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The Superoxide Dismutase Gene *sodM* Is Unique to *Staphylococcus aureus*: Absence of *sodM* in Coagulase-Negative Staphylococci

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Superoxide dismutase (SOD) profiles of clinical isolates of *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) were determined by using whole-cell lysates and activity gels. All *S. aureus* clinical isolates exhibited three closely migrating bands of activity as previously determined for laboratory strains of *S. aureus*: SodM, SodA, and a hybrid composed of SodM and SodA (M. W. Valderas and M. E. Hart, J. Bacteriol. 183:3399–3407, 2001). In contrast, the CoNS produced only one SOD activity, which migrated similarly to SodA of *S. aureus*. Southern analysis of eight CoNS species identified only a single *sod* gene in each case. A full-length *sod* gene was cloned from *Staphylococcus epidermidis* and determined to be more similar to *sodA* than to *sodM* of *S. aureus*. Therefore, this gene was designated *sodA*. The deduced amino acid sequence of the *S. epidermidis sodA* was 92 and 76% identical to that of the SodA and SodM proteins of *S. aureus*, respectively. The *S. epidermidis sodA* gene expressed from a plasmid complemented a *sodA* mutation in *S. aureus*, and the protein formed a hybrid with SodM of *S. aureus*. Both hybrid SOD forms as well as the SodM and SodA proteins of *S. aureus* and the *S. epidermidis* SodA protein exist as dimers. These data indicate that *sodM* is found only in *S. aureus* and not in the CoNS, suggesting an important divergence in the evolution of this genus and a unique role for SodM in *S. aureus*.

The staphylococci are a diverse group of species that are routinely categorized in the clinical setting as either coagulase positive or coagulase negative (3). In most cases, coagulase-positive staphylococci isolated from humans are *Staphylococcus aureus*, while the coagulase-negative staphylococci (CoNS) may include any of the remaining 32 species that constitute the genus *Staphylococcus* (34). A notable exception to this axiom is *Staphylococcus intermedius*, which is coagulase positive (33). However, only about 15 of the coagulase-negative species are indigenous to humans, with *Staphylococcus epidermidis* being the species most frequently isolated from bloodstream infections (27, 34).

S. aureus has always been considered a human pathogen with a wide array of disease syndromes, ranging from minor skin abscesses to life-threatening endocarditis, osteomyelitis, and pneumonia (51). Contributing to this array of diseases is the capacity of this organism to produce numerous proteins with cytotoxic and immunogenic properties as well as surface-associated factors that promote adherence and evade host defenses (28, 45). In contrast, the CoNS have been regarded as apathogenic commensals residing on human skin and frequent contaminants of clinical samples (27). However, the increasing use of invasive medical devices in recent years has made the CoNS the pathogens most commonly isolated from bloodstream infections in intensive care units (11). While the determination of virulence factors has not been pursued as vigorously for CoNS as for *S. aureus*, it is evident that capsular polysaccharides are a major factor contributing to attachment to foreign bodies (27). Therefore, a more complete understanding of the mechanisms of disease caused by staphylococci is drastically needed.

The staphylococci reside primarily on the skin and mucous membranes of warm-blooded animals (33). In humans, approximately 30% of healthy individuals and up to 90% of health care workers are carriers of staphylococci (51). Once the bacteria enter the human body through a break in the skin or mucous membranes, they are confronted by the professional phagocytes (50). These host immune cells utilize reactive oxygen intermediates (ROIs), such as superoxide, hydrogen peroxide, and hydroxyl ions, to aid in the killing of phagocytosed bacteria (17, 32, 44). In addition, bacteria must also prevent damage to nucleic acids, proteins, and cell membranes from ROIs that arise from incomplete reduction of oxygen during aerobic respiration (reviewed in references 22 and 47). Most microorganisms that utilize aerobic respiration produce a number of enzymes that counteract the deleterious effects of ROIs (19, 29). For example, superoxide dismutase (SOD) converts superoxide to hydrogen peroxide and oxygen and catalase converts hydrogen peroxide to water and oxygen (22, 47).

SOD has been shown to be important in several bacteria for defense against killing by professional phagocytes of vertebrate hosts. Extracellular SODs, such as those from *Mycobacterium tuberculosis* and *Nocardia asteroides*, as well as the periplasmically located Cu/Zn SOD from *Escherichia coli* protect these microorganisms from phagocytic killing (2, 5, 6, 7). Inactivation of the cytoplasmically located SODs of *Shigella flexneri* and *E. coli* K-12 results in increased sensitivity to killing by serum and neutrophils (21, 38). In addition, *sod* mutations in *Streptococ-*

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TABLE 1. Bacterial strains used in this study

Strain	Relevant characteristics	Source[a]
S. aureus		
RN6390	Prototypic strain	M. S. Smeltzer, University of Arkansas for Medical Sciences
ATCC 25923		ATCC
HAC1	Blood	OMCT
HAC2	Skin abscess	OMCT
HAC3	Peritoneal fluid	OMCT
HAC4	Respiratory secretions	OMCT
HAC6	Surgical wound	OMCT
UAB1	Sputum	UAB
UAB2	Sputum	UAB
UAB4	Sputum	UAB
UAB5	Sputum	UAB
CoNS		
S. epidermidis ATCC 12228		ATCC
S. epidermidis HAC33	Blood	OMCT
S. epidermidis HAC36	Surgical wound	OMCT
S. epidermidis HAC94	Unknown	OMCT
S. epidermidis HAC111	Triple lumen catheter	OMCT
S. epidermidis HAC112	Wound isolate	OMCT
S. epidermidis HAC113	Blood	OMCT
S. epidermidis HAC114	Triple lumen catheter	OMCT
S. epidermidis HAC115	Blood	OMCT
S. auricularis HAC140	Unknown	OMCT
S. auricularis HAC145	Deep tissue wound	OMCT
S. capitis HAC138	Tissue wound	OMCT
S. carnosus KSI2019	Food isolate	J. J. Iandolo, University of Oklahoma Health Science Center
S. haemolyticus HAC146	Arterial line catheter	OMCT
S. hominis HAC79	Blood	OMCT
S. hominis HAC139	Tissue wound	OMCT
S. hominis HAC142	Urine	OMCT
S. hominis HAC144	Blood	OMCT
S. lugdunensis HAC51	Abscess	OMCT
S. lugdunensis HAC141	Deep tissue wound	OMCT
S. simulans HAC44	Deep tissue wound	OMCT
S. simulans HAC143	Blood	OMCT

[a] ATCC, American Type Culture Collection, Manassas, Va.; OMCT, Osteopathic Medical Center of Texas; UAB, University of Alabama at Birmingham.

cus pneumoniae, *Campylobacter coli*, *Yersinia enterocolitica*, and *Haemophilus influenzae* result in attenuation of virulence, reduced colonization of the chicken stomach, decreased survival in the spleens and livers of mice, and the inability to colonize the rat nasopharynx, respectively (15, 41, 42, 53).

Apart from earlier conflicting reports (31, 37) regarding the importance of staphylococcal SOD in disease, the role of SOD in *S. aureus* has only recently been addressed. This organism contains two genes, *sodM* and *sodA*, that account for three SOD activities (13, 40, 48). The *sodM* and *sodA* gene products are important for the viability of *S. aureus* when grown under oxidative stress conditions (13, 48). In addition, the ability to survive amino acid starvation during aerobic growth is reduced in a *S. aureus sodA* mutant (13, 52). However, the *sodA* mutation did not affect the organism's ability to recover from starvation (13, 52). While a *sodA* mutation in *S. aureus* was demonstrated to have no effect on virulence in a mouse abscess model (13), the effect of *sodM* and *sodM sodA* mutations on virulence has not been determined.

Because *S. aureus* produces three SODs, a characteristic unique among the gram-positive bacteria, the purpose of the present study was to determine if SOD activities are different among species of *Staphylococcus*. Results from this study indicate that unlike *S. aureus*, the CoNS produced only one SOD activity, which is most closely related to SodA of *S. aureus* with respect to migration on activity gels, Southern analysis, and amino acid similarities. Because *S. aureus* is considered a primary pathogen and the CoNS are typically recognized as opportunistic pathogens, the presence of a second SOD (namely, SodM) in *S. aureus* may be related to this organism's ability to cause disease.

MATERIALS AND METHODS

Staphylococcal strains and growth conditions. Staphylococcal strains used in this study are listed in Table 1. Strains were routinely grown overnight (15 to 18 h) in tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C with rotary aeration (180 rpm) or on tryptic soy agar plates (tryptic soy broth containing 1.5% agar). Clinical isolates were provided by Larry Kemp of the Osteopathic Medical Center of Texas, Fort Worth, Tex., and by Ken Waites, Division of Laboratory Medicine, University of Alabama at Birmingham. Gram-positive cocci possessing catalase activity were categorized as coagulase positive or negative by inoculating 0.5 ml of reconstituted rabbit plasma (Difco Laboratories) with a single isolated colony and incubating at 37°C for 3 h. Tubes were observed for the presence of a fibrin clot. Species identification and characterization were carried out by using positive combination type 6 panels (Dade International, Inc., West Sacramento, Calif.) read after a 16- to 24-h incubation at 35°C with a

TABLE 2. Subunit composition of staphylococcal SODs

SOD	Predicted mol wt[a]	Mol wt of active enzyme[b]	No. of subunits/active enzyme[c]
S. aureus SodM	23,015	45,512	1.98
S. aureus SodA	22,686	38,572	1.70
S. epidermidis SodA	22,679	38,572	1.70
S. aureus SodM/SodA		41,953	1.82/1.85
S. aureus SodM/*S. epidermidis* SodA		41,953	1.82/1.85

[a] The predicted molecular weight of each SOD was calculated from the deduced amino acid composition of the respective gene.
[b] The molecular weight of each active enzyme was estimated by Ferguson plot (20) as described in Materials and Methods.
[c] Calculated by dividing the molecular weight of the active enzyme by the predicted molecular weight. Values for the hybrid forms were calculated using the predicted molecular weights of SodM and SodA, respectively.

Microscan Walkaway automated instrument (Dade). These panels use the results of 18 separate biochemical tests and the susceptibility to 18 different antibiotics to identify and characterize *Staphylococcus* species (Dade).

Preparation of cell lysates and SOD activity assay. Whole-cell lysates of staphylococcal strains were prepared by using the procedure of Valderas and Hart (48). Total protein of whole cell lysates was determined by the Bradford assay (Bio-Rad Laboratories, Richmond, Calif.). Cell protein (5 or 50 μg) was loaded onto 15% (wt/vol) nondenaturing polyacrylamide gels and separated by electrophoresis in buffer lacking sodium dodecyl sulfate. SOD activity was determined by the nitroblue tetrazolium negative staining method of Beauchamp and Fridovich (8).

SOD subunit composition. Whole-cell lysates of *S. aureus* RN6390, *S. epidermidis* ATCC 12228, and the *sodA* mutant of *S. aureus* RN6390 containing the *S. epidermidis sod* gene on a plasmid (pCL15 *epi-sod*) were prepared as described above. Total protein (5 μg) from each strain along with standard proteins of known molecular weights (Sigma) were loaded on nondenaturing polyacrylamide gels of various concentrations (12, 15, 18, and 21%). Proteins were separated by electrophoresis and stained for SOD activity as previously described (8). The relative mobility (R_f) of each band of activity was determined. Gels were then rinsed in water overnight and stained with Coomassie brilliant blue (Fisher) (4), and the R_f values for the standard proteins were determined. R_f values for standard proteins and SOD activity bands were used to generate Ferguson plots (20) as per Sigma technical bulletin no. MKR-137. The correlation (r) of slopes versus the molecular weights of standard proteins was 0.987.

Chromosomal DNA analysis, cloning, and complementation. Chromosomal DNA was isolated from staphylococci by the guanidine-HCl-CsCl method described by Dyer and Iandolo (16). DNA was digested with either *Eco*RI or *Hin*dIII, resolved by agarose gel electrophoresis, and transferred by passive diffusion to neutral nylon membranes (MagnaGraph; Micron Separations Inc., Westborough, Mass.). Membranes were hybridized overnight (18 to 24 h) at 65°C with PCR products containing either *sodM*, *sodA*, or *S. epidermidis* ATCC 12228 *sodA* labeled with digoxigenin-11-UTP (Roche Molecular Biochemicals, Indianapolis, Ind.) as described by Smeltzer et al. (46) and Hart et al. (25). Hybridizing probes were detected by autoradiography with alkaline phosphatase-conjugated antidigoxigenin $F(ab')_2$ antibody fragments (Roche Molecular Biochemicals) and the chemiluminescent substrate CDP-*Star* (Roche Molecular Biochemicals).

Sequence from the 5′ end of the putative *sod* gene reported by Heidrich et al. (26) for *S. epidermidis* BN280 (GenBank accession no. X97011) was used to perform a BLAST search of the genomic DNA database (in progress) for *S. epidermidis* RP62A maintained by The Institute for Genomic Research (TIGR) (http://www.tigr.org). The oligonucleotide primers 5′-AGGCCATTGGTCGTA TTT-3′ and 5′-GCAAATCATCTAAGGGCTATG-3′ were designed and used to amplify an approximately 0.9-kbp region containing the *sodA* gene from *S. epidermidis* ATCC 12228 by PCR. The PCR product was ligated into pCR2.1 (Invitrogen, Carlsbad, Calif.) and used to transform *E. coli* INVαF′, as recommended by the manufacturer (Invitrogen). Plasmid DNA from antibiotic-resistant transformants was isolated using a plasmid miniprep kit (Bio-Rad Laboratories) and digested with *Eco*RI to verify the presence of an approximately 0.9-kbp insert. Plasmid DNA containing the *S. epidermidis sodA* gene (pCR2.1 *epi-sod*) was sequenced at the University of Arkansas for Medical Sciences DNA Sequencing Core Facility (Little Rock) with a DNA sequencer (Perkin-Elmer Biosystems, Foster City, Calif.).

The *Eco*RI fragment containing the *S. epidermidis sodA* gene was also ligated into the shuttle vector pCL15 (kindly provided by Chia Lee at the University of Kansas Medical Center) and transformed into *E. coli* HB101. Plasmid DNA (pCL15 *epi-sod*) isolated from antibiotic-resistant colonies was used to transform the *S. aureus* RN4220 *sodA* mutant (48) by electroporation (35). Plasmid isolation and Southern analysis were used to confirm the presence of pCL15 *epi-sod* in chloramphenicol-resistant transformants. Plasmid pCL15 was also transformed into the *sodA* mutant as a vector control.

Nucleotide sequence accession number. The full-length *sodA* gene from *S. epidermidis* ATCC 12228 has been assigned GenBank accession number AF410177.

RESULTS AND DISCUSSION

SOD subunit composition. Most procaryotic SOD proteins studied thus far exist as either dimers or tetramers (10). Genetic evidence from a previous study demonstrated that at least the middle band of activity found in cell lysates of *S. aureus* consists of a multimeric form composed of SodM and SodA (48). In the present study, we compared the relative mobility of each band of SOD activity to that of proteins of known molecular weight by nondenaturing polyacrylamide gel electrophoresis (PAGE) and SOD staining. These values were used to generate Ferguson plots (20), which allowed the calculation of the molecular weight of each band of SOD activity. Results indicate that the *S. aureus* SodM and SodA, the *S. epidermidis* SodA and the hybrids composed of SodM and SodA exist as dimers (Table 2).

SOD profiles among clinical isolates of *S. aureus*. Because the SOD profile of laboratory strains of *S. aureus* appears to be unique among the gram-positive bacteria (23, 24, 30, 39, 48, 49, 53), we examined the SOD profiles of other staphylococci, in particular, clinical isolates of *S. aureus* and CoNS. Clinical *S. aureus* strains isolated from two different geographical locations were analyzed for SOD activity using nondenaturing PAGE and SOD staining (Fig. 1). Cell lysates from all eight *S. aureus* isolates (Fig. 1, lanes 2 to 6 and 8 to 11) as well as ATCC 25923 (lane 7) demonstrated a SOD profile similar to that of *S. aureus* RN6390 (lane 1). Originally we loaded 5 μg of total protein from each clinical strain and observed only two bands of SOD activity that exhibited a migration pattern identical to that of the SodA and SodM/SodA hybrid proteins of *S. aureus* RN6390. Analysis of 50 μg of total protein revealed, in each case, a third band of activity that migrated to a position similar to that of SodM of *S. aureus* RN6390 (Fig. 1). Interestingly, the level of activity for SodM ranged from detectable to approximately half of that observed for *S. aureus* RN6390. In addition, in all *S. aureus* strains (including laboratory strains) examined thus far, the SodM homodimer band of activity has always been less than the heterodimeric hybrid band composed of SodM and SodA (48). It is not known at present why the SOD heterodimer would exhibit more activity than the homodimer

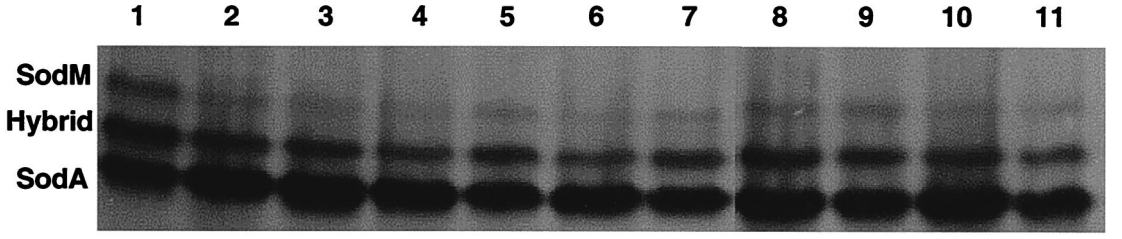

FIG. 1. Activity gel analysis of *S. aureus* SOD. Lane 1, RN6390; lane 2, HAC1; lane 3, HAC2; lane 4, HAC3; lane 5, HAC4; lane 6, HAC6; lane 7, ATCC 25923; lane 8, UAB1; lane 9, UAB2; lane 10, UAB4; lane 11, UAB5. Each lane contains approximately 50 μg of protein (see Results and Discussion). Stained gels were scanned with an AlphaImager 2000 (Alpha Innotech Corp.) imaging system, and the inverse image was generated with NIH Image software.

SodM protein. Perhaps the heterodimer is more stable than the SodM homodimer in cell lysates or the SodM homodimer is either secreted or associated with the cytoplasmic membrane, which has been observed with some bacterial SODs (1, 7, 14, 18, 24). However, we have compared SOD activity from *S. aureus* spent media to that of cell lysates and determined that the specific activity for all three SODs is approximately the same for both preparations, thereby suggesting that the SOD activity associated with spent media is not the result of secretion (data not shown).

SOD profiles among CoNS. Given the *S. aureus* characteristic three bands of SOD activity, we decided to determine whether CoNS have similar SOD activities. Cell lysate total protein (5 μg) from each of 22 CoNS encompassing eight different species was resolved by nondenaturing PAGE and stained for SOD activity (Fig. 2). All 22 CoNS exhibited a single band of SOD activity migrating to a position similar to that of SodA of *S. aureus* RN6390 (Fig. 2, lanes 1). Activity of the single band ranged from barely detectable to equaling the SodA band of *S. aureus* RN6390 (Fig. 2). Levels of activity among the nine *S. epidermidis* strains (Fig. 2A, lanes 2 to 10), which included ATCC 12228, were approximately the same. These data indicate that CoNS contain only one SOD activity with a migratory pattern similar to that of the *S. aureus* SodA

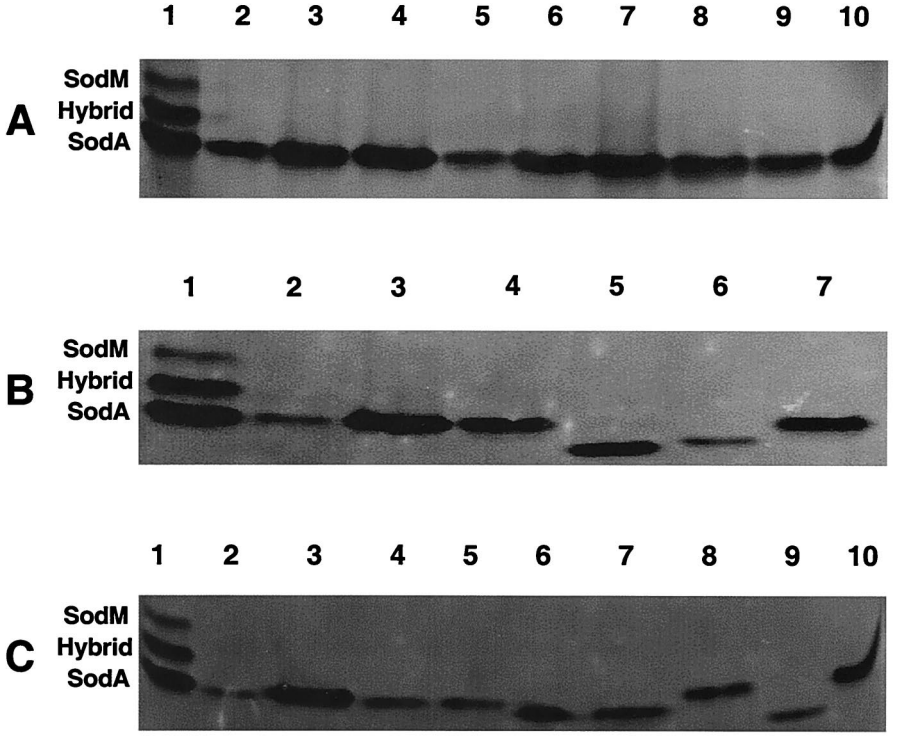

FIG. 2. Activity gel analysis of CoNS. (A) *S. epidermidis* isolates. Lane 1, *S. aureus* RN6390; lane 2, HAC33; lane 3, HAC36; lane 4, HAC94; lane 5, HAC111; lane 6, HAC112; lane 7, HAC113; lane 8, HAC114; lane 9, HAC115; lane 10, ATCC 12228. (B and C) Other CoNS. (B) Lane 1, *S. aureus* RN6390; lane 2, *S. auricularis* HAC140; lane 3, *S. auricularis* HAC145; lane 4, *S. capitis* HAC138; lane 5, *S. carnosus* KSI2019; lane 6, *S. haemolyticus* HAC146; lane 7, *S. epidermidis* ATCC 12228. (C) Lane 1, *S. aureus* RN6390; lane 2, *S. hominis* HAC79; lane 3, *S. hominis* HAC139; lane 4, *S. hominis* HAC142; lane 5, *S. hominis* HAC144; lane 6, *S. lugdunensis* HAC51; lane 7, *S. lugdunensis* HAC141; lane 8, *S. simulans* HAC44; lane 9, *S. simulans* HAC143; lane 10, *S. epidermidis* ATCC 12228. Each lane contains approximately 5 μg of protein (see Results and Discussion). The gels were analyzed and the image was generated as described for Fig. 1.

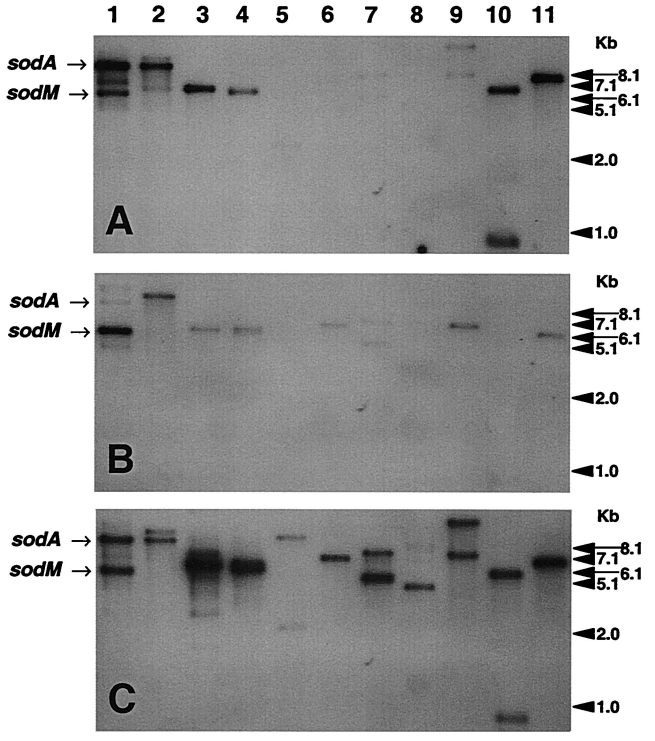

FIG. 3. Southern analysis of *S. aureus* and CoNS chromosomal DNA hybridized with probes specific for *S. aureus sodA* (A), *S. aureus sodM* (B), or *S. epidermidis sod* (C). Lane 1, *S. aureus* RN6390; lane 2, *S. aureus* UAB1; lane 3, *S. epidermidis* ATCC 12228; lane 4, *S. simulans* HAC143; lane 5, *S. hominis* HAC 79; lane 6, *S. carnosus* KSI2019; lane 7, *S. capitis* HAC138; lane 8, *S. auricularis* HAC140; lane 9, *S. lugdunensis* HAC141; lane 10, *S. haemolyticus* HAC146; lane 11, *S. epidermidis* HAC33. Arrows show the positions of the *S. aureus* RN6390 *Eco*RI fragment-hybridizing bands that contain the *sodM* and *sodA* genes.

protein. While we were unable to isolate sufficient amounts of total protein from all CoNS strains, for those that we were able to isolate, no additional bands of activity were observed when 50 μg of protein was resolved by nondenaturing PAGE and stained for SOD activity (data not shown).

Southern analysis of clinical isolates of CoNS. To determine whether the lack of additional bands of SOD activity among the CoNS was due to the absence of a *S. aureus sodM* gene equivalent, we isolated chromosomal DNA from a representative strain of each of the eight species of CoNS examined. The DNA was digested with either *Eco*RI (Fig. 3) or *Hin*dIII (data not shown) and hybridized with probes generated from the *S. aureus* RN6390 *sodM* and *sodA* genes and from the *sodA* gene isolated from *S. epidermidis* ATCC 12228. The nucleic acid sequences of all three *sod* genes are 74% identical (data not shown), and as expected, at a hybridization temperature of 65°C, some cross-hybridization occurred. This is particularly evident with *S. aureus* strains RN6390 and UAB1 (Fig. 3, lanes 1 and 2). All three probes were able to hybridize with *Eco*RI fragments containing the *sodA* and *sodM* genes, although the fragment containing the *sodA* gene was barely detectable when probed with *sodM* (Fig. 3, lanes 1 and 2). Nevertheless, Southern analysis using these probes indicates that the CoNS examined in this study contain only one *sod* gene. A single *Eco*RI fragment-hybridizing band was observed for *S. epidermidis* ATCC 12228 and clinical isolates, *Staphylococcus carnosus*, *Staphylococcus simulans*, and *Staphylococcus auricularis*, while *Staphylococcus hominis*, *Staphylococcus capitis*, *Staphylococcus lugdunensis*, and *Staphylococcus haemolyticus* exhibited two *Eco*RI fragment-hybridizing bands (Fig. 3). In all cases, the hybridizing bands were identical in size regardless of which probe was used, and the intensity of the hybridizing bands was always greater with the *S. aureus sodA* or *S. epidermidis sod* probe than with the *sodM* probe. In addition, species exhibiting two hybridizing bands with *Eco*RI-digested DNA exhibited only one band when digested with *Hin*dIII and hybridized with the *sod* probes, indicating only one *sod* gene in these species (data not shown). Chromosomal DNA hybridized with the *sodM* probe and in some cases those probed with *sodA* required extended exposure times in order to detect hybridizing bands. No bands were detected for *S. auricularis* and *S. haemolyticus* when hybridized with the *sodM* probe (Fig. 3B, lanes 8 and 10). While the clinical isolate of *S. epidermidis* (Fig. 3C, lane 11) exhibited a single hybridizing band when probed with the *S. epidermidis sod*, *S. epidermidis* ATCC 12228 exhibited a number of less intense bands (Fig. 3C, lane 3). These hybridizing bands may share a level of nucleic acid relatedness to the *S. epidermidis sod* probe. However, it is unlikely that these fragments represent additional *sod* genes due to the appearance of only one band of SOD activity (Fig. 2A, lane 10) and the absence of additional genes identified through searches of the DNA database. In addition, the *sodA* probe also hybridized to additional *Eco*RI chromosomal fragments of *S. aureus*, albeit less intensely than the *Eco*RI fragments containing either the *sodM* or *sodA* genes (Fig. 3A and C, lanes 1 and 2). Again, it is unlikely that these hybridizing fragments represent additional *sod* genes, since SOD activity is undetectable in a *sodM sodA* mutant of *S. aureus* (48). However, it is possible that additional *sod* genes were not expressed due to the growth conditions employed in our study or that the genes contained mutations. In addition, the products of these genes could be unstable in cell lysates. The failure to find the *E. coli* Cu/Zn SOD until recently has been attributed to the loss of the protein during isolation primarily due to its periplasmic location but also due to instability during the isolation procedures employed (9).

***sod* gene from *S. epidermidis*.** To assess the relatedness of the *sod* gene from *S. epidermidis* ATCC 12228 to the *sodM* and *sodA* genes of *S. aureus*, the nucleic acid sequence of the 5′ end of the putative *sod* gene of *S. epidermidis* BN280 (26) (GenBank accession no. X97011) was used to search the *S. epidermidis* RP62A genomic sequence maintained by TIGR (http://www.tigr.org) to find the entire open reading frame of the *sod* gene. PCR primers designed from this sequence were used to amplify an approximately 0.9-kbp fragment from chromosomal DNA of *S. epidermidis* ATCC 12228, which was cloned and sequenced. The deduced amino acid sequence of the ORF was 100% identical to the sequence reported for *S. epidermidis* RP62A by TIGR (data not shown). The predicted amino acid sequence of the *S. epidermidis sod* ORF is 92 and 76% identical to the amino acid sequences of SodA and SodM of *S. aureus* RN6390, respectively (Fig. 4A). In addition, the predicted SOD amino acid sequences from *S. carnosus* (AJ295150) and *Staphylococcus xylosus* (AJ276960) and the recently reported

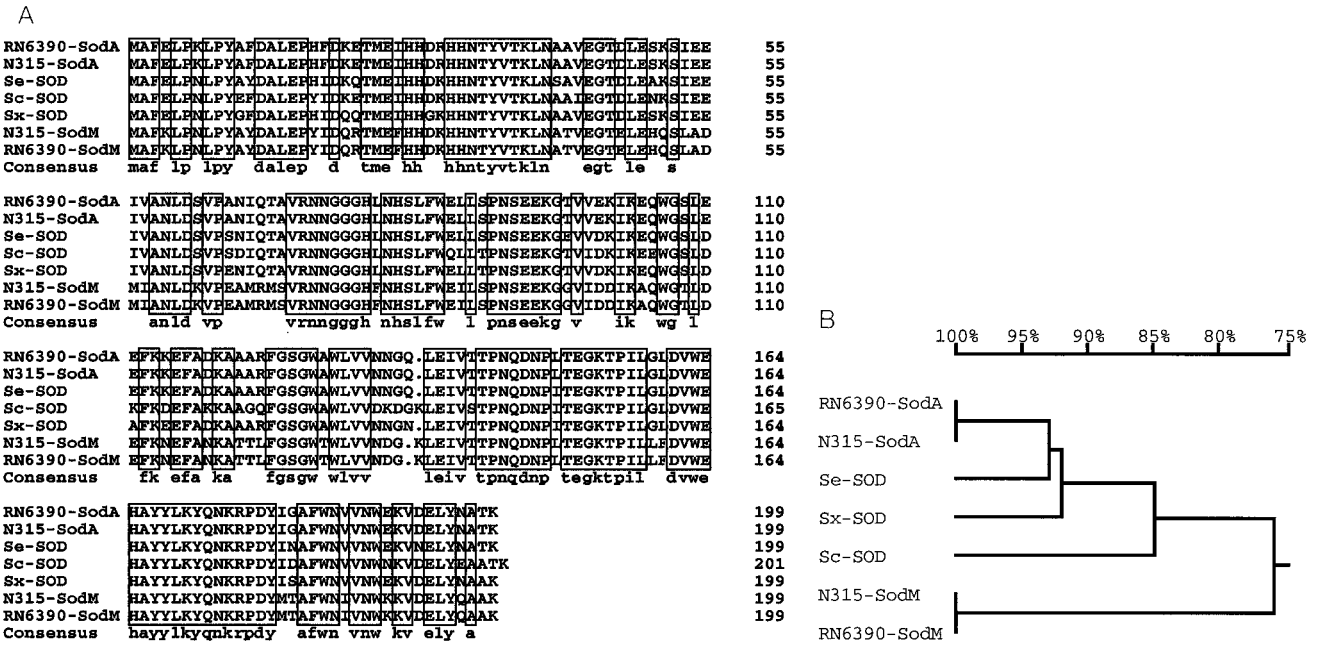

FIG. 4. (A) Amino acid sequence alignments of staphylococcal SODs. The *S. aureus* RN6390 SodA (48) and SodM (48) (AF273269) proteins, the *S. aureus* N315 SodA and SodM proteins (36) (AP003129 and AP003134, respectively), and the Sod proteins of *S. epidermidis* (Se) ATCC 12228 (AF410177), *S. carnosus* (Sc) (CAC14833), and *S. xylosus* (Sx) (CAB95744) are shown. Consensus sequences are boxed. A gap at position 136 of the SodM sequences was manually inserted. (B) Phylogenetic tree. Amino acid sequences were analyzed with DNAMAN (Lynnon BioSoft, Vaudreuil, Quebec, Canada) software, which uses the neighbor-joining method described by Saitou and Nei (43).

sequences from *S. aureus* N315 (AP003129 and AP003134 for *sodM* and *sodA*, respectively) (36) were included for comparison (Fig. 4A). The homologs share 67.8% identity as a group.

These sequences were also used to generate a phylogenetic tree (Fig. 4B) to determine their relatedness. Data from this analysis suggest that the SODs in *S. epidermidis*, *S. carnosus*, and *S. xylosus* are closely related to SodA of *S. aureus*, whereas these proteins along with SodA of *S. aureus* are only distantly related to SodM, exhibiting 51% similarity to it.

In addition, the *sod* gene from *S. epidermidis* ATCC 12228 was cloned into the staphylococcal shuttle vector pCL15 and transformed into the *sodA* mutant of *S. aureus* (Fig. 5). Cell lysates from the parental strain, *S. aureus* RN4220 (Fig. 5, lane 1), and *S. epidermidis* ATCC 12228 (Fig. 5, lane 2) exhibited the expected patterns of SOD activity. Only the SodM band of activity was observed in the *sodA* mutant (48) (Fig. 5, lane 3) and the *sodA* mutant containing pCL15 (Fig. 5, lane 4). However, the *sodA* mutant containing pCL15 *epi-sod* exhibited a band of SOD activity comparable to that of *S. epidermidis* and a hybrid band of activity similar to that observed for the parental *S. aureus* strain (48) (Fig. 5, lane 5). These data demonstrate that the *S. epidermidis sodA* gene expressed in *S. aureus* and the gene product formed a hybrid with SodM of *S. aureus* and, like the hybrid SOD band seen with wild-type *S. aureus*, exist as a heterodimer (Table 2). The formation of a hybrid of two SOD proteins is seen not only in *S. aureus* (48) but also in *E. coli* (12). In *E. coli* the hybrid is a dimeric protein consisting of one subunit each of SodA (Mn-containing enzyme) and SodB (Fe-containing enzyme). In *E. coli* as well as in *S. aureus*, it is not known whether the formation of a hybrid SOD protein has physiological relevance or is the result of subunit exchange between two related proteins.

Originally, it was reported that *sodM* from *S. aureus* RN6390 encoded a 187-amino-acid protein with 76% identity to SodA (48) (GenBank accession no. AF273269). We have now determined that the *sodM* sequence that was reported earlier con-

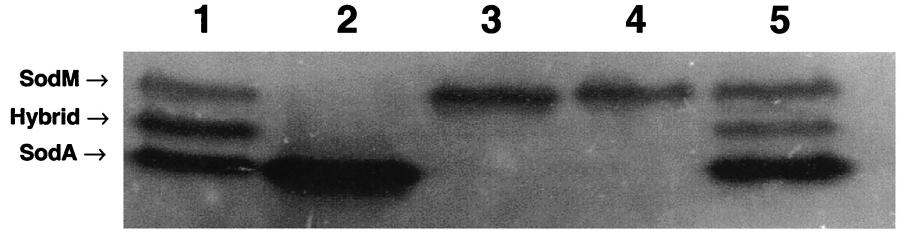

FIG. 5. SOD activity gel of *S. aureus sodA* mutant complemented with *S. epidermidis sod*. Lane 1, *S. aureus* RN4220; lane 2, *S. epidermidis* ATCC 12228; lane 3, *S. aureus* RN4220 *sodA* mutant; lane 4, *S. aureus* RN4220 *sodA* mutant containing pCL15; lane 5, *S. aureus* RN4220 *sodA* mutant containing pCL15 *epi-sod*. The gel was analyzed and the image was generated as described for Fig. 1.

tained an incorrect base that resulted in a 12-amino-acid truncation of the predicted *sodM* ORF. The correct base was verified by sequencing the region containing the base in question, and we now report that the *S. aureus* RN6390 *sodM* ORF encodes a protein of 199 amino acids with a predicted molecular mass of 22.7 kDa (Fig. 4A).

In summary, we have determined that eight representative species of the CoNS contain only one *sod* gene that yields one band of SOD activity as determined by nondenaturing PAGE and staining for SOD activity. This is in contrast to *S. aureus*, which contains two genes responsible for three bands of activity (48). The putative amino acid sequence from three CoNS *sod* genes indicates that these genes are more similar to the *sodA* gene than the *sodM* gene of *S. aureus*. Therefore, the *S. epidermidis* gene isolated in this study is designated *sodA*. Furthermore, the CoNS SOD proteins migrate on activity gels to a position similar to that of the *S. aureus* SodA protein. Whether the differences observed with the SOD profiles between *S. aureus* and the CoNS represent an important divergence in the evolution of the staphylococci is not known at present. However, the origin of the *S. aureus sodM* gene is an intriguing question. We recently demonstrated that the *sodM* gene is important in maintaining viability under oxidative stress conditions in an *S. aureus* strain containing a *sodA* mutation (48). Expression of *sodM* increased as cells entered the postexponential and stationary phases of growth (48) similar to that observed for *sodA* (13). However, while SodA is the most abundant of the three SOD activities observed, the increase in total SOD activity as cells entered the postexponential and stationary phases of growth is attributed to the increased production of SodM (48). These data suggest a regulatory mechanism for *sodM* independent of *sodA* and a unique role for SodM. Results of the present study support a unique role for *sodM* in that all *S. aureus* isolates, including those isolated from the clinical environment, contain *sodM* while the CoNS do not. As a pathogen, *S. aureus* is certainly better equipped than the CoNS, as it produces numerous toxins, enzymes, and cell wall-associated proteins, which concertedly cause a wide variety of disease syndromes in humans (28). Perhaps the *S. aureus* SodM protein is yet another important factor that contributes to the disease-causing ability of this organism. Studies addressing this question are in progress.

ADDENDUM

During the review of this article, a paper that described the use of PCR to amplify an internal fragment of the *sodA* gene in 40 CoNS type strains was published (40a). In that study, protein (50 μg) from cell lysates of the 40 CoNS strains as well as 25 unrelated clinical strains of *S. aureus* was resolved by nondenaturing PAGE and stained for SOD activity. All CoNS type strains exhibited a single band of SOD activity while all of the *S. aureus* isolates exhibited three closely migrating bands of SOD activity. Our data are in agreement with the finding of Poyart et al. that CoNS strains express only one SOD while *S. aureus* strains express three.

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