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Rapid and Accurate Species-Level Identification of Coagulase-Negative Staphylococci by Using the *sodA* Gene as a Target

CLAIRE POYART,* GILLES QUESNE, CLAIRE BOUMAILA, AND PATRICK TRIEU-CUOT

Laboratoire Mixte Pasteur-Necker de Recherche sur les Streptocoques et Streptococcies and Unité INSERM 411, Faculté de Médecine Necker-Enfants Malades, 75730 Paris Cedex 15, France

Received 20 August 2001/Accepted 28 September 2001

Simple PCR and sequencing assays that utilize a single pair of degenerate primers were used to characterize a 429-bp-long DNA fragment internal (*sodA*$_{int}$) to the *sodA* gene encoding the manganese-dependent superoxide dismutase in 40 coagulase-negative staphylococcal (CNS) type strains. The topology of the phylogenetic tree obtained was in general agreement with that which was inferred from an analysis of their 16S rRNA or *hsp60* gene sequences. Sequence analysis revealed that the staphylococcal *sodA* genes exhibit a higher divergence than does the corresponding 16S ribosomal DNA. These results confirm that the *sodA* gene constitutes a highly discriminative target sequence for differentiating closely related bacterial species. Clinical isolates that could not be identified at the species level by phenotypical tests were identified by use of this database. These results demonstrate the usefulness of this method for rapid and accurate species identification of CNS isolates, although it does not allow discrimination of subspecies. The *sodA* sequence polymorphisms observed with staphylococcal species offer good opportunities for the development of assays based on DNA chip technologies.

Coagulase-negative staphylococci (CNS), which are part of the normal skin flora, have emerged as predominant pathogens in hospital-acquired infections (8, 15). They are associated with the presence of foreign bodies, such as prosthetic valves, cerebrospinal fluid shunts, and orthopedic prostheses, as well as intravascular, urinary, and dialysis catheters. Therefore, it has become increasingly important to accurately identify these isolates to the species level in order to define the clinical significance of these bacteria, to carry out a proper epidemiologic surveillance, and to manage patients infected with CNS in case of relapse. A variety of manual and automated methods have been developed for the identification of CNS that are important in human medicine (6, 7, 19, 24). These methods, based on phenotypic characteristics, include conventional identifications and several commercial kits. Unfortunately, the overall accuracy of these systems is low, ranging from 50 to 70% (6, 7, 19, 24). Several genotypic methods based on the analysis of PCR products derived from selected DNA targets have thus been developed for species-level identification of CNS, including electrophoretic analysis (2) and determination (12) of the 16S ribosomal DNA (rDNA) sequence. In the latter case, however, the interpretation of these data may be complicated by the fact that closely related species may have identical 16S rDNA sequences or, alternatively, that divergent 16S rDNA sequences may exist within a single organism (26). To solve this problem, it is possible to use alternative monocopy target sequences which exhibit a higher divergence than those of the 16S rDNA. Recently, partial sequencing of the highly conserved and ubiquitous *hsp60* and *tuf* genes have been found to be useful for identification and taxonomic classification of species of the genus *Staphylococcus* (5, 17, 18). It was previously reported that PCR and sequencing of the *sodA* gene of the gram-positive cocci which encodes the manganese-dependent superoxide dismutase (Mn-SOD), with the use of a single pair of degenerate primers, constitute a valuable approach to the genotypic identification of streptococcal (22) and enterococcal (23) species. In the present study, we report the use of the same universal primers (21) to construct a *sodA* database of 40 staphylococcal type species and we demonstrate the usefulness of this library for a rapid sequence-based identification method for CNS isolates.

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* Corresponding author. Mailing address: Laboratoire Mixte Pasteur-Necker de Recherche sur les Streptocoques et Streptococcies, Faculté de Médecine Necker-Enfants Malades, 75730 Paris Cedex 15, France. Phone: (33) (1) 40 61 56 79. Fax: (33) (1) 40 61 55 92. E-mail: cpoyart@pasteur.fr.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The main characteristics of the staphylococcal strains used in this study, including the type strains, are listed in Tables 1 and 2. Type strains were obtained from the Collection de l'Institut Pasteur (CIP). All cultures were grown at 37°C in brain heart infusion broth and subcultured on brain heart infusion agar for examination of the purity and the colony characteristics. Clinical isolates of CNS were identified by the ID 32 Staph system (API-bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions and by use of APILAB ID 32 software.

DNA manipulations. Rapid extraction of bacterial genomic DNA collected from 2 ml of an overnight culture was performed with the InstaGen Matrix (Bio-Rad) according to the manufacturer's instructions. The *sodA* degenerate primers *d1* (5′-CCITAYICITAYGAYGCIYTIGARCC-3′) and *d2* (5′-ARRTA RTAIGCRTGYTCCCAIACRTC-3′) were used to amplify an internal fragment, designated *sodA*$_{int}$, representing approximately 83% of the *sodA* gene. PCRs were performed on a Gene Amp System 2400 thermal cycler (Perkin-Elmer Cetus, Courtaboeuf, France) in a final volume of 50 μl containing 150 ng of DNA as the template, 0.5 μM each primer, a 200 μM concentration of each deoxynucleoside triphosphate, and 1 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer) in a 1× amplification buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$). The PCR mixtures were denatured (3 min at 95°C) and then subjected to 30 cycles of amplification (60 s of annealing at 37°C, 45 s of elongation at 72°C, and 30 s of denaturation at 95°C). PCR products were resolved by electrophoresis on a 1% agarose gel stained with ethidium bromide. PCR products were purified on an S-400 Sephadex (Pharmacia, Uppsala, Swe-

TABLE 1. Staphylococcal type strains used in this study

Strain	Other designation[a]	$sodA_{int}$ accession no.
S. aureus subsp. *anaerobius* CIP 103780 T	ATCC 35844	Not determined
S. aureus subsp. *aureus* CIP 65.8 T	ATCC 12600	Not determined
S. arlettae CIP 103501 T	ATCC 43959	AJ34894
S. auricularis CIP 103587 T	ATCC 33753	AJ34895
S. capitis subsp. *capitis* CIP 81.53 T	ATCC 27840	AJ34896
S. capitis subsp. *ureolyticus* CIP 104192 T	ATCC 49326	AJ34897
S. caprae CIP 104000 T	ATCC 35538	AJ34898
S. carnosus subsp. *carnosus* CIP 103274 T	ATCC 51365	AJ34899
S. carnosus subsp. *utilis* CIP 105758 T	DSM 11676	AJ34900
S. chromogenes CIP 81.59 T	DSM 20454	AJ34901
S. cohnii subsp. *cohnii* CIP 81.54 T	ATCC 29974	AJ34902
S. cohnii subsp. *urealyticum* CIP 104024 T	ATCC 49330	AJ34903
S. condimenti CIP 105760 T	DSM 11674	AJ34904
S. delphini CIP 103732 T	ATCC 49171	AJ34905
S. epidermidis CIP 81.55 T	ATCC 14990	AJ34906
S. equorum CIP 103502 T	ATCC 43958	AJ34907
S. felis CIP 103366 T	ATCC 49168	AJ34908
S. gallinarum CIP 103504 T	ATCC 35539	AJ34909
S. haemolyticus CIP 81.56 T	ATCC 29970	AJ34910
S. hominis subsp. *hominis* CIP 81.57 T	ATCC 27844	AJ34911
S. hominis subsp. *novobiosepticus* CIP 105719 T	ATCC 700236	AJ34912
S. hyicus CIP 81.58 T	ATCC 11249	AJ34913
S. intermedius CIP 81.60 T	ATCC 29663	AJ34914
S. kloosii CIP 103503 T	ATCC 43959	AJ34915
S. lentus CIP 8163 T	ATCC 29070	AJ34916
S. lugdunensis CIP 103642 T	ATCC 43809	AJ34917
S. lutrae CIP 105399 T	ATCC 700373	AJ34918
S. muscae CIP 103641 T	ATCC 49910	AJ34919
S. pasteuri CIP 103540 T	ATCC 51129	AJ34920
S. piscifermentans CIP 103958 T	DSM 7373	AJ34921
S. pulvereri CIP 104364 T	DSM 9930	AJ34922
S. saccharolyticus CIP 103275 T	ATCC 14953	AJ34923
S. saprophyticus subsp. *bovis* CIP 105260 T		AJ34924
S. saprophyticus subsp. *saprophyticus* CIP 76.125 T	ATCC 15305	AJ34925
S. schleiferi subsp. *coagulans* CIP 104370 T	ATCC 49545	AJ34926
S. schleiferi subsp. *schleiferi* CIP 103643 T	ATCC 43808	AJ34927
S. sciuri subsp. *carnaticus* CIP 105826 T	ATCC 700058	AJ34928
S. sciuri subsp. *sciuri* CIP 81.62 T	ATCC 29062	AJ34929
S. simulans CIP 81.64 T	ATCC 27848	AJ34930
S. vitulus CIP 104850 T	ATCC 51145	AJ34931
S. warneri CIP 81.65 T	ATCC 27836	AJ34932
S. xylosus CIP 81.66 T	ATCC 29971	AJ34933
Macrococcus caseolyticus[b] CIP 100755 T	ATCC 13548	AJ34934

[a] ATCC, American Type Culture Collection; DSM, Deutsche Sammlung Von Mikrooganismen.
[b] Formerly designated *Staphylococcus caseolyticus*.

TABLE 2. Identification of various staphylococcal strains by sequencing the $sodA_{int}$ fragment

Strain	Origin	Bacterial species (% identity)[a]	$sodA_{int}$ accession no.
NEM1997	Cheese	*M. caseolyticus* (100)	AJ34935
NEM1998	Cheese	*M. caseolyticus* (100)	AJ34936
NEM1999	Middle ear fluid	*S. auricularis* (99.8)	AJ34937
NEM2000	Middle ear fluid	*S. auricularis* (99.8)	AJ34938
NEM2001	Urine	*S. capitis* (99.8)	AJ34939
NEM2002	Blood	*S. capitis* (99.8)	AJ34940
NEM2003	Blood	*S. capitis* (100)	AJ34941
NEM2004	Urine	*S. caprae* (100)	AJ34942
NEM2005	Blood	*S. caprae* (100)	AJ34943
NEM2006	Cheese	*S. chromogenes* (99.8)	AJ34944
NEM2007	Cheese	*S. chromogenes* (99.8)	AJ34945
NEM2008	Blood	*S. epidermidis* (100)	AJ34946
NEM2009	Catheter	*S. epidermidis* (100)	AJ34947
NEM2010	Blood	*S. epidermidis* (99.5)	AJ34948
NEM2011	Catheter	*S. haemolyticus* (100)	AJ34949
NEM2012	Blood	*S. haemolyticus* (99.5)	AJ34950
NEM2013	Cutaneous abcess	*S. lugdunensis* (99.8)	AJ34951
NEM2014	Synovial fluid	*S. lugdunensis* (99.8)	AJ34952
NEM2015	Nasal swab	*S. pasteuri* (100)	AJ34953
NEM2016	Urine	*S. saprophyticus* (99.8)	AJ34954
NEM2017	Blood	*S. schleiferi* (99.8)	AJ34955
NEM2018	Vagina	*S. simulans* (100)	AJ34956
NEM2019	Cheese	*S. vitulus* (99.8)	AJ34957
NEM2020	Blood	*S. warneri* (99.8)	AJ34958
NEM2021	Blood	*S. xylosus* (99.3)	AJ34959
NEM2022	Cheese	*S. xylosus* (99.5)	AJ34960

[a] The species identification was based on the phylogenetic position of the $sodA_{int}$ fragment of the strain studied relative to those of the type strains, as shown in Fig. 1. The numbers in parentheses indicate the percentages of identity of the $sodA_{int}$ fragments with that of the corresponding type strains.

den) column, and both strands were directly sequenced with the oligonucleotides *d1* and *d2* by using an ABI-PRISM Big Dye terminator sequencing kit on an ABI-PRISM 310 Genetic Analyzer (Perkin-Elmer) as previously described (23). All precautions to prevent carryover of amplified DNA were used.

Sequence analysis. Nucleotide sequences were analyzed with Perkin-Elmer software programs (Sequence Analysis, Sequence Navigator, and Autoassembler). Multiple alignment of *sod* genes was carried out by the CLUSTAL X program (9). The construction of the unrooted phylogenetic tree was performed by the neighbor-joining method (25). The topology of the phylogenetic tree was evaluated by bootstrap analyses to give the degree of confidence intervals for each node on the phylogenetic tree. The confidence values were determined for branches which showed possible monophyletic clades of related organisms separated at each node. It is generally accepted that the monophyly of a clade can be accepted if the clade occurs in more than 95% of the bootstrapped trees (4).

Protein extraction and SOD activity assay. Crude cell lysates of staphylococcal strains were prepared as follows. Cells from 10 ml of overnight cultures were harvested by centrifugation, washed with an equal volume of Tris-EDTA buffer (50 mM Tris HCl [pH 7.6], 50 mM EDTA), suspended in 2 ml of TELL lysis buffer (50 mM Tris HCl [pH 7.6], 50 mM EDTA, 200 mg of lysozyme/liter and 30 mg of lysostaphin/liter), and incubated for 1 h at 37°C. After ultrasonic disruption for 3 min in 30-s pulses at 4°C, the lysates were cleared by centrifugation (16, 170 × *g*, for 10 min at 4°C). The supernatants were recovered and stored at −20°C until needed. Fifty micrograms of total proteins was electrophoresed through a 10% nondenaturing polyacrylamide gel which was stained for SOD activity by the method of Beauchamp and Fridovich (1).

Nucleotide sequence accession numbers. The sequences determined were submitted to the EMBL gene bank and assigned the accession numbers listed in Tables 1 and 2.

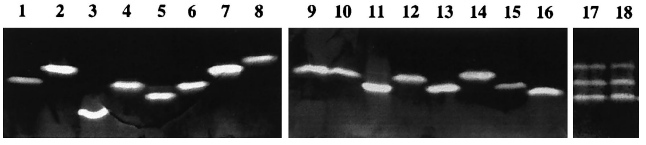

FIG. 1. SOD activity gel. Crude extracts (50 μg) of various staphylococcal type strains were loaded onto a nondenaturing 10% polyacrylamide gel stained for SOD activity. Lanes 1 to 18, *S. capitis* subsp. *capitis*, *S. chromogenes*, *S. cohnii* subsp. *cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis* subsp. *hominis*, *S. hyicus; S. intermedius*, *S. lentus*, *S. lugdunensis*, *S. saprophyticus* subsp. *saprophyticus*, *S. schleiferi* subsp. *schleiferi*, *S. simulans*, *S. vitulus*, *S. warneri*, *S. xylosus*, *S. aureus* subsp. *aureus*, and *S. aureus* subsp. *anaerobius*, respectively.

RESULTS AND DISCUSSION

CNS express a single Mn-SOD. It has recently been reported that *Staphylococcus aureus* possesses two genes encoding Mn-SOD, designated *sodA* and *sodM* (3, 21, 28). In order to confirm these results, we analyzed the SOD activities of the type strains *S. aureus* subsp. *aureus* and *S. aureus* subsp. *anaerobius* and 25 unrelated clinical isolates of *S. aureus* following electrophoresis of protein extracts in a nondenaturing polyacrylamide gel. All strains of *S. aureus* produce three closely migrating bands of SOD activity (Fig. 1 and data not shown). Previous work demonstrated that the upper band of activity corresponds to SodM, the lowest band corresponds to SodA, and the middle band was proposed to result from the formation of a hybrid protein composed of SodM and SodA (28). This analysis indicates that, unlike all the low-GC-content, gram-positive bacilli and cocci described so far, *S. aureus* carries genes encoding two unrelated SODs. The fact that *S. aureus* strains possess two different *sod* genes impedes direct sequencing of PCR products with the primers *d1* and *d2*, since both genes coamplify with this pair of oligonucleotides. Therefore, to determine if CNS possess one gene encoding Mn-SOD, the SOD activities of crude bacterial extracts of the 40 type strains of CNS were analyzed following electrophoresis in nondenaturing polyacrylamide gels. This analysis revealed that all the strains studied produce a single band of SOD activity (Fig. 1 and data not shown), which suggests that CNS, as opposed to *S. aureus*, express a single type of Mn-SOD.

Amplification and sequencing of the $sodA_{int}$ gene from various CNS type strains. By using the primers *d1* and *d2* in a PCR assay, we amplified an internal fragment representing approximately 85% of the *sodA* gene in 40 type strains of CNS (*Staphylococcus arlettae*, *Staphylococcus auricularis*, *Staphylococcus capitis* subsp. *capitis*, *Staphylococcus capitis* subsp. *urealyticus*, *Staphylococcus caprae*, *Staphylococcus carnosus* subsp. *carnosus*, *Staphylococcus carnosus* subsp. *utilis*, *Staphylococcus chromogenes*, *Staphylococcus cohnii* subsp. *cohnii*, *Staphylococcus cohnii* subsp. *urealyticus*, *Staphylococcus condimenti*, *Staphylococcus delphini*, *Staphylococcus epidermidis*, *Staphylococcus equorum*, *Staphylococcus felis*, *Staphylococcus gallinarum*, *Staphylococcus haemolyticus*, *Staphylococcus hominis* subsp. *hominis*, *Staphylococcus hominis* subsp. *novobiosepticus*, *Staphylococcus hyicus*, *Staphylococcus intermedius*, *Staphylococcus kloosii*, *Staphylococcus lentus*, *Staphylococcus lugdunensis*, *Staphylococcus lutrae*, *Staphylococcus muscae*, *Staphylococcus pasteuri*, *Staphylococcus piscifermentans*, *Staphylococcus pulvereri*, *Staphylococcus saccharolyticus*, *Staphylococcus saprophyticus* subsp. *bovis*, *Staphylococcus saprophyticus* subsp. *saprophyticus*, *Staphylococcus schleiferi* subsp. *coagulans*, *Staphylococcus schleiferi* subsp. *schleiferi*, *Staphylococcus sciuri* subsp. *carnaticus*, *Staphylococcus sciuri* subsp. *sciuri*, *Staphylococcus simulans*, *Staphylococcus vitulus*, *Staphylococcus warneri*, *Staphylococcus xylosus*). We also included in this study *Macrococcus caseolyticus*, which was formerly designated *Staphylococcus caseolyticus* (13). A single amplification product having the expected size of 480 bp was observed with all staphylococcal species (data not shown). Direct sequencing of these amplicons gave rise to electropherograms devoid of overlapping peaks, which confirms that these strains contain a single type of *sod* gene. Sequence analysis of these amplicons revealed that they were actual $sodA_{int}$ fragments, since the corresponding deduced polypeptides all possessed the amino acids characteristic of the Mn-SOD at the expected positions (data not shown). Multiple alignment of the staphylococcal $sodA_{int}$ DNA sequences was carried out by the CLUSTAL X program. The sequences of the degenerated primers *d1* and *d2* and alignment gaps were not taken into consideration for calculations. Although some differences could be observed, the topology of the phylogenetic tree obtained (Fig. 2) was in general agreement with that which was inferred from an analysis of their 16S rRNA or *hsp60* gene sequences (17, 27). The phylogenetic position of *M. caseolyticus* is the most distant from any other CNS species (Fig. 2 and data not shown), an observation consistent with the decision to remove this species from the genus *Staphylococcus* (13). It is worth noting, however, that most staphylococcal species groups are not supported by significant bootstrap values (i.e., ≥95%). In fact, if this critical value is used, only three major clusters corresponding to species groups *S. sciuri*, *S. intermedius*, and *S. simulans* are defined (Fig. 2).

The *S. sciuri* group includes *S. sciuri*, *S. lentus*, *S. pulvereri*, and *S. vitulus*. These four species differ from the other *Staphylococcus* species by several remarkable features. They, are novobiocin resistant and oxidase positive, they are the sole species possessing cytochrome *c* in their electron transport systems, and they all share the same characteristic pattern of amino acid substitution in their HSP60 proteins (14, 17). Interestingly, we observed that their $sodA_{int}$ sequences, when compared to other CNS sequences, contained an additional codon which codes for a prolyl residue at position 78 of the corresponding 143-amino-acid-long partial SodA protein (data not shown). It is notable that the $sodA_{int}$ sequences of *S. pulvereri* and *S. vitulus* display 99.5% identity, which confirms that they constitute a single species (20).

An *S. intermedius* group consisting of *S. intermedius*, *S. delphini*, and *S. lutrae* was recovered in 98.1% of the bootstrap trees. The fact that *S. schleiferi* was not included in this cluster is in disagreement with the results from DNA annealing studies or from a phylogenetic analysis of their 16S rDNA or *hsp60* gene sequences (17, 27). Moreover, the related species *S. hyicus*, *S. muscae*, and *S. chromogenes* did not cluster to form a *S. hyicus* species subgroup (Fig. 2).

The *S. simulans* group species, defined in 100% of the bootstrap trees (Fig. 2), consists of *S. simulans*, *S. piscifermentans*, *S. condimenti*, and *S. carnosus*.

The *S. saprophyticus* group, as defined by 16S rDNA sequence analysis (91% of bootstrap value), includes the novo-

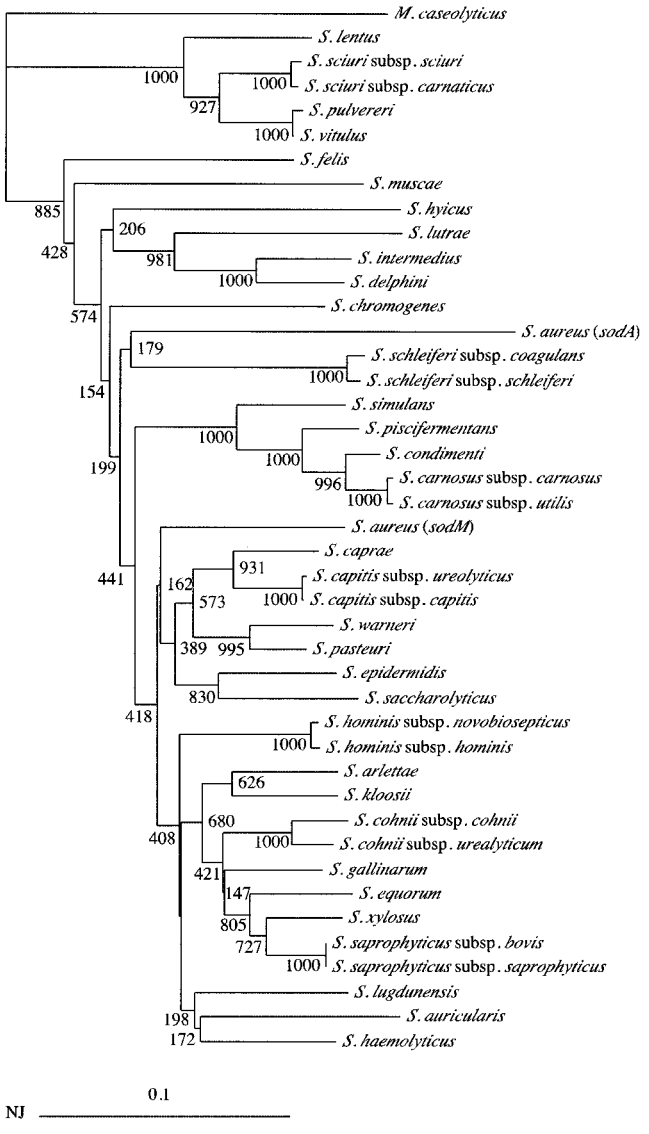

FIG. 2. Phylogenetic unrooted tree showing relationships among the $sodA_{int}$ fragments from various staphylococcal type strains. The tree was established from an analysis of the sequences listed in Table 1 by using the neighbor-joining method. The $sodA_{int}$ sequence of *M. caseolyticus* type strains included in this work was used as an outgroup sequence to root the tree. The value on each branch is the estimated confidence limit (expressed as a percentage) for the position of the branch as determined by bootstrap analysis. Only the bootstrap values superior to 95% were considered significant (4). The scale bar (neighbor-joining distance) represents 10% differences in nucleotide sequences. The accession numbers of *sodA* and *sodM* were AF121672 and Z49245, respectively.

biocin-resistant and oxidase-negative species *S. saprophyticus*, *S. arlettae*, *S. kloosi*, *S. cohnii*, *S. gallinarum*, *S. equorum*, and *S. xylosus* (27). In our analysis, however, the monophyly of this clade is uncertain since it is associated with a bootstrap value of 68% (Fig. 2). Similarly, the *S. epidermidis* group (*S. epidermidis*, *S. capitis*, *S. caprae*, and *S. saccharolyticus*), which constitutes a monophyletic clade supported by a high bootstrap value (97%) on the basis of 16S rDNA sequence analysis (27), did not form a clearly distinct lineage in our study (38.9% of bootstrap value). On the other hand, association of *S. pasteuri* and *S. warneri* to the *S. epidermidis* group was inferred from our treeing analysis (Fig. 2).

As reported in an analysis of their 16S rDNA sequences (27), we found that the branching order of the species *S. auricularis*, *S. haemolyticus*, *S. hominis*, and *S. lugdunensis* was uncertain in our $sodA_{int}$-based phylogenetic analysis.

Evidence for horizontal transfer of a *sod* gene from CNS to *S. aureus*. The *sodA* and *sodM* genes from *S. aureus* display 77.5% sequence identity, which indicates that the presence of these two isofunctional genes in this bacterium is not due to a recent duplication event. In a $sodA_{int}$ phylogenetic tree that includes the sequences of *Bacillus subtilis*, *Clostridium perfringens*, *Enterococcus faecalis*, *Enterococus faecium*, *Lactococcus lactis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*, both the *sodM* and *sodA* genes from *S. aureus* were clearly positioned within the staphylococcal lineage (data not shown). However, it is worth noting that the phylogenetic position of the *sodM* gene in the *sod* tree (Fig. 2) is similar to that of *S. aureus* in 16S rDNA or *hsp60* gene trees (17, 27). This might indicate that *sodM* is the indigenous *S. aureus sod* gene whereas *sodA* was acquired by horizontal gene transfer from an as-yet-uncharacterized CNS. This hypothesis is based on the fact that *S. aureus* possesses a remarkable ability to acquire useful genes from various bacteria by lateral gene transfer, as revealed by genome sequence analysis (16). Accordingly, it has been proposed that the *mecA* homologue present in *S. sciuri* is the evolutionary precursor of the *S. aureus* methicillin resistance gene *mecA* (29). Efforts are currently being made to track the original host of the *S. aureus sodA* gene.

Species identification of clinical and environmental isolates of staphylococci by sequencing the $sodA_{int}$ gene. Pairwise comparison of the staphylococcal $sodA_{int}$ sequences revealed that their mean identity (81.5%) is inferior to that calculated from a comparison of their 16S rDNA genes (mean identity, 98%) but is similar to that computed from a comparison of their *hsp60* genes (mean identity, 82%) (17). These results confirm that *sodA* might constitute a more discriminative target sequence than does the 16S RNA to differentiate closely related bacterial species, as already demonstrated for differentiating closely related species belonging to the genera *Streptococcus* and *Enterococcus* (11, 22, 23). The $sodA_{int}$ fragments of *S. cohnii* subsp. *cohnii* and *S. cohnii* subsp. *urealyticum* display 4% sequence divergence, enabling the distinction between these two subspecies (Table 3). However, the $sodA_{int}$ fragments of the remaining pairs of type strain subspecies (*S. capitis* subsp. *capitis* and *S. capitis* subsp. *ureolyticus*, *S. carnosus* subsp. *carnosus* and *S. carnosus* subsp. *utilis*, *S. hominis* subsp. *hominis* and *S. hominis* subsp. *novobiosepticus*, *S. saprophyticus* subsp. *bovis* and *S. saprophyticus* subsp. *saprophyticus*, *S. schleiferi* subsp. *coagulans* and *S. schleiferi* subsp. *schleiferi, S. sciuri* subsp. *carnaticus* and *S. sciuri* subsp. *sciuri*) display more than 99.3% sequence identity. This finding is consistent with the observation that the *hsp60* genes of *S. schleiferi* subsp. *coagulans* and *S. schleiferi* subsp. *schleiferi* display 98% sequence identity (17). We therefore concluded that the *sodA* gene, like the *hsp60* gene, does not allow discrimination at the subspecies level.

Twenty-six unrelated CNS isolates were identified by con-

TABLE 3. Identity matrix based on pairwise comparisons of *sodA*$_{int}$ fragments of CNS type strains

Strain[a]	% Identity with the following strain[b]:																																						
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1. *S. arlettae*	82.3	86.5	86.2	84.6	80.7	80.4	79.3	86.2	88.1	81.4	76.5	84.6	88.1	77.4	89.7	86.9	87.6	87.9	74.8	77.6	91.1	71.8	85.8	76.9	73.9	85.3	82.1	71.1	83.7	88.8	88.8	77.6	77.9	71.8	71.8	81.6	71.6	85.1	89.0
2. *S. auricularis*		83.2	83.0	83.0	79.9	79.9	81.1	83.0	82.8	80.6	74.6	80.9	82.8	75.1	86.0	84.1	83.0	83.0	73.2	74.4	80.4	65.7	83.7	72.3	72.3	83.0	79.6	69.2	78.6	83.7	83.7	75.8	76.2	69.2	69.2	79.6	69.2	82.8	82.1
3. *S. capitis* subsp. *capitis*			99.8	93.7	79.0	79.0	84.8	84.1	84.8	79.3	78.8	89.3	85.3	78.3	85.1	87.2	86.9	86.5	79.3	77.9	85.1	72.5	84.4	74.4	75.1	90.2	81.4	74.4	87.2	86.2	86.2	81.1	81.8	73.0	73.2	83.9	74.8	89.5	85.3
4. *S. capitis* subsp. *ureolyticus*				93.9	78.8	78.8	84.6	84.4	84.6	79.0	79.0	89.0	85.1	78.1	84.6	86.9	86.7	86.2	79.0	78.1	85.3	72.3	84.1	74.8	74.8	90.0	81.1	74.1	86.9	86.0	86.0	80.9	81.6	72.7	73.0	83.7	74.6	89.3	84.8
5. *S. caprae*					76.5	76.5	83.0	83.0	83.0	77.9	79.7	88.1	86.0	77.2	82.5	89.7	86.5	86.0	77.2	79.7	85.5	71.1	84.6	75.5	76.9	90.4	79.0	72.5	87.4	87.2	87.2	80.0	80.9	71.3	71.6	81.4	73.0	89.7	84.8
6. *S. carnosus* subsp. *carnosus*						99.5	73.9	78.3	78.8	96.8	73.7	77.6	79.0	74.8	80.7	75.1	78.8	79.7	69.7	71.8	79.0	69.9	79.5	70.9	71.8	79.0	93.8	69.0	75.8	78.1	78.1	76.5	76.9	70.8	70.6	88.0	69.4	77.6	78.8
7. *S. carnosus* subsp. *utilis*							73.7	78.1	78.6	96.8	73.7	77.4	79.0	74.6	80.7	74.8	78.6	79.5	69.7	71.8	79.0	69.9	78.8	70.6	71.6	79.0	93.8	69.0	75.8	78.1	78.1	76.0	76.5	70.8	70.6	87.7	69.4	77.6	78.8
8. *S. chromogenes*								80.0	79.3	74.4	77.9	80.4	80.0	75.1	82.8	80.4	80.4	80.2	77.6	78.6	78.3	69.5	80.4	77.2	76.2	83.7	77.9	70.2	78.6	81.6	81.6	78.8	79.3	70.9	70.9	80.0	70.6	81.6	80.9
9. *S. cohnii* subsp. *cohnii*									95.6	80.2	78.3	85.5	88.3	75.1	90.2	85.3	87.9	88.3	76.7	77.2	86.2	70.2	85.3	75.5	74.4	85.3	80.9	72.7	82.3	90.4	90.4	79.0	79.0	73.4	73.4	80.4	73.2	85.1	89.5
10. *S. cohnii* subsp. *urealyticum*										80.2	78.8	85.1	90.4	75.1	91.6	86.5	87.9	88.3	76.5	78.1	87.6	70.9	86.7	76.5	74.1	87.6	80.2	71.8	83.7	91.8	91.8	77.6	77.6	72.7	72.7	80.0	72.3	86.7	90.0
11. *S. condimenti*											74.8	78.8	79.3	75.3	81.8	76.2	80.0	80.9	69.7	72.3	79.3	69.9	79.3	71.3	72.0	79.3	94.7	69.7	75.1	78.6	78.6	75.5	76.0	71.5	71.3	89.1	70.1	78.6	79.5
12. *S. delphini*												77.4	78.6	75.1	78.1	78.3	78.1	78.3	75.8	91.8	79.5	69.7	78.1	81.8	73.0	80.4	75.1	70.2	75.5	80.9	80.9	80.9	80.9	69.5	69.5	76.7	70.2	79.3	78.3
13. *S. epidermidis*													83.4	79.5	84.1	84.6	87.9	88.1	76.2	78.1	84.1	69.5	82.8	74.4	75.8	86.5	79.0	72.5	88.6	83.7	83.7	80.0	79.7	73.7	73.7	80.2	73.0	85.8	84.1
14. *S. equorum*														75.5	89.3	86.9	85.8	85.8	74.1	76.9	89.7	70.2	85.1	73.2	73.9	86.0	81.1	70.6	83.9	93.0	93.0	76.9	77.2	70.6	70.6	83.0	71.1	85.8	91.4
15. *S. felis*															75.1	76.9	76.5	76.5	76.7	74.4	79.7	69.9	79.0	75.1	76.5	76.9	74.1	71.8	75.1	76.9	76.9	76.7	76.7	72.3	71.8	73.4	72.3	76.5	76.7
16. *S. gallinarum*																86.7	88.1	88.6	74.8	79.5	90.0	71.3	87.2	75.8	74.6	87.4	83.7	72.5	82.8	91.8	91.8	77.4	76.9	72.3	72.3	81.6	73.0	86.0	91.4
17. *S. haemolyticus*																	87.6	87.9	74.6	78.1	86.0	69.0	87.6	73.9	78.1	86.7	78.8	70.2	83.7	88.6	88.6	78.6	78.8	70.4	70.6	77.4	70.2	86.0	86.9
18. *S. hominis* subsp. *hominis*																		99.3	75.8	79.0	86.7	70.9	87.4	75.5	79.0	86.0	81.4	73.4	83.7	85.5	85.5	80.0	80.0	73.2	73.0	81.1	73.4	85.5	86.7
19. *S. hominis* subsp. *novobiosepticus*																			75.5	79.0	86.2	71.1	87.6	75.5	78.8	85.5	82.3	73.4	83.4	86.0	86.0	79.5	79.5	73.2	73.0	81.1	73.4	85.5	86.7
20. *S. hyicus*																				75.3	73.9	66.4	73.9	73.7	70.4	76.7	69.5	68.1	75.3	74.8	74.8	74.8	74.8	68.8	69.0	71.1	68.5	76.0	74.4
21. *S. intermedius*																					80.2	68.3	79.5	81.1	74.1	79.5	72.5	68.5	76.9	80.4	80.4	77.4	77.9	67.4	67.4	74.8	68.5	79.3	78.3
22. *S. kloosii*																						72.0	87.6	76.9	75.1	87.4	80.0	73.4	84.8	90.9	90.9	78.1	77.9	73.0	73.0	81.6	73.9	85.3	90.9
23. *S. lentus*																							69.7	68.3	69.5	73.9	68.5	90.0	66.9	72.0	72.0	66.9	67.6	88.9	88.7	69.0	90.0	73.4	70.6
24. *S. lugdunensis*																								76.5	74.8	84.4	79.5	70.4	82.3	86.9	86.9	78.6	79.0	70.2	69.9	77.6	70.9	84.4	84.6
25. *S. lutrae*																									71.3	75.8	72.0	69.7	73.7	74.4	74.4	77.9	78.1	68.1	68.1	70.6	69.7	72.7	74.6
26. *S. muscae*																										76.0	71.3	70.4	74.4	74.4	74.4	74.6	74.4	71.6	71.1	72.3	70.9	74.4	74.4
27. *S. pasteuri*																											80.7	73.7	86.2	88.1	88.1	81.6	82.1	75.5	75.8	83.7	74.1	94.4	86.5
28. *S. piscifermentans*																												70.4	75.8	80.2	80.2	78.1	78.1	70.8	70.6	90.7	70.8	79.5	80.0
29. *S. pulvereri*																													69.9	73.2	73.2	69.5	69.9	93.5	93.3	68.3	99.5	74.1	73.0
30. *S. saccharolyticus*																														85.1	85.1	81.1	80.9	71.6	71.8	78.1	70.4	84.4	84.8
31. *S. saprophyticus* subsp. *bovis*																															100.0	79.0	79.0	72.7	72.7	80.7	73.7	87.6	94.4
32. *S. saprophyticus* subsp. *saprophyticus*																																79.0	79.0	72.7	72.7	80.7	73.7	87.6	94.4
33. *S. schleiferi* subsp. *coagulans*																																	98.8	69.5	69.7	78.3	69.9	79.7	78.6
34. *S. schleiferi* subsp. *schleiferi*																																		69.0	69.2	79.0	70.4	80.4	78.6
35. *S. sciuri* subsp. *carnaticus*																																			99.3	69.0	93.5	74.4	72.0
36. *S. sciuri* subsp. *sciuri*																																				69.0	93.3	74.4	72.0
37. *S. simulans*																																					68.8	82.5	81.1
38. *S. vitulus*																																						74.6	73.4
39. *S. warneri*																																							84.4
40. *S. xylosus*																																							

[a] The main characteristics of the strains are listed in Table 1.
[b] The strain numbers correspond to the strains identified by the numbers on the left.

ventional microbiological tests, the ID 32 Staph system, and the $sodA_{int}$ system (Table 2). In all cases, the $sodA_{int}$ sequences of the isolates displayed less than 1.5% divergence with that of the corresponding type strain (Table 2). For 14 strains (NEM1999, NEM2000, NEM2002, NEM2004, NEM2006, NEM2008, NEM2009, NEM2010, NEM2011, NEM2013, NEM2014, NEM2016, NEM2021, and NEM2022), the two methods gave the same results. The remaining 12 isolates were not identified at the species level, or were misidentified, by the conventional microbiological phenotypic tests. These strains were correctly identified by the $sodA_{int}$ system. For example, NEM1997 and NEM1998 were identified by the ID 32 Staph system as *S. capitis* although they were novobiocin resistant and oxidase positive. Both strains were identified as *M. caseolyticus* following $sodA_{int}$-based sequence analysis. NEM2001, NEM2003, NEM2004, NEM2015, and NEM2018 were formerly misidentified by conventional methods as *S. hominis*, *S. epidermidis*, *S. haemolyticus*, *S. warneri*, and *S. epidermidis*, respectively. They were subsequently unambiguously identified with the *sodA* system as *S. capitis*, *S. caprae*, *S. pasteuri*, and *S. simulans*, respectively. These latter species are often misidentified by conventional methods (10), and genotypic methods are often necessary to identify these uncommon species. Lastly, NEM2005, NEM2012, NEM2017, NEM2019, and NEM2020, which were not identified by the ID 32 Staph system, were identified by the $sodA_{int}$ method as *S. caprae*, *S. haemolyticus*, *S. schleiferi*, *S. vitulus*, and *S. warneri*, respectively.

Conclusions. We have determined the $sodA_{int}$ sequences of 40 type strains of CNS and demonstrated the usefulness of this database for species-level identification of staphylococcal isolates. This method consists of a PCR carried out with a single pair of degenerate oligonucleotides for amplification of a staphylococcal $sodA_{int}$ fragment and direct sequencing of the PCR product with the same degenerate primers. Under these conditions, the delay required for bacterial identification is less than 24 h. This method might be useful in reference laboratories for characterization of strains that could not be assigned to a species on the basis of their conventional phenotypic reaction. Furthermore, the *sodA* sequence polymorphisms observed with staphylococcal species offer good opportunities for the development of assays based on DNA chip technologies.

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