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Identification of *Streptococcus sanguinis* with a PCR-Generated Species-Specific DNA Probe

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The objective of the present study was to design a PCR-generated DNA probe and determine the specificity of the probe for the identification of clinical isolates of *Streptococcus sanguinis*. To do this, we examined over 200 arbitrarily primed PCR (AP-PCR) amplicon patterns obtained with DNA from clinical isolates of *S. sanguinis*. A 1.6-kb DNA amplicon that was common to all AP-PCR profiles was extracted from agarose gels and then cloned and sequenced. A search for a similar sequence in the GenBank database with the BLASTN program revealed that the 1.6-kb DNA fragment comprised an intergenic region between two housekeeping genes, *uncC* (proton-translocating ATPase) and *murA* (UDP-*N*-acetylglucosamine enolpyruvyl transferase). Three digoxigenin-labeled DNA probes were synthesized on the basis of the sequence of the 1.6-kb fragment: the sequence of probe SSA-1 contained the proton-translocating ATPase (*uncC*) and the entire intergenic region, the sequence of probe SSA-2 contained only the intergenic region, and the sequence of probe SSA-3 contained an internal region of the *murA* gene. Dot blot hybridization showed that the three probes displayed signals for hybridization to both *S. sanguinis* strain ATCC 10556 and the *S. sanguinis* clinical isolates. Probe SSA-1, however, hybridized to DNA from *S. oralis* and *S. mitis*. Probe SSA-3 hybridized to DNA from *S. gordonii*, *S. mitis*, *S. oralis*, *S. parasanguinis*, and *S. vestibularis*. The probe SSA-2-specific intergenic region appeared to be specific for *S. sanguinis*. The results from this study suggest that probe SSA-2 may serve as a species-specific DNA probe for the identification of clinical isolates of *S. sanguinis*.

The heterogeneous group of oral streptococci collectively named *Streptococcus sanguinis* (formerly *S. sanguis*) are members of the indigenous oral biota colonizing dental plaque (6, 25). *S. sanguinis* first colonizes an infant's oral cavity at about 9 months of age (8) and may serve a protective or antagonistic role against the cariogenic bacterium *S. mutans* (8, 26, 27). On the other hand, *S. sanguinis* may also cause life-threatening bacterial endocarditis (10) and septicemia (15).

Previous methods for identifying *S. sanguinis* are based primarily on physiological and biochemical characteristics. The reliability and reproducibility of the conventional phenotypic identification, however, varied among methodologies and investigators (5, 21). For example, previous studies were unable to demonstrate agreement between the genotypic and phenotypic methods for identifying clinical *S. sanguinis* isolates (8, 30). Accordingly, other methods are being examined, including those that combine PCR with nucleic acid probes for detection and identification of *S. sanguinis* and other oral bacteria (14, 17, 19, 22, 23, 29, 35, 36, 37).

In the present study, we used a PCR-based approach to develop a DNA probe for identifying *S. sanguinis* based on a common amplicon present on arbitrarily primed PCR (AP-PCR) profiles. The specificity of this probe was tested against a panel of previously confirmed clinical isolates of *S. sanguinis* (8, 30). The results of the study suggest that this species-specific probe may serve as a useful tool in the identification of *S. sanguinis* from clinical samples.

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MATERIALS AND METHODS

Bacterial strains. Sixteen reference strains obtained from the American Type Culture Collection (ATCC; Manassas, Va.) were included in this study (Table 1). Two other reference strains, *S. pneumoniae* WU2 and *Escherichia coli* JM109, were obtained from J. Yother at the University of Alabama at Birmingham. *S. sanguinis* strain ATCC 10556 was selected as a positive control. Strains representing other species served as negative controls. An additional 78 clinical isolates of *S. sanguinis* that were confirmed to be *S. sanguinis* strains by biochemical tests and 16S rRNA gene (rDNA) sequence analyses in a previous study (8, 30) were also included. The 78 clinical isolates with unique genotypes were collected from 16 individuals who visited the maternity and pediatric clinics at the Jefferson County Department of Health in Birmingham, Alabama. The details of the sample collection procedure and *S. sanguinis* isolation have been reported in previous studies (8, 30). Briefly, saliva samples and dental plaques were collected, dispersed, and plated onto MM10-sucrose agar (41). *S. sanguinis* was initially identified on the basis of its distinct colony morphology on MM10-sucrose medium (8, 25, 41), and its identity was then confirmed by biochemical tests (30). Sixteen (20%) of those clinical *S. sanguinis* isolates (listed in Table 1) were randomly selected and further confirmed to be *S. sanguinis* according to their 16S rDNA sequences.

Genomic DNA isolation. Genomic DNA was isolated from overnight cultures grown in Todd-Hewitt broth at 37°C in an anaerobic chamber (85% N_2, 10% CO_2, 5% H_2) with a commercially available DNA extraction kit (Wizard Genomic DNA Purification Kit; Promega Corp. Madison, Wis.), according to the instructions of the manufacturer. Purified DNA was dissolved in 10 mM Tris-HCl buffer containing 1 mM EDTA (pH 8.0); the final concentrations were adjusted spectrophotometrically to 50 μg/ml.

AP-PCR experiments. AP-PCR was performed with all DNA samples by previously described methods (22, 23, 30). A total of 40 commercially available single-stranded 10-mer oligonucleotide primers (Kit A and Kit B; Operon Technologies, Inc., Alameda, Calif.) were screened for their suitabilities in differentiating *S. sanguinis* strains from non-*S. sanguinis* strains. Because primer OPA-02

TABLE 1. Bacterial species and clinical isolates tested in this study

Bacterial species and strain	Source of isolation
Reference species	
S. sanguinis ATCC 10556	Patient with bacterial endocarditis
S. oralis ATCC 10557	Patient with bacterial endocarditis
S. oralis ATCC 35037	Human mouth
S. oralis ATCC 9811	Human mouth
S. gordonii ATCC 10558	Patient with bacterial endocarditis
S. cristatus ATCC 49999	Coronal dental plaque
S. mitis ATCC 903 (biovar 2)	Ulcerated sore throat
S. parasanguinis ATCC 15911	Human throat
S. pneumoniae[a] WU2	Normal mouse serum
S. mutans ATCC 25175	Human carious dentine
S. salivarius ATCC 7073	Patient with acute articular rheumatism
S. sobrinus ATCC 33478	Human dental plaque
S. ratti ATCC 19645	Caries lesion in rat
S. vestibularis ATCC 49124	Human oral cavity
Lactobacillus acidophilus ATCC 4356	Human mouth
Actinomyces naeslundii ATCC 12104	Human sinus
E. coli JM109	
S. sanguinis clinical isolates[b]	
UASa14	18-mo-old boy
UASa22	24-mo-old girl
UASa25	24-mo-old boy
UASa30	24-mo-old boy
UASa33	18-mo-old boy
UASa38	24-mo-old boy
UASa39	15-mo-old boy
UASa42	18-mo-old girl
UASa43	24-mo-old boy
UASa44	18-mo-old boy
UASa49	18-mo-old girl
UASa51	24-mo-old girl
UASa56	24-mo-old boy
UASa57	24-mo-old boy
UASa70	15-mo-old girl
UASa88	24-mo-old girl

[a] The 16S rRNA sequence of *S. pneumoniae* was used as a reference (20).
[b] All clinical isolates of *S. sanguinis* were isolated from the dental plaque and initially identified on the basis of their phenotypes and their identities were then confirmed by biochemical and genotypic tests (30).

(5′-TGCCGAGCTG-3′) was able to produce discriminative amplification patterns (data not shown), it was selected for use in this study. All amplification reactions were conducted in a thermal cycler (GeneAmp 2400; Applied Biosystems, Foster City, Calif.) with a total volume of 50 μl containing 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl [pH 8.3]), 200 μM each nucleotide, 100 pmol of primer OPA-02, 2.5 U of *Taq* polymerase, 3.5 mM $MgCl_2$, and 50 ng of purified template DNA. The temperature profile was 40 cycles at 94°C for 1 min, 36°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 5 min. The resulting AP-PCR amplicons were separated on a 1.5% agarose gel in TBE (Tris-borate-EDTA) buffer and stained with an ethidium bromide solution (1 μg/ml). The final images of the gels were captured with a digital camera and saved in the tagged image file format for further comparisons.

DNA cloning and sequencing. DNA amplification demonstrated that all *S. sanguinis* strains tested contained a 1,653-bp fragment (Fig. 1). This fragment was excised from the agarose gel and eluted by using the QIAquick gel extraction kit (QIAGEN Inc., Santa Clarita, Calif.). The DNA fragment was cloned (TA cloning kit; Invitrogen, Carlsbad, Calif.) and transformed into *E. coli*, and the insert was sequenced in both directions. A sequence similarity search of the nonredundant GenBank database was preformed by using BLASTN program (National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Md.) (2). Putative open reading frames (ORFs) were identified by using the ORF Finder program (National Center for Biotechnology Information).

Generation of DNA-based probes and dot hybridization. The results of the BLASTN search followed by the identification of ORFs with the ORF Finder revealed that the 1,653-bp amplicon contained three regions: portions of two housekeeping genes and an intergenic region. Accordingly, three DNA probes were designed for PCR amplification of these three regions, as illustrated in Fig. 2. The sequences and positions of the primers used to construct the probes are listed in Table 2. The three PCR products were labeled with digoxigenin, and dot hybridization was conducted according to the instructions of the manufacturer (Digoxigenin-High Prime DNA Labeling and Detection Starter Kit I; Roche Molecular Biochemicals, Indianapolis, Ind.). Briefly, heat-denatured chromosomal DNA (1 μg) was applied to a positively charged nylon membrane through the wells of Bio-Dot apparatus (Bio-Rad Laboratories, Hercules, Calif.). The filter was baked at 120°C for 30 min and then hybridized with a digoxigenin-labeled probe at 68°C (no formamide) or at 42°C (containing 50% formamide), washed at 68°C in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% sodium dodecyl sulfate (highly stringent conditions), and visualized by colorimetric detection with nitroblue tetrazolium–5-bromo-4-chloro-3-indolylphosphate.

Primer design. Three sets of primers were designed on the basis of the sequence of the 1,653-bp fragment from *S. sanguinis* type strain ATCC 10556. The forward primer, primer F1 (5′-GATTGACCAAGAACGCCGGGCT-3′), was derived from nucleotides (nt) 36 to 57 of the fragment. The reverse primer, primer R3 (5′-CGCATGATATCAGAGATGCAACCC-3′), was derived from nt 1618 to 1640 of the fragment. The specificities of the primers for all 20 reference and 78 clinical strains were tested. All amplification reactions were performed by a standardized PCR protocol, as described above, except that the temperature profile consisted of 30 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. The PCR products were separated in 1% agarose gels and stained with ethidium bromide.

Three clinical isolates (isolates UASa11, UASa22, and UASa33) representing three *S. sanguinis* biovars (30) were selected for sequencing after the amplification. The 1,653-bp amplicons generated by PCR were isolated with the QIAquick PCR purification kit (QIAGENE, Valencia, Calif.) and sequenced in both directions. The sequences were aligned (MacVector, version 7.1.1; Accelrys, Madison, Wis.) to compare the 1,653-bp sequences of the *S. sanguinis* strains of different biovars with that of *S. sanguinis* type strain ATCC 10556.

Nucleotide sequence accession number. The 1,653-bp sequence from *S. sanguinis* ATCC 10556 is available in the GenBank database under accession number AF343003. The sequence of the intergenic region of *S. sanguinis* is available in the GenBank database under accession number AY277586.

RESULTS

The AP-PCR fingerprints demonstrated that a 1.6-kb amplicon could be consistently observed in the PCR profiles of type strain ATCC 10556 and all other strains of *S. sanguinis* tested (Fig. 1). In contrast, this fragment was not present in the AP-PCR fingerprints of other mitis group streptococcal species. Sequencing of the 1,653-bp DNA fragment revealed two partial ORFs with high degrees of similarity to other *Streptococcus* species; one was from nt 1 to 111 and the other was from nt 583 to 1653. A search with the BLASTN program found that the region from nt 1 to 111 bore a high degree of similarity (92%) to the distal portion of the proton-translocating ATPase *uncC* gene of *S. sanguinis* reported by Quivey et al. (34) (GenBank accession no. AF001955). The second ORF (nt 583 to 1653) closely resembled *murA* (UDP-*N*-acetylglucosamine enolpyruvyl transferase) from *S. pneumoniae*, *S. pyogenes*, and *S. mutans*, among others. A putative noncoding intergenic region was found between the two housekeeping genes.

On the basis of the sequence of the 1.6-kb fragment, three digoxigenin-labeled DNA probes were synthesized: the sequence of probe SSA-1 contained the proton-translocating ATPase (*uncC*) and the entire intergenic region, the sequence

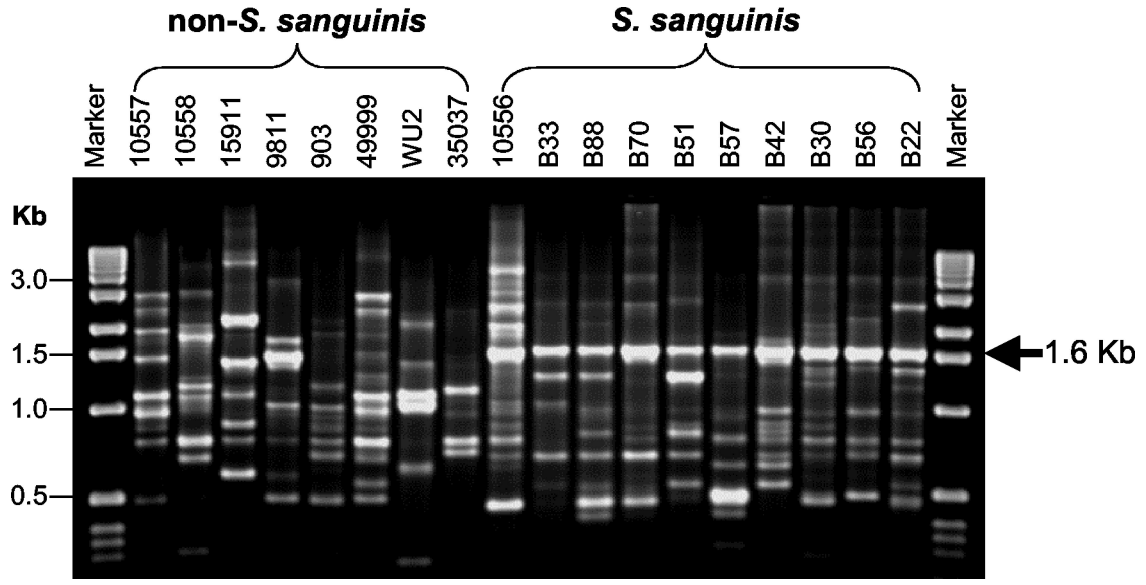

FIG. 1. AP-PCR fingerprint profiles generated from *S. sanguinis*, other oral mitis group species, and *S. sanguinis* clinical isolates. AP-PCR results were obtained by amplification of genomic DNA with primer OPA-02. A 1.6-kb amplicon was observed for all *S. sanguinis* strains. Other reference strains of the mitis group showed different AP-PCR patterns and the absence of a 1.6-kb amplicon. The non-*S. sanguinis* strains tested were as follows: 10557, *S. oralis*; 10558, *S. gordonii*; 15911, *S. parasanguinis*; 9811, *S. oralis*; 903, *S. mitis*; 49999, *S. cristatus*; WU2, *S. pneumoniae*; 35037, *S. oralis*.

of probe SSA-2 contained only the intergenic region, and the sequence of probe SSA-3 contained an internal region of the *murA* gene. The stringency of hybridization was examined by dot blot hybridization with the genomic DNA of *S. sanguinis* and the other species listed in Table 1. The results showed that all three probes hybridized to the DNA of the type strain *S. sanguinis* ATCC 10556 (Fig. 3A to C). Probe SSA-1 (Fig. 3A) and probe SSA-3 (Fig. 3C) weakly hybridized to most of the *Streptococcus* species, including *S. oralis* (ATCC 10557), *S. gordonii* (ATCC 10558), *S. oralis* (ATCC 9811), *S. parasanguinis* (ATCC 15911), and *S. vestibularis* (ATCC 49124) but did not hybridize to actinomyces, lactobacilli, or *E. coli*.

Probe SSA-2, whose sequence spans the intergenic region, hybridized to the genomic DNA of *S. sanguinis* ATCC 10556 (Fig. 3B) as well as to the genomic DNA of all clinical *S. sanguinis* isolates (Fig. 3D). No cross-hybridization was observed with other representative isolates of common oral streptococci, indicating a high degree of specificity of probe SSA-2 to *S. sanguinis*. An extensive search of the GenBank database with the probe SSA-2 sequence failed to reveal similarities to any DNA, with *E* value greater than 0.2. The ORF Finder suggested the presence of a putative ORF within the intergenic region (nt 285 to 515) which resembled a histidine kinase from *S. pneumoniae* and *S. pyogenes* and a hypothetical protein from *S. pyogenes*, *S. mutans*, and *S. agalactiae* (*E* value less than 2e-05). Nonetheless, the identity between the theoretical protein sequences of the probe and those predicted from genome sequences did not exceed 31%, and the nucleotide match was even less. Indeed, the nucleotide sequence of the intergenic region bore little or no similarity to sequences in the GenBank and failed to hybridize to probe SSA-2, whose sequence contains the entire intergenic region.

A set of primers (primers F1 and R3) was designed to test whether the presence of the 1.6-kb fragment could be used to identify strains of *S. sanguinis*. The results showed that only *S. sanguinis* isolates (78 strains) exhibited the 1,653-bp fragment

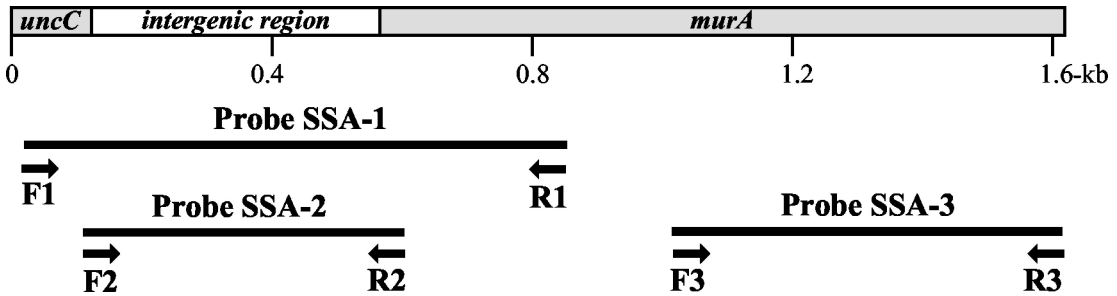

FIG. 2. Locus and composition of DNA-based probes used for dot hybridization. The three probes were designed on the basis of the sequence of a 1,653-bp fragment from strains of *S. sanguinis.* The sequence of probe SSA-1 comprised a portion of the first ORF (*uncC* gene) and the entire intergenic region, the sequence of probe SSA-2 comprised only the intergenic region, and the sequence of probe SSA-3 comprised an internal region of the second ORF (*murA* gene).

TABLE 2. Sequences and positions of primers for PCR-based probe construction

Probe	Primer sequence	Sequence position
SSA-1	F1: 5′-GATTGACCAAGAACGCCGGGCT-3′	36–57
	R1: 5′-CCTCCTCAGACAAAGGCTGGGTGGCAT-3′	841–815
SSA-2	F2[a]: 5′-GAAGCCATTTTGCCTAGATTGATGG-3′	112–136
	R2: 5′-CCATACCGATTCCTTACTCTAAATTT-3′	586–561
SSA-3	F3: 5′-CGAAGCAAAGGCAGAGCGGCTCAAGGG-3′	1026–1052
	R3: 5′-CGCATGATATCAGAGTGCAACCC-3′	1640–1618

[a] F2 is 2 nucleotides outside the ATPase coding sequence.

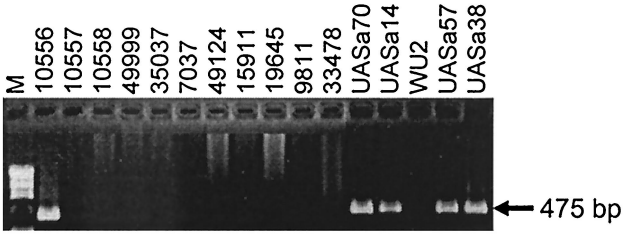

FIG. 4. Specific amplification of the 475-bp intergenic region from *S. sanguinis* with primers F2 and R2. The agarose gel shows the presence of the PCR amplicon for ATCC 10556 and the *S. sanguinis* clinical isolates and the absence of the PCR amplicon for the other non-*S. sanguinis* strains tested.

on 1% agarose gels, as predicted, and no PCR products were detected among the 20 other species strains tested (data not shown). Furthermore, a specific set of PCR primers (primers F2 and R2) was designed to amplify the 475-bp intergenic region, as shown in Fig. 4. DNA from three biovars of *S. sanguinis* clinical isolates (30) was amplified by PCR with these primers and then sequenced. Although identical in size, the sequences of the three PCR amplicons showed the presence of 12 to 18 polymorphic sites compared with the sequence of ATCC 10556, with a 4% variation in nucleotide sequence found among the three biovars of *S. sanguinis* isolates (data not shown).

DISCUSSION

The accurate identification of *S. sanguinis* has been problematic. Conventional identification protocols are usually time-consuming and exhibit ambiguities when they are used to differentiate among the members of the mitis group (21, 32). Several new PCR-based methods show promise in identifying isolates to the species level (1, 11, 12, 20, 35, 43). For example, Garnier and coworkers (11) described PCR primers based on internal fragments of the genes encoding D-alanine-D-ligases for the identification of clinically relevant viridans group streptococcal species. Recently, Rudney and Larson (35) developed AP-PCR protocols for the identification of members of the mitis group, including strains of *S. sanguinis*. However, the use of only the PCR-based typing method proved ineffective for differentiation of the members of the mitis group. Thus, the combination of a PCR-based typing method and DNA-based probe hybridization might increase the overall accuracy of bacterial species delineation (35).

The 16S rDNA locus in bacteria has been widely used as a target for PCR primers or probes for the identification of numerous microorganisms in cerebrospinal fluid, the gastrointestinal tract, and other sources (12, 43). Several studies have included the rDNA locus to identify *S. sanguinis* from clinical specimens (8, 20, 30, 33, 35). The probes that were used were usually based on variable regions within the 16S rDNA (3), but such regions often differ by only a few base pairs, especially among the members of the mitis group (20). In fact, Jacobs et

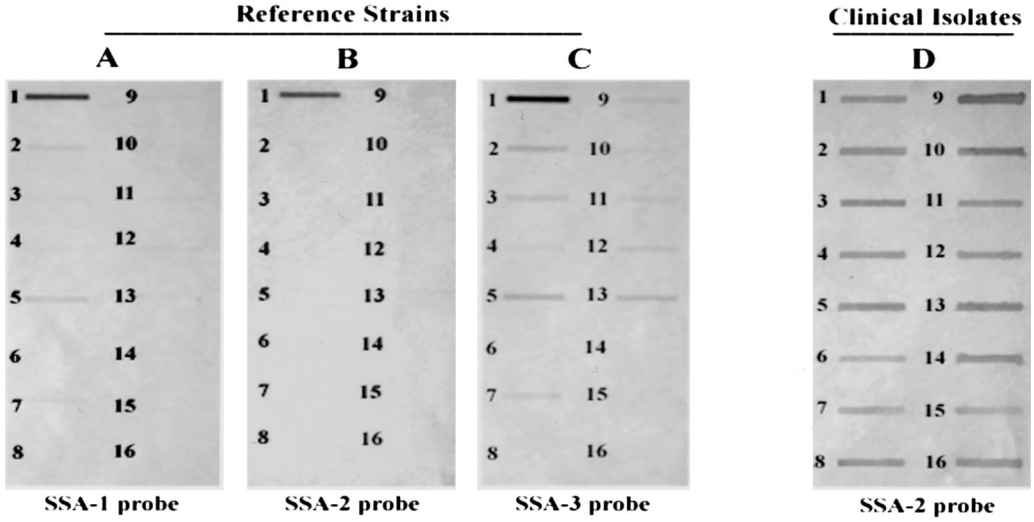

FIG. 3. Dot blot hybridization shows the specificities of the three probes hybridized with different *Streptococcus* reference strains as well as with *S. sanguinis* clinical isolates. Probe SSA-2 was specific for type strain ATCC 10556 (B) and all *S. sanguinis* clinical isolates (D). SSA-1 and SSA-3 showed different degrees of hybridization to other species (A and C). The bacterial strains tested were as follows: 1, *S. sanguinis*; 2, *S. oralis*; 3, *S. gordonii*; 4, *S. cristatus*; 5, *S. oralis*; 6, *S. mitis*; 7, *S. parasanguinis*; 8, *S. pneumoniae*; 9, *S. mutans*; 10, *S. salivarius*; 11, *S. sobrinus*; 12, *S. ratti*; 13, *S. vestibularis*; 14, *A. naeslundii*; 15, *L. acidophilus*; 16, *E. coli* JM109.

al. (18) reported that the use of oligonucleotide probes specific for the 16S rRNA sequences of *S. anginosus*, *S. constellatus*, and *S. intermedius* resulted in the reaction of a large number of strains with both the *S. constellatus*- and the *S. intermedius*-specific probes. Among the members of the mitis group, the degrees of similarity, based on comparison of 16S rDNA sequences, between the species ranged from 96 to 99% (20), indicating an increased risk of false-positive results in hybridization reactions. Therefore, the development of a highly sensitive and specific DNA-based probe assay for the identification of *S. sanguinis* adds a potentially valuable new tool for the early detection and identification of *S. sanguinis* infection and colonization. Similar approaches have previously been reported by other investigators and successfully applied to the species identification of several microorganisms, such as *Prevotella*, *Porphyromonas*, *Legionella*, *Candida*, and *Bacteroides* (13, 14, 28, 40, 44).

In our study, we demonstrated that a specific PCR-generated probe and a set of primers could identify *S. sanguinis* from a subset of clinical isolates previously suggested to be members of the *S. sanguinis* complex. Although our findings show that the specifically designed probe SSA-2 possessed accuracy in discriminating *S. sanguinis* from the mitis group, further research is needed to determine whether the *S. sanguinis*-specific PCR probe or primer set described here could yield both a high degree of sensitivity and a high degree of specificity in detecting *S. sanguinis* from whole plaque samples containing hundreds of different phylotypes or species. One strategy would be to examine plaque samples from pre- and postdentate infants for *S. sanguinis*, as *S. sanguinis* has been shown to colonize the oral cavity before the emergence of teeth (6, 8, 24, 38), and then to monitor the oral cavities of infants for colonization with *S. sanguinis*. This, in turn, might then be used to predict the time to colonization with the mutans group of streptococci (8) and, perhaps, the risk for dental caries (7, 9, 24). Incorporation of a signature sequence unique to *S. sanguinis* into a microarray-type or reverse checkerboard assay (4, 31, 42) might be a convenient way to assay plaque samples for the presence or absence of *S. sanguinis*. Also noteworthy is the fact that the presence of *S. sanguinis* has been shown to be associated with health in periodontal diseases (16), suggesting that its activity is antagonistic against periodontopathogens, similar to that against mutans group streptococci shown previously (8, 16, 39).

In conclusion, because the PCR-generated DNA probes can precisely identify *S. sanguinis* at the species level, application of these species-specific DNA markers will provide valuable tools for the early detection of *S. sanguinis* colonization and facilitate epidemiological study of its interaction with other oral microbes associated with caries progression and periodontal diseases.

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REFERENCES

1. **Alam, S., S. R. Brailsford, R. A. Whiley, and D. Beighton.** 1999. PCR-based methods for genotyping viridans group streptococci. J. Clin. Microbiol. **37:** 2772–2776.
2. **Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
3. **Amann, R. I., L. Krumholz, and D. A. Stahl.** 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J. Bacteriol. **172:**762–770.
4. **Becker, M. R., B. J. Paster, E. J. Leys, M. L. Moeschberger, S. G. Kenyon, J. L. Galvin, S. K. Boches, F. E. Dewhirst, and A. L. Griffen.** 2002. Molecular analysis of bacterial species associated with childhood caries. J. Clin. Microbiol. **40:**1001–1009.
5. **Beighton, D., J. M. Hardie, and R. A. Whiley.** 1991. A scheme for the identification of viridans streptococci. J. Med. Microbiol. **35:**367–372.
6. **Carlsson, J., H. Grahnen, G. Jonsson, and S. Wikner.** 1970. Establishment of *Streptococcus sanguis* in the mouths of infants. Arch. Oral Biol. **15:**1143–1148.
7. **Caufield, P. W., G. R. Cutter, and A. P. Dasanayake.** 1993. Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. J. Dent. Res. **72:**37–45.
8. **Caufield, P. W., A. P. Dasanayake, Y. Li, Y. Pan, J. Hsu, and J. M. Hardin.** 2000. Natural history of *Streptococcus sanguinis* in the oral cavity of infants: evidence for a discrete window of infectivity. Infect. Immun. **68:**4018–4023.
9. **De Stoppelaar, J. D., J. Van Houte, and O. Backer Dirks.** 1969. The relationship between extracellular polysaccharide-producing streptococci and smooth surface caries in 13-year-old children. Caries Res. **3:**190–199.
10. **Douglas, C. W., J. Heath, K. K. Hampton, and F. E. Preston.** 1993. Identity of viridans streptococci isolated from cases of infective endocarditis. J. Med. Microbiol. **39:**179–182.
11. **Garnier, F., G. Gerbaud, P. Courvalin, and M. Galimand.** 1997. Identification of clinically relevant viridans group streptococci to the species level by PCR. J. Clin. Microbiol. **35:**2337–2341.
12. **Greisen, K., M. Loeffelholz, A. Purohit, and D. Leong.** 1994. PCR primers and probes for the 16S rRNA gene of most species of pathogenic bacteria, including bacteria found in cerebrospinal fluid. J. Clin. Microbiol. **32:**335–351.
13. **Guillot, E., and C. Mouton.** 1996. A PCR-DNA probe assay specific for *Bacteroides forsythus*. Mol. Cell. Probes **10:**413–421.
14. **Guillot, E., and C. Mouton.** 1997. PCR-DNA probe assays for identification and detection of *Prevotella intermedia* sensu stricto and *Prevotella nigrescens*. J. Clin. Microbiol. **35:**1876–1882.
15. **Herzberg, M. C., K. Gong, G. D. MacFarlane, P. R. Erickson, A. H. Soberay, P. H. Krebsbach, G. Manjula, K. Schilling, and W. H. Bowen.** 1990. Phenotypic characterization of *Streptococcus sanguis* virulence factors associated with bacterial endocarditis. Infect. Immun. **58:**515–522.
16. **Hillman, J. D., S. S. Socransky, and M. Shivers.** 1985. The relationships between streptococcal species and periodontopathic bacteria in human dental plaque. Arch. Oral Biol. **30:**791–795.
17. **Ida, H., T. Igarashi, A. Yamamoto, N. Goto, and R. Sasa.** 1999. A DNA probe specific to *Streptococcus sobrinus*. Oral. Microbiol. Immunol. **14:**233–237.
18. **Jacobs, J. A., C. S. Schot, A. E. Bunschoten, and L. M. Schouls.** 1996. Rapid species identification of "*Streptococcus milleri*" strains by line blot hybridization: identification of a distinct 16S rRNA population closely related to *Streptococcus constellatus*. J. Clin. Microbiol. **34:**1717–1721.
19. **Kaufhold, A., A. Podbielski, G. Baumgarten, M. Blokpoel, J. Top, and L. Schouls.** 1994. Rapid typing of group A streptococci by the use of DNA amplification and non-radioactive allele-specific oligonucleotide probes. FEMS Microbiol. Lett. **119:**19–25.
20. **Kawamura, Y., X. G. Hou, F. Sultana, H. Miura, and T. Ezaki.** 1995. Determination of 16S rRNA sequences of *Streptococcus mitis* and *Streptococcus gordonii* and phylogenetic relationships among members of the genus *Streptococcus*. Int. J. Syst. Bacteriol. **45:**406–408.
21. **Kilian, M., L. Mikkelsen, and J. Henrichsen.** 1989. Taxonomic study of viridans streptococci: description of *Streptococcus gordonii* sp. nov. and emended descriptions of *Streptococcus sanguis*, *Streptococcus oralis*, and *Streptococcus mitis*. Int. J. Syst. Bacteriol. **39:**471–484.
22. **Li, Y., and P. W. Caufield.** 1998. Arbitrarily primed polymerase chain reaction fingerprinting for the genotypic identification of mutans streptococci from humans. Oral Microbiol. Immunol. **13:**17–22.
23. **Li, Y., P. W. Caufield, I. R. Emanuelsson, and E. Thornqvist.** 2001. Differentiation of *Streptococcus mutans* and *Streptococcus sobrinus* via genotypic and phenotypic profiles from three different populations. Oral Microbiol. Immunol. **16:**16–23.
24. **Loesche, W. J., S. Eklund, R. Earnest, and B. Burt.** 1984. Longitudinal investigation of bacteriology of human fissure decay: epidemiological studies in molars shortly after eruption. Infect. Immun. **46:**765–772.
25. **Loesche, W. J., J. Rowan, L. H. Straffon, and P. J. Loos.** 1975. Association of *Streptococcus mutans* with human dental decay. Infect. Immun. **11:**1252–1260.
26. **Loesche, W. J., and S. A. Syed.** 1973. The predominant cultivable flora of carious plaque and carious dentine. Caries Res. **7:**201–216.
27. **Loesche, W. J., A. Walenga, and P. Loos.** 1973. Recovery of *Streptococcus*

mutans and *Streptococcus sanguis* from a dental explorer after clinical examinations of single human teeth. Arch. Oral Biol. **18:**571–575.
28. **Lo Presti, F., S. Riffard, F. Vandenesch, and J. Etienne.** 1998. Identification of *Legionella* species by random amplified polymorphic DNA profiles. J. Clin. Microbiol. **36:**3193–3197.
29. **Menard, C., P. Gosselin, J. F. Duhaime, and C. Mouton.** 1994. Polymerase chain reaction using arbitrary primer for the design and construction of a DNA probe specific for *Porphyromonas gingivalis*. Res. Microbiol. **145:**595–602.
30. **Pan, Y. P., Y. Li, and P. W. Caufield.** 2001. Phenotypic and genotypic diversity of *Streptococcus sanguis* in infants. Oral Microbiol. Immunol. **16:** 235–242.
31. **Papapanou, P. N., P. N. Madianos, G. Dahlen, and J. Sandros.** 1997. "Checkerboard" versus culture: a comparison between two methods for identification of subgingival microbiota. Eur. J. Oral Sci. **105:**389–396.
32. **Pearce, C., G. H. Bowden, M. Evans, S. P. Fitzsimmons, J. Johnson, M. J. Sheridan, R. Wientzen, and M. F. Cole.** 1995. Identification of pioneer viridans streptococci in the oral cavity of human neonates. J. Med. Microbiol. **42:**67–72.
33. **Poyart, C., G. Quesne, S. Coulon, P. Berche, and P. Trieu-Cuot.** 1998. Identification of streptococci to species level by sequencing the gene encoding the manganese-dependent superoxide dismutase. J. Clin. Microbiol. **36:** 41–47.
34. **Quivey, R. G., Jr., R. C. Faustoferri, W. A. Belli, and J. S. Flores.** 1991. Polymerase chain reaction amplification, cloning, sequence determination and homologies of streptococcal ATPase-encoding DNAs. Gene **97:**63–68.
35. **Rudney, J. D., and C. J. Larson.** 1999. Identification of oral mitis group streptococci by arbitrarily primed polymerase chain reaction. Oral Microbiol. Immunol. **14:**33–42.
36. **Rudney, J. D., E. K. Neuvar, and A. H. Soberay.** 1992. Restriction endonuclease-fragment polymorphisms of oral viridans streptococci, compared by conventional and field-inversion gel electrophoresis. J. Dent. Res. **71:**1182–1188.
37. **Schmidhuber, S., W. Ludwig, and K. H. Schleifer.** 1988. Construction of a DNA probe for the specific identification of *Streptococcus oralis*. J. Clin. Microbiol. **26:**1042–1044.
38. **Smith, D. J., J. M. Anderson, W. F. King, J. van Houte, and M. A. Taubman.** 1993. Oral streptococcal colonization of infants. Oral Microbiol. Immunol. **8:**1–4.
39. **Socransky, S. S., A. D. Haffajee, C. Smith, and S. Dibart.** 1991. Relation of counts of microbial species to clinical status at the sampled site. J. Clin. Periodontol. **18:**766–775.
40. **Steffan, P., J. A. Vazquez, D. Boikov, C. Xu, J. D. Sobel, and R. A. Akins.** 1997. Identification of *Candida* species by randomly amplified polymorphic DNA fingerprinting of colony lysates. J. Clin. Microbiol. **35:**2031–2039.
41. **Syed, S. A., and W. J. Loesche.** 1973. Efficiency of various growth media in recovering oral bacterial flora from human dental plaque. Appl. Microbiol. **26:**459–465.
42. **Tanner, A. C., P. M. Milgrom, R. Kent, Jr., S. A. Mokeem, R. C. Page, C. A. Riedy, P. Weinstein, and J. Bruss.** 2002. The microbiota of young children from tooth and tongue samples. J. Dent. Res. **81:**53–57.
43. **Tannock, G. W., A. Tilsala-Timisjarvi, S. Rodtong, J. Ng, K. Munro, and T. Alatossava.** 1999. Identification of *Lactobacillus* isolates from the gastrointestinal tract, silage, and yoghurt by 16S-23S rRNA gene intergenic spacer region sequence comparisons. Appl. Environ. Microbiol. **65:**4264–4267.
44. **Teng, L. J., P. R. Hsueh, J. C. Tsai, F. L. Chiang, C. Y. Chen, S. W. Ho, and K. T. Luh.** 2000. PCR assay for species-specific identification of *Bacteroides thetaiotaomicron*. J. Clin. Microbiol. **38:**1672–1675.