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Sequence Analysis of the *Staphylococcus aureus srrAB* Loci Reveals that Truncation of *srrA* Affects Growth and Virulence Factor Expression▽

Alexa A. Pragman, Lisa Herron-Olson, Laura C. Case, Sara M. Vetter, Evan E. Henke, Vivek Kapur, and Patrick M. Schlievert*

Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455

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The SrrAB system regulates metabolism and virulence factors in *Staphylococcus aureus*. We sequenced the *srrAB* loci of 21 isolates and performed a phylogenetic analysis. Vaginal and bovine isolates clustered together, while skin isolates were genetically diverse. Few nucleotide polymorphisms were observed, and most were synonymous. Two strains (N2 and N19) with N-terminal truncations in SrrA displayed defects in growth and abnormally upregulated virulence factor expression under low-oxygen conditions.

Staphylococcus aureus is a gram-positive coccus that colonizes the skin and mucous membranes of humans and animals. Coordinated expression of virulence factors may result in serious infections, such as toxic shock syndrome (TSS), sepsis (10, 16, 20), and bovine mastitis (12). Differences in virulence factor regulation contribute to the variable pathogenic potential of the organism in humans or other animals. *S. aureus* encodes many global regulators of virulence, including a quorum-sensing system, the Sar family of virulence regulators, two-component systems, and transcriptional regulators (5–7, 13–15, 18, 22). The staphylococcal respiratory response (SrrAB) two-component system regulates energy metabolism as well as the genes *tst* (toxic shock syndrome toxin 1 [TSST-1]), *spa* (staphylococcal protein A), and *icaR* (intercellular adhesion locus repressor) in response to oxygen (17, 19, 25, 25a, 26). Although the effects of SrrAB have been investigated for four different *S. aureus* strains to date and the *srrAB* loci have been found in all sequenced isolates of *S. aureus*, the conservation of DNA and protein sequences from isolates of diverse origin is unknown. In order to ascertain the level of conservation of *srrAB* among human skin, human vaginal, and bovine udder isolates, PCR amplification and sequencing methods were used. The DNA and amino acid sequences were analyzed for phylogenetic relatedness. The strains and primers used in this study are described in Tables 1 and 2, respectively. For sequencing, *S. aureus* was grown in Todd-Hewitt medium (Difco Laboratories, Sparks, MD) in laboratory aerobic atmosphere with shaking. Genomic DNA was isolated by digestion with lysostaphin (Sigma-Aldrich Corp., St. Louis, MO), followed by purification using the DNeasy tissue kit (QIAGEN Corp., Valencia, CA). *srrAB* was PCR amplified using a high-fidelity enzyme (ABgene, Rochester, NY). For each strain, 30 *srrAB* sequence reads were performed using the primers shown in Table 2, and the sequences were assembled with DNASTAR SeqMan (LaserGene, Madison, WI). Assemblies were analyzed for weak or disparate residues and manually corrected, trimmed, and aligned by nucleotide and amino acid homology using ClustalW. Alignments were analyzed by parsimony analysis using PAUP with hierarchical clustering and a bootstrap value of 1000 (Sinauer Associates, Sunderland, MA) (23).

Strain growth as well as hemolysin activity and TSST-1 expression in *srrA* mutants and two wild-type strains (MN8 and CDC587) was determined as follows: *S. aureus* was grown in beef heart medium at 37°C under aerobic conditions (with shaking at 200 rpm), low-oxygen conditions (<0.3% oxygen, without shaking), or anaerobically in BBL GasPak jars (Becton Dickinson and Company, Franklin Lakes, NJ) without shaking. Cell densities after 24 h were determined by plate counts, hemolysin activity was determined by bioassay (lysis of rabbit erythrocytes incorporated into 0.8% agarose) (21), and TSST-1 expression was determined by quantitative Western immunoblotting (3). Strain N19 was complemented with pJMY11, a multicopy plasmid with wild-type *srrAB* (26). The resultant strain was also assayed for growth and hemolysin production.

The results of the *srrAB* nucleotide sequence comparison appear in the phylogenetic tree shown in Fig. 1. Nucleotide sequences were compared due to the large proportion of synonymous mutations compared to nonsynonymous mutations among the sequences. Two clusters are immediately apparent. The bovine mastitis isolates (PSA6, PSA10, PSA20) cluster together, as do the vaginal isolates (1956, 2000, T35). The skin isolates appear scattered throughout the phylogram. MRSA252, an isolate from the United Kingdom, clusters near the U.S. vaginal isolates. This is not surprising, as the genomic backbone of MRSA252 is conserved relative to a recently sequenced TSS-associated isolate from the United States (Lisa Herron-Olson, personal communication). The oldest isolate, from a mild skin infection in 1926, clusters closely with several of the more recent vaginal and skin isolates.

Alignments of SrrAB amino acids show that the protein sequences are well conserved among diverse isolates. Most of the DNA polymorphisms resulted in synonymous mutations; of the six nonconserved residues in SrrAB, only two (SrrB A/T322 and A/D502) resulted in a change to a dissimilar amino acid. Of note, strains N2 and N19 have N-terminal truncations in their *srrA* sequences. The first 22 amino acids of the N2 SrrA sequence are absent, with the majority of the phosphate receiver domain still present. In strain N19, the first 78 amino

* Corresponding author. Mailing address: Department of Microbiology, University of Minnesota Medical School, 420 Delaware St. SE, MMC 196, Minneapolis, MN 55455. Phone: (612) 624-9471. Fax: (612) 626-0623. E-mail: schli001@umn.edu.

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TABLE 1. *S. aureus* strains sequenced

Strain name(s)	Source or reference	Yr isolated	Type of infection[a]
1926 (26665)	Dennis W. Watson Culture Collection	1926	Mild skin infection
1956	William Altemeier (1)	1956	Typical vaginal isolate (nonmenstrual) from a healthy person
2000	Patrick M. Schlievert	2004	Toxic shock syndrome (menstrual)
USA400	Patrick M. Schlievert (10)	2000	Purpura fulminans caused by *S. aureus*
K15A	Patrick M. Schlievert	1981	Axillary culture from a healthy child
T35	Patrick M. Schlievert (21)	1981	Typical menstrual isolate from a healthy person
N2, N6, N7, N9, N12, N19	N Study	2003	Persistent skin infections in patients with atopic dermatitis
PSA 6, PSA 10, PSA 20	Vivek Kapur	1992	Bovine mastitis strains from the United States
COL	8	Early 1960s	Early MRSA from a wound infection in the United Kingdom
MW2	2	1998	Community-acquired MRSA, septicemia, and septic arthritis in North Dakota patient
N315	11	1982	MRSA pharyngeal isolate from a Japanese patient
MRSA252	9	1997	Hospital-acquired MRSA from a case of postoperative septicemia in the United Kingdom
MSSA276	9	1998	Invasive community-acquired MSSA from a case of osteomyelitis and bacteremia in the United Kingdom
Mu50	11	1997	MRSA, surgical wound infection in a Japanese patient

[a] MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

acids are absent, including an aspartate residue at position 56 that is predicted to be the site of phosphorylation in SrrA (4, 24). Due to this truncation, N19 SrrA may be incapable of acting as a phosphoacceptor or may exhibit unregulated DNA-binding activity. Although both N2 and N19 have ribosomal binding sites upstream of their SrrA translational start sites, it is not known if SrrA is translated in these strains. We do not predict that SrrB translation is altered in N2 and N19, as these strains exhibit no sequence changes near the SrrB translational start site.

TABLE 2. Primers used in *srrAB* sequencing

Primer	Sequence
srrAB sense	5′-ATGTATTTATCACAAAGTTTGA-3′
srrAB antisense	5′-ATTTAATAGTTGATATTCGCAA-3′
Forward 1	5′-GTGAAGAAACAAACCGTGTTGAAGG-3′
Forward 2	5′-GTTAAATCGTGTGTCTAGTGAAGCTG-3′
Forward 3	5′-TGACGATGTGTTTGATAAAGGTAAATCTG-3′
Forward 4	5′-TTGGTCAATTATCGCAGGCATTTA-3′
Forward 5	5′-TGTTGTTGTGACAGTTCGTGATATG-3′
Forward 6	5′-GAAGCGTGTTTGGAGTTATGATATGGA-3′
Forward 7	5′-TTCAGAACGCATGTTATTGATTAGAGA-3′
Forward 8	5′-GAAAAGCATGTGTGGGAGGTATGA-3′
Forward 9	5′-AAGAAGAACGCAATCTACAACTG-3′
Forward 10	5′-TGATGAGCCGGCTAAATAGTGTCG-3′
Forward 11	5′-GGCTATCCAACAAAAGCACAGA-3′
Forward 12	5′-GCATGTCGACGCATTATCCA-3′
Forward 13	5′-CGTACACCGATATCATTACTTCAAGG-3′
Forward 14	5′-ATACGAAACCTGGAGATGAAAT-3′
Forward 15	5′-TTTTTGATTGATGTGGGGAAT-3′
Reverse 1	5′-GGTGCAATGCCTGTACCTGTATCTT-3′
Reverse 2	5′-CAGCTTCACTAGACACACGATTTAAC-3′
Reverse 3	5′-CAGATTTACCTTTATCAAACACATCGTCA-3′
Reverse 4	5′-TAAATGCCTGCGATAATTGACCAA-3′
Reverse 5	5′-CATATCACGAACTGTCACAACAACA-3′
Reverse 6	5′-TCCATATCATAACTCCAAACACGCTTC-3′
Reverse 7	5′-TCTCTAATCAATAACATGCGTTCTGAA-3′
Reverse 8	5′-TCATACCTCCCACACATGCTTTTC-3′
Reverse 9	5′-CAGTTGTAGATTGCGTTCTTCTT-3′
Reverse 10	5′-CGACACTATTTAGCCGGCTCATCA-3′
Reverse 11	5′-TCTGTGCTTTTGTTGGATAGCC-3′
Reverse 12	5′-TGGATAATGCGTCGACATGC-3′
Reverse 13	5′-CCTTGAAGTAATGATATCGGTGTACG-3′
Reverse 14	5′-ATTTCATCTCCAGGTTTCGTAT-3′
Reverse 15	5′-ATTCCCCACATCAATCAAAAA-3′

The effect of *srrA* mutation on growth and virulence factor production was assessed for strains N2 and N19. Strains N2 and N19 have growth defects under low-oxygen and anaerobic conditions, in comparison to MN8 and CDC587, two strains

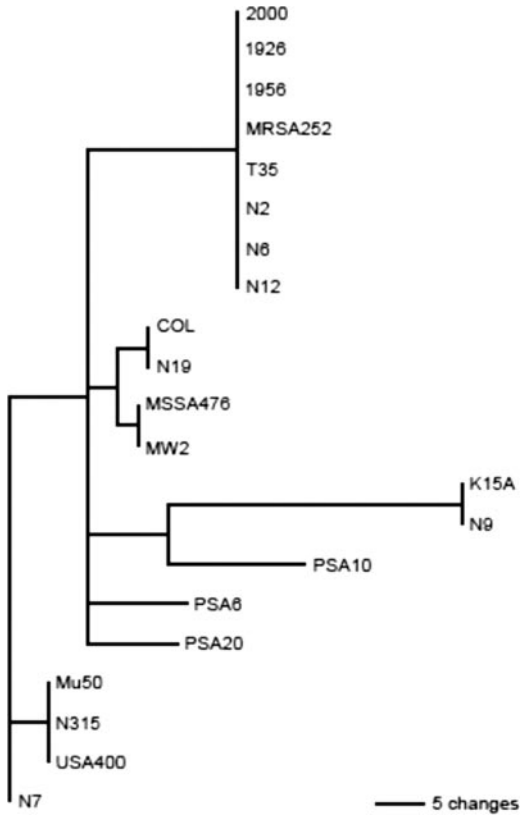

FIG. 1. *srrAB* phylogenetic tree. The nucleotide coding sequences of the 21 *srrAB* loci were used to create a phylogenetic tree. Distinct clusters include the vaginal isolates (1956, 2000, and T35) and the bovine mastitis isolates (PSA 6, 10, 20). Human skin isolates are scattered throughout the phylogram.

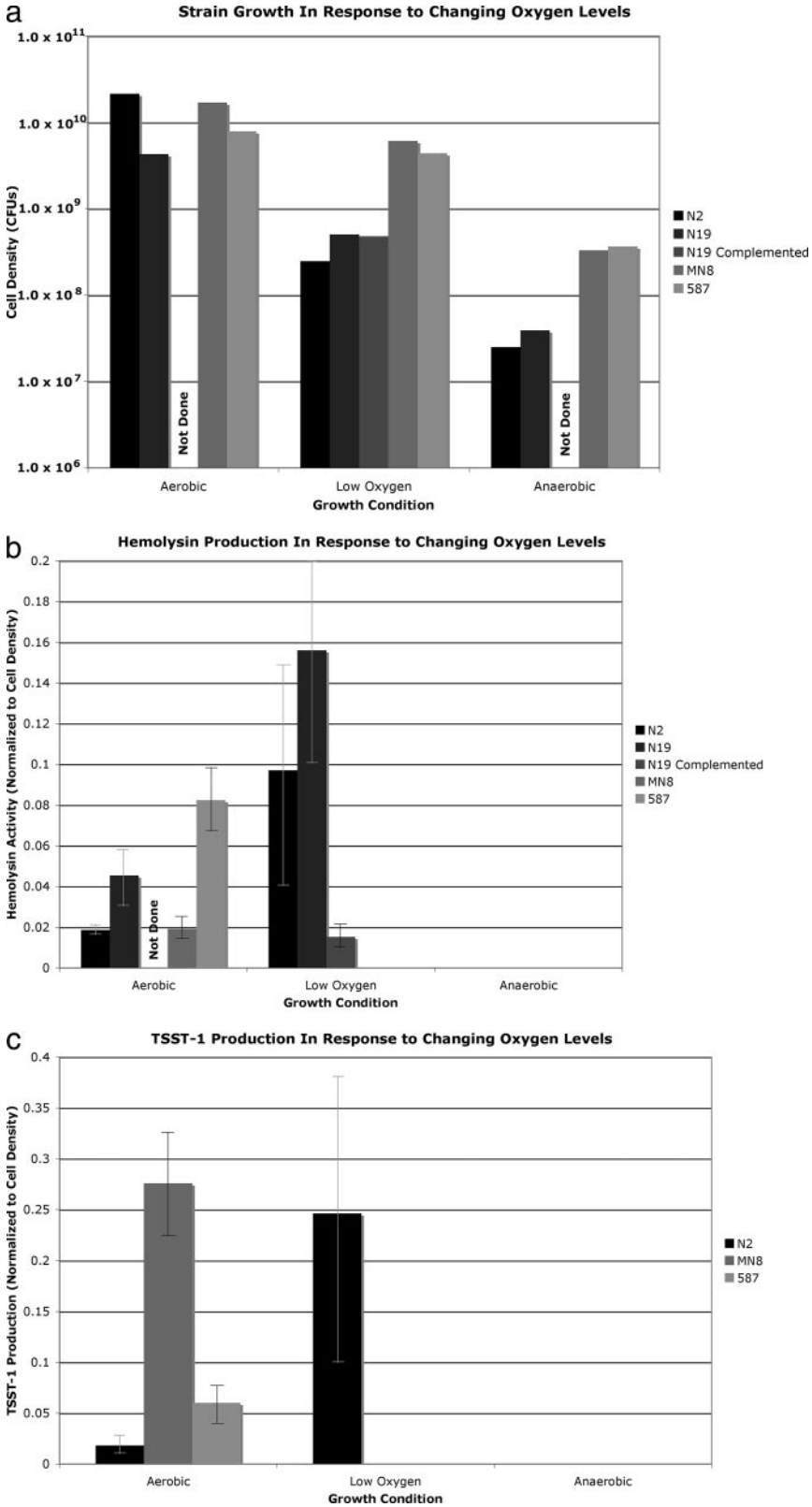

FIG. 2. N-terminal truncations of *srrA* affect growth and virulence factor production. All data points represent an average of results of four separate experiments. (a) Strain growth in response to change in oxygen levels. Strains N2 and N19 demonstrate growth defects under low-oxygen and anaerobic conditions, in comparison to control strains MN8 and CDC587. Complementation of N19 with wild-type *srrAB* did not affect growth. (b) Hemolysin activity. Strains N2 and N19 demonstrate increased hemolysin levels under low-oxygen conditions, while control strains demonstrate no production. Complementation of N19 with wild-type *srrAB* repressed hemolysin production. No strains produced hemolysin during anaerobic growth. Activity is reported in micrograms of hemolysin/1.0×10^8 CFU. (c) TSST-1 activity. Strain N2 demonstrates increased production of TSST-1 under low-oxygen conditions, while neither control strain is able to make TSST-1 under low-oxygen conditions. Strain N19 lacks the gene for TSST-1 and is therefore unable to make TSST-1. TSST-1 was not made by any strain under anaerobic conditions. Activity is reported in micrograms of TSST-1/1.0×10^8 CFU.

with intact SrrAB that are capable of expressing hemolysin and TSST-1 (Fig. 2a). N2 and N19 display normal hemolysin activity under aerobic conditions and increased activity under low-oxygen conditions; wild-type strains MN8 and 587 did not express hemolysin under low-oxygen conditions. Complementation of N19 with a multicopy plasmid containing wild-type *srrAB* resulted in a dramatic repression of hemolysin production. This suggests that while the wild-type SrrAB system represses hemolysin under low-oxygen conditions, the truncated SrrAB system in strains N2 and N19 is unable to repress hemolysin production under low-oxygen conditions. None of the strains produced hemolysin under anaerobic conditions (Fig. 2b). N2 demonstrates TSST-1 production under aerobic conditions with enhanced TSST-1 production under low oxygen. Strains MN8 and 587 demonstrated no TSST-1 production under low-oxygen conditions. These findings suggest that the N2 SrrAB system is incapable of repressing TSST-1 production under low-oxygen conditions. N19 lacks the gene for TSST-1 and is therefore unable to express it. No strain produced TSST-1 under anaerobic conditions (Fig. 2c). The SrrAB system has been shown to repress virulence factors such as hemolysin and TSST-1 under low-oxygen conditions. N2 and N19 display an increase in hemolysin and TSST-1 production under low-oxygen conditions that is consistent with a loss of repression due to a nonfunctional SrrA. Complementation of N19 with wild-type *srrAB* resulted in restoration of hemolysin repression under low-oxygen conditions. Both N2 and N19 were isolated from patients with chronic skin infections in the setting of atopic dermatitis. This superficial and chronic skin infection environment may favor strains that are deficient in sensing oxygen.

In summary, our alignments of *srrAB* sequences from disparate isolates demonstrate relatively few changes in the sequences at the nucleotide level and no focal points of increased polymorphism. The phylogenetic tree demonstrates clustering of bovine mastitis isolates and clustering of human vaginal isolates, while human skin isolates do not cluster. The separate niches inhabited by the vaginal and bovine strains may account for the divergence. In this study, truncations in *srrA* affected growth and virulence factor regulation. N2 demonstrated decreased growth and increased hemolysin and TSST-1 activity with oxygen limitation, while N19 showed decreased growth and increased hemolysin activity with oxygen limitation. Complementation of N19 with wild-type *srrAB* resulted in restoration of hemolysin repression under low-oxygen conditions. These findings are consistent with a loss of SrrAB-mediated virulence factor repression under low-oxygen conditions in strains N2 and N19.

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