Cloning of the *Streptococcus gordonii* PK488 Gene, Encoding an Adhesin Which Mediates Coaggregation with *Actinomyces naeslundii* PK606

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Coaggregation between *Streptococcus gordonii* PK488 and *Actinomyces naeslundii* PK606 is mediated by a 38-kDa streptococcal protein, designated ScaA. The gene, scaA, which encodes this protein has been cloned into *Escherichia coli*. A genomic *S. gordonii* PK488 library (in Lambda ZAP II) was screened with anti-*S. gordonii* immunoglobulin G absorbed with *S. gordonii* PK1804, an isogenic coaggregation-defective mutant of strain PK488. A positive recombinant phage was isolated, and a phagemid designated pRA1 was obtained which contained a 6.6-kb insert. Expression of *scaA* from pRA1 and from a subcloned internal 2.1-kb fragment was observed. The absorbed antiserum cross-reacted with a 34.7-kDa protein, SsaB, from *S. sanguis* 12, also a coaggregation partner of *A. naeslundii* PK606. Absorbed antiserum to *S. gordonii* PK488 and antiserum to SsaB both reacted with 38-kDa proteins in supernatants from mildly sonicated preparations from 11 other coaggregation partners of *A. naeslundii* PK606. Putative adhesin genes were identified in each of these coaggregation partners by Southern analysis of their genomic DNA with the cloned 2.1-kb fragment as a probe. A 30-base oligonucleotide probe based on the sequence of *ssaB* of *S. sanguis* 12 hybridized in an identical manner. These data extend the notion that most of the viridans streptococci that coaggregate with actinomyces are capable of expressing ScaA-related proteins.

Viridans streptococci play a prominent role during the primary colonization of freshly cleaned teeth (28, 31). Coaggregation, the ability of one strain to adhere to another, has been surveyed extensively among the subgingival oral streptococci (17), and nearly all strains coaggregate with specific partner cells. Most oral streptococci coaggregate intergenerically with other early colonizers, namely, *Actinomyces, Haemophilus*, and *Veillonella* spp. and, intragenerically, with genetically distinct streptococci (18, 20). The ability of certain streptococci to adhere to saliva-costed hydroxyapatite (6) and to proline-rich proteins, components of saliva (12), suggests that they are also able to attach to the salivary pellicle in vivo. This duality of adherence traits equips the streptococci to serve as anchors during early colonization of the tooth surface and the commencement of dental plaque accretion.

Six groups of streptococci, coaggregation groups 1 to 6, and six groups of actinomyces, coaggregation groups A to F, have been delineated on the basis of various coaggregation properties, including inhibition of coaggregation by lactose (5, 16, 21-23). The current study focuses on *Streptococcus gordonii* PK488 (group 6 streptococcal reference strain) coaggregation with *Actinomyces naeslundii* PK606 (group D actinomyces reference strain). This lactose-insensitive interaction is mediated by a protease- and heat-sensitive adhesin on the streptococcus which binds to a complementary heat-insensitive receptor on the actinomyces partner.

We have previously reported that the *S. gordonii* PK488 adhesin is a 38-kDa protein (19). A similar antigen was found in all six of the streptococcal coaggregation group reference strains. An antigenic cross-reactivity also was noted between the 38-kDa protein of *S. gordonii* PK488 and the saliva-binding 34.7-kDa SsaB protein of *S. sanguis* 12 (10). The *ssaB* gene, encoding the latter protein, has been cloned and sequenced (10, 11), and the *ssaB* sequence is 87% identical to a saliva-binding fimbrial protein from *S. paraspenguis* (*S. sanguis*) FW213 (9, 10, 33).

Here, we report the cloning of a gene encoding the 38-kDa protein from *S. gordonii* PK488 and examine the prevalence of similar genes and putative coaggregation-mediating adhesins among other human oral streptococci. The antiserum used to detect the cloned protein specifically blocks lactose-insensitive coaggregation between most of these streptococci and *A. naeslundii* PK606 (19).

**MATERIALS AND METHODS**

**Strains and culture conditions.** All strains used in this study are listed in Table 1. Streptococci were cultured in a medium consisting of tryptone, yeast extract, Tween 80, and glucose buffered to pH 7.5 with K₂HPO₄ (27). Streptococcal cultures were grown at 37°C under anaerobic conditions with the GasPak system (BBL Microbiology Systems, Cockeysville, Md.). *Escherichia coli* XL1-Blue (Stratagene Cloning Systems, La Jolla, Calif.) was cultured aerobically at 37°C in Luria-Bertani (LB) broth or on agar (Gibco-BRL, Gaithersburg, Md.) with or without 100 µg of ampicillin (Sigma Chemical Co., St. Louis, Mo.) per ml.

**Antiserum production.** The preparation of the immunoglobulin G (IgG) against *S. gordonii* PK488 and the absorption with cells of the coaggregation-defective (Cog-) mutant *S. gordonii* PK1804 have been described previously (19). IgG against the cloned SsaB protein has been described previously (11) and was a gift from B. C. McBride.

**DNA preparation.** *S. gordonii* PK488 chromosomal DNA was prepared as described previously (11). An adaptation of this method was used to obtain DNA from other streptococci.
for Southern blot analysis. Five milliliters of an overnight culture was washed twice with 5 ml of TES buffer, pH 7.0 (20 mM Tris-HCl, 5 mM disodium EDTA, 100 mM NaCl). The pellet was resuspended in 0.5 ml of TES and treated with 28 μl of mutanolysin (3,300 U/ml; Sigma) at 37°C for 1 h. After the addition of 41 μl of pronase (700 U/ml; Calbiochem, San Diego, Calif.) and 40 μl of 20% sodium lauryl sarcosine, the mixture was incubated for an additional 30 min at 37°C. The lysate was extracted three times with phenol and once with chloroform. The nucleic acids were precipitated with ethanolic precipitation and centrifugation in a 10 to 30% sucrose linear gradient (3). Fragments of 5 to 10 kb were selected to construct a library, using the Lambda ZAP II vector system and E. coli XLI-Blue as host cells (Stratagene). This library was screened with Cot-1 mutant-absorbed anti-S. gordonii PK488 IgG according to the protocol described by Stratagene. Several immunoreactive plaques were cored from agar plates, and the plaque was allowed to diffuse overnight into SM buffer (30) at 4°C. Plaques were purified, amplified, and subjected to in vivo excision to obtain plasmid derivatives. DNA ligation, packaging, and amplification, as well as the in vivo excision of the recombinant plasmid, were done according to the Stratagene Lambda ZAP II instruction manual.

Subcloning. Plasmid DNA was digested with various restriction enzymes. Fragments were separated on 0.7% agarose gels and excised (30). The DNA was purified by using the GeneClean II Kit (Bio 101, Inc., La Jolla, Calif.). Following self-ligation or ligation to dephosphorylated Blue-script KS II+ plasmid, the products were used to transform E. coli XLI-Blue made competent by the calcium chloride method (2). The transformed cells were plated on LB agar-containing ampicillin. Individual colonies were subcultured in LB broth containing ampicillin and examined for expression of the 38-kDa protein by Western blot (immunoblot) analysis.

**Western blotting.** Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed with precast 4 to 20% Tris–glycine gradient gels (Novex, San Diego, Calif.). Proteins separated on gels were either Western blotted or stained by using the Pro-Blue system (Integrated Separation Systems, Natick, Mass.). Western blot transfers to nitrocellulose filters were performed in the Mini-PROTEAN II transfer chamber (Bio-Rad). Following the transfer, the filters were treated with a 1,000-fold dilution of either Cog-1 mutant-absorbed anti-PK488 rabbit IgG or rabbit IgG against the SsaB protein from S. sanguis 12 at a final concentration of, respectively, 4 or 1 μg of protein per ml. Immune complexes were visualized with alkaline phosphatase-conjugated anti-rabbit IgG and a dye indicator system supplied by Promega. Prestained markers (Bio-Rad) were used to calibrate molecular weights in gels and blots.

**Southern blotting.** Between 2 and 4 μg of restriction enzyme-treated DNA was electrophoresed in a 0.7% agarose gel on a model MPH horizontal gel apparatus (IBI, New Haven, Conn.) at 60 V for 4 h. The gel was (i) depurinated in 0.25 M HCl for 10 min, (ii) denatured in a solution of 0.5 M NaOH containing 1.5 M NaCl for 30 min, and (iii) neutralized in a solution of 1 M Tris-HCl, pH 7.5, containing 0.5 M NaCl for 30 min. DNA fragments were transferred to nitrocellulose membranes by capillary action (32). The DNA was cross-linked in a model 1800 Stratalinker (Stratagene). Radioactively labeled probes were generated by nick translation of a 2.1-kb ClaI-ParI fragment from S. gordonii PK488 or by end labeling of a 30-base oligonucleotide (TACATC TGGGAAATCAACACCGAAGAAGAA). This oligonucleotide was synthesized on a PCR-MATE model 391 DNA synthesizer (Applied Biosystems) and purified on an oligonucleotide purification cartridge (Applied Biosystems). The oligonucleotide was based on the previously reported sequence of the ssaB gene (10) and corresponds to nucleotides 1709 to 1738, which encode amino acids 222 to 231 of SsaB. Hybridization of 32P-labeled probes to the transferred DNA was carried out by the method of Church and Gilbert (4) at

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### Table 1. Strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Characteristic(s)</th>
<th>Reference or source</th>
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</thead>
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<tr>
<td>S. gordonii DL1</td>
<td>Reference strain for coaggregation group 1</td>
<td>5, 19</td>
</tr>
<tr>
<td>S. oralis H1</td>
<td>Reference strain for coaggregation group 2</td>
<td>5, 19</td>
</tr>
<tr>
<td>S. oralis 34</td>
<td>Reference strain for coaggregation group 3</td>
<td>5, 19</td>
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<td>S. oralis 322</td>
<td>Reference strain for coaggregation group 5</td>
<td>19, 21</td>
</tr>
<tr>
<td>Streptococcus SM PK509</td>
<td>Reference strain for coaggregation group 6</td>
<td>19, 21</td>
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<td>Coaggregation-defective mutant of PK488</td>
<td>19</td>
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<td>Coaggregates with actinomyces (20)</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>S. gordonii ATCC 10557</td>
<td>Type strain</td>
<td>5</td>
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<td>S. sanguis 12</td>
<td>Adherence to S-HA*</td>
<td>B. C. McBride</td>
</tr>
<tr>
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<td>Formerly S. sanguis FW213 (33); adherence to S-HA</td>
<td>P. Fives-Taylor</td>
</tr>
<tr>
<td>S. crista PK1408</td>
<td>Formerly S. sanguis CC5A (13); corncob formation</td>
<td>C. Mouton</td>
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<td>S. milleri K44Y</td>
<td>Coaggregates with actinomyces (1)</td>
<td>J. Mizuno</td>
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<tr>
<td>E. faecalis GF590</td>
<td>Noncoaggregating control strain</td>
<td>D. Clewell</td>
</tr>
<tr>
<td>E. coli XLI-Blue</td>
<td>recoA strain used for cloning</td>
<td>Stratagene</td>
</tr>
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</table>

* S-HA, saliva-coated hydroxypatite.
65°C for the 2.1-kb probe and at 50°C for the 30-mer probe. The hybridization buffer consisted of 0.25 M Na₂HPO₄, 7H₂O, 36 mM H₃PO₄, 1 mM EDTA, pH 8.0, 1% PENTEX bovine serum albumin fraction V (Miles Inc., Kankakee, Ill.), and 7% SDS. The 17-h hybridization was followed by (i) two 5-min washes in 2x SSC buffer (1x SSC is 150 mM NaCl plus 15 mM sodium citrate), (ii) a 30-min wash in 2x SSC, and (iii) a 10-min wash in 1x SSC. All washes were done at the temperature used for the hybridization. Following the washes the nitrocellulose sheets were blotted partially dry, enclosed in clear plastic wrap, and exposed to Kodak X-Omat AR film at −70°C prior to development.

Preparation of bacterial extracts. Streptococcal extracts for Western blots were obtained by mild sonication as follows. Cells from 100-ml overnight cultures were pelleted and washed three times with phosphate-buffered saline. The pellets were resuspended in 1 ml of distilled water, placed in an ice bath, and sonicated for 1 min at maximum output with a Microdisruptor KT40 (Kontes, Vineland, N.J.). The mildly sonicated cell suspensions were centrifuged for 15 min at 14,000 × g, and the resultant supernatants were assayed for protein content with the BCA reagent (Pierce Chemical Co., Rockford, Ill.). The average amount of protein released from the streptococci was 150 µg/100-ml culture. E. coli extracts were also prepared in this manner, and the extracts contained 25 times more protein than the streptococcal extracts, suggesting significant cell breakage with E. coli. Alternatively, to determine the expression of putative transformants, small aliquots of overnight cultures of recombinant E. coli were washed, resuspended in water, and boiled for 5 min with SDS sampling buffer containing 5% 2-mercaptoethanol prior to loading onto an SDS-PAGE gel for Western blotting.

RESULTS

Isolation of recombinant clones. S. gordonii PK488 DNA was digested with EcoRI, and a lambda phage library was constructed and plated on E. coli XL1-Blue. Plaque lifts were screened for expression of the 38-kDa protein by using Cog mutant–absorbed anti-PK488 rabbit IgG. One immunoreactive plaque was purified, amplified, and rescued as a phagemid which was designated pRA1.

E. coli XL1-Blue harboring pRA1 expressed a protein (Fig. 1A, lane 3) similar in size to the 38-kDa adhesin from S. gordonii PK488 (Fig. 1A, lane 2) and the SsAB adhesin from S. sanguis (Fig. 1A, lane 5). However, E. coli XL1-Blue with the vector only did not express the protein (Fig. 1A, lane 4). Antiserum raised against the SsAB protein also reacted with the 38-kDa protein expressed by pRA1 (Fig. 1B, lane 3). A corresponding gel stained for protein is shown in Fig. 1C.

Subcloning. Restriction and deletion analyses were performed with pRA1 to localize the region which expresses the 38-kDa protein (Fig. 2). Cleavage with EcoRI did not release the entire insert; apparently one of two EcoRI sites within the multiple cloning region of the vector had been lost. However, double digests of pRA1 with EcoRI and then SpeI released the 2.9-kb vector and established the size of the insert as approximately 6.6 kb. Additional restriction enzyme digests were done, and a number of deletion derivatives were subcloned and examined for expression of the 38-kDa protein (Fig. 2). The gene, scaA, was localized to a 2.1-kb ClaI-PstI fragment.

Southern blot analysis of streptococcal genomic DNA. Chromosomal DNA from the six coaggregation-group-representative streptococci and several other oral streptococci was digested with PstI, Southern blotted, and hybridized with the labeled 2.1-kb ClaI-PstI fragment (Fig. 3A). The labeled probe reacted with a 3.9-kb fragment from S. gordonii PK488 (Fig. 3A, lane 6). The probe also reacted with a 3.9-kb fragment from S. gordonii DL1 (Fig. 3A, lane 1) and S. gordonii ATCC 10558 (Fig. 3A, lane 11); a 4.1-kb fragment from S. oralis 34 (Fig. 3A, lane 3), S. oralis J22 (Fig. 3A, lane 4), and S. oralis ATCC 10557 (Fig. 3A, lane 10); a 5.2-kb fragment from S. oralis H1 (Fig. 3A, lane 2), S. parasangues FW213 (Fig. 3A, lane 8), and S. milleri K44Y (Fig. 3A, lane 12); a 6.5-kb fragment from Streptococcus SM PK509 (Fig. 3A, lane 5); and a 1.9-kb fragment from S. sanguis 12 (Fig. 3A, lane 7) and S. sanguis ATCC 10556 (Fig. 3A, lane 9). The probe did not react with two noncoaggregating streptococci, S. sobrinus 6715-10 (Fig. 3A, lane 13) and Enterococcus faealis GF590 (Fig. 3A, lane 14). Incomplete digestion of DNA from strains DL1 and ATCC 10558 (Fig. 3A, lanes 1 and 11, respectively) is the probable cause of the multiple bands.

Since the antiserum raised against the 38-kDa adhesin cross-reacted with the S. sanguis 12 SsAB adhesin, a 30-base oligonucleotide probe was derived from the ssaB sequence (10). The 30 nucleotides were 93% homologous to a 30-base sequence of fimA of S. parasangues FW213, but the amino acid sequences of the corresponding regions were identical (9). This 30-mer reacted in a manner identical to that observed with the 2.1-kb ClaI-PstI fragment from S. gordoni PK488 (Fig. 3B). Furthermore, both the 30-mer probe and the 2.1-kb probe reacted with two other streptococci (data not shown) S. cristà PK1408 (S. sanguis CC5A; associated with coaggregation formation) and S. sanguis CI04 (a reference strain for streptococcal coaggregation group 3), that coaggregate with A. naeslundii PKK60 (1, 19).

Western blot analysis. Each of the viridans streptococci tested by Southern analysis was examined by Western analysis to check for the expression of a ScaA-like protein similar to that of S. gordonii PK488. The presence of these
proteins in the reference strains of the other five streptococcal coaggregation groups (those shown in the Southern analysis in Fig. 3A, lanes 1 to 5) was reported earlier (19). The supernatants from mild sonicates of the remaining streptococci were electrophoresed, blotted, and probed with anti-PK488 mutant-absorbed rabbit IgG (Fig. 4A). The 38-kDa protein was evident in S. sanguis 12 (Fig. 4A, lane 2), S. parasanguis FW213 (Fig. 4A, lane 3), S. sanguis ATCC 10556 (Fig. 4A, lane 4), S. oralis ATCC 10557 (Fig. 4A, lane 5), and S. gordonii ATCC 10558 (Fig. 4A, lane 6) and weakly reactive in S. milleri K44Y (Fig. 4A, lane 7). It was not detectable in S. sobrinus 6715-10 (Fig. 4A, lane 8) or E. faecalis GF590 (Fig. 4A, lane 9), but a strong band was observed with S. cristia PK1408 (Fig. 4A, lane 10). All of the streptococci that reacted in the Southern analysis (Fig. 3A) also expressed a protein with a molecular size similar to that of ScaA. Except for S. parasanguis FW213, all positive strains coaggregated with A. naeslundii PK606.

A matching Western blot, but including the reference strains of the six streptococcal coaggregation groups, was developed with anti-SsaB serum (Fig. 4B). Nearly identical results were evident. The reactivity with S. oralis 34 (Fig. 4B, lane 4) was weaker than with the other reference strains (Fig. 4B, lanes 2, 3, 5, 6, and 7), as had been observed earlier with anti-PK488 mutant-absorbed rabbit IgG (19). Though not included in Fig. 4, S. sanguis C104, a reference strain for streptococcal coaggregation group 3, reacted positively with both antisera (1).

FIG. 2. Partial restriction map and characteristics of deletion derivatives subcloned from pRA1. The expression of the 38-kDa protein by each subclone is indicated.

FIG. 3. Southern blot analysis of PstI-digested genomic DNA from various streptococcal strains. (A) Southern blot was probed with a 2.1-kb Clal-PstI fragment from S. gordonii PK488. (B) Southern blot was probed with a 30-mer derived from the savaI sequence from S. sanguis 12 as described in Materials and Methods. The strains were S. gordonii DL1 (lane 1), S. oralis H1 (lane 2), S. oralis 34 (lane 3), S. oralis J22 (lane 4), Streptococcus SM PK509 (lane 5), S. gordonii PK488 (lane 6), S. sanguis 12 (lane 7), S. parasanguis FW213 (lane 8), S. sanguis ATCC 10556 (lane 9), S. oralis ATCC 10557 (lane 10), S. gordonii ATCC 10558 (lane 11), S. milleri K44Y (lane 12), S. sobrinus 6715-10 (lane 13), and E. faecalis GF590 (lane 14).

DISCUSSION

The cloned 2.1-kb Clal-PstI fragment from S. gordonii PK488 expressed the 38-kDa adhesin, termed ScaA for streptococcal coaggregation adherence. Because isogenic Cog− mutants of S. gordonii PK488 (the reference strain for streptococcal coaggregation group 6) are deficient in this protein and the Cog− mutant-absorbed antiserum recognizing this protein specifically blocks coaggregation with A. naeslundii PK606, we proposed that ScaA mediates coaggregation with A. naeslundii PK606 (19). S. gordonii PK488 exhibits only lactose-insensitive coaggregation with A. naeslundii PK606. Reference strains for streptococcal coaggregation groups 1, 3, 4, and 5 exhibit both a lactose-sensitive and lactose-insensitive type of coaggregation with A. naeslundii PK606. When these streptococci were preincubated with this Cog− mutant-absorbed antiserum, their lactose-insensitive coaggregation was blocked and only the lactose-sensitive coaggregations were observed (19). These results provide strong evidence for the specific recognition
FIG. 4. Immunoblots of proteins released by sonication from various streptococci and *E. faecalis* GS90. (A) Developed with Cog− mutant-absorbed IgG prepared from anti-PK488 serum. The strains were *S. gordonii* PK488 (lane 1), *S. sobrinus* 12 (lane 2), *S. parasanguis* FW213 (lane 3), *S. sanguis* ATCC 10556 (lane 4), *S. oralis* ATCC 10557 (lane 5), *S. gordonii* ATCC 10558 (lane 6), *S. milleri* K44Y (lane 7), *S. sobrinus* 6715-10 (lane 8), *E. faecalis* GS90 (lane 9), and *S. crista* PK1408 (lane 10). (B) Developed with anti-SsaB IgG from *S. sanguis* 12. The strains were *S. gordonii* DL1 (lane 2), *S. oralis* H1 (lane 3), *S. oralis* 34 (lane 4), *S. oralis* J22 (lane 5), *Streptococcus* SM PK509 (lane 6), *S. gordonii* PK488 (lane 7), *S. sobrinus* 12 (lane 8), *S. parasanguis* FW213 (lane 9), *S. sanguis* ATCC 10556 (lane 10), *S. oralis* ATCC 10557 (lane 11), *S. gordonii* ATCC 10558 (lane 12), *S. milleri* K44Y (lane 13), *S. sobrinus* 6715-10 (lane 14), *E. faecalis* GS90 (lane 15), and *S. crista* PK1408 (lane 16). Lane 1 (panel B) contains standards as described in the Fig. 1 legend.

by the absorbed antiserum of the ScaA molecule responsible for lactose-insensitive coaggregation with *A. naeslundii* PK606. The fact that the coaggregation patterns characteristic of the six streptococcal coaggregation groups represent greater than 90% of the coaggregation patterns observed in surveys of more than 100 fresh streptococcal isolates (17, 21) also supports the notion that a ScaA-like protein may mediate this lactose-insensitive coaggregation with actinomycetes.

The two DNA probes used in this study hybridized to *PstI*-generated fragments of genomic DNA from all coaggregation-positive oral streptococci, indicating the possibility of widespread occurrence of scaA-homologous DNA. Restriction fragment length polymorphisms were evident. Each of these streptococci also expressed a protein immunoreactive with both antiserum probes, the anti-PK488 mutant-absorbed rabbit IgG and the anti-SsaB IgG from *S. sanguis* 12. Thus, it appears that all streptococci that coaggregate with *A. naeslundii* PK606 also express a ScaA-related protein.

This study examined all three streptococcal strains that were previously shown to possess either immuno-cross-reactive adhesins (*S. gordonii* PK488 and *S. sanguis* 12) (10) or saliva-binding proteins (*S. sanguis* 12 and *S. parasanguis* FW213) (10). We have shown here that these three streptococci share homologous DNA sequences. Restriction fragment length polymorphisms were evident with the probes prepared from both *S. gordonii* PK488 and *S. sanguis* 12. The three streptococci also express an immunoreactive protein of about 38 kDa. All streptococci which reacted positively with probes in the Southern and Western analyses are coaggregation partners of *A. naeslundii* PK606, with the exception of *S. parasanguis* FW213, an atypical viridans streptococcus (33). In fact, *S. parasanguis* FW213 does not coaggregate with any of the reference strains of the six actinomycoses coaggregation groups (1). The FimA protein expressed by *S. parasanguis* FW213 may have diverged evolutionarily and lost its ability to mediate coaggregation but not its ability to immunoreact with antisera to related adhesins. It remains to be determined whether ScaA, like SsaB and FimA, can mediate binding of streptococci to salivary molecules and be involved in the adherence of the streptococci to saliva-coated hydroxyapatite, a model surface for teeth.

Apparently, these adherence functions are very important to the streptococci, since all the adherent strains express them. Two other strains, *S. sobrinus* 6715-10 and *E. faecalis* GS90, do not exhibit these adherence functions and do not react with the probes used here in the Southern and Western analyses.

Unlike other oral streptococci that coaggregate with several actinomycoses, *S. gordonii* PK488 coaggregates only with *A. naeslundii* PK606. Because of this apparently simple kind of adherence activity spectrum, it was chosen for initiation of the studies on the genetic organization of coaggregation and other adherence-related genes in oral bacteria. Only a few such coaggregation-relevant genes or gene products have been examined. The 75-kDa adhesin from *Prevotella loescheii* PK1295 has been purified and shown to possess adherence- and coaggregation-related functions (26). Both a 34-kDa outer membrane protein from *Haemophilus parainfluenzae* HP-28 and Fab fragments from anti-34-kDa-protein antibody inhibit coaggregation between *H. parainfluenzae* and *S. sanguis* SA-1; they also inhibit adherence of *H. parainfluenzae* to experimental salivary pellicle (24). Of special interest is the gene encoding a 76-kDa cell surface lipoprotein, SarA, from *S. gordonii* DL1-Challis, which has been cloned and partially sequenced and is associated with coaggregation of actinomycoses (14, 15).

The proteins encoded by adherence-relevant genes that have been cloned from other oral bacteria include (i) a 162-kDa sialic acid-binding lectin called SSP-5 from *S. gordonii* M5 (7), (ii) an adhesin-antigen 80-kDa complex from *S. gordonii* G9B consisting of 80-, 62-, and 52-kDa polypeptides (25, 29), (iii) a 59-kDa type 2 fimbrial subunit, FimA, from *A. naeslundii* (*A. viscosus*) T14V (8), (iv) a similar type 2 fimbrial subunit gene from *A. naeslundii* WVU45 (35), and (v) the 56.9-kDa type 1 fimbrial subunit, FimP, from *A. naeslundii* (*A. viscosus*) T14V (34).

None of these adherence-relevant proteins is similar in molecular size to ScaA, the 38-kDa adhesin from *S. gordonii* PK488 described here, SsaB, the 34.7-kDa adhesin from *S.
sanguis 12, or the 34.3-kDa type 1 fimbrial protein from *S. parasanguis* FW213. Therefore, it appears that this family of 34- to 38-kDa proteins is a distinct class. These proteins may mediate (i) coaggregation with actinomyces, a group of early colonizing bacteria like the streptococci, and (ii) binding to salivary molecules in the acquired pellicle, another surface exposed only at times of early colonization. Or, they may have lost one or the other function. The mechanisms involved in accretion on freshly cleaned tooth surfaces are the subject of intense investigation in several laboratories. Clearly, streptococci have evolved a highly efficient set of mechanisms to adhere, since 60 to 80% of the early colonizers are streptococci (28). We propose that many and perhaps all viridans streptococci that coaggregate with *A. naeslundii* PK606 express one of the 34- to 38-kDa class of proteins that exhibit dual functions of coaggregation mediator and salivary-binding protein.

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ADDENDUM IN PROOF

The nucleotide sequence of *scaA* has been completed. The deduced amino acid sequence of ScaA is 310 amino acids long and is 91 and 80% identical to SasB (10) and FimA (9), respectively. ScaA has the lipoprotein consensus sequence (Leu-X-X-Cys) recently reported for SarA (14) and SasB (N. Ganeshkumar, N. Arora, and P. E. Kolenbrander, J. Bacteriol. 175:572–574, 1993) and present in the FimA sequence (9).

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