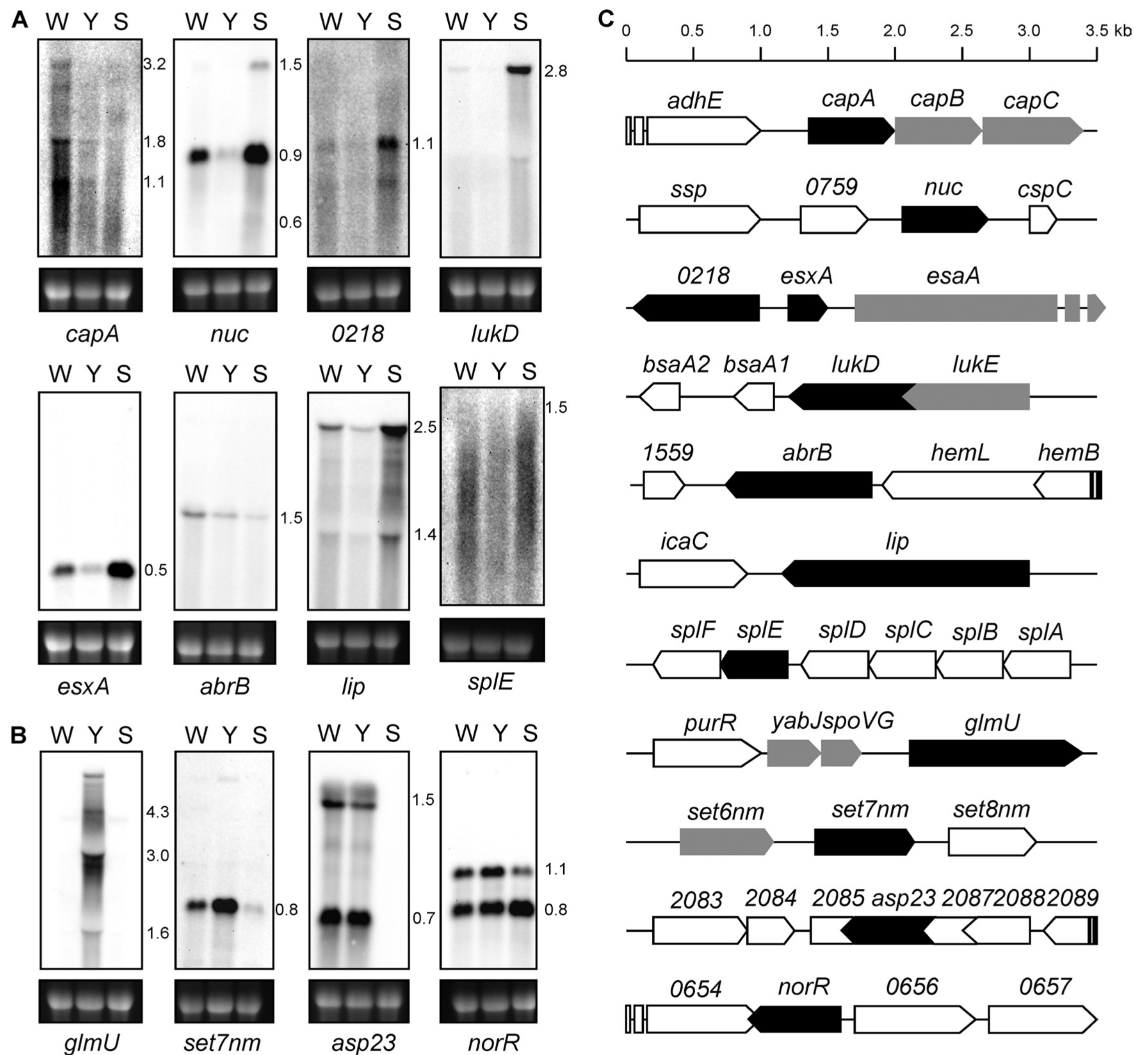

FIG. 1. Northern blot analysis of *yabJ-spoVG*-dependent genes. Northern blots of total RNA from *S. aureus* Newman (W), Δ*yabJ-spoVG* mutant SM148 (Y), and Δ*sigB* mutant MS64 (S) for selected genes that were found to be down- (A) or upregulated (B) in the microarray in SM148. Transcript masses are indicated in kb. The ethidium bromide-stained 16S rRNA pattern is shown as an indication for the RNA loading. (C) Locus maps of ORFs selected for Northern blots (black). ORFs appearing in the microarray are highlighted in gray. *adhE*, encoding iron-containing alcohol dehydrogenase; *ssp*, encoding extracellular matrix and plasma binding protein; *cspC*, encoding cold-shock protein CSD family protein; *bsaA1/2*, encoding lantibiotic precursors; *hemL*, encoding glutamate-1-semialdehyde-2,1-aminomutase; *hemB*, encoding delta-aminolevulinic acid dehydratase; *icaC*, encoding intercellular adhesion protein IcaC; *splABCDF*, encoding serine proteases; *purR*, encoding *pur* operon repressor; *set8 nm*, encoding exotoxin NM8.

in the Δ*yabJ-spoVG* mutant SM148 to the level seen with the wild type, while p*yabJ* did not complement this phenotype (Fig. 2). In line with previous findings (32), the σ^B mutant showed a higher transcription of *nuc* than the wild type. Since the *nuc* gene and its vicinity do not contain any σ^B consensus sequences, the high *nuc* transcriptional response must be under indirect σ^B-dependent negative control. The σ^B-dependent repression of *nuc* transcription in the wild type functions presumably over σ^B-activated SarA, reported to downregulate *nuc* (10, 19). Since transcription of *yabJ-spoVG* is virtually abolished in σ^B mutants (7, 36, 46), SpoVG could not contribute to *nuc* regulation in the σ^B mutant. When SpoVG, however, was expressed constitutively from plasmid p*spoVG* or p*yabJspoVG*, it was able to further activate *nuc* transcription in the σ^B mutant. These data suggest that SpoVG has an activating effect on *nuc* transcription that is antagonized and/or overruled by the repressing effect of σ^B (Fig. 2B).

SpoVG inactivation reduces excreted virulence factors. The transcriptional response of *nuc* was mirrored by the nuclease activity measured in bacterial supernatants (Fig. 3A). The Δ*yabJ-spoVG* mutant expressed lower nuclease activity than the wild type, while the Δ*sigB* mutant exhibited higher nuclease activity. Again, nuclease activity in the Δ*yabJ-spoVG* mutant was increased by complementation with SpoVG with either plasmid p*spoVG* or p*yabJspoVG*, but not by p*yabJ*. The extracellular lipase and protease activities followed the same pattern, being reduced in the Δ*yabJ-spoVG* mutant and enhanced in the Δ*sigB* mutant (Fig. 3B and C). As described above, complementation of the Δ*yabJ-spoVG* mutant with SpoVG increased their activity. Interestingly, the nuclease, lipase, and protease activities in the supernatant of a Δ*sigB* mutant were

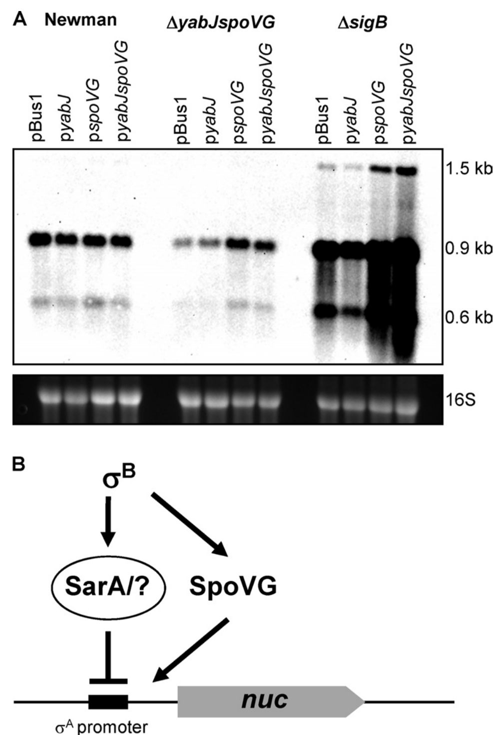

FIG. 2. *trans*-complementation of *nuc* expression. (A) Northern blot analysis of *nuc* in *S. aureus* Newman, its isogenic Δ*yabJ-spoVG* mutant SM148, and Δ*rsbUVW-sigB* mutant IK184 with pBus1 (empty plasmid), p*yabJ* (P_{bacA}-*yabJ*), p*spoVG* (P_{bacA}-*spoVG*), and p*yabJspoVG* (P_{bacA}-*yabJ-spoVG*) after 5 h of growth in LB broth. Transcript masses are indicated. The ethidium bromide-stained 16S rRNA pattern is shown as an indication of the RNA loading. (B) Postulated regulatory pattern of *nuc* in *S. aureus* Newman. σ^B downregulates through activation of SarA and/or other repressors the transcription of *nuc*. At the same time σ^B activates SpoVG expression, which in turn counteracts SarA and activates *nuc* transcription.

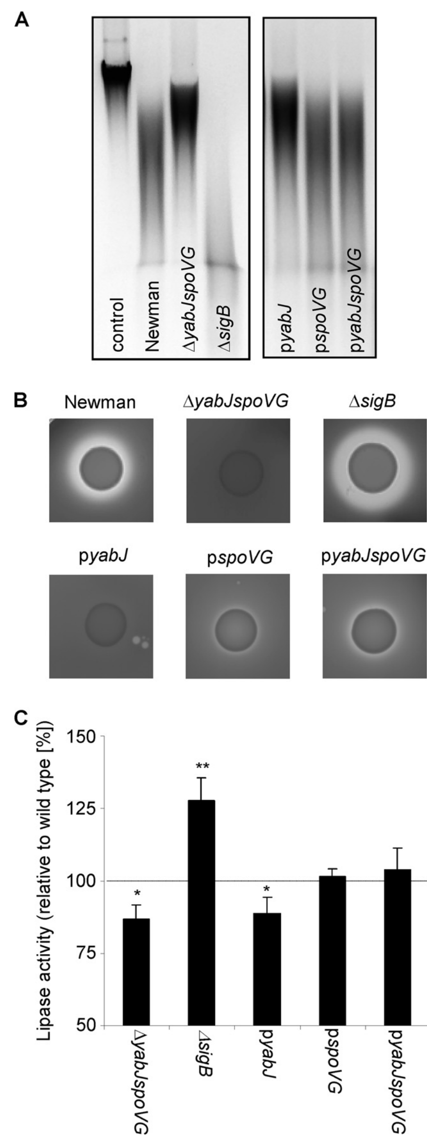

FIG. 3. Influence of σ^B and the σ^B-dependent *yabJ-spoVG* operon on excreted virulence factors. The nuclease, protease, and lipase activities of *S. aureus* Newman, the Δ*yabJ-spoVG* mutant SM148, the Δ*sigB* mutant MS64, and SM148 complemented with p*yabJ*, p*spoVG*, and p*yabJspoVG* were compared. (A) Representative agarose gels of calf thymus DNA after incubation with bacterial culture supernatants to detect nuclease activity. Whereas the DNA is not degraded with sterile LB broth (control), samples incubated with culture supernatants show different stages of DNA degradation. (B) Proteolytic activity of the strains after growth on skim milk agar plates. (C) Lipase activity monitored by hydrolysis of 4-nitrophenyl octanoate by culture supernatants. Data shown are the means ± SD of four independent experiments and are presented as percentage of lipase activity compared with that of the wild-type Newman (100%). Statistical significances between wild-type and mutant supernatants were assessed with a paired, two-tailed Student *t* test (*, $P < 0.05$; **, $P < 0.01$).

not further enhanced by any of the three complementing plasmids, as would have been expected from the transcriptional data (data not shown), possibly due to a bottleneck between transcription and secretion.

Role of σ^B and the *yabJ-spoVG* operon in oxacillin resistance. We previously showed that deletion of the *yabJ-spoVG* operon reduces oxacillin and glycopeptide resistance of MRSA and GISA strains to the same extent as σ^B inactivation, which led to the assumption that σ^B might affect antibiotic resistance of MRSA and GISA via the expression of YabJ and/or SpoVG (46). However, the plasmids used in our previous study to complement the Δ*yabJ-spoVG* mutants harbored *yabJ-spoVG* under the control of their native σ^B-dependent promoter and thus did not allow the complementation of a Δ*sigB* mutant to support this hypothesis. Here, we complemented the MRSA Δ*yabJ-spoVG* mutant SM165 with plasmids p*yabJ*, p*spoVG*, and p*yabJspoVG* (Fig. 4A), expressing *yabJ* and *spoVG* from the heterologous σ^B-independent *bacA* promoter, which also allowed us now to complement the Δ*sigB* mutant MRSA strain BS128 (Fig. 4B). In line with our previous findings, SpoVG was sufficient to complement the SM165 resistance phenotype, while YabJ had no apparent effect on the resistance level. However, neither YabJ nor SpoVG, nor both YabJ and SpoVG, were able to restore the oxacillin resistance of the Δ*sigB* mutant, indicating that SpoVG is not the only mediator of the σ^B effect on antibiotic resistance in MRSA.

Complementation of the *S. aureus yabJ-spoVG* deletion mutant with *B. subtilis spoVG*. SpoVG in *S. aureus* was originally discovered and named after its sequence homologue in *B.*

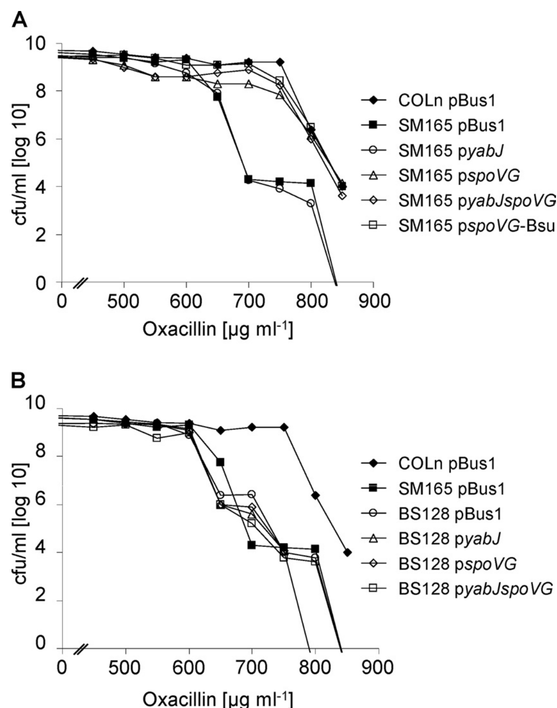

FIG. 4. Role of YabJ and SpoVG in oxacillin resistance in MRSA strain COLn. Population analysis profiles for oxacillin are shown for COLn and its isogenic Δ*yabJ-spoVG* (SM165) (A) and Δ*rsbUVW-sigB* (BS128) (B) mutants with pBus1 (empty plasmid), p*yabJ* (P_{bacA}-*yabJ*), p*spoVG* (P_{bacA}-*spoVG*), p*yabJspoVG* (P_{bacA}-*yabJ-spoVG*), and p*spoVG-Bsu* (P_{bacA}-*spoVG* from *B. subtilis*).

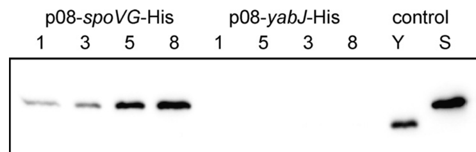

FIG. 5. Expression of YabJ and SpoVG. Western blot analysis of SpoVG-His6 and YabJ-His6 in cytoplasmic protein fractions of strain SM148 with p08-*spoVG*-His and p08-*yabJ*-His, respectively, grown for 1, 3, 5, and 8 h (indicated at top of lanes) in LB broth at 37°C and as a control of strain SM148 with p*yabJ*-His (Y) or p*spoVG*-His (S) after 5 h of growth. SpoVG-His6 has a molecular mass of 12.3 kDa (43). YabJ-His6 was smaller, although its theoretical molecular mass (14.8 kDa) was higher than that of SpoVG-His6, most likely due to a degradation of YabJ-His6 at the N terminus.

subtilis, showing an amino acid identity of 61% and a similarity of 76%. To test whether *B. subtilis spoVG* can functionally complement the *spoVG* deletion in *S. aureus*, we constructed a fusion plasmid which harbors *B. subtilis spoVG* under the control of the constitutive *bacA* promoter (p*spoVG-Bsu*). *trans*-complementation of the MRSA Δ*yabJ-spoVG* mutant SM165 with this plasmid restored the oxacillin resistance to the wild-type level (Fig. 4A), signaling that not only the sequence but also functional characteristics were conserved.

Only SpoVG appears translated under *in vitro* conditions. To analyze the expression profiles of YabJ and SpoVG, we fused a C-terminal 6-histidine tag to *yabJ* and *spoVG*, respectively, in pSTM08, expressing the *yabJ-spoVG* operon from its native promoter. The resulting constructs p08-*yabJ*-His and p08-*spoVG*-His were expressed in *S. aureus* SM148 during growth over 8 h in LB broth. While the SpoVG-His6 expression followed the observed transcriptional profile and increased within time, no signal for YabJ-His6 could be detected (Fig. 5), indicating that YabJ was not expressed or expressed only in very small amounts under these *in vitro* growth conditions.

The possibility that YabJ-His6 might be unstable or not accessible by anti-his6 antibodies was ruled out by expressing YabJ-His6 and as a control also SpoVG-His6 from the constitutive *bacA* promoter (constructs p*yabJ*-His and p*spoVG*-His). These plasmids allowed us to detect YabJ-His6 and SpoVG-His6 in the cell extracts obtained from the respective derivatives (Fig. 5). A direct fusion of the luciferase reporter gene luc^+ to P_{yabJ} exhibited luciferase activity (46), excluding further that elements required for translation initiation were missing in the region upstream of the *yabJ* ORF. However, mRNA structure predictions of the sequences around the *yabJ* and *spoVG* ribosome binding site (RBS) indicate that the *yabJ* RBS might be sequestered by a hairpin structure, whereas the *spoVG* RBS is freely accessible (data not shown), suggesting that the mRNA secondary structure preceding the *yabJ* ORF might impede the initiation of YabJ translation (51). The conditions under which YabJ is produced, if at all, as well as the function of YabJ, remain to be identified.

Conclusion. We showed that the deletion of the σ^B-dependent *yabJ-spoVG* operon in *S. aureus* Newman strongly reduced the expression and activities of important virulence determinants, such as extracellular nuclease, protease, and lipase. Complementation experiments confirmed SpoVG as the main effector of the *yabJ-spoVG* operon, as shown earlier for capsule production and antibiotic resistance (46). Although the crystal structure of SpoVG from *S. aureus* has recently been solved, its mode of action remains to be elucidated (30). Transcriptomic analysis of the role of the *yabJ-spoVG* operon indicated that SpoVG counteracts a subset of σ^B-regulated genes. In *B. subtilis*, for which SpoVG was originally described, it is characterized as a regulator of sporulation and asymmetric septation (34, 42). Despite the functional discrepancy between SpoVG from the sporulating *B. subtilis* and the nonsporulating *S. aureus*, there seems to be a conserved underlying molecular function, as *spoVG* inactivation in *B. subtilis* also influenced gene expression directed by an alternative sigma factor, however, not by the respective σ^B homolog (39). This hypothesis is further supported by our finding that SpoVG from *B. subtilis*, like SpoVG from *S. aureus*, could complement the reduced methicillin resistance level of a Δ*yabJ-spoVG* MRSA mutant. Future studies will have to reveal the mode of action of SpoVG as well as the function of YabJ and the environmental conditions required for its expression.

ACKNOWLEDGMENTS

This study was carried out with financial support from the Forschungskredit of the University of Zurich to B.S., from the Swiss National Science Foundation grant 31-117707 to B.B.-B., and from the HOMFOR grant T201000365 to M.B.

REFERENCES

1. **Adhikari, R. P., and R. P. Novick.** 2008. Regulatory organization of the staphylococcal *sae* locus. Microbiology **154:**949–959.

2. **Ausubel, F. M., et al.** 1987. Current protocols in molecular biology. John Wiley & Sons, Inc., New York, NY.
3. **Baba, T., et al.** 2002. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet **359:**1819–1827.
4. **Becher, D., et al.** 2009. A proteomic view of an important human pathogen—towards the quantification of the entire S*taphylococcus aureus* proteome. PLoS One **4:**e8176.
5. **Berends, E. T., et al.** 2010. Nuclease expression by S*taphylococcus aureus* facilitates escape from neutrophil extracellular traps. J. Innate Immun. **2:**576–586.
6. **Bischoff, M., and B. Berger-Bächi.** 2001. Teicoplanin stress-selected mutations increasing σ^B activity in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **45:**1714–1720.
7. **Bischoff, M., et al.** 2004. Microarray-based analysis of the *Staphylococcus aureus* σ^B regulon. J. Bacteriol. **186:**4085–4099.
8. **Bronner, S., H. Monteil, and G. Prevost.** 2004. Regulation of virulence determinants in *Staphylococcus aureus*: complexity and applications. FEMS Microbiol. Rev. **28:**183–200.
9. **Burlak, C., et al.** 2007. Global analysis of community-associated methicillin-resistant *Staphylococcus aureus* exoproteins reveals molecules produced *in vitro* and during infection. Cell Microbiol. **9:**1172–1190.
10. **Cassat, J., et al.** 2006. Transcriptional profiling of a *Staphylococcus aureus* clinical isolate and its isogenic *agr* and *sarA* mutants reveals global differences in comparison to the laboratory strain RN6390. Microbiology **152:** 3075–3090.
11. **Chan, P. F., and S. J. Foster.** 1998. Role of SarA in virulence determinant production and environmental signal transduction in *Staphylococcus aureus*. J. Bacteriol. **180:**6232–6241.
12. **Charbonnier, Y., et al.** 2005. A generic approach for the design of whole-genome oligoarrays, validated for genomotyping, deletion mapping and gene expression analysis on *Staphylococcus aureus*. BMC Genomics **6:**95.
13. **Cheung, A. L., A. S. Bayer, G. Zhang, H. Gresham, and Y. Q. Xiong.** 2004. Regulation of virulence determinants *in vitro* and *in vivo* in *Staphylococcus aureus*. FEMS Immunol. Med. Microbiol. **40:**1–9.
14. **Cheung, A. L., K. J. Eberhardt, and V. A. Fischetti.** 1994. A method to isolate RNA from gram-positive bacteria and mycobacteria. Anal. Biochem. **222:** 511–514.
15. **Churchill, G. A.** 2004. Using ANOVA to analyze microarray data. Biotechniques **37:**173–175, 177.
16. **Clarke, S. R., and S. J. Foster.** 2006. Surface adhesins of *Staphylococcus aureus*. Adv. Microb. Physiol. **51:**187–224.
17. **Diep, B. A., et al.** 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. Lancet **367:**731–739.
18. **Dinges, M. M., P. M. Orwin, and P. M. Schlievert.** 2000. Exotoxins of *Staphylococcus aureus*. Clin. Microbiol. Rev. **13:**16–34.
19. **Dunman, P. M., et al.** 2001. Transcription profiling-based identification of *Staphylococcus aureus* genes regulated by the *agr* and/or *sarA* loci. J. Bacteriol. **183:**7341–7353.
20. **Duthie, E. S., and L. L. Lorenz.** 1952. Staphylococcal coagulase; mode of action and antigenicity. J. Gen. Microbiol. **6:**95–107.
21. **Fujimoto, S., and Y. Ike.** 2001. pAM401-based shuttle vectors that enable overexpression of promoterless genes and one-step purification of tag fusion proteins directly from *Enterococcus faecalis*. Appl. Environ. Microbiol. **67:** 1262–1267.
22. **Giachino, P., S. Engelmann, and M. Bischoff.** 2001. σ^B activity depends on RsbU in *Staphylococcus aureus*. J. Bacteriol. **183:**1843–1852.
23. **Gill, S. R., et al.** 2005. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. J. Bacteriol. **187:**2426–2438.
24. **Goda, S. K., and N. P. Minton.** 1995. A simple procedure for gel electrophoresis and northern blotting of RNA. Nucleic Acids Res. **23:**3357–3358.
25. **Hempel, K., et al.** 2010. Quantitative cell surface proteome profiling for σ^B-dependent protein expression in the human pathogen *Staphylococcus aureus* via biotinylation approach. J. Proteome Res. **9:**1579–1590.
26. **Holden, M. T., et al.** 2004. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc. Natl. Acad. Sci. U. S. A. **101:**9786–9791.
27. **Homerova, D., M. Bischoff, A. Dumolin, and J. Kormanec.** 2004. Optimization of a two-plasmid system for the identification of promoters recognized by RNA polymerase containing *Staphylococcus aureus* alternative sigma factor σ^B. FEMS Microbiol. Lett. **232:**173–179.
28. **Horsburgh, M. J., et al.** 2002. σ^B modulates virulence determinant expression and stress resistance: characterization of a functional *rsbU* strain derived from *Staphylococcus aureus* 8325-4. J. Bacteriol. **184:**5457–5467.
29. **Katayama, Y., H. Z. Zhang, and H. F. Chambers.** 2004. PBP 2a mutations producing very-high-level resistance to beta-lactams. Antimicrob. Agents Chemother. **48:**453–459.
30. **Kim, H. H., B. J. Lee, and A. R. Kwon.** 2010. Expression, crystallization, and preliminary X-ray crystallographic analysis of putative SpoVG from *Staphylococcus aureus*. Arch. Pharm. Res. **33:**1285–1288.
31. **Kullik, I., P. Giachino, and T. Fuchs.** 1998. Deletion of the alternative sigma factor σ^B in *Staphylococcus aureus* reveals its function as a global regulator of virulence genes. J. Bacteriol. **180:**4814–4820.
32. **Kullik, I. I., and P. Giachino.** 1997. The alternative sigma factor σ^B in *Staphylococcus aureus*: regulation of the *sigB* operon in response to growth phase and heat shock. Arch. Microbiol. **167:**151–159.
33. **Kuroda, M., et al.** 2001. Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. Lancet **357:**1225–1240.
34. **Matsuno, K., and A. L. Sonenshein.** 1999. Role of SpoVG in asymmetric septation in *Bacillus subtilis*. J. Bacteriol. **181:**3392–3401.
35. **McCallum, N., et al.** 2006. *In vivo* survival of teicoplanin-resistant *Staphylococcus aureus* and fitness cost of teicoplanin resistance. Antimicrob. Agents Chemother. **50:**2352–2360.
36. **Meier, S., et al.** 2007. σ^B and the σ^B-dependent *arlRS* and *yabJ-spoVG* loci affect capsule formation in *Staphylococcus aureus*. Infect. Immun. **75:**4562–4571.
37. **Morikawa, K., et al.** 2001. Overexpression of sigma factor σ^B urges *Staphylococcus aureus* to thicken the cell wall and to resist beta-lactams. Biochem. Biophys. Res. Commun. **288:**385–389.
38. **Pané-Farré, J., B. Jonas, K. Forstner, S. Engelmann, and M. Hecker.** 2006. The σ^B regulon in *Staphylococcus aureus* and its regulation. Int. J. Med. Microbiol. **296:**237–258.
39. **Perez, A. R., A. Abanes-De Mello, and K. Pogliano.** 2006. Suppression of engulfment defects in *Bacillus subtilis* by elevated expression of the motility regulon. J. Bacteriol. **188:**1159–1164.
40. **Renzoni, A., et al.** 2006. Transcriptomic and functional analysis of an autolysis-deficient, teicoplanin-resistant derivative of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **50:**3048–3061.
41. **Rollof, J., J. H. Braconier, C. Soderstrom, and P. Nilsson-Ehle.** 1988. Interference of *Staphylococcus aureus* lipase with human granulocyte function. Eur. J. Clin. Microbiol. Infect. Dis. **7:**505–510.
42. **Rosenbluh, A., C. D. Banner, R. Losick, and P. C. Fitz-James.** 1981. Identification of a new developmental locus in *Bacillus subtilis* by construction of a deletion mutation in a cloned gene under sporulation control. J. Bacteriol. **148:**341–351.
43. **Rossi, J., M. Bischoff, A. Wada, and B. Berger-Bächi.** 2003. MsrR, a putative cell envelope-associated element involved in *Staphylococcus aureus sarA* attenuation. Antimicrob. Agents Chemother. **47:**2558–2564.
44. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, NY.
45. **Scherl, A., et al.** 2006. Exploring glycopeptide-resistance in *Staphylococcus aureus*: a combined proteomics and transcriptomics approach for the identification of resistance-related markers. BMC Genomics **7:**296.
46. **Schulthess, B., et al.** 2009. Functional characterization of the σ^B-dependent *yabJ-spoVG* operon in *Staphylococcus aureus*: role in methicillin and glycopeptide resistance. Antimicrob. Agents Chemother. **53:**1832–1839.
47. **Senn, M. M., et al.** 2005. Molecular analysis and organization of the σ^B operon in *Staphylococcus aureus*. J. Bacteriol. **187:**8006–8019.
48. **Singh, V. K., J. L. Schmidt, R. K. Jayaswal, and B. J. Wilkinson.** 2003. Impact of *sigB* mutation on *Staphylococcus aureus* oxacillin and vancomycin resistance varies with parental background and method of assessment. Int. J. Antimicrob. Agents **21:**256–261.
49. **Sinha, S., et al.** 1999. Crystal structure of *Bacillus subtilis* YabJ, a purine regulatory protein and member of the highly conserved YjgF family. Proc. Natl. Acad. Sci. U. S. A. **96:**13074–13079.
50. **Talaat, A. M., et al.** 2002. Genomic DNA standards for gene expression profiling in *Mycobacterium tuberculosis*. Nucleic Acids Res. **30:**e104.
51. **Unoson, C., and E. G. Wagner.** 2007. Dealing with stable structures at ribosome binding sites: bacterial translation and ribosome standby. RNA Biol. **4:**113–117.
52. **Wang, L., J. D. Trawick, R. Yamamoto, and C. Zamudio.** 2004. Genome-wide operon prediction in *Staphylococcus aureus*. Nucleic Acids Res. **32:** 3689–3702.
53. **Wu, S., H. de Lencastre, and A. Tomasz.** 1996. Sigma-B, a putative operon encoding alternate sigma factor of *Staphylococcus aureus* RNA polymerase: molecular cloning and DNA sequencing. J. Bacteriol. **178:**6036–6042.
54. **Ziebandt, A. K., et al.** 2004. The influence of *agr* and σ^B in growth phase dependent regulation of virulence factors in *Staphylococcus aureus*. Proteomics **4:**3034–3047.