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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-coated 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. parasanguis* (*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 K_2HPO_4 (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

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TABLE 1. Strains used

Strain	Characteristic(s)	Reference or source
S. gordonii DL1	Reference strain for coaggregation group 1	5, 19
S. oralis H1	Reference strain for coaggregation group 2	5, 19
S. oralis 34	Reference strain for coaggregation group 3	5, 19
S. sanguis C104	Reference strain for coaggregation group 3	5, 19
S. oralis J22	Reference strain for coaggregation group 4	5, 19
Streptococcus SM PK509	Reference strain for coaggregation group 5	19, 21
S. gordonii PK488	Reference strain for coaggregation group 6	19, 21
S. gordonii PK1804	Coaggregation-defective mutant of PK488	19
S. sanguis ATCC 10556	Type strain	American Type Culture Collection
S. oralis ATCC 10557	Coaggregates with actinomyces (20)	American Type Culture Collection
S. gordonii ATCC 10558	Type strain	American Type Culture Collection
S. sanguis 12	Adherence to S-HA[a]	B. C. McBride
S. parasanguis FW213	Formerly *S. sanguis* FW213 (33); adherence to S-HA	P. Fives-Taylor
S. crista PK1408	Formerly *S. sanguis* CC5A (13); corncob formation	C. Mouton
S. milleri K44Y	Coaggregates with actinomyces (1)	J. Mizuno
S. sobrinus 6715-10	Noncoaggregating control strain	5
E. faecalis GF590	Noncoaggregating control strain	D. Clewell
E. coli XL1-Blue	*recA* strain used for cloning	Stratagene

[a] S-HA, saliva-coated hydroxyapatite.

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 ethanol, vacuum dried, resuspended in 50 μl of TE buffer (30) containing 3 μl of RNase (500 μg/ml; Boehringer Mannheim, Indianapolis, Ind.), and incubated for 30 min at 37°C prior to storage at 4°C. Plasmid DNA was prepared by using the Magic Minipreps DNA Purification System (Promega Corp., Madison, Wis.).

Recombinant DNA methods. *S. gordonii* PK488 DNA (10 μg in 50 μl) was digested with *Eco*RI (0.2 U/μl), with digestion times varying from 30 s to 5 min. Reactions were stopped at the desired times by adding 5 μl of 0.5 M EDTA. Partial digests were pooled and fractionated by 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* XL1-Blue as host cells (Stratagene). This library was screened with Cog^- mutant-absorbed anti-*S. gordonii* PK488 IgG according to the protocol described by Stratagene. Several immunoreactive plaques were cored from agar plates, and the phage 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 Bluescript KS II+ plasmid, the products were used to transform *E. coli* XL1-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^- 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 0.5 M Tris-HCl, pH 7.5, containing 1.5 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 *Cla*I-*Pst*I 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 ^{32}P-labeled probes to the transferred DNA was carried out by the method of Church and Gilbert (4) at

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 $Na_2HPO_4 \cdot 7H_2O$, 36 mM H_3PO_4, 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 2× SSC buffer (1× SSC is 150 mM NaCl plus 15 mM sodium citrate), (ii) a 30-min wash in 2× SSC plus 1% SDS, and (iii) a 10-min wash in 1× 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 Microdisrupter 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 *Eco*RI, 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* 12 (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 *Eco*RI did not release the entire insert; apparently one of two *Eco*RI sites within the multiple cloning region of the vector had been lost. However, double digests of pRA1 with *Eco*RI and then *Spe*I 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 *Cla*I-*Pst*I fragment.

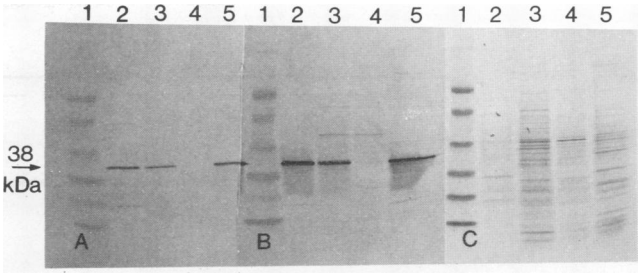

FIG. 1. Western blot (immunoblot) analysis and SDS-PAGE of proteins released by sonication from *S. gordonii* PK488, recombinant *E. coli* XL1 containing pRA1, *E. coli* XL1 containing the vector only, and *S. sanguis* 12 (lanes 2 to 5, respectively). An aliquot containing 1.5 μg of protein was loaded onto each lane. (A) Immunoblot was developed with Cog^- mutant-absorbed IgG prepared from anti-PK488 serum. (B) Immunoblot was developed with anti-SsaB IgG from *S. sanguis* 12. (C) Corresponding gel stained with ProBlue. Lane 1 in each panel contains prestained low-molecular-weight standards (Bio-Rad) with apparent molecular masses (daltons) of 110,000 (phosphorylase *b*, rabbit muscle), 84,000 (bovine serum albumin), 47,000 (ovalbumin, hen egg white), 33,000 (carbonic anhydrase, bovine), 24,000 (soybean trypsin inhibitor), and 16,000 (lyzozyme).

Southern blot analysis of streptococcal genomic DNA. Chromosomal DNA from the six coaggregation-group-representative streptococci and several other oral streptococci was digested with *Pst*I, Southern blotted, and hybridized with the labeled 2.1-kb *Cla*I-*Pst*I 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. parasanguis* 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 faecalis* 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. parasanguis* 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 *Cla*I-*Pst*I fragment from *S. gordonii* PK488 (Fig. 3B). Furthermore, both the 30-mer probe and the 2.1-kb probe reacted with two other streptococci (data not shown) *S. crista* PK1408 (*S. sanguis* CC5A; associated with corncob formation) and *S. sanguis* C104 (a reference strain for streptococcal coaggregation group 3), that coaggregate with *A. naeslundii* PK606 (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

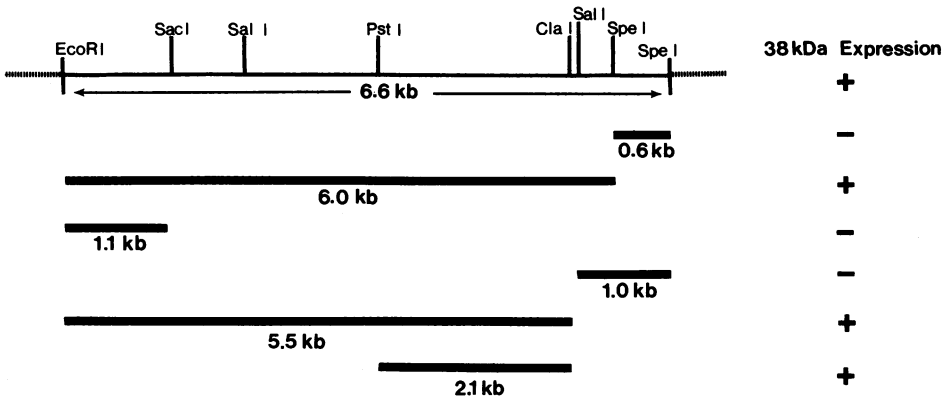

FIG. 2. Partial restriction map and characteristics of deletion derivatives subcloned from pRA1. The expression of the 38-kDa protein by each subclone is identified on the right. The DNA fragment length of each deletion derivative is indicated.

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. crista* 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.

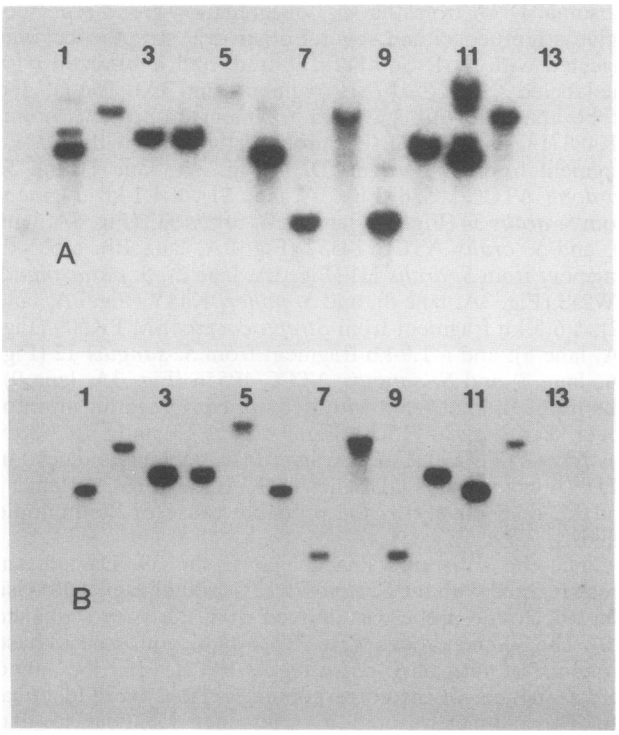

FIG. 3. Southern blot analysis of *Pst*I-digested genomic DNA from various streptococcal strains. (A) Southern blot was probed with a 2.1-kb *Cla*I-*Pst*I fragment from *S. gordonii* PK488. (B) Southern blot was probed with a 30-mer derived from the *ssaB* sequence from *S. sanguis* 12 as described in 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).

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).

DISCUSSION

The cloned 2.1-kb *Cla*I-*Pst*I 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

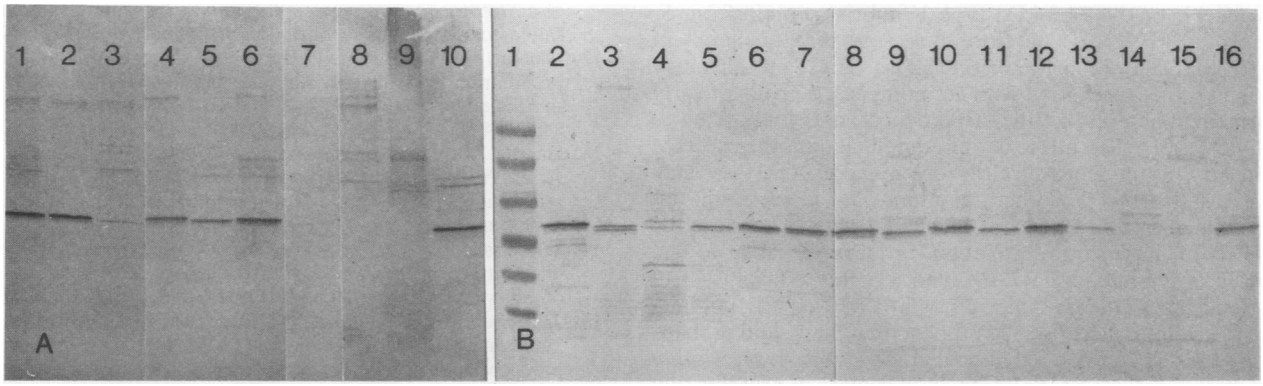

FIG. 4. Immunoblots of proteins released by sonication from various streptococci and *E. faecalis* GF590. (A) Developed with Cog^- mutant-absorbed IgG prepared from anti-PK488 serum. The strains were *S. gordonii* PK488 (lane 1), *S. sanguis* 12 (lane 2), *S. parasanguis* FW213 (lane 3), *S. sanguis* 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* GF590 (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. sanguis* 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* GF590 (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 actinomyces.

The two DNA probes used in this study hybridized to *Pst*I-generated fragments of genomic DNA from all coaggregation-positive human 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 actinomyces 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* GF590, 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 actinomyces, *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 actinomyces (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 saliva-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 SsaB (10) and FimA (9), respectively. ScaA has the lipoprotein consensus sequence (Leu-X-X-Cys) recently reported for SarA (14) and SsaB (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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