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Effect of Mild Acid on Gene Expression in *Staphylococcus aureus*

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During staphylococcal growth in glucose-supplemented medium, the pH of a culture starting near neutrality typically decreases by about 2 units due to the fermentation of glucose. Many species can comfortably tolerate the resulting mildly acidic conditions (pH, ~5.5) by mounting a cellular response, which serves to defend the intracellular pH and, in principle, to modify gene expression for optimal performance in a mildly acidic infection site. In this report, we show that changes in staphylococcal gene expression formerly thought to represent a glucose effect are largely the result of declining pH. We examine the cellular response to mild acid by microarray analysis and define the affected gene set as the mild acid stimulon. Many of the genes encoding extracellular virulence factors are affected, as are genes involved in regulation of virulence factor gene expression, transport of sugars and peptides, intermediary metabolism, and pH homeostasis. Key results are verified by gene fusion and Northern blot hybridization analyses. The results point to, but do not define, possible regulatory pathways by which the organism senses and responds to a pH stimulus.

Facultative pathogens such as staphylococci produce a wide variety of accessory proteins. Many of these are involved in, or required for, infectivity and are collectively referred to as the virulon. It is widely believed that the genes encoding these proteins are regulated according to the exigencies of the local sites in which the organism is able to establish an infection. The evidence in support of this very logical view is, at best, rather sketchy, largely for two reasons. Firstly, it has, until very recently, been unapproachably difficult to determine the putative locale-specific gene expression patterns. Secondly, it has been no easy matter to identify the specific environmental factors that would determine these patterns. As a preamble to the ultimate objective of defining in vivo environmental factors that influence the expression of accessory genes involved in local pathogenesis, we have begun to reexamine the effect of certain nutrients and other chemicals, including glucose, acidic pH, salt, salicylic acid, and subinhibitory concentrations of antibiotics, on the expression of virulence genes during growth in vitro. These substances have widespread effects on the transcription of virulence genes and other accessory genes, which are presumably initiated through signaling elements and mediated through the complex regulatory network that governs the expression of all accessory genes (34). Our entrée into the transduction of environmental signals was provided by the observation that all of these substances affected transcription of a key signaling locus, *sae*, which is involved in the regulation of the expression of many exoproteins (36). In that study, which was initiated by a determination of the effect of glucose, it was shown that *sae* has a complex transcriptional pattern that undergoes a very striking switch during in vitro growth at neutral pH, in which a 2.1-kb transcript, present initially, is replaced by three other, of 3.2, 2.6, and 0.5 kb (36). When the *Staphylococcus aureus* strain tested is grown in the presence of glucose, the switch occurs but the new transcripts are quickly turned off. At this point the glucose is exhausted and the pH has dropped from 7.5 to 5.5, suggesting that the drop in pH rather than the presence of glucose is responsible for the turn-off (36). Here, we confirm that pH affects the expression of *sae* and show that a modest reduction in pH, at which the organisms survive and grow, probably without activating the full-scale acid stress response, affects the expression of many *sae*-regulated genes, as well as many genes that do not belong to the *sae* regulon. To define this newly identified mild acid stimulon (MAS), we have used transcriptome profiling by microarray. The MAS does not represent an acid-shock response; the cultures used were pregrown in pH-adjusted media such that at the first time point the cultures were in steady-state exponential growth. As described here, the MAS consists of all *S. aureus* genes whose transcript level differs by at least twofold during growth at pH 5.5 versus that at pH 7.5.

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids used in this study are listed in Table 1. The standard strain used in most of the work is RN6734, a φ13 lysogen of RN6390B. These two *agr* group I strains are in the NCTC8325 lineage and therefore have an *rsbU* defect, leading to a partial σ^B-negative phenotype (23).

Media and growth conditions. *S. aureus* was cultured in Casamino Acids-yeast extract-glycerophosphate (CYGP) broth (35), without glucose, and which was adjusted to pH 5.5 or 7.5. Broth was inoculated to an initial density of 5×10^7 CFU ml^{-1} with *S. aureus* from GL agar plates with appropriate antibiotic and grown overnight at 37°C. Cultures were grown in Erlenmeyer flasks at a 1/10 volume and incubated with shaking at ~220 rpm at 37°C. Cultures were monitored turbidometrically with a Klett-Summerson (New York, N.Y) colorimeter at 540 nm. After reaching ~3 times the initial inoculum (growth from ~15 to ~50 Klett units), cultures were diluted twofold and grown again to 50 Klett units. Cultures were again diluted twofold. The time zero (T0) time point for all time course experiments was defined when the density reached 50 Klett units for the

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TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Genotype or description	Reference or source
Strains		
DH5α	F^- ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 hsdR17* (r_K^- mK^+)	Promega
RN4220	Restriction-deficient mutant of strain 8325-4	Kreiswirth et al. (27a)
RN6734	phi13 lysogen of RN6390B	Vojtov et al. (52)
PC1839	8325-4 *sarA*::Km	Chan & Foster (8)
RN9388	RN6734 *sarA*::Km; transductant of PC1839	Tegmark et al. (48a)
KT201	8325-4 *sarH1*::pKT200 Em (aka *sarS*)	This work
RN9897	RN6734 *sarS*::Em; transductant of KT201	This work
ALC1905	RN6390 *sarT*::Em	Schmidt et al. (45a)
RN9899	RN6734 *sarT*::Em; transductant of ALC1905	This work
RN9375	8325-4 *sigB*::Tc	Nicholas et al. (33a)
RN9898	RN6734 *sigB*::Tc; transductant of RN9375	This work
CYL807	COL *arlR*::Tn*551;* gift of C. Lee (Kansas City, Kans.)	This work
RN9896	RN6734 *arlR*::Tn*551;* transductant of CYL807	This work
PM614	PM466 rot::Tn*917*	McNamara et al. (32)
RN9886	RN6734 rot::Tn*917;* transductant of PM614	This work
RN9360	Replacement of *rsbU* deletion in 8325-4, adjacent Tc; gift of I. Kullik	Novick and Jiang (36)
RN10029	RN6734 *rsbU*$^+$ Tc; transductant of RN9360	This work
RN7206	phi13 lysogen of RN6911 (Δ*agr*::tetM)	Novick et al. (36a)
Plasmids		
pRN7044	Ptst::blaZ transcriptional fusion	Vojtov et al. (52)
pRN7166	pRN7044 with Em^r replaced by Cm^r	This work
pRN6827	pSA3800 Phla::*blaZ*	Vandenesch and Novick (unpub.)
pRN7041	PsspA::*blaZ* transcriptional fusion	Vojtov et al.
pCN50	(Source of Cm cassette, 1-kb ApaI/XhoI fragment)	Charpentier et al. (submitted)

third time (~1.5×10^8 CFU ml^{-1}). For cultures grown without glucose, the pH at the end of 6 h was always within 0.2 units of the starting pH.

RNA purification. Cells were harvested by centrifugation at specified time points and treated with RNA Protect reagent from QIAGEN (Valencia, Calif.). Cells were mechanically disrupted by agitation with glass beads using a Fast-Prep apparatus (Q-Biogene, Carlsbad, Calif.), and RNA was purified using the RNeasy kit from QIAGEN. RNA integrity was checked by agarose gel electrophoresis in Tris-acetate buffer, and RNA was quantitated by determination of absorbance at 260 nm.

Northern blot hybridization. RNA from equal numbers of cells was separated by denaturing gel electrophoresis (morpholinepropanesulfonic acid-formaldehyde) on 1% agarose gels. RNA was transferred to Hybond N^+ membranes from Amersham by vacuum and UV cross-linked. [α-^{32}P]dATP-labeled (Amersham) probes were generated by PCR and hybridized to the blots overnight. Washed blots were exposed to phosphorimager screens, which were read by using a Molecular Dynamics PhosphorImager. PCR primers were obtained from Integrated DNA Technologies (Coralville, Iowa), and their sequences are listed in Table 2.

Microarray analysis. A custom DNA microarray prepared by Affymetrix for Wyeth was used. The microarray contains probe sets for all conserved, nonredundant open reading frames from six published *S. aureus* genome sequences, unique GenBank entries, as well as strain N315 intergenic regions. Design of the chip has been detailed by Dunman et al. (19). All microarray procedures were performed as described by Beenken et al. (3). Briefly, biotinylated cDNA was prepared and hybridized to the microarray. The arrays were read with an Affymetrix GS3000 scanner, and data were analyzed with GeneSpring software version 6.1 (Silicon Genetics, Redwood City, Calif.). All procedures were done in duplicate, and results are reported for genes with a twofold or greater expression differential ($P \leq 0.5$) between the two conditions.

Exoprotein analyses. Exoproteins were analyzed by the method of Laemmli et al. (28). Briefly, culture supernatants from equal numbers of cells were precipitated with 10% trichloroacetic acid, and the pellets were resuspended in 2% sodium dodecyl sulfate (SDS) and boiled for 3 min. After separation by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), gels were stained with Coomassie blue and scanned. β-Lactamase was assayed by the nitrocefin method adapted for use in microtiter plates (52).

Plasmid construction. The ~1-kb chloramphenicol resistance cassette was removed from plasmid pCN50 by digestion with ApaI and XhoI. The fragment was isolated by gel electrophoresis in Tris-acetate buffer and extracted with a QIAquick gel extraction kit (QIAGEN). Likewise, the vector backbone of pRN7044 was isolated by digestion with ApaI and XhoI followed by gel electrophoresis and gel extraction. The chloramphenicol cassette was ligated into the pRN7044 backbone with T4 DNA ligase. The ligation product was used to transform chemically competent *Escherichia coli* DH5α. DNA of the resulting plasmid, pRN7166, was isolated with a QIAprep spin miniprep kit, introduced into RN4220 by electroporation, and transferred to other *S. aureus* strains by transduction with phage ϕ11.

TABLE 2. Oligonucleotide primers used in this study

Primer	Sequence (5′–3′)	N315 locus
16S F	GGTGAGTAACACGTGGATAA	SArRNA01
16S R	ATGTCAAGATTTGGTAAGGTT	SArRNA01
RNAIII F	ATGATCACACAGAGATGTGA	SAS065
RNAIII R	CTGAGTCCTAGGAAACTAACTC	SAS065
sarA F	GAGTTGTTATCAATGGTCACTTATGCTG	SA0573
sarA R	GTGATTCGTTTATTTACTCGACTC	SA0573
hla F	TTAGCCTGGCCTTCAGCC	SA1007
hla R	TGCCATATACCGGGTTC	SA1007
saeS F	GGCTTCTGAAATTACGCAACAAATG	SA0660
saeS R	GTTACAGTCACCGTAGTTCCCAC	SA0660
rot F	CTCTACTTGCAATCGCATCACTG	SA1583
rot R	GGGATTGTTGGGATGTTTGTTAATAC	SA1583
spa F	GGCACTACTGCTGACAAAATTGCTGCAG	SA0107
spa R	GTTCGCGACGACGTCCAGCTAATAACGCTGC	SA0107
sspA F	GACAACAGCGACACTTG	SA0901
sspA R	CTGAATTACCACCAGTTG	SA0901
cap5b F	GTACAGTTGTATCGAATGTAGCG	SA0145
cap5b R	GTGCATCAGTCACAGTATTAAC	SA0145
IrgAB F	GCCGGATCCGAAGTGAGCCATCTATA	SA0252
IrgAB R	GCCGAATTCGATAATAACAATGGCTC	SA0252

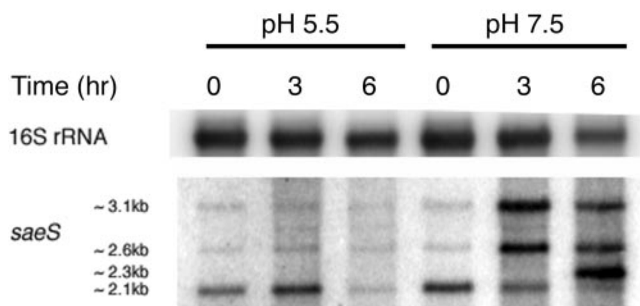

FIG. 1. Northern blot analysis of the effect of pH on *saeS* transcripts. Whole-cell RNA was extracted from RN6734 grown in CYGP broth without glucose and adjusted to pH 5.5 or 7.5. The RNA was separated on a denaturing gel and vacuum blotted; the blot was hybridized with a radiolabeled DNA probe complementary to *saeS*. The probe was detected with a PhosphorImager. Equalization was determined by subsequent blotting with a probe complementary to 16S rRNA.

RESULTS

Effect of pH on *sae* transcription. To study the effect of pH on *sae* expression independently of glucose, we grew cultures of strain RN6734 at pH 5.5 and 7.5 in CYGP broth without glucose and prepared Northern blots of whole-cell RNA from culture samples taken at three standard time points, T0, T3, and T6, as defined in Materials and Methods. pH did not vary by more than 0.1 to 0.2 units throughout the 6-h period. The Northern blot obtained with an *saeS* probe for these RNA samples (Fig. 1) demonstrates that the transition in the *sae* transcription pattern occurs at pH 7.5 but not at pH 5.5 and that the level of transcription is decreased at the lower pH.

Response of exoprotein gene promoters to changes in pH. If the *sae* locus is involved in a global response to this pH differential, *sae*-regulated genes would be predicted to respond to changes in pH in this range. Accordingly, transcriptional fusions using staphylococcal β-lactamase (*blaZ*) as a reporter were used to test for promoter activity in response to changes in pH. The *blaZ* reporter, under a constitutive P_{bla} promoter, was insensitive to changes in pH in the range of pH 5.5 to 7.5 (data not shown). Plasmid-carried β-lactamase fusions involving P_{hla}, P_{tst}, and P_{sspA} were introduced into various strains and tested for β-lactamase activity during growth at pH 5.5 versus 7.5. The promoters for these three exoprotein genes were selected because, although postexponentially up-regulated by *agr*, they are regulated differentially by *sar* and *sae* (8, 36, 52; for a review, see the report of Novick 34). Results for the β-lactamase fusions are shown in Fig. 2A to C. The three promoters responded to changes in pH generally in parallel with their response to *sae*. Thus, at pH 7.5, P_{hla}-*blaZ* shows the typical postexponential induction; however, at pH 5.5, there is a gradual increase of β-lactamase activity but no clear postexponential induction pattern. P_{tst}-*blaZ* also shows postexponential induction typical of *agr*-regulated exoprotein genes at pH 7.5 (34). This pattern is completely absent at pH 5.5, even though *agr* expression is induced at this pH. Finally, P_{sspA}-*blaZ* shows a postexponential induction pattern that is similar at the two pH levels. These results imply that one or more pH-sensitive regulatory gene products may control expression from these promoters.

Effects of regulatory genes. To test known regulatory genes for possible roles in pH-dependent regulation, we transferred null mutations in *sarA, sarS, sarT, rot, arlRS*, and *sigB* and a molecular repair of *rsbU* into RN6734 and tested for their effects using the β-lactamase fusion plasmids. The expectation was that a mutation affecting a required regulatory gene would abolish the pH-dependent expression differential, with respect both to expression levels and to kinetics. As shown in Fig. 2 and 3, none of the regulatory mutations tested eliminated the observed pH differential. Nevertheless, several informative results were obtained: (i) from the results shown in Fig. 2C and D, the *sarA* mutation dramatically increased the pH-dependent differential expression of the P_{sspA}-*blaZ* reporter at pH 5.5, but had little or no effect at pH 7.5. This result may be consistent with previous experiments done in the presence of glucose, demonstrating a major increase in *sspA* expression in a *sarA* mutant background (8, 27), because these would have had a low pH at the time the samples were taken. Additionally, the *sarA* mutation did not eliminate the temporal up-regulation of *sspA*. (ii) Figure 2E and F show that *hla* expression was pH dependent in both wild-type and *sigB* mutant cells and was sharply up-regulated at pH 7.5 but not at 5.5. (iii) The mutation affecting *sarA*, but not those affecting *sarS* or *sarT*, profoundly decreased P_{tst}-*blaZ* expression and greatly reduced temporal induction (Fig. 3). (iv) The *arl* mutation appears to up-regulate the P_{tst}-*blaZ* reporter at all time points at pH 5.5 but only at the latest time point at pH 7.5. Finally, the *rot* mutation enhanced the temporal induction of the P_{tst}-*blaZ* reporter at pH 7.5. This result is consistent with the reported repressor activity of Rot (32), which was presumably determined at pH 7.5.

Identification of the MAS. Given the apparent complexity of the response of exoprotein gene expression to changing pH, it seemed worthwhile to examine the entire MAS at the transcriptional level. For this purpose, we employed a staphylococcal DNA microarray (Affymetrix) (3, 6, 19, 20).

To perform this analysis, we prepared whole-cell RNA from two cultures of RN6734 in CYGP broth without glucose, each at three different time points. The cultures were started with an inoculum taken from an overnight GL plate (pH, ~7.2). One culture was started at pH 5.5, the other at pH 7.5, and the two were grown as described in Materials and Methods, with samples taken at T0, T3, and T6 for RNA preparation. Comparable amounts of cDNA were hybridized to the microarrays for each experimental point. In Table 3 are shown the results for all genes whose transcript level differed by at least twofold between the two pH conditions, at any of the time points. In this presentation, to avoid any implications of regulatory mechanism, we refer to up or up-regulated and down or down-regulated in comparing transcript levels between the two conditions. In all, 424 genes were identified; approximately twice as many were up-regulated at pH 5.5 as were down-regulated. In addition to genes encoding proteins ostensibly involved in pH homeostasis, there were many encoding proteins involved in amino acid uptake or metabolism, carbohydrate uptake or metabolism, virulence (encoding either secreted or surface proteins), and transcriptional regulation and, curiously, a considerable number of prophage genes were found to be pH regulated. Among the prophage-encoded genes, a much greater number (31 of 32) were down-regulated at pH 5.5 than

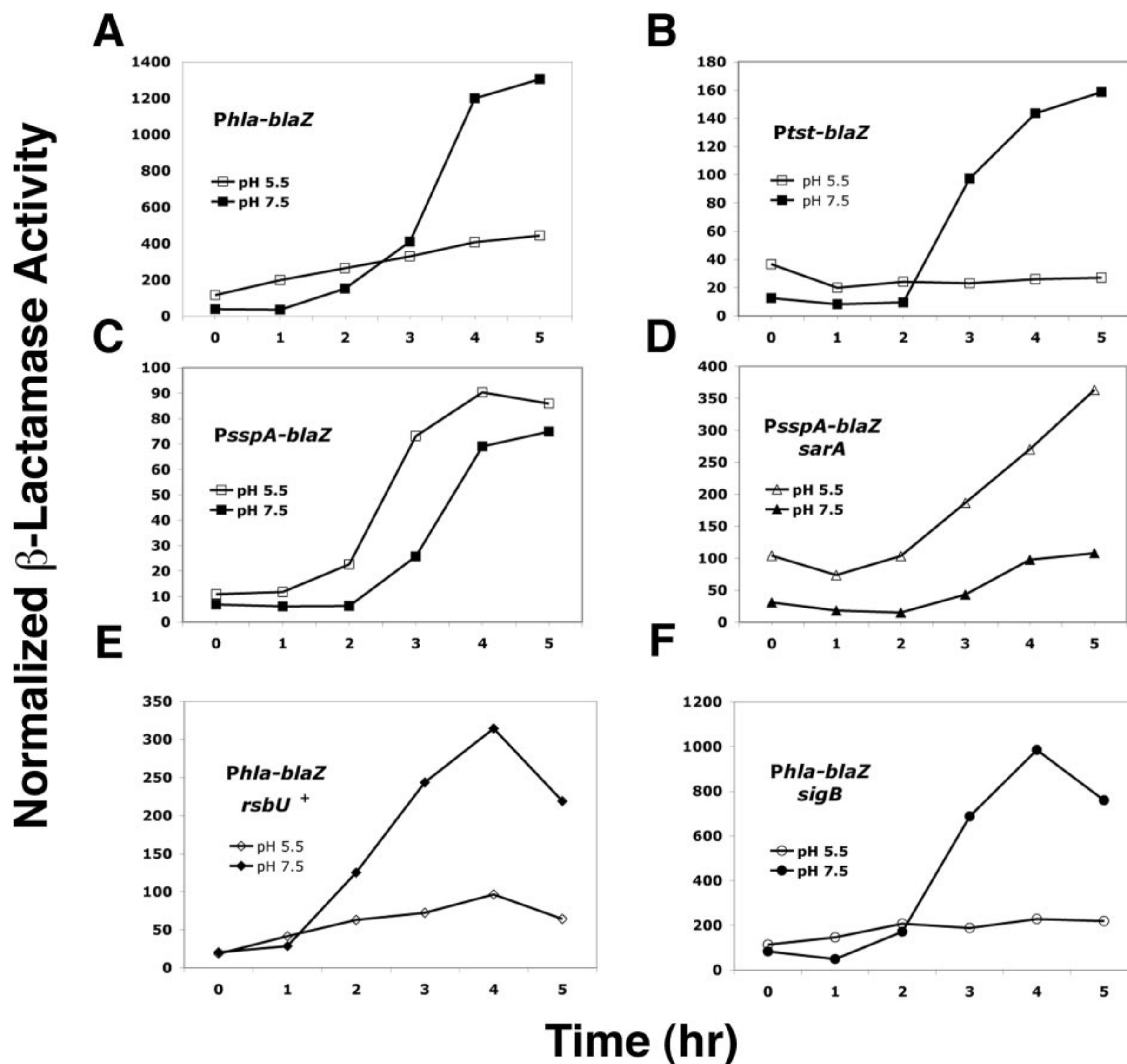

FIG. 2. Transcriptional reporter analysis of the effect of pH on transcription of genes coding for several exoproteins, the effect of *sarA* on pH-dependent *sspA* transcription, and the effect of *rsbU* and *sigB* on pH-dependent *hla* transcription. RN6734 transduced with pRN7044 (P_{tst}::*blaZ*) (A), pRN6827 (P_{hla}::*blaZ*) (B), or pRN7041 (P_{sspA}::*blaZ*) (C) was assayed for β-lactamase activity during growth at pH 5.5 or pH 7.5. (D) pH-dependent β-lactamase activity of RN9388 (RN6734 Δ*sarA*) transduced with pRN7041, measured during growth. (E) pH-dependent β-lactamase activity of RN10029 (RN6734 *rsbU*$^+$) transduced with pRN6827, measured during growth. (F) pH-dependent β-lactamase activity of RN9898 (RN6734 Δ*sigB*) transduced with pRN6827, measured during growth.

were up-regulated. Given that prophage genes are not generally expressed in the prophage state, this result could indicate that spontaneous prophage induction occurs more frequently at pH 7.5. Among the surface proteins, a much greater number (72 of 84) were up-regulated at pH 5.5 than were down-regulated. Of the genes encoding secreted proteins, about the same number were up- as down-regulated. As a functional category, transporters with roles in nutrient accumulation and maintenance of homeostasis were differentially expressed in a ratio that paralleled that of the stimulon as a whole, with approximately twice as many up-regulated at pH 5.5 as at pH 7.5. Some genes involved in homeostasis have roles in maintenance of intracellular pH (*ure* operon), others are osmoprotective (*opuC* operon), and some contribute to protection against both low pH and high osmolarity (*kdp* operon) (7, 47).

We have analyzed the microarray data on the basis of individual genes. However, the MAS includes several clusters of adjacent genes that may represent single transcription units. The individual genes in each cluster are oriented in the same direction and have the same temporal patterns and pH responses. Thus, the conclusions would have been the same if the analysis had been based on transcription units rather than individual genes.

Northern blot hybridization analysis. Northern blot hybridization was used to validate the microarray data for several

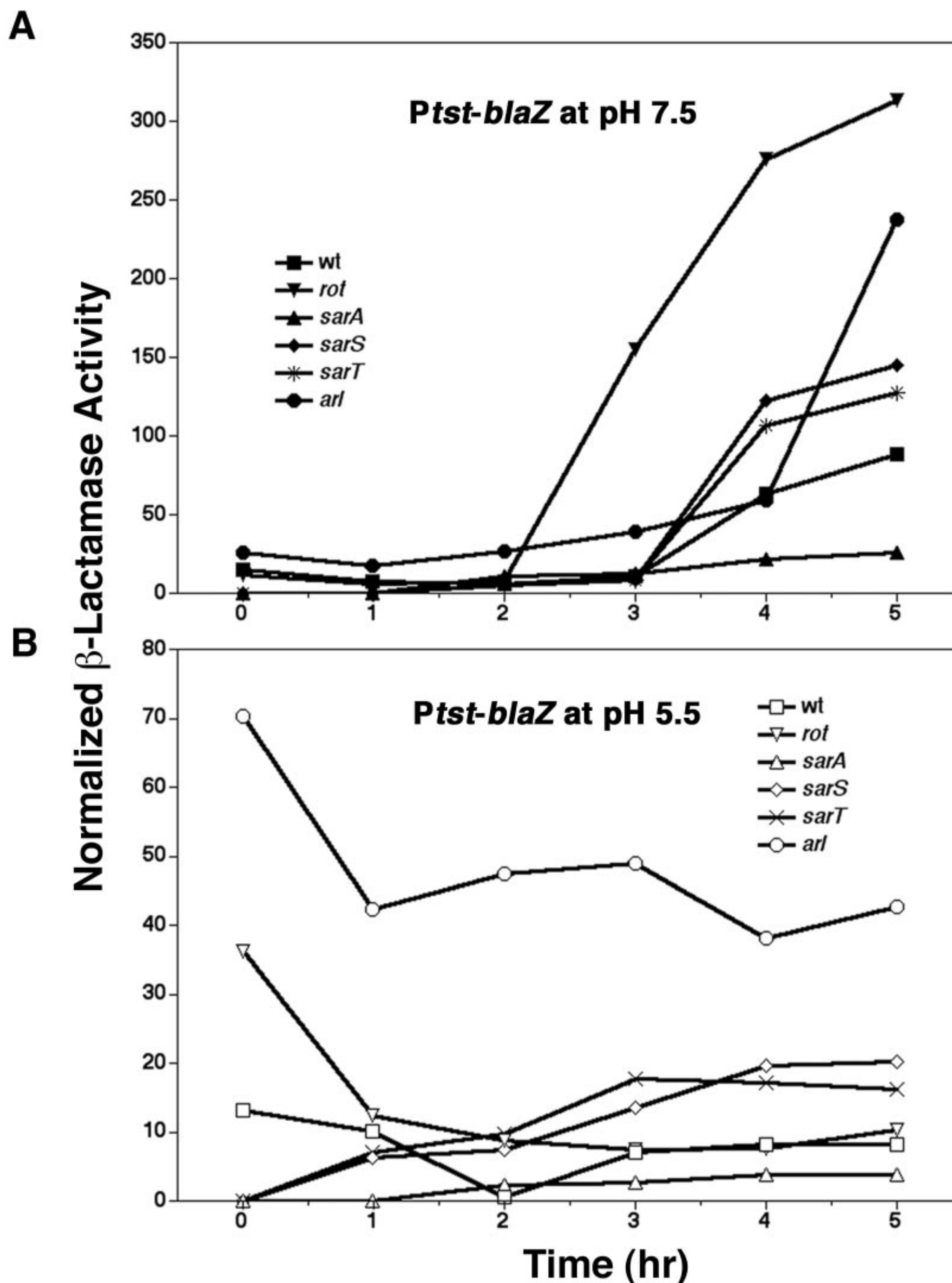

FIG. 3. Transcriptional reporter analysis of the effect of pH on the transcription of genes coding for Tsst at pH 7.5 (A) and pH 5.5 (B) in various genetic backgrounds. In Em^r-marked mutants, P_{tst}::*blaZ* reporter pRN7166 was used.

genes, and it provided an important comparison with gene fusion data. In addition to several extensively studied genes, including *hla*, *spa*, *sspA, sarA*, and *rnaIII*, we analyzed three loci, *rot*, *cap5b*, and *lrgAB*, whose pH-dependent expression was first revealed by the microarray data. Northern blot hybridization results are presented in Fig. 4, from which the following conclusions are apparent. (i) The blotting data for *cap5b* and *lrgAB* were consistent in direction if not magnitude with the profiling data. The *cap5b* transcript level was increased at pH 5.5, whereas *lrgAB* was increased at pH 7.5. Two or three transcripts are visible in the *cap* blot and seem to behave somewhat differently. It is planned to determine their sizes and investigate their differential abundance. (ii) *hla* data from the two techniques could not be compared because expression of *hla* reached the GeneChip upper-threshold values at T3 and T6 and was so low at T0 that the *hla* transcript could

TABLE 3. Genes with a ≥2-fold change in transcript level, pH 7.5 versus 5.5[a]

ORF no.	Gene	Description	Fold change at pH 5.5			Effect of regulator			Functional category
			T0	T3	T6	*agr*	*sarA*	*rot*	
Genes up-regulated at pH 5.5									
N315-SA0021	*yycJ*	Conserved HP			2.0		Down		
N315-SA0091	*plc*	1-Phosphatidylinositol phosphodiesterase precurosr	2.4						
N315-SA0092		HP	2.3						
N315-SA0111	*sirA*	Lipoprotein			**3.8**				Surface
N315-SA0112		HP, similar to cysteine synthase	2.1						
N315-SA0121		HP			2.3				
N315-SA0131	*deoD*	Purine nucleoside phosphorylase		2.9					
N315-SA0144	*cap5A*	Capsular polysaccharide synthesis enzyme Cap5A	4.0	3.6	2.6				Surface
N315-SA0145	*cap5B*	Capsular polysaccharide synthesis enzyme Cap5B	3.3	3.3	2.1				Surface
N315-SA0146	*cap5C*	Capsular polysaccharide synthesis enzyme Cap8C	4.2	3.2					Surface
N315-SA0147	*cap5D*	Capsular polysaccharide synthesis enzyme Cap5D	3.2	3.1					Surface
N315-SA0148	*cap5E*	Capsular polysaccharide synthesis enzyme Cap8E	3.3	2.9					Surface
N315-SA0149	*cap5F*	Capsular polysaccharide synthesis enzyme Cap5F	3.3	2.9					Surface
N315-SA0150	*cap5G*	Capsular polysaccharide synthesis enzyme Cap5G	3.6	3.6					Surface
N315-SA0151	*cap5H*	Capsular polysaccharide synthesis enzyme O-acetyl transferase Cap5H	2.8	3.2					Surface
N315-SA0152	*cap5I*	Capsular polysaccharide synthesis enzyme Cap5I	2.7	3.5					Surface
N315-SA0153	*cap5J*	Capsular polysaccharide synthesis enzyme Cap5J		3.2		Up	Up		Surface
N315-SA0154	*cap5K*	Capsular polysaccharide synthesis enzyme Cap5K	2.0						Surface
N315-SA0155	*cap5L*	Capsular polysaccharide synthesis enzyme Cap5L	2.1	2.8					Surface
N315-SA0156	*cap5M*	Capsular polysaccharide synthesis enzyme Cap5M		2.9					Surface
N315-SA0157	*cap5N*	Capsular polysaccharide synthesis enzyme Cap5N		2.9					Surface
N315-SA0158	*cap5O*	Capsular polysaccharide synthesis enzyme Cap8O		2.6					Surface
N315-SA0162	*aldA*	Aldehyde dehydrogenase homologue		6.3			Down		
N315-SA0170		Conserved HP	3.4			Up			
N315-SA0171	*fdh*	NAD-dependent formate dehydrogenase	2.8	4.5	4.0				
N315-SA0173		HP, similar to surfactin synthetase	2.1			Up		Up	Secreted
N315-SA0184		Conserved HP		3.0		Up			
N315-SA0185		Conserved HP		3.7		Up			
N315-SA0186		HP, similar to sucrose phosphotransferase enzyme II		5.0			Down		Transport
N315-SA0187		HP, similar to transcription regulator		5.2		Up	Down		Regulator
N315-SA0223	*atoB*	Acetyl-CoA acetyltransferase homologue		3.8					
N315-SA0224	*fadB*	HP, similar to 3-hydroxyacyl-CoA dehydrogenase		3.6					
N315-SA0225	*fadD*	HP, similar to glutaryl-CoA dehydrogenase		3.1					
N315-SA0226	*fadE*	HP, similar to acid-CoA ligase		2.8					
N315-SA0227	*fadX*	Conserved HP		2.4					
N315-SA0229		HP, similar to nickel ABC transporter nickel-binding protein	9.8	6.3	3.9				Transport
N315-SA0244	*tagF*	HP, similar to teichoic acid biosynthesis protein F			2.1		Up		Surface
N315-SA0258	*rbsK*	Probable ribokinase		3.3					Transport
N315-SA0259	*rbsD*	Ribose permease		3.3					Transport
N315-SA0260		HP, similar to ribose transporter RbsU		3.8					Transport
N315-SA0262		HP			**3.1**				
N315-SA0270		HP, similar to secretory antigen precursor SsaA			2.8				Secreted
N315-SA0291		HP			2.1			Up	
N315-SA0304	*nanA*	*N*-Acetylneuraminate lyase subunit		2.7					
N315-SA0305		HP, similar to glucokinase		2.4					
N315-SA0318		HP, similar to transport protein SgaT		4.2	2.3				Transport
N315-SA0319		Conserved HP		3.9	2.5				
N315-SA0320		HP, similar to PTS fructose-specific enzyme IIBC component		4.0	2.6				Transport
N315-SA0321		HP, similar to transcription antiterminator BglG family		3.3	2.9				Regulator
N315-SA0322		HP, similar to transcription regulator			**2.6**				Regulator
N315-SA0325	*glpT*	Glycerol-3-phosphate transporter		4.1	2.3				Transport
N315-SA0428		Conserved HP			2.6			Up	
N315-SA0432	*treP*	PTS enzyme II, phosphoenolpyruvate dependent			3.8	Down			Transport
N315-SA0433		Alpha-glucosidase		2.2	3.7	Down			
N315-SA0510	*araB*	Probable L-ribulokinase		2.2					
N315-SA0519	*sdrC*	Ser-Asp rich fibrinogen-binding, bone sialoprotein-binding protein	5.7	5.6	6.9				Surface
N315-SA0547	*mvk*	Mevalonate kinase			2.1				
N315-SA0548	*mvaD*	Mevalonate diphosphate decarboxylase			2.1				
N315-SA0549	*mvaK2*	Phosphomevalonate kinase			2.0			Down	
N315-SA0550		Conserved HP			2.5				
N315-SA0587		Lipoprotein, streptococcal adhesin PsaA homologue	2.1						Surface

Continued on following page

TABLE 3—*Continued*

ORF no.	Gene	Description	Fold change at pH 5.5			Effect of regulator			Functional category
			T0	T3	T6	*agr*	*sarA*	*rot*	
N315-SA0599	*abcA*	ATP-binding cassette transporter A	2.1						Transport
N315-SA0605		HP, similar to dihydroxyacetone kinase		2.8			Down		
N315-SA0606		Conserved HP		2.6					
N315-SA0607		Conserved HP		3.1					
N315-SA0612		Conserved HP	2.1						
N315-SA0620		Secretory antigen SsaA homologue, similar to autolysin			2.7			Up	Secreted
N315-SA0622		HP, similar to AraC/Xy|S family transcriptional regulator			2.1				Regulator
N315-SA0623		HP		2.1	2.2				
N315-SA0636		Conserved HP			2.4				
N315-SA0637		Conserved HP			2.9				
N315-SA0651		HP			4.2			Up	
N315-SA0666		6-Oyruvoyl tetrahydrobiopterin synthase homologue		2.4			Up		
N315-SA0667		Conserved HP		2.4	2.7				
N315-SA0711		Conserved HP			2.5				
N315-SA0738		HP	2.7	2.0	3.7				
N315-SA0739		Conserved HP	4.2	2.2	3.6			Up	
N315-SA0820	*glpQ*	Glycerophosphoryl diester phosphodiesterase		2.3					
N315-SA0835	*clpB*	ClpB chaperone homologue			2.3				
N315-SA0893		Conserved HP			2.1				
N315-SA0899	*sspC*	Cysteine protease	2.2			Up	Down	Down	Secreted
N315-SA0900	*sspB*	Cysteine protease precursor	2.3				Down	Down	Secreted
N315-SA0901	*sspA*	Serine protease; V8 protease; glutamyl endopeptidase	2.4						Secreted
N315-SA0974		Conserved HP			2.3				
N315-SA0978		LPXTG-containing conserved HP			**3.2**				Surface
N315-SA0979		Conserved HP			3.2				
N315-SA0980		HP, similar to ferrichrome ABC transporter			**3.1**				Transport
N315-SA0981		HP, similar to ferrichrome ABC transporter			2.6				Transport
N315-SA0982	*srtB*	Conserved HP			2.1				Transport
N315-SA0983		Conserved HP			**3.1**				
N315-SA0996	*sdhB*	Succinate dehydrogenase iron-sulfur protein subunit		2.3					
N315-SA1007	*hla*	Alpha-hemolysin precursor	4.4			Up	Up	Down	Secreted
N315-SA1041	*pyrR*	Pyrimidine operon repressor chainA		2.0		Up			Regulator
N315-SA1042	*pyrP*	Uracil permease		2.9					Transport
N315-SA1043	*pyrB*	Aspartate transcarbamoylase chain A		2.3	2.1				
N315-SA1044	*pyrC*	Dihydroorotase		2.2					
N315-SA1045	*carA*	Carbamoyl-phosphate synthase small chain		2.6	2.0	Up	Up		
N315-SA1046	*carB*	Carbamoyl-phosphate synthase large chain		2.4					
N315-SA1047	*pyrF*	Orotidine-5-phosphate decarboxylase		2.6	2.2				
N315-SA1048	*pyrF*	Orotate phosphoribosyltransferase	2.0	2.8	2.0				
N315-SA1056		HP			2.2			Up	
N315-SA1076	*rnc*	RNase III			2.3				
N315-SA1081	*rpsP*	30S ribosomal protein S16			2.1				
N315-SA1089	*sucD*	Succinyl-CoA synthetase		2.3					
N315-SA1120		HP, similar to transcription regulator GntR family			2.2	Up	Up		Regulator
N315-SA1140	*glpF*	Glycerol uptake facilitator		2.3		Down	Down		Transport
N315-SA1141	*gplK*	Glycerol kinase		2.6					Transport
N315-SA1149	*glnR*	Glutamine synthetase repressor			2.1				Regulator
N315-SA1154		Conserved HP	**Up**	2.2					
N315-SA1162		HP		2.1					
N315-SA1176		Conserved HP			2.6				
N315-SA1211	*opp-2E*	Oligopeptide transporter putative ATPase domain	2.5	2.2					Transport
N315-SA1212	*opp-2D*	Oligopeptide transport ATPase	3.7	3.0					Transport
N315-SA1213	*opp-2C*	Oligopeptide transporter membrane permease domain	3.9	2.8	2.0				Transport
N315-SA1214	*opp-2B*	Oligopeptide transporter membrane permease domain	4.5	3.6	2.7	Up	Up		Transport
N315-SA1215		HP			2.4				
N315-SA1235		Conserved HP			3.2				
N315-SA1245	*odhA*	2-Oxoglutarate dehydrogenase E1		2.3		Up			
N315-SA1252		Conserved HP			2.2				
N315-SA1265		Conserved HP		2.2					
N315-SA1275		Conserved HP	2.2	**2.9**		Up	Up		
N315-SA1310	*ansA*	Probable L-asparaginase			2.1				
N315-SA1318		HP			2.1				
N315-SA1321		HP			2.0				
N315-SA1410	*grpE*	GrpE protein			2.1				
N315-SA1434		Acetyl-CoA carboxylase (biotin carboxylase subunit)		2.1					

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TABLE 3—*Continued*

ORF no.	Gene	Description	Fold change at pH 5.5			Effect of regulator			Functional category
			T0	T3	T6	*agr*	*sarA*	*rot*	
N315-SA1476		HP		2.6					
N315-SA1531	*ald*	Alanine dehydrogenase		3.3					
N315-SA1551	*sgtA*	Probable transglycosylase			2.1				
N315-SA1554	*acs*	Acetyl-CoA synthetase		2.3					
N315-SA1601	*crcB*	Conserved HP			**2.0**				
N315-SA1609	*pckA*	Phosphoenolpyruvate carboxykinase		5.0		Up			
N315-SA1630	*splB*	Serine protease SplB	**2.3**			Up	Up	Down	Secreted
N315-SA1631	*splA*	Serine protease SplA	**Up**			Up	Up	Down	Secreted
N315-SA1844	*agrA*	Accessory gene regulator A	2.8			Up	Up		Regulator
N315-SA1879	*kdpC*	Probable potassium-transporting ATPase C chain		9.2	3.0			Down	Transport
N315-SA1880	*kdpB*	Probable potassium-transporting ATPase B chain	3.3	11.0	2.9				Transport
N315-SA1881	*kdpA*	Probable potassium-transporting ATPase A chain	5.7	17.6	3.4			Down	Transport
N315-SA1882	*kdpD*	Sensor protein KdpD	12.3						Regulator
N315-SA1883	*kdpE*	KDP operon transcriptional regulatory protein KdpE	10.2						Regulator
N315-SA1889		HP			2.0		Up		
N315-SA1890		Conserved HP			2.4				
N315-SA1896	*thiD*	Phosphomethylpyrimidine kinase		2.0					
N315-SA1929	*pyrG*	CTP synthase		2.2	2.7				
N315-SA1931		HP, similar to spermine/spermidine acetyltransferase bit		2.1			Up		
N315-SA1937		Conserved HP		2.0			Down		
N315-SA1938	*pyn*	Pyrimidine nucleoside phosphorylase		2.1					
N315-SA1947	*czrA*	Repressor protein		2.6	2.3				Regulator
N315-SA1948	*czrB*	Cation-efflux system membrane protein homolog	2.1	2.3					Transport
N315-SA1968	*arg*	Arginase		2.1					
N315-SA1976		Conserved hypothetical protein	2.5						
N315-SA1979		HP, similar to ferrichrome ABC transporter (binding protein)			2.7				Transport
N315-SA2006		HP, similar to MHC class II analog		2.0	2.3	Up	Up		Surface
N315-SA2007		HP, similar to alpha-acetolactate decarboxylase	4.5	**2.2**				Up	
N315-SA2008	*budB*	Alpha-acetolactate synthase	7.8	**3.1**					
N315-SA2060		HP, similar to transcription regulator MarR family	2.1	2.5	2.1				Regulator
N315-SA2075	*fdhD*	FdhD protein homologue			2.1				
N315-SA2079		HP, similar to ferrichrome ABC transporter fhuD precursor			2.4	Up	Up		Transport
N315-SA2081		HP, similar to urea transporter	**2.3**						Transport
N315-SA2082	*ureA*	Urease gamma subunit	3.9	10.4	6.3			Down	
N315-SA2083	*ureAB*	Urease beta subunit	5.7	12.8	**8.4**			Down	
N315-SA2084	*ureC*	Urease alpha subunit	4.5	10.2	4.8			Down	
N315-SA2085	*ureE*	Urease accessory protein UreE	2.8	5.6	3.8			Down	
N315-SA2086	*ureF*	Urease accessory protein UreF	2.7	6.1	4.0			Down	
N315-SA2087	*ureG*	Urease accessory protein UreG	2.0	3.6	3.0			Down	
N315-SA2088	*ureD*	Urease accessory protein UreD		3.6	2.7			Down	
N315-SA2093	*ssaA*	Secretory antigen precursor SsaA homolog		2.1	2.7	Down			Secreted
N315-SA2097		HP, similar to secretory antigen precursor SsaA			3.9				Secreted
N315-SA2101		Conserved HP	2.0						
N315-SA2120		HP, similar to amino acid amidohydrolase			2.1				
N315-SA2126		HP			2.6				
N315-SA2142	*semB*	HP, similar to multidrug resistance protein	3.9	5.3	4.2				Transport
N315-SA2143		Conserved HP	4.0	6.3	5.7				
N315-SA2146	*tcaA*	TcaA protein			2.0				Regulator
N315-SA2159		HP, similar to transcription repressor of sporulation			2.3				Regulator
N315-SA2165		HP, similar to transcriptional regulator tetR-family		2.1	2.5				Regulator
N315-SA2166		HP, similar to cationic transporter		2.4	2.4				Transport
N315-SA2168		HP		2.4					
N315-SA2173		HP			2.2				
N315-SA2204	*gpm*	Phosphoglycerate mutase	3.0						
N315-SA2208	*hlgC*	Gamma-hemolysin component C	2.3			Up	Up	Down	Secreted
N315-SA2216		HP, similar to ABC transporter			**2.4**				Transport
N315-SA2217		HP, similar to lipoprotein inner membrane ABC-transporter			**2.2**				Transport
N315-SA2227		Truncated HP, similar to D-serine/D-alanine/glycine transporter		4.3					Transport
N315-SA2229		Conserved HP	3.4						
N315-SA2231		HP, similar to glucose epimerase			2.2				
N315-SA2234	*opuCD*	Probable glycine betaine/carnitine/choline ABC transporter (membrane p) opuCD	2.6	2.2					Transport

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TABLE 3—*Continued*

ORF no.	Gene	Description	Fold change at pH 5.5			Effect of regulator			Functional category
			T0	T3	T6	*agr*	*sarA*	*rot*	
N315-SA2235	*opuCC*	Glycine betaine/carnitine/choline ABC transporter (osmoprotective) opuCC	2.8	2.5					Transport
N315-SA2236	*opuCB*	Probable glycine betaine/carnitine/choline ABC transporter (membrane p) opuCB	3.3						Transport
N315-SA2237	*opuCA*	Glycine betaine/carnitine/choline ABC transporter (ATP-bindin) opuCA	4.9	2.4					Transport
N315-SA2238		Conserved HP			2.1				
N315-SA2239		HP, similar to amino acid transporter	7.7	4.6	2.4				Transport
N315-SA2246		HP			2.2				
N315-SA2296		HP, similar to transcriptional regulator MerR family		3.0					Regulator
N315-SA2297		HP, similar to GTP-pyrophosphokinase		3.1	2.3			Down	
N315-SA2298		Conserved HP	2.8						
N315-SA2299		Conserved HP	3.3						
N315-SA2300		HP, similar to glucarate transporter		2.2		Down			Transport
N315-SA2304	*fbp*	Fructose-bisphosphatase		2.5					
N315-SA2311		HP, similar to NAD(P)H-flavin oxidoreductase			2.0				
N315-SA2316	*srtA*	Sortase			2.4				Transport
N315-SA2318	*sdhA*	HP, similar to L-serine dehydratase		4.4	2.8	Up			
N315-SA2319	*sdhB*	HP, similar to beta-subunit of L-serine dehydratas		5.3	2.3	Up	Up		
N315-SA2320		HP, similar to regulatory protein pfoR		4.6	2.2				Regulator
N315-SA2326	*ptsG*	PTS system, glucose-specific IIABC component	2.1					Down	Transport
N315-SA2328	*cidB*	Conserved HP	2.4	2.6					
N315-SA2329	*cidA*	Conserved HP	5.3	**3.1**					
N315-SA2330	*cidR*	HP, similar to transcription regulator	2.4						Regulator
N315-SA2332		HP, similar to secretory antigen precursor SsaA			**2.8**				Secreted
N315-SA2338		HP		**3.1**	**2.6**				
N315-SA2341	*rocA*	1-Pyrroline-5-carboxylate dehydrogenase		3.0		Up	Down		
N315-SA2343		HP		2.7		Up	Up		
N315-SA2355		Conserved HP		2.4	**4.1**				
N315-SA2356	*isaA*	Immunodominant antigen A			2.5				Surface
N315-SA2405	*betA*	Choline dehydrogenase			2.5				
N315-SA2406	*gbsA*	Glycine betaine aldehyde dehydrogenase gbsA			3.6				
N315-SA2408	*cudT*	Choline transporter		2.5					Transport
N315-SA2411	*citM*	HP, similar to magnesium citrate secondary transporter	2.1						
N315-SA2413	*cysJ*	Sulfite reductase (NADPH) flavoprotein			2.0				
N315-SA2414	*gpxA*	HP, similar to glutathione peroxidase	3.2	2.6	2.3			Down	
N315-SA2423	*clfB*	Clumping factor B			2.8				Surface
N315-SA2428	*arcA*	Arginine deiminase	2.1	**2.6**		Up			
N315-SA2430	*aur*	Zinc metalloproteinase aureolysin	2.0			Up	Down		Secreted
N315-SA2434		Fructose phosphotransferase system enzyme fruA homolog		2.3				Down	
N315-SA2443		HP		3.1					
N315-SA2444		HP	2.5	3.0					
N315-SA2445		HP	3.1	3.2					
N315-SA2446	*secY*	HP, similar to preprotein translocase secY	**3.1**	3.4					Transport
N315-SA2447		HP, similar to streptococcal hemagglutinin protein	5.8	2.2		Up	Up		Surface
N315-SA2459	*icaA*	Intercellular adhesion protein A	3.0						Surface
N315-SA2460	*icaD*	Intercellular adhesion protein D	2.2						Surface
N315-SA2463	*lip*	Triacylglycerol lipase precursor	4.1	2.0		Up	Down		Secreted
N315-SA2480	*drp35*	Drp35		2.8					
N315-SA2481		Conserved hypotehtical protein		2.1					
N315-SA2494	*cspB*	Cold shock protein cspB			2.6				
N315-SAS014		HP	**Up**						
N315-SAS017		HP			**2.3**				
N315-SAS039		HP			2.0				
N315-SAS064		HP (bacteriophage phiN315)			2.2				
	agrC		3.6						Regulator
		LPXTG-motif cell wall achor domain protein, similar to ClfA	**3.6**						Surface
		Similar to surface protein Pls	**Up**						Surface
		Similar to Staphylococcus lugdunensis gene Fbl	**Up**	**3.0**					
COL-SA0109		HP		2.1					
COL-SA0484		HP			**2.1**				
COL-SA0492		HP	**Up**						
COL-SA0794		HP		2.9					
COL-SA0850		HP	2.9	2.1	3.9				

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TABLE 3—*Continued*

ORF no.	Gene	Description	Fold change at pH 5.5			Effect of regulator			Functional category
			T0	T3	T6	*agr*	*sarA*	*rot*	
COL-SA0852		HP	**5.6**		**7.8**				
COL-SA0933		HP			2.7				
COL-SA1042		HP			2.4				
COL-SA1043		Glycosyl transferase group 1			2.5				
COL-SA1170		HP		2.3					
COL-SA1177		HP		2.3					
COL-SA1186		Antibacterial protein	23.5						Secreted
COL-SA1187		Antibacterial protein	28.7						Secreted
COL-SA1332		HP			**2.4**				
COL-SA1339		HP			**2.0**				
COL-SA1343		HP			**3.4**				
COL-SA1347		HP			**2.3**				
COL-SA1528		HP			2.2				
COL-SA1529		HP			2.0				
COL-SA1865	*splE*	Serine protease SplE	2.3						Secreted
COL-SA1877	*epiB*	Lantibiotic epidermin biosynthesis protein EpiB	2.2						Secreted
COL-SA2027		HP	3.4						
COL-SA2065		HP		**9.5**	**2.8**				
COL-SA2069		HP		**13.9**	**3.2**				
COL-SA2629		HP			**3.1**				
COL-SA2637		HP			2.2				
COL-SA2676		LPXTG-motif cell wall anchor domain protein	5.2	2.3					Surface
COL-SA2693		HP	3.9	3.7					
Mu50-SAV0850		HP			2.2				
Mu50-SAV0866		HP			**Up**				
Mu50-SAV0888		HP			3.1				
Mu50-SAV1991		HP			**2.2**				
Mu50-SAV1996		HP			**2.2**				
MW1600				2.4					
MW1681				**Up**					
MW1896		HP			**2.3**				
MW1926		HP			**2.4**				
MW2575		Ser/Thr repeat surface, similar to hemagglutinin		**3.1**					Surface
Genes downregulated at pH 5.5									
N315-SA0100		Conserved HP	5.7				Up		
N315-SA0107	*spa*	Immunoglobulin G-binding protein A precursor	**Down**			Down	Down	Up	Surface
N315-SA0129		LPXTG-containing HP			2.1				Surface
N315-SA0131	*deoD*	Purine nucleoside phosphorylase	2.1						
N315-SA0162	*aldA*	Aldehyde dehydrogenase homologue	2.3				Down		
N315-SA0206	*msmX*	multiple sugar-binding transport ATP-binding protein	2.5						Transport
N315-SA0207		HP, similar to maltose/maltodextrin-binding protein	3.6						Transport
N315-SA0208		Maltose/maltodextrin transport permease homologue	5.2						Transport
N315-SA0209		Maltose/maltodextrin transport permease homologue	5.9						Transport
N315-SA0210		HP, similar to NADH-dependent dehydrogenase	5.0						
N315-SA0211		HP, similar to NADH-dependent dehydrogenase	4.4						
N315-SA0212		Conserved HP	3.9			Down	Down		
N315-SA0214	*uhpT*	Hexose phosphate transport protein	4.1						Transport
N315-SA0218	*pflB*	Formate acetyltransferase	2.6		2.9				
N315-SA0219	*pflA*	Formate acetyltransferase activating enzyme	2.0		2.2				
N315-SA0232	*ldh*	L-Lactate dehydrogenase	2.2						
N315-SA0233		PTS enzyme (EC 2.7.1.69), factor II homologue, maltose and glucose specific	2.3						Transport
N315-SA0252	*lrgA*	Holin-like protein LrgA	5.2	16.9	7.3				Surface
N315-SA0253	*lrgB*	Holin-like protein LrgB	5.7	20.5	10.2		Down		Surface
N315-SA0265	*lytM*	Peptidoglycan hydrolase	2.7						Secreted
N315-SA0303		HP, similar to sodium-coupled permease	2.7						Transport
N315-SA0304	*nanA*	*N*-Acetylneuraminate lyase subunit	2.9						
N315-SA0357		HP, similar to exotoxin 2		6.1	**2.6**				Secreted
N315-SA0388	*set12*	Exotoxin 12		**2.2**					Secreted
N315-SA0395		HP (pathogenicity island SaPIn2)		10.5					
N315-SA0478		Conserved HP		2.2					
N315-SA0562	*adh*	Alcohol dehydrogenase I			3.7				
N315-SA0639		HP, similar to ABC transporter required for expression of cytochrome *bd*			2.2				Transport

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TABLE 3—*Continued*

ORF no.	Gene	Description	Fold change at pH 5.5			Effect of regulator			Functional category
			T0	T3	T6	*agr*	*sarA*	*rot*	
N315-SA0640		HP, similar to ABC transporter required for expression of cytochrome *bd*			3.0				Transport
N315-SA0650	*norA*	Quinolone resistance protein	2.7						Transport
N315-SA0660	*saeS*	Histidine protein kinase		2.1	2.3				Regulator
N315-SA0661	*saeR*	Response regulator		2.3	2.3				Regulator
N315-SA0662		HP		5.2	3.0				
N315-SA0663		HP		9.0	3.4			Down	
N315-SA0691	*sstD*	Lipoprotein, similar to ferrichrome ABC transporter	2.9						Transport
N315-SA0744	*ssp*	Extracellular ECM and plasma binding protein		**2.6**					Surface
N315-SA0746	*nuc*	Staphylococcal nuclease		2.2			Down		Secreted
N315-SA0754		HP, similar to lactococcal prophage ps3 protein 05			3.0		Up		
N315-SA0773		Conserved HP	3.4						
N315-SA0807	*mnhG*	Na^+/H^+ antiporter subunit			2.0				Transport
N315-SA0808	*mnhF*	Na^+/H^+ antiporter subunit			2.3	Down			Transport
N315-SA0810	*mnhD*	Na^+/H^+ antiporter subunit			2.0				Transport
N315-SA0820	*glpQ*	Glycerophosphoryl diester phosphodiesterase	2.4						
N315-SA0889		HP	31.1	**17.6**	**10.3**				
N315-SA0890		Conserved HP	6.2	32.8	9.5				
N315-SA0937		Cytochrome *d* ubiquinol oxidase subunit I homologue	2.6						
N315-SA0938		Cytochrome *d* ubiquinol oxidase subunit II homologue	3.0						
N315-SA0963	*pyc*	Pyruvate carboxylase		2.0					
N315-SA1000		HP, similar to fibrinogen-binding protein	4.1	3.9					Surface
N315-SA1001		HP			4.6				
N315-SA1003				**12.2**	4.1				
N315-SA1004	*fib*	HP, similar to fibrinogen-binding protein	2.4	**13.1**	4.7				Surface
N315-SA1270		HP, similar to amino acid pearmease			**3.5**	Down			Transport
N315-SA1272	*ald*	Alanine dehydrogenase			4.0	Down			
N315-SA1490	*yhjN*	Conserved HP			2.2				
N315-SA1583	*rot*	Repressor of toxins Rot	2.7	3.0	4.0			Up	Regulator
N315-SA1591	*arsR*	Arsenical resistance operon repressor homologue	2.0						Regulator
N315-SA1628	*splD*	Serine protease SplD			2.5	Up	Up	Down	Secreted
N315-SA1629	*splC*	Serine protease SplC			2.7			Down	Secreted
N315-SA1630	*splB*	Serine protease SplB			2.2	Up	Up	Down	Secreted
N315-SA1631	*splA*	Serine protease SplA				Up	Up	Down	Secreted
N315-SA1637	*lukD*	Leukotoxin, LukD (pathogenicity island SaPIn3)		16.1	9.9				Secreted
N315-SA1638	*lukS*	Leukotoxin LukE		16.6	9.5				Secreted
N315-SA1750	*mapW_1*	Truncated map-w protein		9.7	7.6				Surface
N315-SA1751	*mapW_2*	Truncated map-w protein		7.8	6.1				Surface
N315-SA1753		HP (bacteriophage phiN315)		2.4					
N315-SA1755		HP (bacteriophage phiN315)		27.2	11.8				
N315-SA1758	*sak*	Staphylokinase precursor			2.4				Secreted
N315-SA1759		Lytic enzyme	2.5						Secreted
N315-SA1784		HP (bacteriophage phiN315)	2.2						
N315-SA1789		HP (bacteriophage phiN315)	2.0						
N315-SA1790		HP (bacteriophage phiN315)	2.8						
N315-SA1792		Single-strand DNA-binding protein	3.1						
N315-SA1795		HP (Bacteriophage phiN315)	2.6						
N315-SA1796		HP (Bacteriophage phiN315)	2.8						
N315-SA1812	*lukE*	HP, similar to synergohymenotropic toxin precursor		12.3	5.9			Down	Secreted
N315-SA1813	*lukM*	HP, similar to leukocidin chain lukM precursor		11.7	5.2				Secreted
N315-SA2095		HP, similar to D-octopine dehydrogenase		2.3					
N315-SA2108		HP, similar to transcription regulator RpiR family		2.1	3.3				Regulator
N315-SA2114	*glvC*	PTS system, arbutin-like IIBC component	2.3						Transport
N315-SA2135	*gltS*	HP, similar to sodium/glutamate symporter	3.9				Up		Transport
N315-SA2147	*tcaR*	TcaR transcription regulator		2.1	2.5				Regulator
N315-SA2156		L-Lactate permease lctP homologue	3.2						Transport
N315-SA2194		HP, similar to Zn-binding lipoprotein adcA	3.6						Surface
N315-SA2206	*sbi*	IgG-binding protein SBI		6.0	4.2				Surface
N315-SA2207	*hlgA*	Gamma-hemolysin chain II precursor		3.1	16.2				Secreted
N315-SA2208	*hlgC*	Gamma-hemolysin component C		6.7	2.7	Up	Up	Down	Secreted
N315-SA2209	*hlgB*	Gamma-hemolysin component B		7.4	2.8	Up	Up	Down	Secreted
N315-SA2210	*bioX*	HP, similar to BioX protein			**5.9**				
N315-SA2211	*bioW*	HP, similar to 6-carboxyhexanoate–CoA ligase			3.6				
N315-SA2251	*opp-1F*	Oligopeptide transporter putative ATPase domain	2.1			Up			Transport
N315-SA2253	*opp-1C*	Oligopeptide transporter putative membrane permease domain	2.1						Transport

Continued on following page

TABLE 3—*Continued*

ORF no.	Gene	Description	Fold change at pH 5.5			Effect of regulator			Functional category
			T0	T3	T6	*agr*	*sarA*	*rot*	
N315-SA2254	*opp-1B*	Oligopeptide transporter putative membrane permease domain	2.2						Transport
N315-SA2255	*opp-1A*	Oligopeptide transporter putative substrate binding domain	2.1						Transport
N315-SA2300		HP, similar to glucarate transporter	2.7			Down			Transport
N315-SA2302	*stpC*	HP, similar to ABC transporter	5.8	5.1	3.0				Transport
N315-SA2302	*stpC*	HP, similar to ABC transporter	5.8	5.1	3.0				Transport
N315-SA2321		HP	2.9	2.3		Up	Up		
N315-SA2430	*aur*	Zinc metalloproteinase aureolysin		2.5		Up	Down		Secreted
N315-SAS025		HP		2.6					
N315-SAS035		HP		2.2	2.4				
N315-SAS051		HP			2.3				
N315-SAS058		HP (bacteriophage phiN315)		2.4					
	splF			4.6					Secreted
COL-SA0256		HP	**Down**						
COL-SA0334		Bacteriophage L54a, conserved HP	2.4						
COL-SA0472	exotoxin 2				**2.3**				Secreted
COL-SA0478	exotoxin 3	Exotoxin 3	3.4						Secreted
COL-SA0906		Terminase small subunit	2.2						
COL-SA1165		HP		2.1					
COL-SA1477	*ilvA*	Threonine dehydratase catabolic		2.7	4.1				
COL-SA1865	*splE*	Serine protease SplE			2.4				Secreted
COL-SA1870		HP		2.2					
COL-SA2014		Terminase small subunit	2.1						
COL-SA2420		HP, RpiR family		**2.1**	**10.4**				Regulator
COL-SA2480		HP	2.8						
COL-SA2511	*fnbA*	Fibronectin-binding protein A		**2.2**					Surface
COL-SA2568		HP	**2.1**						
Mu50-SAV0861		HP, bacteriophage?	**Down**						
Mu50-SAV0883		HP, bacteriophage?	**Down**						
Mu50-SAV0888		HP, bacteriophage?	2.4						
Mu50-SAV0889		HP, bacteriophage?	2.6						
Mu50-SAV0893		HP, bacteriophage?	2.6						
Mu50-SAV0894		HP, bacteriophage?	2.8						
Mu50-SAV0895		HP, bacteriophage?	2.0						
Mu50-SAV0898		HP, bacteriophage?	2.2						
Mu50-SAV0899		HP, bacteriophage?	2.2						
Mu50-SAV0900		HP, bacteriophage?	2.6						
Mu50-SAV0902		HP, bacteriophage?	2.6						
Mu50-SAV0903		HP, bacteriophage?	2.3						
Mu50-SAV0905		HP, bacteriophage?	2.3						
Mu50-SAV0906		HP, bacteriophage?	2.6						
Mu50-SAV0907		HP, bacteriophage?	2.7						
Mu50-SAV0909		HP, bacteriophage?	2.5						
Mu50-SAV0910		HP, bacteriophage?	2.7						
Mu50-SAV0911		HP, bacteriophage?	2.9						
Mu50-SAV1985		HP, bacteriophage?	2.8						
Mu50-SAV1988		HP, bacteriophage?	3.8						
MW1382		HP	3.3						
MW1441		HP			2.4				
MW1920		HP	2.2						
MW1927		HP	**3.1**						
MWP018					**Down**				

[a] "Fold change" indicates increase or decrease for up-regulated and down-regulated genes, respectively. Values in bold indicate low expression under one condition introduced uncertainty in the fold difference value. "Down" denotes saturation under this condition or absence under other condition such that no fold difference could be calculated; "Up" denotes saturation under this condition or absence under other condition such that no fold difference could be calculated.

not be detected by blotting. Comparison of the *hla* Northern blotting data with the gene fusion results revealed that transcript levels and promoter activity were similar in direction if not magnitude. (iii) With the exception of the T6 time point, the Northern blotting data for *sspA* were relatively consistent with both the profiling data and with the gene fusion data. Although at T3 the *sspA* transcript appeared higher at pH 5.5 and at T6 it appeared higher at pH 7.5, these differences were less than twofold. One possibility for the differences observed between gene fusion and Northern blot data could be that the gene fusion construct used may not have included all 5′ regulatory elements (Fig. 2 and 4). At T0, the transcription level was too low to be evaluated at either pH. (iv) The *spa* data could not be confirmed by Northern blotting of RN6734 RNA

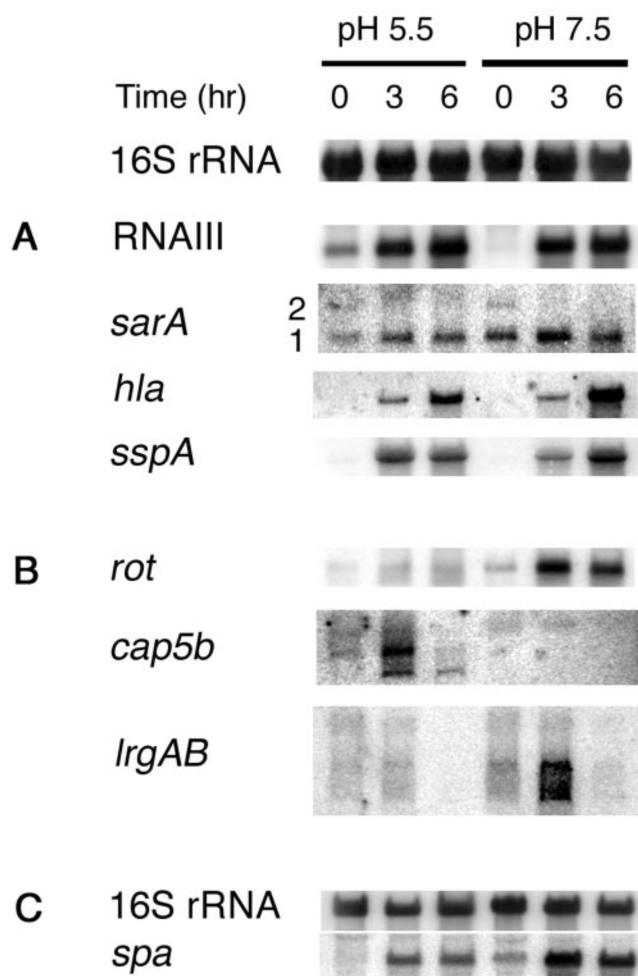

FIG. 4. Northern blot analysis of the effect of pH on the transcript levels of genes coding for several exoproteins and their regulators. (A and B) Blots of whole-cell RNA from RN6734 grown in CYGP broth without glucose and adjusted to pH 5.5 or 7.5. (B) Blots of transcripts identified by microarray analysis as pH dependent. The 16S rRNA blot above panels A and B shows equal loading. (C) pH-dependent expression of *spa* in RN7206 (RN6734 Δ*agr*); equal loading is shown by the adjacent 16S rRNA blot.

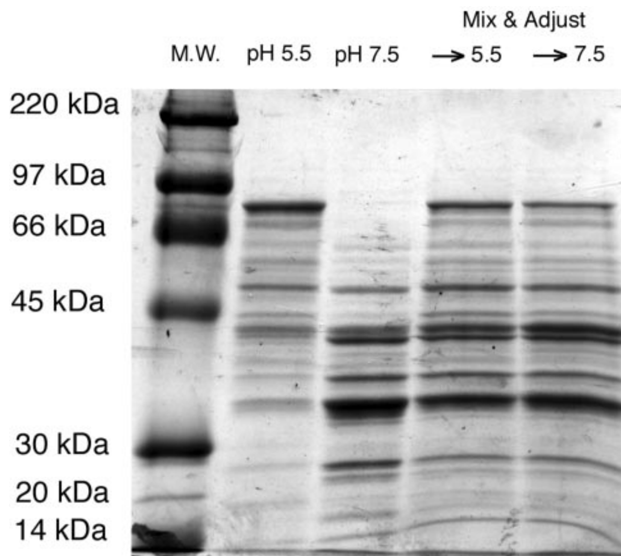

FIG. 5. Exoprotein profiles of cultures grown at pH 5.5 or 7.5, and the effect of pH-dependent protease activity on exoprotein profiles. Exoprotein was prepared from 6-h cultures grown in CYGP broth adjusted to either pH 5.5 or pH 7.5. Supernatants from equal numbers of cells were precipitated with trichloroacetic acid and subjected to SDS-PAGE. The right two lanes show exoprotein from both of the left lanes combined, adjusted to each pH, and incubated at 37°C for 30 min to investigate the effect of differential protease activity.

because the level of expression was too low at most time points in this *agr*$^+$ strain. Therefore, we prepared blots with RNA from the isogenic *agr*-null strain, RN7206. Although the blots from one strain cannot be directly compared with the microarray data from another strain, *spa* expression in RN7206 was greater at pH 7.5 throughout growth and, in RN6734, it was greater at pH 7.5 only at the earliest time point. (v) Although the pH regulation of *rot* seen in the microarray was confirmed by Northern blot hybridization, *rot* transcription increased postexponentially at both pHs rather than being constitutive as has been reported elsewhere (32). (vi) The Northern blots for *agr-rnaIII* and *sae* were consistent with the microarray results.

Although *sarA* does not belong to the MAS based on analysis of RN6734, interpretation of *sarA* results is problematic; *sarA* is transcribed from three separate promoters (12), one of which, P3, uses σ^B and is not active in RN6734 and other derivatives of NCTC8325, owing to the *rsbU* defect in these strains. In an *rsbU*$^+$ strain this promoter is much more active at pH 5.5 than at pH 7.5 (unpublished data). Conversely, the P1 promoter appeared to be considerably less active at pH 5.5 than at pH 7.5 (the level of the P2 transcript was too low to be relevant). Thus, the role of pH in the overall production of SarA protein, which may be highly strain specific, remains to be determined.

Effect of pH on overall exoprotein patterns. Given that the MAS seems large and complex and that it includes several exoprotein genes, including those encoding known virulence factors, we have begun to analyze the effects of pH on the extracellular proteome. For this purpose, supernatants were collected from cultures of RN6734 grown at pH 5.5 versus pH 7.5 in the absence of glucose at the T6 time point. Supernatant exoprotein profiles, as determined by SDS-PAGE, are shown in Fig. 5. Several exoproteins were observed to be more abundant at pH 7.5 but were considerably reduced or absent at pH 5.5, and vice versa; additionally, there was a considerable asymmetry in the size distributions of the bands corresponding to the two pH conditions, with higher-molecular-weight species predominant at pH 5.5 and lower-weight species at pH 7.5. To test for the possibility that this asymmetry could represent differential proteolytic activity, we mixed equal quantities of the two supernatants, adjusted the mixtures to either pH 5.5 or 7.5, incubated them for 30 min at 37°C, and analyzed them by SDS-PAGE. The resulting exoprotein patterns, shown in the two rightmost lanes in Fig. 5, were indistinguishable with the exception of a high-molecular-weight band, which we have identified as *geh* lipase (unpublished data). This band is somewhat weaker after incubation of the mixture at pH 7.5 than at 5.5, presumably owing to differential proteolysis (30, 42, 48).

Overall, these results are consistent with transcription-level regulation of exoprotein production, and it is clear that proteolysis is not responsible for most of the pH-dependent differences in the exoprotein profile.

Identification of differentially abundant exoproteins. We identified several of the differentially abundant exoproteins by mass spectrometry. Due to differences in loading and resolution between the gel from which the bands were excised and the gel shown in Fig. 5, these proteins are not labeled in Fig. 5. In addition to the cleaved form of lipase, these included an immunodominant surface antigen, IsaA (more abundant at pH 5.5), the precursor form of autolysin Atl (more abundant at pH 5.5), and the serine protease-like SplF (more abundant at pH 7.5). Comparing these and the microarray results, some of the differential abundances of these proteins are a reflection of pH-dependent differences in transcription (IsaA and SplF); others are likely the result of posttranscriptional mechanisms (Geh and Atl).

FIG. 6. pH ranges of various niches *S. aureus* can colonize in the human host. Blood, pH 7.4 (Robinson 1975); vagina, 4.2 to 6.6 (Wagner and Ottesen 1982); abscess, 6.2 to 7.3 (Bessman, Page et al. 1989); urinary tract (UT), 4.6 to 7 (McClatchey 1994); lung, 6.8 to 7.6 (Cheng, Rodriguez et al. 1998); mouth, 5 to 7 (Dong, Pearce et al. 1999); nose, 6.5 to 7 (England, Homer et al. 1999); skin, 4.2 to 5.9 (Ehlers, Ivens et al. 2001).

DISCUSSION

This study began with the observation that the glucose-induced down-regulation of certain staphylococcal virulence genes is largely a result of the pH reduction resulting from glucose fermentation. The end point of this pH reduction is generally around pH 5.5 in standard glucose-supplemented broth cultures of *S. aureus*; in cultures grown at this pH the classical *agr*-dependent postexponential induction of virulence genes such as *hla* and *tst*, seen at pH 7.5, is eliminated. Microarray analysis revealed that the transcript levels of over 400 *S. aureus* genes were affected, and the overall pattern is here defined as the MAS. More than twice as many genes are up-regulated at pH 5.5 than are down-regulated, relative to effects at pH 7.5.

It is well known that bacteria have elaborate mechanisms by which they maintain their internal pH within tight limits. Major, potentially lethal reductions in external pH activate an acid stress response, which mobilizes a variety of resources in an attempt to defend the cytoplasmic pH (14, 37, 38). At the same time, bacteria such as staphylococci can grow and divide normally over a considerable pH range—at least 5 to 9. In the present work we have analyzed the lower half of this range and found that there is a large set of genes that are differentially regulated between pH 5.5 and 7.5—the MAS. We suggest that this modulation of gene expression is likely to represent an adaptation to pH-variable environments, rather than a global acid stress response. In other words, pH could be one of the factors that determine locale-specific gene expression patterns, since certain body sites are characterized by variations in pH from the homeostatic 7.4 (Fig. 6) (5, 10, 16, 21, 22, 31, 41, 53). Given the breadth of the MAS, plus the fact that the intracellular pH is maintained within very narrow limits, the pH differential must be sensed by one or more surface receptors and transduced to the interior of the cell. As there is a complex regulatory network that coordinates the expression of accessory genes (34), the MAS must interact with one or more regulatory genes involved in this network. For any regulatory genes that belong to the MAS, it is predicted that their regulons will follow suit, insofar as the regulation is direct. That is, if transcription of a regulatory gene is reduced at one pH, the genes that it up-regulates will also be reduced at that pH, and vice versa. Genes that do not follow this rule must be regulated indirectly. For those regulatory genes that do not belong to the MAS, any target genes that do belong must also be regulated indirectly. Therefore, examination of stimulons and their overlaps with regulons is expected to aid greatly in the understanding of the overall regulatory network. Results presented here may be viewed as the beginning of this analysis.

A number of tantalizing relationships between the MAS and certain other stimulons and regulons have been revealed; these relationships are noted here and are clearly in need of further study.

One example is the stimulon defined by the response to subinhibitory concentrations of cell wall-active antibiotics (bacitracin, D-cycloserine, and oxacillin) (50). The MAS appears to overlap significantly with the cell wall-stress stimulon. Twenty-five of the 158 genes in this stimulon are also in the MAS, and for these genes the two stimulons are concordant, raising the question of whether there is some common adaptivity between the two, such as the involvement of any of these 25 genes in transducing the pH stimulus. Seventeen of these 25 genes were both up-regulated by cell wall-active antibiotics and were up-regulated at pH 5.5. Seven of the remaining eight genes were down-regulated by antibiotics and were also down-regulated at pH 5.5. The only exception was a hypothetical gene that could correspond to an unidentified regulator (a repressor) of the MarR type. Because the microarray used in defining the cell wall-stress stimulon was less comprehensive than the one used in the present study, it is possible that the relationship between the two stimulons is even greater than observed here.

Another stimulon that was recently defined is the set of genes that are differentially expressed during growth in a mature flow cell-resident biofilm (3). The biofilm state has been shown to be relevant to several different infection niches for growth, persistence, and resistance to antibiotic treatment (17). Although the strain used by Beenken et al. is not of the

NCTC8325 lineage and the cultures were grown with glucose, genes they identified as differentially expressed between exponential-phase planktonic cultures, in which the pH may have not yet dropped below pH 6.0, and mature biofilms may be relevant for comparison to the MAS. Of the 95 genes that are reported up-regulated in a biofilm, well over half (55) are also up-regulated under the mild acid condition. The operons up-regulated under both conditions include those coding for capsular polysaccharide biosynthesis, pyrimidine biosynthesis, the potassium-proton antiporter system *kdp*, the urease system, and the *ssp* operon. Among the 17 genes down-regulated under both conditions are *rot*, *spa*, *nuc*, *pycA*, and the *opp1* and *spl* operons. None of the mild-acid-down-regulated genes were up-regulated in the biofilm, and only 17 of the 278 biofilm down-regulated genes were up-regulated in mild acid. The results presented here support the suggestion of Beenken et al. that a mature biofilm is an acidic environment. The incomplete concordance of the biofilm and mild acid stimulons may reveal sets of genes specific to biofilm or planktonic lifestyles. In sum, the mild acid-biofilm correlation provides an example of an important niche where pH-dependent gene expression may be critical for growth and persistence.

A third example involves the conditionally essential two-component signal transduction system (TCS), YycG/YycF. Five of 12 genes recently reported to be controlled by YycG/YycF were identified in the MAS, although the TCS itself was not. YycF recognizes a pair of directly repeated hexamers within the promoter region of target genes (18). Two of the target genes with their direct repeats oriented in one direction were down-regulated at pH 5.5; three with their repeats in the opposite direction were up-regulated at pH 5.5. This difference in the orientation of *cis* regulatory elements in the promoters of genes belonging to the YycG/YycF TCS regulon correlates with the differential pH effects on these genes, suggesting an interesting possible relationship between this TCS and transduction of the pH signal.

Among the genes belonging to the MAS are 26 known or putative regulatory genes. The response of target gene subsets should, to a first approximation, follow the response of the controlling regulatory gene. The *sae* regulon appears to fit this scheme, at least with respect to the few target genes that have been individually analyzed—for example, *sae* up-regulated leukotoxin and gamma hemolysin are down-regulated at pH 5.5, like *sae*. The generality of this effect will be reported elsewhere in connection with an ongoing analysis of the *sae* regulon. The transcript levels of 19 other known or putative regulatory genes are increased at pH 5.5, and those of six are decreased. In Table 3 are compiled data on known regulatory interactions involving two of the better studied of these regulators, *agr* and *rot*, whose regulons have been determined (20, 43). We note that the RNA samples used for determination of the *agr* and *rot* regulons were all prepared from bacteria grown in the presence of glucose, and the *rot* regulon was determined with an *agr*-null strain. Additionally, they were determined with an earlier version of the microarray lacking many important genes. Nevertheless, most of the *agr*-regulated and *rot*-regulated genes adhere to the principle that target genes follow the response of the controlling regulator. For genes that do not, the simplest possibility is that the regulatory connection is indirect. Thus, *agr* is up-regulated 3.6-fold at pH 5.5 and T0 (but not at the other time points). At this time point, when *agr* is a member of the MAS, the *agr*-regulated members of the MAS follow the regulation of *agr*, whereas at other time points there is no consistency to the response of *agr*-regulated genes. Interestingly, although *agr* belongs to the MAS, no known regulators of *agr*, including *svrA*, *sarA*, *sarU*, *ssrAB*, and *arlRS*, belong to the stimulon. Of course, any of these regulators could be affected at the posttranscriptional level.

rot was identified as a member of the MAS and was shown to be down-regulated at pH 5.5 at all three time points. Thirty-nine genes (9.3% of the stimulon) are *rot* regulated, of which 28 demonstrated higher transcript titers at pH 5.5. This is largely consistent with the reported role of *rot* as a repressor (32). Genes encoding surface proteins, such as *spa*, which is up-regulated by *rot*, are down-regulated at pH 5.5. *rot* regulates *spa* via SarS (43), which is not listed in the MAS. However, the stimulon list was generated by using an arbitrary cutoff value of a twofold change in transcription. Further analysis indicated that although it is not decreased twofold, *sarS* is indeed moderately down-regulated at the lower pH tested. Only one gene, SA0173, a hypothetical gene similar to surfactin synthetase of *Bacillus subtilis*, is regulated in the same direction by *agr* and *rot* (up).

Microarray data for the *sarA* regulon have also been published (20) and are included in Table 3, although *sarA* does not appear to belong to the MAS in strains of the NCTC8325 lineage, such as RN6734. Forty-six genes in the stimulon are SarA regulated. Most of these have increased transcript levels at pH 5.5, and most SarA-regulated genes with pH-sensitive expression are SarA up-regulated. These genes are regulated predominantly through *agr*.

It has been suggested that SarA is important in the transduction of the pH signal and, superficially, the data on the relation of SarA to *sspA* shown in Fig. 2C and D would seem to bear this out. SarA seems to be a repressor of *sspA* only at pH 5.5. However, a closer look at these data suggests that it is not SarA but a separate positive regulator that may be responsible. We note that at pH 7.5, deletion of *sarA* has no effect on *sspA* expression in the NCTC8325-derived background tested here; i.e., *sarA* cannot be a repressor of *sspA* at pH 7.5. If it were a repressor only at pH 5.5, then the level of *sspA* at pH 7.5 should match the higher expression level at pH 5.5, rather than the lower. The simplest interpretation here is that SarA is not a pH-dependent repressor, but rather that there is a pH-dependent activator that functions at pH 5.5, only in the absence of SarA.

It has also been suggested that σ^B may have a role in transducing the pH signal (9, 39); on the basis of data presented here, it is suggested that there may be two pH-sensing pathways, one independent of σ^B and the other possibly σ^B dependent. The microarray analysis was performed with RN6734 because of the enormous experience and wide use of this and other NCTC8325 derivatives, despite their *rsbU* defect (23, 26). As these strains have only weak residual σ^B activity, the studies reported here will have defined a pH-sensing pathway that is essentially independent of σ^B. This has been confirmed by a recently published DNA microarray analysis of the σ^B regulon (6). Of the 198 genes found to be σ^B up-regulated by transcriptional profiling of three different $rsbU^+$ strains, 25 were also up-regulated by mild acid. None of these 198 genes was

down-regulated by mild acid. Eighteen of the 53 σ^B down-regulated genes were also down-regulated by mild acid; however, 19 of these 53 were up-regulated by mild acid. These results are consistent with the absence of *sigB* or members of the *sigB* operon in the MAS and the pH-insensitive transcription pattern of *sigB* in RN6734 (data not shown). However, *sarA* has a σ^B-dependent promoter that is also pH dependent (unpublished data), so that in an $rsbU^+$ strain, genes regulated by SarA may define a pH-dependent pathway that is σ^B dependent. This interpretation is highly tentative since, as noted above, it is not known whether the interplay of the three *sarA* promoters results in σ^B-dependent differences in SarA protein levels.

In conclusion, our results have pointed to a number of approaches that could help to identify the pathway(s) by which external pH is sensed by the organism. Additionally, they address but do not solve the basic question of the role of pH-dependent gene expression in pathogenesis. On the one hand, as shown here, it is clear that mild acid modulates the expression of a large set of staphylococcal genes, including virulence genes; on the other hand, it is well known that certain sites in an animal host are characterized by pHs different from the homeostatic 7.4. The basic question, then, is whether there is a significant connection between these two separate findings. In other words, is pH-dependent modulation of virulence gene expression adaptive for the organism with respect to different sites in the animal host that are maintained at particular pHs (different from 7.4)? And, if so, would this be an example of bacteria modulating the expression of particular genes for adaptation to particular tissue sites?

The available studies of pH-dependent and host-niche-specific staphylococcal virulence gene expression reveal little that suggests such a connection (1, 2, 4, 9, 11, 24, 25, 29, 33, 36, 40, 44–46, 49, 51, 54, 55). A potentially informative study, however, is that of Coulter et al. (1998), which identified genes required for growth and persistence in various animal models of staphylococcal infection. Mutations in *opp2*, *sspA*, *odhA*, and *epiB* were shown to cause attenuation in a systemic infection model but not in abscess or burn wound models, while those in *opp1*, *pycA*, and *pflB* caused attenuation in an abscess model but not in systemic infection or burn wound models (15). The genes associated with systemic infections were up-regulated, while those associated with abscesses were down-regulated at pH 5.5. A homologous oligopeptide permease (opp) system in *B. subtilis* is required for the uptake of signaling peptides necessary for pH-dependent expression of *srf*, which encodes a surfactin synthetase (13). A surfactin synthetase homologue is also part of the staphylococcal MAS and is up-regulated at pH 5.5, as is *opp2*. This could represent a conserved adaptation to the mild acid condition.

The interesting correlation between tissue-site specificity and pH modulation of these genes raises the possibility that tissue-specific pH variation could impact on pathogenesis. It seems doubtful, however, that the pHs at the sites in question prior to infection are consistent with the observed pH-dependent modulation in bacterial gene expression, because these sites are typically maintained at pH 7.4 (Fig. 6). Perhaps a local pH change as a result of the infection requires an adaptive response, prohibiting bacteria incapable of such a response from maintaining an infection.

A quite different and rather dramatic example of how pH-dependent virulence gene expression could impact human health, but in a manner that is also not consistent with the concept of pH-dependent tissue site adaptation, is menstrual toxic shock. The human vagina is maintained at a pH of <5, a pH at which toxic shock syndrome toxin 1 is not produced (45) because *tst*, the gene encoding the toxin, is essentially silent (Fig. 2). However, during menses the pH rises above 7, a pH at which *tst* is strongly expressed—which could very well account for the well-known relation between toxic shock syndrome and menstruation (4). However, whether pH-dependent expression of *tst* has any adaptive role for the organism or is simply coincidental is far from clear. Indeed, it is not obvious whether causing toxic shock syndrome is in any way advantageous for the organism; instead, maintaining the vaginal pH of <5 could represent a host defense against toxic shock, a defense that the bacteria have clearly not countered by pH-dependent gene expression but one that is inadvertently breached by the host through menstrual bleeding.

The in vivo relevance of the pH-dependent accessory gene expression we report here will require more accurate measurement of the pH of the host environment during infection and testing of knockouts of the members of the signal transduction network in relevant animal models. Regardless of the role of differential expression in an infection, elucidation of the MAS will forward attempts to map the *S. aureus* accessory gene regulatory network. Regulons have great value in establishing epistasis and suggesting functional roles for the corresponding regulator, but their usefulness is limited because regulators often act in concert and effects may be absent or even contradictory in single mutants. Stimulons allow us to perturb genetically complete organisms but require analysis of the relevant mutants to dissect cause and effect. The combination of these methods should in the future allow construction of a comprehensive model of the *S. aureus* accessory gene regulatory network.

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