

Staphylococcus epidermidis saeR Is an Effector of Anaerobic Growth and a Mediator of Acute Inflammation[∇]

L. D. Handke,¹† K. L. Rogers,¹ M. E. Olson,¹ G. A. Somerville,³ T. J. Jerrells,¹ M. E. Rupp,^{1,2}
P. M. Dunman,¹ and P. D. Fey^{1,2*}

Departments of Pathology and Microbiology¹ and Internal Medicine,² University of Nebraska Medical Center, Omaha, Nebraska 68198, and Department of Veterinary and Biomedical Sciences, University of Nebraska–Lincoln, Lincoln, Nebraska 68583³

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The *saeRS* two-component regulatory system regulates transcription of multiple virulence factors in *Staphylococcus aureus*. In the present study, we demonstrated that the *saePQRS* region in *Staphylococcus epidermidis* is transcriptionally regulated in a temporal manner and is arranged in a manner similar to that previously described for *S. aureus*. Studies using a mouse foreign body infection model demonstrated that the virulence of strain 1457 and the virulence of a mutant, strain 1457 *saeR*, were statistically equivalent. However, histological analyses suggested that the polymorphonuclear neutrophil response at 2 days postinfection was significantly greater in 1457-infected mice than in 1457 *saeR*-infected mice, demonstrating that SaeR influences the early, acute phases of infection. Microarray analysis demonstrated that a *saeR* mutation affected the transcription of 65 genes (37 genes were upregulated and 28 genes were downregulated); in particular, 8 genes that facilitate growth under anaerobic conditions were downregulated in 1457 *saeR*. Analysis of growth under anaerobic conditions demonstrated that 1457 *saeR* had a decreased growth rate compared to 1457. Further metabolic experiments demonstrated that 1457 *saeR* had a reduced capacity to utilize nitrate as a terminal electron acceptor and exhibited increased production of lactic acid in comparison to 1457. These data suggest that in *S. epidermidis* SaeR functions to regulate the transition between aerobic growth and anaerobic growth. In addition, when grown anaerobically, 1457 *saeR* appeared to compensate for the redox imbalance created by the lack of electron transport-mediated oxidation of NADH to NAD⁺ by increasing lactate dehydrogenase activity and the subsequent oxidation of NADH.

Prokaryotic two-component regulatory systems modulate gene expression in response to different environmental signals. Analyses of the available *Staphylococcus aureus* genomes have revealed that *S. aureus* encodes 16 putative two-component regulatory systems (14), 6 of which have been characterized (3, 10, 13, 31, 36, 43, 53). One two-component regulatory system, encoded by *saeRS*, was originally identified as a transposon mutant system deficient in the synthesis of several exoproteins, including α - and β -hemolysin, and coagulase (21). SaeRS has subsequently been shown to regulate staphylococcal immune evasion proteins (48), adhesins (26), and γ -hemolysin (58). Importantly, *sae* inactivation reduces invasion of *S. aureus* in several animal models and cell lines (20, 35, 52, 57).

The *sae* operon in *S. aureus* is comprised of four genes, *saePQRS*, with *saeR* encoding the response regulator and *saeS* encoding the sensor kinase (18). The functions of SaeP and SaeQ are unknown, but it has been postulated that they have important roles in *sae*-dependent regulation (44, 52). Transcriptional studies revealed that the *sae* genes in *S. aureus* are translated from three or four different transcripts and that *saeRS* is present in three of these transcripts (44, 47, 52).

Transcription of the *sae* mRNAs is growth phase dependent, activated by *agr* (19, 44, 52), and regulated by SaeR autoactivation (44). Although several environmental conditions affect *sae* expression, including low pH, salt, glucose, or subinhibitory concentrations of antibiotics (44), the specific activating signal remains unknown.

In this work, we demonstrated that the *sae* locus is present in the closely related, opportunistic pathogen *Staphylococcus epidermidis*, the preeminent cause of biomaterial-related infections in hospital settings. In contrast to *S. aureus*, which produces a large array of toxins and adherence factors, *S. epidermidis* produces very few virulence factors. Of the virulence factors that have been described (32, 54, 60, 61), a major virulence mechanism associated with *S. epidermidis* is its ability to form a biofilm, which is largely composed of polysaccharide intercellular adhesin (PIA) (38). PIA, which is synthesized by enzymes encoded in the four-gene *icaADBC* operon (27), dramatically reduces the effectiveness of antibiotics and the host immune system (55, 56). Importantly, *S. epidermidis* *icaADBC* operon mutants are less virulent in animal models of biomaterial infection (49, 50). SaeRS is required for transcription of several virulence genes in *S. aureus*, which led us to speculate that the SaeRS system has a similar function in *S. epidermidis*.

* Corresponding author. Mailing address: University of Nebraska Medical Center, 986280 Nebraska Medical Center, Omaha, NE 68198-6280. Phone and fax: (402) 559-4077. E-mail: pfey@unmc.edu.

† Present address: Department of Molecular, Biology and Microbiology, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111.

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MATERIALS AND METHODS

Bacterial strains and plasmids used in study. Bacterial strains and plasmid constructs used in the current study are listed in Table 1.

Culture media and conditions. Staphylococcal strains were cultured on tryptic soy agar (TSA) (Difco, Detroit, MI) or in tryptic soy broth (TSB) (Difco) at 37°C

TABLE 1. Bacterial strains, bacteriophage, and plasmids used in the current study

| Bacterial strain, bacteriophage, or plasmid | Relevant characteristic(s) ^a | Source or reference |
|---|---|---------------------|
| Strains | | |
| <i>E. coli</i> DH5 α | Chemically competent plasmid cloning host | Invitrogen |
| <i>S. epidermidis</i> 1457 | Biofilm positive strain, transducible with phage 71 | 39 |
| <i>S. epidermidis</i> 1457 NR | 1457 resistant to novobiocin (1 μ g/ml) and rifampin (10 μ g/ml) | This study |
| <i>S. carnosus</i> TM300 | <i>ica</i> -negative strain, negative control in the biofilm assay | 24 |
| <i>S. aureus</i> RN4220 | Restriction-negative, modification-positive isolate | 34 |
| 1457 <i>saeR</i> | <i>saeR</i> knockout mutant of strain 1457, Tmp ^r | This study |
| 1457 <i>saeR</i> /pNF155 | 1457 <i>saeR</i> complemented with <i>saeRS</i> | This study |
| Bacteriophage | | |
| Phage 71 | <i>S. epidermidis</i> transducing phage | 40 |
| Plasmids | | |
| pUC19 | High-copy-number plasmid with multiple cloning site, Amp ^r | Invitrogen |
| pGO558 | <i>dhfr</i> cloned into the <i>S</i> alI site of pUC19, Amp ^r Tmp ^r | G. Archer |
| pROJ6448 | pE194 with pC221 nick site for mobilization, temperature-sensitive replicon, Erm ^r | 46 |
| pNF38 | <i>sae</i> allelic replacement vector, Amp ^r Tmp ^r Erm ^r | This study |
| pNF41 | RNAIII allelic replacement vector, Amp ^r Tmp ^r Erm ^r | This study |
| pGO1 | Conjugative plasmid, Gen ^r | 2 |
| pC221 | Mobilizeable plasmid, Cam ^r | 46 |

^a Abbreviations: Amp, ampicillin; Tmp, trimethoprim; Gen, gentamicin; Cam, chloramphenicol; Erm, erythromycin.

except where indicated otherwise. Luria-Bertani Miller broth or agar (Difco) was used to propagate *Escherichia coli* strain DH5 α . Antibiotics and medium additives were purchased from Sigma (St. Louis, MO) and used at the following concentrations: ampicillin, 50 μ g/ml; erythromycin, 10 μ g/ml for staphylococci and 500 μ g/ml for *E. coli*; trimethoprim, 10 μ g/ml; and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal), 50 μ g/ml. Aerobic growth studies of strains 1457 and 1457 *saeR* were performed by diluting overnight cultures 1/200 (normalized for growth) into TSB, which was incubated at 37°C at 225 rpm with a flask-to-volume ratio of 10:1. Anaerobic growth studies were performed by diluting an overnight culture (grown in pre-reduced TSB) 1/50 in TSB containing 3 mg/liter resazurin (Acros Organics, Geel, Belgium), 1.0 g/liter L-cysteine hydrochloride monohydrate (MP Biomedicals, Solon, OH), and 3 mM potassium

nitrate (Sigma). Growth was monitored in an anaerobic chamber (model 1029; Thermo Scientific, Waltham, MA) at 37°C.

Molecular analyses and assembly of *saeR* allelic replacement vectors. PCR primers for amplification of *saePQRS* from *S. epidermidis* 1457 were designed using the genome sequence of *S. epidermidis* RP62A (GenBank accession number CP000029.) The cloning host for the *saeR* allelic replacement experiments was *E. coli* DH5 α (Invitrogen, Carlsbad, CA). First, a 965-bp piece of DNA encompassing *saeQ* and the 5' end of *saeR* was amplified using forward primer 373 (5'-GGAATTCGAAAGAGAGTGTAAATCATGAAG-3') and reverse primer 374 (5'-CGGGATCCGAAGCAAGATACCATAGCAATTC-3') (Fig. 1). Primers 373 and 374 contained EcoRI and BamHI restriction sites, respectively (underlined). This piece of DNA was subsequently ligated to the EcoRI

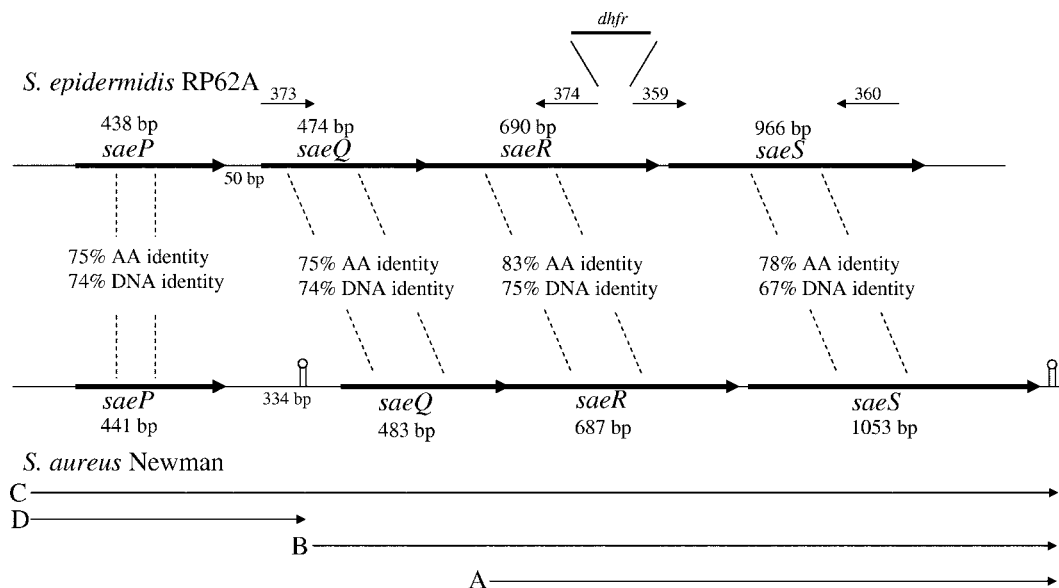


FIG. 1. Comparison of *sae* nucleotide and protein sequences from *S. aureus* Newman (GenBank accession number AJ556794) and *S. epidermidis* RP62A. The DNA identity percentages indicate the level of nucleotide sequence identity for each of the *sae* genes, as well as the *saePQ* intergenic region. Percentages of amino acid (AA) identity are also indicated. The positions of primers 373, 374, 359, and 360 (see text) are indicated by arrows. *sae* transcripts, as defined by Novick and Jiang (44) and Steinhuber et al. (52), are labeled A, B, C, and D.

TABLE 2. RT-PCR primers

| Gene | Forward primer | Reverse primer |
|-------------|------------------------------------|------------------------------|
| SE1977 | 1285 (TTTTTGATCGTGTAAACGGACAG) | 1286 (TGAAATGATTCTCAAATAGGC) |
| SE2170 | 1287 (AATTGCGGCATGAGATTAGG) | 1288 (AAACGCGCATTAACTGCTTC) |
| SE2171 | 1289 (CGCATCGTCTTGCTGTTTC) | 1290 (TGAAGGTGTTTCGATGCAGTG) |
| SE2172 | 1291 (TCACCAAGCAGTTCCACAAG) | 1292 (TGCAAACGTTTCAAGCAGTC) |
| SE0640 | 1293 (TGCTTCATATCCTCTTTAATTTTCATC) | 1294 (TTTGTGTTCTTGGAGCTTTG) |
| SE0227 | 1295 (AAGGCCCACTCATATGTTGC) | 1296 (CGCTTTTCGCTTACCCTTC) |
| SE0214 | 1297 (TTAGGTCCAGCACCAGAACC) | 1298 (CGATACCAGTTGCCATTGTG) |
| SE0215 | 1299 (TGAAATCTTGCCGTACAAACC) | 1300 (TCAGGCGGTTCAACATTTTC) |
| <i>gyrA</i> | 5 (GGGTAAATATCACCTCATGG) | 6 (GCAGTTGGGAAATCAGGACC) |

and BamHI sites of pUC19 (59), which was renamed pNF30. Next, a 949-bp piece of DNA encompassing the 3' end of *saeR* and the 5' end of *saeS* was amplified using forward primer 359 (5'-ACGCGTCGACCCCTACACTATTAC AACTGTG-3') and reverse primer 360 (5'-AACTGCAGCTAAACGGTTCTTT CAAAGATACG-3') (Fig. 1). Primers 359 and 360 contained SalI and PstI sites, respectively (underlined). This piece of DNA was subsequently ligated into the SalI and PstI sites of pNF30, which was then called pNF32. *dhfr*, encoding trimethoprim resistance, was excised from pGO558 using SalI and cloned into the SalI site of pNF32, yielding pNF34. Finally, pROJ6448 (46), a temperature-sensitive derivative of pE194 (28), was ligated into the PstI site of pNF34, yielding pNF38.

pNF38 was subsequently electroporated into RN4220, a restriction-deficient, modification-proficient strain of *S. aureus* (34), using previously described protocols (51). Modified pNF38 DNA was isolated from RN4220 using a Wizard plus MidiPrep kit (Promega, Madison, WI) and subsequently electroporated into *S. epidermidis* 1457 (39) using the electroporation protocol developed by Augustin and Götz (4). To detect chromosomal integration of pNF38 into *saeR*, strain 1457 containing pNF38 was grown to mid-exponential phase in 10 ml TSB (Difco, Sparks, MD) containing 10 µg/ml erythromycin (Sigma, St. Louis, MO) at 30°C. The culture was then diluted 1:100 in fresh TSB (100 ml) and was incubated at 43°C (nonpermissive temperature) overnight with shaking at 100 rpm. This step was repeated the following day. The culture was then plated (with incubation at 43°C) onto TSA containing trimethoprim (10 µg/ml; Sigma) and replica plated onto TSA containing 10 µg/ml erythromycin to count colonies that were trimethoprim resistant and erythromycin susceptible (i.e., colonies with double recombination in *saeR* and subsequent loss of erythromycin resistance). Following confirmation of gene knockouts by PCR and Southern blot hybridization, knockout alleles were backcrossed into wild-type *S. epidermidis* 1457 using transducing phage 71, as described previously (40). The allelic replacement of *saeR* with *dhfr* resulted in the loss of 134 bp.

Mouse foreign body infection model. The mouse foreign body infection model was used to evaluate the virulence of *S. epidermidis* 1457 and 1457 *saeR* (50). Both strains were inoculated into 100 ml TSB and were incubated for 16 h at 37°C with shaking at 250 rpm. Bacteria were harvested by centrifugation and suspended in sterile saline (0.9% NaCl) to obtain concentrations of 1×10^7 , 1×10^8 , and 1×10^9 CFU/ml. Following ketamine/xylazine administration, the skin on an animal's back and flank was shaved with surgical clippers and disinfected with povidone-iodine. One-centimeter catheter segments (Jelco 16G FEP catheter; Johnson and Johnson, New Brunswick, NJ) were implanted into the subcutaneous space in 25- to 30-g male Swiss-Webster mice. One hundred microliters of saline (0.9%) containing either 10^6 , 10^7 , or 10^8 CFU of 1457 or 1457 *saeR* was inoculated into the flank of each mouse containing an implanted catheter. The mice were housed for 7 days with unrestricted access to water and rodent diet. Animals were euthanized by carbon dioxide inhalation, and the catheters were explanted.

To obtain *S. epidermidis* cell counts, the catheter was first removed from a mouse flank and placed into 1 ml of saline. Next, a standardized amount of pericatheter tissue, excluding the overlying cutaneous tissue, was removed from the animal and placed into 1 ml of saline. Both samples were mixed with a vortex mixer to dislodge adherent cells, and the cells were enumerated after dilution and plating on TSA. Tissue sample results were standardized by determining the number of cells per gram of tissue. A statistical analysis (Mann-Whitney test) was performed with GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, CA).

Tissues used for histological examination were harvested at 2 and 7 days postinfection and were stored in 10% formalin. Embedding, cutting, and eosin and hematoxylin staining of these tissues were performed by the Eppley Histol-

ogy Core Laboratory at the University of Nebraska Medical Center. For enumeration of the mononuclear cell populations at the site of infection, tissue from the catheterized site was collected at 2 and 7 days postinfection and was placed in a 15-ml conical centrifuge tube containing 10 ml RPMI 1640 supplemented with 5% heat-inactivated fetal bovine serum (Mediatech, Herndon, VA), 400 IU/ml collagenase IV, and 30 µg/ml DNase I (Sigma). Tissue was placed on a GyroMini nutating mixer (Labnet, Edison, NJ) and incubated at 37°C for 2 h. To obtain a suspension of single cells, tissue was passed through a stainless steel mesh, and the entire cell fraction was centrifuged at $300 \times g$ for 5 min at 4°C. Cells were washed in phosphate-buffered saline, and the pellet was resuspended in Hanks balanced salt solution (Mediatech, Herndon, VA). The mononuclear cell population was enriched by using a density gradient (Lympholyte-M; Cedarlane Labs, Burlington, Canada) as directed by the manufacturer. Briefly, the cell pellet was suspended in 5 ml Hanks balanced salt solution, underlaid with 5 ml Lympholyte-M, and centrifuged at $1,000 \times g$ for 20 min at room temperature. Dead cells and red blood cells were pelleted, and mononuclear cells were collected at the interface. Cytochrome preparations of the isolated mononuclear cell populations were obtained with a Cytopro 7620 (Wescor, Logan, UT). Differential cell counts were obtained by staining slides with a Diff-Quick kit (ThermoFisher Scientific, Waltham, MA) as directed by the manufacturer. A minimum of 200 cells per slide were counted.

RNA isolation and Northern analysis. Cultures of *S. epidermidis* 1457 were grown overnight in TSB, diluted 1:200 into fresh TSB (flask-to-volume ratio, 5:1; shaking at 200 rpm [microaerobic conditions]), and grown at 37°C to optical densities at 600 nm (OD_{600}) of 0.25 (early exponential growth), 3.0 (mid-exponential growth), and 8.2 (late exponential growth). RNA was then isolated as described by Luong et al. (37). The following primer sets were used to amplify regions of the *sae* locus that were used as DNA probes for the Northern blots: for *saeP* (379 bp), forward primer AGCGCCACCAAAAATTACAT and reverse primer TTACATTAGGCGCATGTGGA; and for *saeS* (285 bp), forward primer CGATTGGAGGTCGATACTGG and reverse primer TCTGAAGGTTTACG GGATGG. DNA probes were labeled using digoxigenin-labeled dUTP (Roche, Indianapolis, IN).

Transcriptional profiling. Overnight cultures of *S. epidermidis* 1457 and 1457 *saeR* were diluted 1:100 into fresh TSB and grown at 37°C to an OD_{600} of 2.7 (flask-to-volume ratio, 5:1; shaking at 200 rpm [microaerobic conditions]). RNA was converted to cDNA, and microarray analysis was performed according to the manufacturer's instructions (Affymetrix expression analysis technical manual; Affymetrix, Inc., Santa Clara, CA) for antisense prokaryotic arrays essentially as described previously by Beenken and colleagues (7). To ensure reproducibility, two cDNA samples from each strain were prepared from two separate experiments. Each cDNA sample was hybridized to an *S. epidermidis* GeneChip. Signal intensity values for each qualifier (predicted open reading frame [ORF] and intergenic region) were normalized to the median signal intensity value for each GeneChip. Sample values were then averaged. Genes for which there was at least a twofold difference ($P \leq 0.05$, *t* test) in RNA titer between 1457 and 1457 *saeR* were considered differentially expressed in a *saeR*-dependent manner.

Reverse transcriptase PCR (RT-PCR) was used to confirm the microarray data for genes involved in anaerobic metabolism (Table 2). RNA was isolated as described above, and 10 ng was used in each reaction mixture. A One Tube RT-PCR kit (Roche Diagnostics) was used according to the manufacturer's recommendations. Oligonucleotides used for detection of specific genes are listed in Table 2. All reactions were allowed to proceed for 22 cycles with an annealing temperature of 50°C. *gyrA* was used as an internal standard as described previously by Conlon et al. (16). Amplified products were visualized on a 1.5% agarose (Sigma, St. Louis, MO) gel. Five of the seven genes (SE214, SE1977, SE2170, SE2171, and SE2172) were further confirmed by Northern blot

analysis. Primer sets described in Table 2 were used to amplify regions of each gene that were used as DNA probes.

Biofilm and PIA assay. Assays for the production of biofilm were performed using the method of Christensen et al. (15). Cells for PIA immunoblot assays were cultured in TSB overnight with shaking. The next day, cells were diluted 1:100 into TSB and grown to an OD_{600} of 1.0. Cell extracts from 3×10^8 CFU were prepared, spotted on 0.45- μ m-pore-size polyvinylidene difluoride (Immobilon-P; Millipore, Bedford, MA) membranes, and developed as previously described (17). The anti-PIA antibody used in these assays was diluted 1:250,000 (the antibody was a kind gift from D. Mack).

Metabolic analyses. Both 1457 and 1457 *saeR* were grown anaerobically in TSB as described above. Nitrate and lactic acid in the culture medium (TSB; after centrifugation and collection of the supernatant) were quantified using an R-Biopharm metabolic assay kit (R-Biopharm, Marshall, MI). Nitrite in the culture medium was quantified using the Griess reagent (Invitrogen, Carlsbad, CA).

RESULTS

***sae* is present in *S. epidermidis*.** BLAST searches (1) performed with the sequences of *saeR* and *saeS* from *S. aureus* strain Newman (GenBank accession number AJ556795 [52]) indicated that genes encoding SaeR and SaeS homologs were present in the genomes of *S. epidermidis* strains RP62A and ATCC 12228 (GenBank accession numbers CP000029 and AE015929, respectively.) In addition, two ORFs (annotated as SERP0367 and SERP0366 in the RP62A genome sequence and as SE_0481 and SE_0480 in the ATCC 12228 genome sequence) were found upstream of *saeR*. Comparison of the *S. epidermidis* genomic sequence data with the *S. aureus* strain Newman genome indicated that the farthest upstream of these ORFs shared 74% nucleotide sequence identity with *S. aureus saeP* (designated ORF4) (Fig. 1). Similarly, *saeQ* from Newman (designated ORF3) shared 75% nucleotide identity with the second ORF in *S. epidermidis* 1457. The *saeR* and *saeS* genes were also similar in the two species, sharing 75 and 67% nucleotide identity, respectively. As a result, we designated the farthest upstream gene in *S. epidermidis* 1457 *saeP* and the second gene *saeQ* (per Novick and Jiang [44]). Primers designed using the *S. epidermidis* RP62A genome sequence amplified products of the appropriate size (from *saeP*, *saeQ*, *saeR*, and *saeS*) from *S. epidermidis* 1457 (data not shown) (39). Therefore, *S. epidermidis* 1457 was used in further studies as it is amenable to genetic manipulation (25).

Transcriptional analysis of the *saePQRS* region. Previous Northern blot analysis of *S. aureus saePQRS* demonstrated that this region was comprised of four transcriptional units, transcripts A, B, C, and D (44, 52) (Fig. 1). Transcript A is produced early in the exponential phase of growth, whereas transcripts B, C, and D are transcribed late in the exponential growth phase. The transition from producing transcript A to producing transcripts B, C, and D is under the control of *agr* (44). Northern blot analysis of *S. epidermidis* 1457 demonstrated that the transcriptional regulation of the *saePQRS* region was similar to that in *S. aureus*. Using *saeS* as a DNA probe, a single transcript that was approximately 1.7 kb long (corresponding to transcript A) was detected during early exponential growth, whereas two additional transcripts that were approximately 2.1 and 3.0 kb long (corresponding to transcripts B and C) were detected later in the exponential growth phase (Fig. 2). In contrast to the results for *S. aureus*, using *saeP* as a probe, a smaller 0.5-kb transcript corresponding to

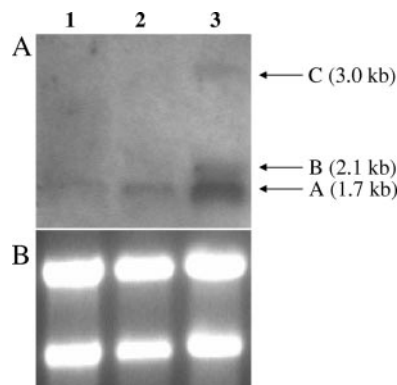


FIG. 2. Northern blot analysis of the *sae* operon hybridized with a *saeS* DNA probe. (A) RNA isolated from 1457 at the following times: lane 1, early exponential growth (OD_{600} , 0.25); lane 2, 1457 mid-exponential growth (OD_{600} , 3.0); lane 3, late exponential growth (OD_{600} , 8.2). The arrows indicate the positions of transcripts A, B, and C as discussed in text. (B) RNA gel hybridized with *saeS* in panel A. Note the equal loading of RNA based on 16S-23S rRNA ethidium bromide staining intensity.

transcript D was never detected at any phase of growth (Fig. 1) (data not shown).

Construction and characterization of a *saeR* mutant in *S. epidermidis*. Because *sae* has been shown to be a regulator of virulence in *S. aureus*, we speculated that *sae* controls virulence factor expression in *S. epidermidis*. To test this possibility, we constructed a *sae* mutant strain of *S. epidermidis* 1457 by insertion of the *dhfr* gene into the predicted effector domain of *saeR*. Following plasmid integration and vector excision by secondary recombination, disruption of *saeR* was confirmed by PCR and Southern blot hybridization (data not shown). To ensure that observations made with this mutant strain were due to *sae* interruption and not due to secondary mutations in other genes, the disrupted *saeR* allele was backcrossed into wild-type strain 1457 with phage 71. Similar to the results for *S. aureus*, disruption of *saeR* did not result in a significant aerobic growth defect (Fig. 3).

In contrast to *S. aureus*, biofilm formation is the primary virulence mechanism in *S. epidermidis*; hence, we assessed the effect of *saeR* inactivation on biofilm formation. Average A_{650} values from six biofilm assays indicated that the *saeR* mutant produced slightly more biofilm than its wild-type counterpart (A_{650} , 2.19 versus 1.90). As expected, two negative control strains, strains 1457 *ica* (25) and *ica*-negative *Staphylococcus carnosus* strain TM300 (24), failed to produce biofilm (A_{650} , 0.33 and 0.28, respectively.) PIA immunoblots indicated that the levels of PIA production were equivalent in 1457 and 1457 *saeR* (data not shown).

Virulence of an *S. epidermidis saeR* mutant in a mouse foreign body infection model. Animal models of infection have demonstrated that *sae* is required for virulence of *S. aureus* (5, 8, 20, 22, 23, 57); therefore, we hypothesized that loss of SaeR in *S. epidermidis* would result in a decrease in virulence. To examine this hypothesis, wild-type strain 1457 and its isogenic *saeR* mutant were compared using the mouse foreign body infection model. For these experiments, infectious doses of 10^6 , 10^7 , and 10^8 bacteria were inoculated into catheter segments implanted in the subcutaneous space of mice. The num-

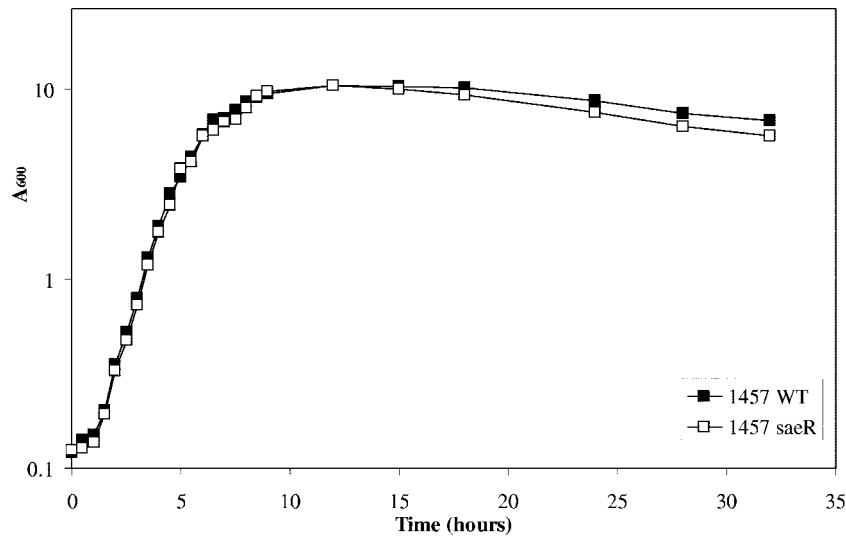


FIG. 3. Comparison of growth of 1457 (WT) and 1457 *saeR*.

ber of catheters analyzed for each inoculum was as follows: for 10^6 CFU of 1457 and 1457 *saeR*, 16 catheters each; for 10^7 CFU of 1457 and 1457 *saeR*, 15 catheters each; and for 10^8 CFU of 1457 and 1457 *saeR*, 16 catheters each. Catheters were explanted 7 days postinfection, and the numbers of *S. epidermidis* cells associated with the catheters were determined following dilution and plating. The numbers of bacteria isolated from the catheters inoculated with strain 1457 and the numbers of bacteria isolated from the catheters inoculated with strain 1457 *saeR* were statistically equivalent for all infectious doses (Fig. 4A) (for 10^6 CFU, $P = 0.24$; for 10^7 CFU, $P = 1.00$; for 10^8 CFU, $P = 0.21$). In addition, tissues directly surrounding the catheters were explanted, and the numbers of bacteria associated with these tissues were also determined. Similar to the bacterial loads on the catheters, the numbers of bacteria per gram of tissue were equivalent for strains 1457 and 1457 *saeR* for each inoculum (Fig. 4B) (for 10^6 CFU, $P = 0.63$; for 10^7 CFU, $P = 0.06$; for 10^8 CFU, $P = 0.40$). Taken together, these data demonstrate that *S. epidermidis* can establish an infection independent of SaeR.

Despite the *in vivo* growth similarities of the wild-type and *saeR* mutant strains, we chose to perform a more thorough histological examination of catheter beds to determine if *saeR* inactivation affected the host response. Indeed, differences in the number of polymorphonuclear neutrophils (PMNs) present at the site of infection were observed for wild-type and *saeR* mutant cells (Fig. 5A and 5B). Eosin and hematoxylin staining of the tissues revealed that darkly staining immune cells were more prevalent adjacent to catheters infected with wild-type strain 1457. Specifically, 2 days after infection, the proportion of cells with morphological characteristics consistent with those of PMNs was higher in the 1457-infected mice ($49.3\% \pm 3\%$) than in the 1457 *saeR*-infected mice ($26.7\% \pm 3\%$) ($P < 0.05$). The numbers of PMNs were similar for the two groups of mice after 7 days of infection ($P \geq 0.05$) (data not shown). These data demonstrate that SaeR directly or indirectly influences the early, acute phase(s) of an infection.

Transcriptional profiling. To ascertain possible causes of the alteration in the innate immune response to *S. epidermidis* with *saeR* inactivated, we performed a transcriptional profile analysis of the wild-type and *saeR* mutant strains. We determined that 65 genes were affected by SaeR (37 genes were upregulated and 28 genes were downregulated) (Table 3). In contrast to the findings for *S. aureus*, in which the large majority of the genes regulated by SaeR code for extracellular toxins and cellular adherence factors (35, 47), genes affected by the loss of SaeR in *S. epidermidis* had multiple functions, including pyruvate metabolism (e.g., *pflA*, *pflB*, *ackA*, and *butA*), amino acid synthesis and metabolism (e.g., *hisG*, *hisD*, *hisH*, *argG*, *argI*, and *argC*), and redox regulation (putative REX regulator, *trxA*, and *trxB*). In a comparison to microarray analyses of three *S. aureus* strains (COL, Newman, and WCUH29), only four genes were identified in *S. epidermidis* that were also regulated by *saeR* in *S. aureus* (35, 47). These four genes were *geh* (lipase), *esp* (*S. epidermidis*)/*sspA* (*S. aureus*) (V8 protease), *lrgA* (holin protein), and the gene encoding a LysM/CHAP domain protein. Interestingly, *geh* was negatively regulated by *saeR* in *S. epidermidis*, but it is positively regulated in *S. aureus*. Of particular interest were a number of genes that were positively regulated by SaeR that may facilitate growth under anaerobic conditions. These genes included the genes encoding the putative NADH:flavin oxidoreductase/fumarate reductase (SE0195), the putative C-4 dicarboxylate anaerobic carrier (SE0227), complex I NADH:ubiquinone oxidoreductase (*snoG*; SE0640), assimilatory nitrite reductase (*nasE*; SE1977), the putative anaerobic C-4 dicarboxylate transporter (SE2170), the putative anaerobic ribonucleoside reductase activator (SE2171), and the class III anaerobic reductase (*nrdD*; SE2172). Due to the close proximity of the SE2170, SE2171, and SE2172 genes, these genes may be members of an operon. Taken together, these data led us to speculate that SaeRS may be involved in sensing and responding to anaerobiosis. RT-PCR was used to confirm that expression of eight genes (SE1977, SE2170, SE2171, SE2172, SE0640, SE0227, SE0214,

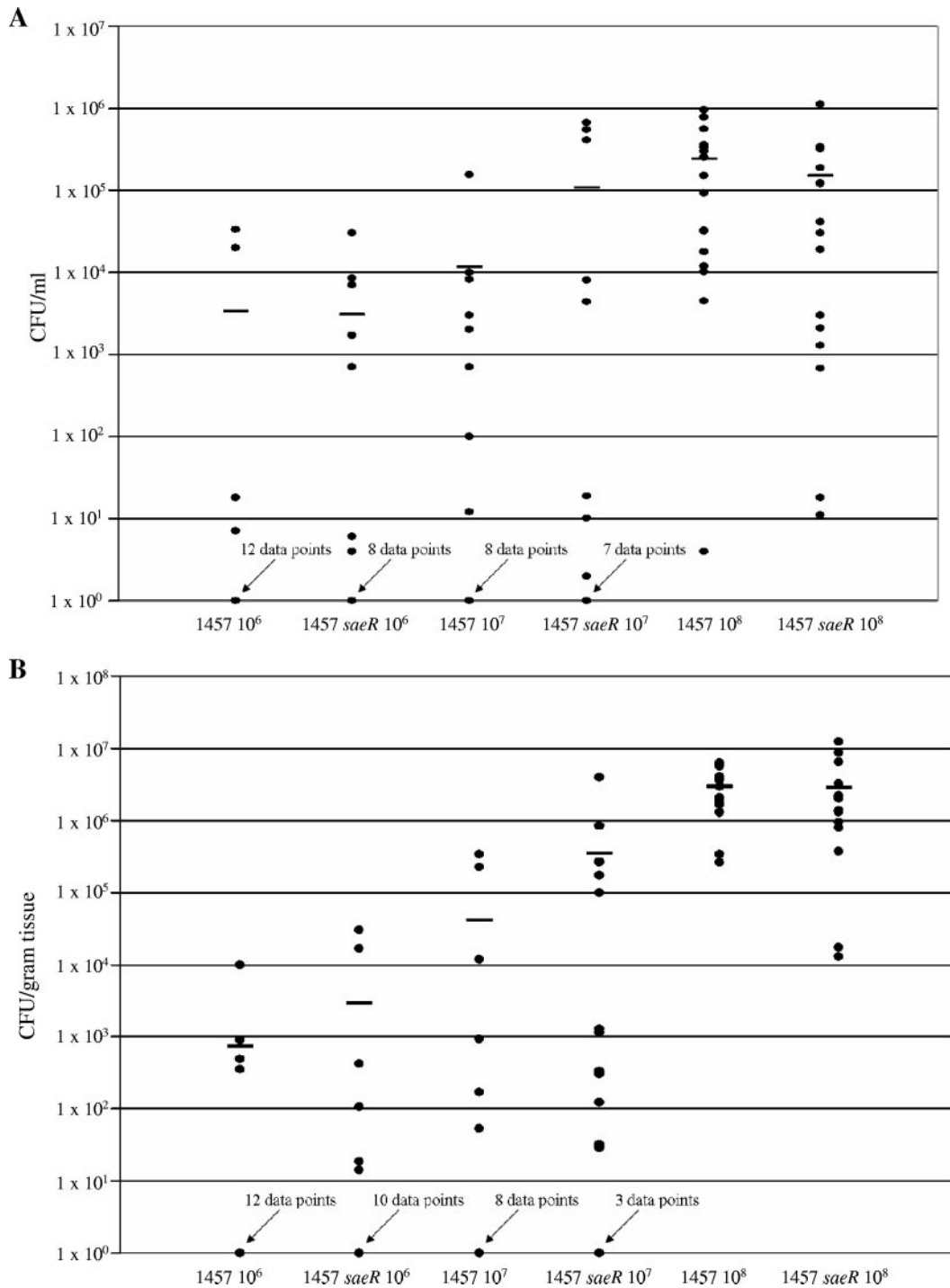


FIG. 4. Animal studies with 1457 and 1457 *saeR*. (A) Numbers of cells obtained from catheters inoculated with 1457 and 1457 *saeR*. The mean values are indicated by bars. The numbers of data points where no bacteria were detected in the samples are indicated above the arrows. (B) Numbers of cells obtained from tissues surrounding catheters inoculated with 1457 and 1457 *saeR*. The mean values are indicated by bars. The numbers of data points where no bacteria were detected in the samples are indicated above the arrows.

and SE0215 [Tables 2 and 3]) possibly involved in anaerobic growth was indeed downregulated in 1457 *saeR* compared to 1457 (Fig. 6). As shown in Fig. 6, we were unable to consistently demonstrate that expression of two of the genes identified by microarray analysis (SE0640 or *snoG* [Fig. 6A, lanes 11

and 12] and SE0227 [Fig. 6A, lanes 13 and 14]) was indeed downregulated in 1457 *saeR*. As the proteins encoded by both of these genes presumably function under anaerobic conditions, the inconsistency in expression levels may be related to variable oxygen tensions in repeat microaerobic cultures. The

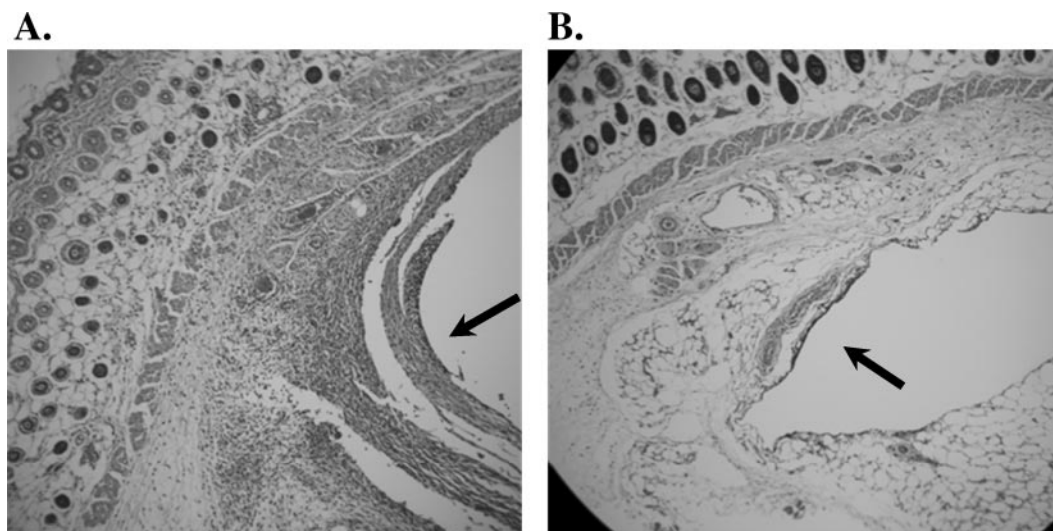


FIG. 5. Hematoxylin- and eosin-stained tissues surrounding catheters infected with 1457 (A) or 1457 *saeR* (B). Note the darkly staining granulocytes in catheter beds (indicated by an arrow) in 1457 (A) compared to 1457 *saeR* (B).

expression of all other genes tested was indeed downregulated in 1457 *saeR* compared to 1457 as assessed by RT-PCR (Fig. 6A) or Northern analysis (Fig. 6B).

Growth of 1457 and 1457 *saeR* under anoxic growth conditions. To assess if SaeR alters anaerobic physiology, strains 1457 and 1457 *saeR* were grown at 37°C in an anaerobic chamber, and the growth was monitored. As expected, the results demonstrated that 1457 *saeR* had a lower growth rate than 1457, although the growth yields were similar (Fig. 7A).

Staphylococci can use nitrate as a terminal electron acceptor under anoxic conditions (11, 12, 41, 45). Since *snoG* is postulated to be an important oxidoreductase that is linked to nitrate reductase activity in electron transport (6), we hypothesized that the growth defect detected in 1457 *saeR* is due to a decreased ability to utilize nitrate as a terminal electron acceptor. To test this possibility, strains 1457 and 1457 *saeR* were grown anaerobically in TSB supplemented with 3 mM potassium nitrate. The results demonstrated that 1457 depleted the nitrate in the culture medium; however, strain 1457 *saeR* had a significantly reduced rate of nitrate utilization (Fig. 7B). Strain 1457 exhausted the nitrate in the culture medium by 30 h after the beginning of growth; however, strain 1457 *saeR* never depleted the nitrate. Furthermore, after 39 h of growth, when the OD₆₀₀ of 1457 and 1457 *saeR* were similar, there was still approximately 2.3 mM nitrate left in the culture supernatant of 1457 *saeR*, whereas no nitrate was detected in the culture supernatant of 1457. Anaerobiosis reduces nitrate to nitrite; therefore, our data demonstrating that nitrate utilization was significantly decreased in strain 1457 *saeR* compared to 1457 led us to speculate that the accumulation of nitrite in the culture medium would be greater in 1457 cultures than in 1457 *saeR* cultures (41). As predicted, nitrite began accumulating coincident with a decrease in the nitrate concentration in the 1457 culture medium. In contrast, the concentration of nitrite in the culture medium of 1457 *saeR* was at the detection limit of the assay (Fig. 7B). Consistent with previous observations, nitrite was extracted from the culture medium only after the concentration of nitrate became limited (41) (Fig. 7B).

The impaired anaerobic growth of strain 1457 *saeR* results in decreased utilization of nitrate as a terminal electron acceptor. This situation can affect redox homeostasis by limiting the ability of the bacterium to oxidize NADH to NAD⁺. We hypothesized that to compensate for the lack of electron transport-mediated oxidation of NADH, more pyruvate would be shunted through lactate dehydrogenase and result in increased lactic acid accumulation in the culture medium of strain 1457 *saeR*. Increasing lactate dehydrogenase (EC 1.1.1.27) activity would help mitigate a redox imbalance by coupling the dismutation of pyruvate to lactic acid with the oxidation of NADH to NAD⁺. As predicted, the pH minimum of the 1457 *saeR* growth medium was significantly lower than the pH minimum of the 1457 culture medium (Fig. 7C), suggesting that more organic acids were produced by 1457 *saeR* than by 1457. Consistent with the pH data, we found a higher concentration of lactic acid in the culture medium of strain 1457 *saeR* than in the culture medium of the isogenic wild-type strain (Fig. 7C). Collectively, these data suggest that the decreased pH observed in the 1457 *saeR* culture medium was due to increased production of lactic acid.

Complementation of 1457 *saeR* with pNF155. 1457 *saeR*/pNF155, which contained full-length *saeRS* on a multicopy plasmid, partially complemented the growth defect observed in 1457 *saeR* grown under anoxic conditions (Fig. 7A). Consistent with these data, pNF155 did not completely restore 1457 *saeR* to the 1457 phenotype with regard to nitrate utilization and lactic acid production. For instance, after 39 h of growth, 1.0 mM nitrate and 11.2 mM lactic acid (mean concentrations) remained in the culture supernatant of 1457 *saeR*/pNF155 ($n = 3$; data not shown).

DISCUSSION

The *sae* two-component regulatory system in *S. aureus* has emerged as an important regulator of virulence. The research described here is the first step in characterization of the *sae* operon in *S. epidermidis* and the first attempt to identify the

TABLE 3. Microarray analysis of wild-type strain *S. epidermidis* 1457 compared to strain 1457 *saeR*

| Gene | Fold change ^a | Locus ^b |
|--|--------------------------|--------------------|
| Genes upregulated in wild-type strain 1457 compared to strain 1457 <i>saeR</i> | | |
| Thiamine biosynthesis lipoprotein (<i>apbE</i>) | 4.7 ↑ | SE0194 |
| Putative NADH:flavin oxidoreductase/fumarate reductase | 5.2 ↑ | SE0195 |
| Putative transmembrane efflux pump protein | 3.7 ↑ | SE0196 |
| Formate acetyltransferase (<i>pflB</i>) | 7.1 ↑ | SE0214 |
| Formate acetyltransferase-activating enzyme (<i>pflA</i>) | 5.4 ↑ | SE0215 |
| Putative C-4 dicarboxylate anaerobic carrier | 2.2 ↑ | SE0227 |
| Putative LysM/CHAP domain protein | 2.2 ↑ | SE0433 |
| Staphylococcal cell division protein (<i>scdA</i>) | 2.3 ↑ | SE0439 |
| <i>Staphylococcus aureus</i> exoprotein (<i>saeR</i>) | 8.2 ↑ | SE0479 |
| <i>Staphylococcus aureus</i> exoprotein (<i>saeP</i>) | 7.0 ↑ | SE0480 |
| <i>Staphylococcus aureus</i> exoprotein (<i>saeQ</i>) | 47.3 ↑ | SE0481 |
| K ⁺ -H ⁺ /Zn-Co-Cd antiporter (<i>czcD</i>) | 2.1 ↑ | SE0577 |
| Complex I NADH:ubiquinone oxidoreductase (<i>snoG</i>) | 2.4 ↑ | SE0640 |
| Peptide binding protein (<i>oppA</i>) | 2.1 ↑ | SE0684 |
| Putative pyruvate ferredoxin oxidoreductase, alpha subunit | 2.4 ↑ | SE0967 |
| Glycyl tRNA synthetase | 4.0 ↑ | SE1252 |
| Acetate kinase (<i>ackA</i>) | 2.0 ↑ | SE1387 |
| Putative REX redox regulator | 2.7 ↑ | SE1647 |
| Putative alanine racemase | 2.2 ↑ | SE1769 |
| Putative SIR2-NAD-dependent deacetylase | 2.4 ↑ | SE1789 |
| Putative D-octopine dehydrogenase | 2.1 ↑ | SE1874 |
| Phosphotransferase system arbutin-like II BC component (<i>ghvC</i>) | 2.4 ↑ | SE1897 |
| Assimilatory nitrite reductase (<i>nasE</i>) | 5.3 ↑ | SE1977 |
| Anti-holin (<i>lrgA</i>) | 2.4 ↑ | SE2013 |
| Anti-holin (<i>lrgB</i>) | 3.3 ↑ | SE2014 |
| Glucose-1-dehydrogenase | 2.6 ↑ | SE2032 |
| Putative Gcn5-related N-acetyltransferase | 2.2 ↑ | SE2069 |
| Putative alcohol dehydrogenase | 5.3 ↑ | SE2098 |
| Putative anaerobic C-4 dicarboxylate transporter | 2.2 ↑ | SE2170 |
| Putative anaerobic ribonucleoside reductase activator | 3.1 ↑ | SE2171 |
| Class III anaerobic reductase (<i>nrdD</i>) | 3.5 ↑ | SE2172 |
| Acetoin reductase (<i>butA</i>) | 8.8 ↑ | SE2225 |
| Putative autolysin/LysM/CHAP domain protein | 2.3 ↑ | SE2319 |
| 5-Methyltetrahydropteroyltriglutamate-homosysteine 5-methyltransferase (<i>metE</i>) | 2.2 ↑ | SE2382 |
| Genes which are downregulated in wild-type strain 1457 compared to strain 1457 <i>saeR</i> | | |
| ATP phosphoribosyltransferase (<i>hisG</i>) | 3.1 ↓ | SE0271 |
| Histidinol dehydrogenase (<i>hisD</i>) | 3.3 ↓ | SE0272 |
| Amidotransferase (<i>hisH</i>) | 2.4 ↓ | SE0274 |
| Lipase (<i>gehC</i>) | 3.1 ↓ | SE0281 |
| Argininosuccinate synthase (<i>argG</i>) | 2.7 ↓ | SE0657 |
| Putative ABC transporter | 2.6 ↓ | SE0998 |
| RecA regulon repressor (<i>lexA</i>) | 2.6 ↓ | SE1022 |
| Thymidylate synthase (<i>thyA</i>) | 3.5 ↓ | SE1120 |
| Thioredoxin reductase (<i>trxB</i>) | 2.1 ↓ | SE1168 |
| Arginine acetyltransferase (<i>argJ</i>) | 2.3 ↓ | SE1211 |
| N-Acetylglutamate gamma-semialdehyde dehydrogenase (<i>argC</i>) | 2.8 ↓ | SE1212 |
| Riboflavin synthase alpha chain (<i>ribB</i>) | 2.4 ↓ | SE1440 |
| Riboflavin-specific deiminase (<i>ribD</i>) | 2.1 ↓ | SE1441 |
| Putative amino acid (possible glutamine) ABC transporter ATP binding protein | 3.2 ↓ | SE1540 |
| Putative amino acid (possible glutamine) binding periplasmic protein | 2.5 ↓ | SE1541 |
| V8 protease (<i>esp</i>) | 2.3 ↓ | SE1543 |
| Putative thioredoxin peroxidase-bacterioferritin | 2.0 ↓ | SE1547 |
| Putative cationic (Mg ²⁺ , Co ²⁺) transporter, CorA family | 2.0 ↓ | SE1958 |
| Thioredoxin (<i>trxA</i>) | 2.2 ↓ | SE2097 |
| Other genes | | |
| Hypothetical protein | 3.6 ↓ | SE0280 |
| Hypothetical protein | 2.2 ↓ | SE0366 |
| Conserved hypothetical protein | 2.1 ↓ | SE0392 |
| Hypothetical protein | 2.4 ↓ | SE0570 |
| Hypothetical protein (possible Na ⁺ /K ⁺ antiporter) | 2.0 ↓ | SE0637 |
| Hypothetical protein | 2.5 ↓ | SE1024 |
| Hypothetical protein | 2.1 ↓ | SE1174 |
| Hypothetical protein | 2.0 ↓ | SE1448 |
| Putative integrase | 2.2 ↓ | SE1472 |
| Hypothetical protein | 2.6 ↑ | SE1903 |
| Hypothetical protein | 2.8 ↑ | SE2173 |
| Hypothetical protein | 5.4 ↑ | SE2331 |

^a An arrow pointing up indicates a gene which is upregulated in wild-type strain 1457 compared to strain 1457 *saeR*. An arrow pointing down indicates a gene which is downregulated in wild-type strain 1457 compared to strain 1457 *saeR*.

^b Locus in the *S. epidermidis* ATCC 12228 genome sequence (GenBank accession number NC 004461).

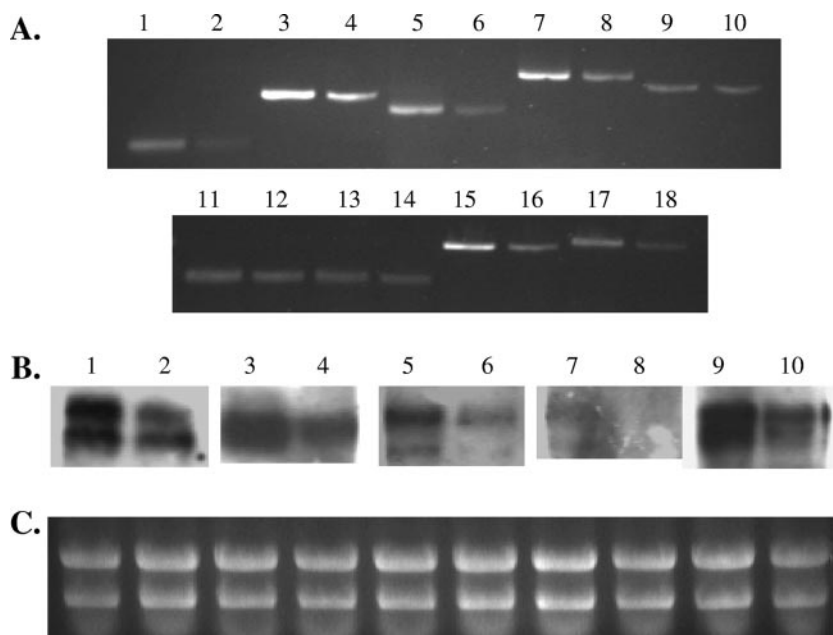


FIG. 6. (A) RT-PCR of genes involved in anaerobic growth for confirmation of transcriptional profiling. Lanes 1 and 2, 1457 (lane 1) and 1457 *saeR* (lane 2) amplified with primers 1285 and 1286 (SE1977); lanes 3 and 4, 1457 (lane 3) and 1457 *saeR* (lane 4) amplified with primers 1287 and 1288 (SE2170); lanes 5 and 6, 1457 (lane 5) and 1457 *saeR* (lane 6) amplified with primers 1289 and 1290 (SE2171); lanes 7 and 8, 1457 (lane 7) and 1457 *saeR* (lane 8) amplified with primers 1291 and 1292 (SE2172); lanes 9 and 10, 1457 (lane 9) and 1457 *saeR* (lane 10) amplified with primers 5 and 6 (*gyrA*); lanes 11 and 12, 1457 (lane 11) and 1457 *saeR* (lane 12) amplified with primers 1293 and 1294 (SE0640); lanes 13 and 14, 1457 (lane 13) and 1457 *saeR* (lane 14) amplified with primers 1295 and 1296 (SE0227); lanes 15 and 16, 1457 (lane 15) and 1457 *saeR* (lane 16) amplified with primers 1297 and 1298 (SE0214); lanes 17 and 18, 1457 (lane 17) and 1457 *saeR* (lane 18) amplified with primers 1299 and 1300 (SE0215). (B) Northern blot analysis of genes involved in anaerobic growth. Lanes 1 and 2, 1457 (lane 1) and 1457 *saeR* (lane 2) hybridized with an SE2170 DNA probe; lanes 3 and 4, 1457 (lane 3) and 1457 *saeR* (lane 4) hybridized with an SE2171 DNA probe; lanes 5 and 6, 1457 (lane 5) and 1457 *saeR* (lane 6) hybridized with an SE1977 DNA probe; lanes 7 and 8, 1457 (lane 7) and 1457 *saeR* (lane 8) hybridized with an SE2172 DNA probe; lanes 9 and 10, 1457 (lane 9) and 1457 *saeR* (lane 10) hybridized with an SE0214 DNA probe. (C) RNA gel hybridized with various probes described above for panel B. Note the equal loading of RNA based on 16S-23S rRNA ethidium bromide staining intensity.

environmental conditions that modulate the regulatory response of *sae*. The *S. epidermidis* *sae* operon was found to be organized in a manner similar to the *S. aureus* operon. Each gene in strain RP62A was found to share at least 67% nucleotide identity to a gene from *S. aureus* strain Newman, and each protein was found to share at least 75% amino acid identity. In addition, Northern analysis demonstrated that the transcriptional regulation of *sae* in *S. epidermidis* strain 1457 was similar to that in *S. aureus* (44, 47, 52) (Fig. 1). Relative to *S. aureus*, *S. epidermidis* has very few defined virulence factors. Of the known virulence factors and mechanisms in *S. epidermidis*, the most extensively studied are the production of PIA/biofilm and the poly- γ -DL-glutamic acid capsule (32, 49, 50). Our data demonstrate that transcription of the genes encoding these virulence factors occurs independent of SaeR in *S. epidermidis*. This observation provided an excellent opportunity to examine the physiological function of *sae* in *S. epidermidis*. Nevertheless, since there is a clear association between virulence and the *sae* operon in *S. aureus*, we utilized a mouse foreign body infection model, a highly pertinent model of *S. epidermidis*-mediated infection, to determine the level of virulence of the *saeR* mutant compared to that of the wild type. Furthermore, this model has been used to demonstrate differences in the virulence of strains that produce similar amounts of PIA/biofilm (32). Interestingly, even though strains 1457 and 1457 *saeR* were equally able to establish an infection in the

mouse foreign body infection model, the host responses to these two strains differed dramatically (Fig. 5A and 5B). At 2 days postinoculation, the PMN response was significantly greater in the tissue of mice infected with 1457 than in the tissue of mice infected with 1457 *saeR*. Our first inclination was to determine whether SaeR regulates the production of phenol-soluble modulins, which are known immune mediators (54). However, no difference in the production of phenol-soluble modulins was detected between the *saeR* mutant and the wild type (data not shown). Further studies are needed to determine which immune modulators are regulated by SaeR during the acute phase of infection.

Transcriptional profiling revealed that SaeR affects metabolic processes, including redox activity, pyruvate metabolism, and amino acid synthesis and metabolism; of particular interest were several genes predicted to be involved in anaerobic metabolism. Two of the genes (SE0227 and SE2170) encode putative anaerobic C-4 dicarboxylate transporters, which facilitate transport of alternative electron acceptors (e.g., fumarate or succinate) (29). In addition, a putative NADH:flavin oxidoreductase/fumarate reductase (SE0195) was also positively regulated by SaeR. The class III anaerobic reductase (*nrdD*; SE2172) is an anaerobic ribonucleotide reductase that catalyzes the formation of DNA from RNA building blocks. *NrdD* is inactivated by molecular oxygen and appears to be essential for anaerobic growth (30, 42). Lastly, *snoG*, encoding part of

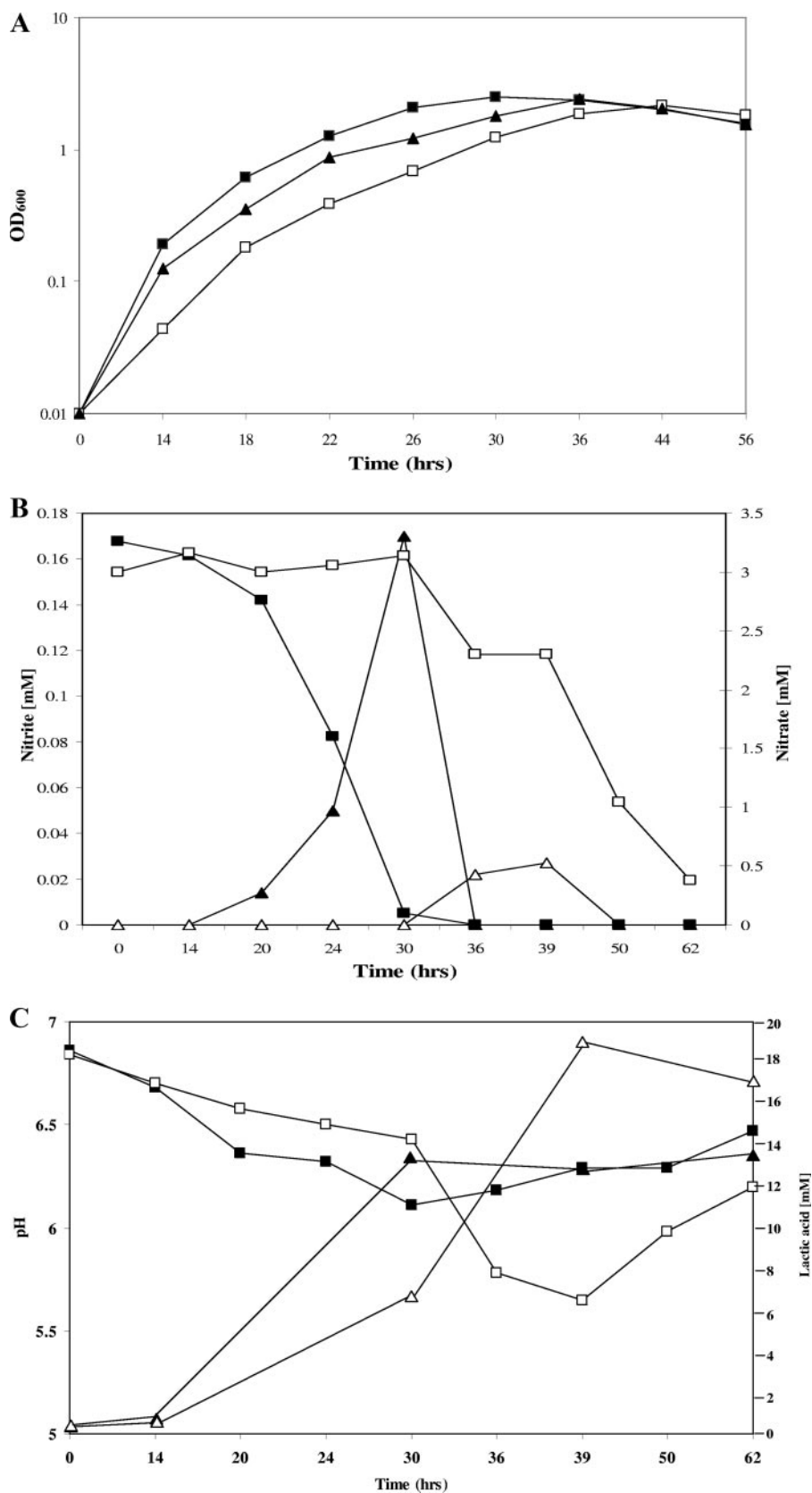


FIG. 7. (A) Effect of anaerobiosis on growth of 1457 (■), 1457 *saeR* (□), and 1457 *saeR*/pNF115 (▲). The results are representative of three separate experiments. (B) Production of nitrite by 1457 (▲) and 1457 *saeR* (△) superimposed on curves showing the utilization of nitrate by 1457 (■) and 1457 *saeR* (□). (C) Production of lactic acid by 1457 (▲) and 1457 *saeR* (△) superimposed on curves showing the pH values of the 1457 growth medium (■) and the 1457 *saeR* growth medium (□). The data in panels B and C are representative of five separate experiments.

the complex I NADH:ubiquinone oxidoreductase, is important in anaerobic respiration linked to nitrate reduction. Taken together, these data suggest that one function of SaeR is to regulate the transition from aerobic growth to anaerobic growth. The fact that *saeR* inactivation did not alter aerobic growth but did result in an anaerobic growth impairment strongly supports this suggestion (Fig. 7A).

Glycolysis generates two molecules of pyruvate for every molecule of glucose consumed; however, in the process it reduces two molecules of NAD⁺ to NADH. Reduction of NAD⁺ to NADH without an equivalent means to oxidize NADH can create a redox imbalance and inhibit growth. Staphylococci primarily reduce pyruvate to lactic acid during anaerobic growth, a process that regenerates NAD⁺ (9, 33). Under anaerobic or microaerobic growth conditions, NAD⁺ can be regenerated from NADH by both the membrane-bound nitrate reductase and the NADH:ubiquinone oxidoreductase (SnoA-G) (6). However, *saeR* inactivation decreased transcription of *snoG*, slowing the utilization of nitrate and likely resulting in the decreased growth rate. To compensate for the redox imbalance, strain 1457 *saeR* shunts pyruvate into lactate dehydrogenase, a reaction allowing for the stoichiometric oxidation of NADH produced during glycolysis (Fig. 7C). In agreement with these data, microarray analysis demonstrated that transcription of both *trxA* and *trxB* is downregulated in the *saeR* mutant, whereas REX, a redox-responsive regulator, is upregulated in the *saeR* mutant (Table 3). The result of this compensation by 1457 *saeR* is a much more acidic extracellular milieu; the consequence of this metabolic switch, especially within a biofilm, is currently being studied in our laboratories.

It is not known why pNF115 is unable to completely restore the wild-type phenotype to 1457 *saeR*. However, the explanation may be related to the fact that the entire two-component regulator (*saeRS*) is present on a multicopy plasmid. Unfortunately, unlike the situation in *S. aureus*, in *S. epidermidis* there is no genetic system to complement mutants in single copy.

In contrast to *S. aureus*, our data suggest that in *S. epidermidis* the *sae* two-component regulatory system functions to control basic metabolic processes but few of the limited number of virulence genes (i.e., lipase and V8 protease genes). Based on the genomic similarities between *S. aureus* and *S. epidermidis*, we speculate that *saeRS* may function to indirectly regulate virulence gene expression by altering the metabolic status of the bacteria, particularly under conditions in which oxygen availability is low, such as the conditions in a deep abscess or biofilm. This hypothesis is actively being tested in our laboratories.

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