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Negative Correlation of Distributions of *Streptococcus cristatus* and *Porphyromonas gingivalis* in Subgingival Plaque▽

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***Porphyromonas gingivalis* is one of the major causative agents of adult periodontitis. One of the features of this periodontal pathogen is its ability to attach to a variety of oral bacterial surfaces and to colonize subgingival dental plaque. We have shown that *Streptococcus cristatus* CC5A inhibits expression of *fimA*, a gene encoding the major protein subunit of long fimbriae in *P. gingivalis*; as a result, *S. cristatus* interrupts formation of *P. gingivalis* biofilms. Here we further demonstrate that the inhibitory activity of *S. cristatus* affects multiple strains of *P. gingivalis* and that optimal inhibitory activity correlates with levels of arginine deiminase expression in *S. cristatus*. More strikingly, the impact of *S. cristatus* on *P. gingivalis* colonization was revealed by comparing levels of *P. gingivalis* and *S. cristatus* in subgingival dental plaque. Spearman correlation analysis indicated a negative correlation between the distributions of *S. cristatus* and *P. gingivalis* ($r = -0.57$; $P < 0.05$). These data suggest that some early colonizers of dental plaque, such as *S. cristatus*, may be beneficial to the host by antagonizing the colonization and accumulation of periodontal pathogens such as *P. gingivalis*.**

Periodontitis is a bacterial biofilm (dental plaque)-related infectious disease. Although over 750 oral bacterial taxa can be detected in the oral cavity, three gram-negative species, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*—known as the red complex—have strong associations with chronic periodontal disease (9, 28). It has been suggested that these gram-negative bacteria are later colonizers of dental plaques and are recruited to microbial communities by earlier colonizers, such as oral streptococci and *Actinomyces* species, via specific interactions of surface molecules (10). While there is no direct evidence that the earlier colonizers are associated with periodontitis, some of these organisms can provide a favorable environment for periodontal pathogens such as *P. gingivalis*. A well-studied interaction of earlier colonizers and later colonizers of dental plaque is coadhesion of *Streptococcus gordonii* and *P. gingivalis*. Specific adherence of *P. gingivalis* to *S. gordonii* strains was demonstrated in in vitro experiments (12). Studies by independent laboratories showed involvement of multiple sets of adhesins in the two bacteria. The first set of adhesins is the *P. gingivalis* long fimbriae (FimA) and glyceraldehyde-3-phosphate dehydrogenase present on the surfaces of streptococci (17). The second set involves the *P. gingivalis* short fimbriae (Mfa1) and the streptococcal SspA/B (antigen I/II) adhesins (4, 23). It is likely that these specific protein-protein interactions promote *P. gingivalis* colonization on existing biofilms consisting of *S. gordonii* and related oral streptococci (4, 27).

We previously reported an antagonistic relationship between *P. gingivalis* and *Streptococcus cristatus* CC5A. *P. gingivalis* was unable to form microcolonies with *S. cristatus*, due to repression of *fimA* expression in the presence of *S. cristatus* (32). Moreover, the long fimbriae are important for aspects of *P. gingivalis* colonization. Previous studies have shown that a *P. gingivalis* strain with a *fimA* deficiency has a diminished capacity to adhere to human gingival fibroblasts and epithelial cells (6) and is deficient in invasion of epithelial cells (35). The *fimA* mutant also is less able to induce periodontal bone loss in a gnotobiotic rat model (18). Our recent study identified an *S. cristatus* surface protein, arginine deiminase (ArcA), responsible for eliciting repression of *fimA* expression in *P. gingivalis* 33277 (34). The *arcA* gene is found in a number of oral bacteria, mainly in streptococci (3). However, *arcA* is differentially expressed among oral streptococcal strains (16). Higher-level expression of *arcA* was observed in *S. cristatus* than in *S. gordonii*, which may contribute to the ability of the organism to prevent *P. gingivalis* colonization in the oral cavity. In this study, we tested an antagonistic role of *S. cristatus* in *P. gingivalis* colonization. We postulate that inhibition of FimA production in *P. gingivalis* by *S. cristatus* requires higher expression of ArcA and that colonization of *S. cristatus* strains expressing an elevated level of ArcA plays an important role in antagonizing *P. gingivalis* colonization.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains used in this study are listed in Table 1. *P. gingivalis* strains were grown from frozen stocks in Trypticase soy broth (TSB) or on TSB blood agar plates, supplemented with yeast extract (1 mg/ml), hemin (5 μg/ml), and menadione (1 μg/ml), at 37°C in an anaerobic chamber (85% N_2, 10% H_2, 5% CO_2). *Streptococcus* strains were grown in Trypticase peptone broth supplemented with 0.5% glucose at 37°C under aerobic conditions.

Partial purification of the *S. cristatus* inhibitory protein. Surface extracts of *S. cristatus* were collected by sonication and centrifugation (13,000 × *g* for 30 min) followed by filtration (0.2-μm pore size). The crude extract of CC5A was partially purified by ammonium sulfate fractionation as described earlier (33, 34), and the fraction precipitated with 66% saturated ammonium sulfate was used for the inhibitory activity.

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TABLE 1. Strains and plasmids used in this study

Strains	Relevant characteristics[a]	Source or reference
P. gingivalis		
33277	Type strain from ATCC, FimA type I	Lab collection
49417	Type strain from ATCC, FimA type II	Lab collection
BH18/10	FimA type I	21
OMZ409	FimA type II	21
W83	FimA type IV	5
HG564	FimA type IV	21
UPF	Derivative of *P. gingivalis* 33277 containing *fim*A::*lac*Z gene fusion in its chromosomal DNA; Em^r	31
S. cristatus		
CC5A	Wild-type strain	20
ARCE	Derivative of *S. cristatus* CC5A containing an insertional mutation in the *arcA* gene; Em^r	This study
CR3	Clinical isolate	7
CR311	Type strain NCTC 12479	8
CH34110	Clinical isolate	Lab collection
PSH1a	Clinical isolate	7
PSH1b	Clinical isolate	7
S. gordonii		
DL1	Wild-type strain	14

[a] Em^r, resistance to erythromycin.

RNA isolation and quantitative RT-PCR. *S. cristatus* cells were harvested and resuspended in 300 μl distilled H_2O and 900 μl Trizol (Invitrogen, Carlsbad, CA). The cells were disrupted by using a Mini-Beadbeater 3110BX (BioSpec Products, Bartlesville, OK). *P. gingivalis* was grown on TSB blood agar plates with or without 10 μg of partially purified ArcA, as described before (34). Bacteria were homogenized in Trizol reagent (Invitrogen). The RNA in the supernatant was then purified using an RNeasy minispin column (Qiagen, Valencia, CA). RNA samples were digested on-column with RNase-free DNase. The total RNA was tested using an Agilent 2100 Bioanalyzer to ensure the quality of the samples. Real-time reverse transcriptase PCR (RT-PCR) analysis was performed by using a QuantiTect SYBR green RT-PCR kit (Qiagen) on an iCycler MyiQTM real-time PCR detection system (Bio-Rad Laboratories, Inc., Redmond, WA) according to the manufacturer's instructions. Amplification reactions consisted of a reverse transcription cycle at 50°C for 30 min, an initial activation at 95°C for 15 min, and 40 cycles of 94°C for 15 s, 58°C for 30 s, and 72°C for 30 s. The melting curve profile was analyzed to verify a single peak for each sample, which indicates primer specificity. The expression levels of the investigated genes for the test sample were determined relative to the untreated calibrator sample by using the comparative cycle threshold (ΔC_T) method. The ΔC_Ts were calculated by subtracting the average C_T of the test sample from the average C_T of the calibrator sample and were then used to calculate the ratio between the two by assuming 100% amplification efficiency. By loading the same amount of total RNA for any comparable samples, the ΔC_T represents the difference in gene expression between the samples. The following primers were used for amplification: CGGAACGAATAACCCAGAGA and CTGACCAAC GAGAACCCACT for the *fimA* gene of 33277, OMZ409, and BH18/10; GGC CTTGACGACTTCTTTGA and ATGCAGTCCCACCAGGATAG for *fimA* of W83; CTAAAATCGCAGCCCTTGTC and GACGCCTCCAATTCGTATGT for *fimA* of HG564; and TGAAGTGACGATGAGCCAGT and GCCAATGA AGCACCGAATAG for *fimA* of 49417. Primers TCCAATGCCAAACCTT TACT and ATACGAGTATCTTCTTCACG were designed to complement the highly conserved regions of *arcA*. All experiments were performed at least three times.

β-Galactosidase assays. *S. cristatus* protein fractions (10 μg) were mixed with 10^5 cells of *P. gingivalis* UPF, which contains a chromosomal *fimA* promoter-*lacZ* reporter construct (34), and spotted onto a TSB blood agar plate. The ability of the fractions to inhibit *fimA* expression in *P. gingivalis* was determined with a β-galactosidase assay. Expression of the *lacZ* gene under the control of the *fim*A promoter was measured by the standard spectrophotometric β-galactosidase assay with ONPG (*o*-nitrophenyl-β-D-galactopyranoside as the substrate, as described previously (31).

Arginine deiminase assay. The arginine deiminase assay was performed in 96-well microplates as previously described (34). Briefly, surface extracts of *S. cristatus* cells were collected by centrifugation and sonication. A 100-μl (5-μg) volume of sample was added to each well and mixed with 50 μl of 0.1 M L-arginine. The mixtures were allowed to react for 1 h at 37°C and were then terminated by the addition of 50 μl of 20% sulfuric acid. Finally, 1% 2,3-butanedione monoxime (Sigma, St. Louis, MO) was added to each well, and the reaction was developed by incubation in the dark for 1 h at 56°C. The resultant gradients of a peach color were quantitated with a Benchmark plus microplate spectrophotometer (Bio-Rad, Hercules, CA) at 492 nm.

Western blot analysis. *S. cristatus* strains were grown in Trypticase peptone broth for 18 h. The surface proteins were collected by sonication and centrifugation as described previously (33). Protein concentrations of the samples were determined by using a Bio-Rad protein assay. The soluble proteins (5 μg) were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, along with prestained molecular weight standards (Bio-Rad), and were transferred to nitrocellulose membranes (Gibco BRL, Rockville, MD) with a mini-Transblot electrophoretic transfer cell (Bio-Rad) at 100 V for 1 h. The membrane was treated with 30 ml of blocking solution (3% bovine serum albumin in phosphate-buffered saline [PBS] containing 0.1% Tween 20, pH 7.4) for 1 h and incubated for 1 h with a polyclonal anti-ArcA antibody diluted 1:1,000 in PBS containing 0.1% Tween 20, pH 7.4. The membrane was then rinsed twice and washed three times for 15 min each with 0.1% Tween 20 in PBS. The membrane was incubated with anti-rabbit immunoglobulin horseradish peroxidase-conjugated secondary antibodies for 1 h and rinsed and washed as described above. Antigen-antibody reactivity was visualized by enhanced chemiluminescence (GE Healthcare Bio-Sciences Corp., Piscataway, NJ).

Dental plaque sampling. Twenty patients with chronic periodontitis were recruited among those newly admitted to the clinic of School of Dental Medicine, State University of New York at Buffalo. The study was approved by the Health Science Institutional Review Board. Patients were informed of the protocols and gave informed consent. Subjects with chronic periodontitis presented with radiographic periodontal bone loss of ≥4 mm at multiple teeth. Additional inclusion criteria were an age of ≥21 years, pocket depth of ≥5 mm and concomitant radiographic evidence of bone loss, no systemic conditions that indicate periodontitis as a manifestation of systemic diseases, no use of systemic antibiotics in the previous 6 months, and no prior periodontal treatment. A total of 20 subjects, 12 males and 8 females, with a mean age of 47.8 years, were enrolled.

All samples were obtained by the same dentist to standardize the sampling procedure. Before sampling, the selected teeth were isolated with cotton rolls. Plaque samples were obtained from one of the deepest periodontal sites by inserting sterile endodontic paper points as deep as possible and keeping them in place for 30 s. The paper points were placed in microcentrifuge tubes containing 500 μl Tris-EDTA buffer. The tubes were then vortexed for 60 s and centrifuged at 12,000 × *g* for 3 min. The pellets in the tubes were stored at −70°C until DNA extraction.

DNA extraction was carried out as follows. The pellets in the microcentrifuge tubes were resuspended in 100 μl Tris-EDTA buffer, boiled for 20 min, and centrifuged at 12,000 × *g* for 2 min. DNA concentration in the supernatants was determined using a NanoDrop spectrophotometer.

P. gingivalis cells and *S. cristatus* cells in the plaque were enumerated by using a QuantiTect SYBR green PCR kit with *P. gingivalis* species-specific 16S rRNA gene primers (TGTAGATGACTGATGGTGAAA and ACTGTTAGCAACTA CCGATGT) (29) or CC5A, CR3, and CR311 strain-specific *arcA* gene primers (CTGACGAAGCGAAAGGTCTG and ATGTGGTTGAGCGATACAGC). Standards used to determine *P. gingivalis* or *S. cristatus* cell numbers were prepared using genomic DNAs from the wild-type strain 33277 or CC5A. A fresh culture of bacteria was mildly sonicated to release single cells from the bacterial clumps, and the bacterial culture was then serially diluted in PBS and plated to enumerate CFU at each dilution. DNA was also isolated from the dilutions, and a quantitative PCR assay was performed by using the QuantiTect SYBR green PCR kit (Qiagen) on an iCycler MyiQ real-time PCR detection system (Bio-Rad Laboratories, Inc., Redmond, WA) according to the manufacturer's instructions, to determine cell number. Three trials were performed on three separate cultures.

Statistical analysis. The Spearman correlation analysis was used to determine the relationship of distributions of *P. gingivalis* and *S. cristatus* in subgingival dental plaque. The role of ArcA in *fimA* expression in *P. gingivalis* strains was analyzed by two-way analysis of variance.

TABLE 2. Quantification of *fimA* expression in *P. gingivalis* strains

P. gingivalis strain (FimA type)	Fold decrease of *fimA* expression in the presence of ArcA[a]
ATCC33277 (I)	12.8 ± 0.7 (a)
BH18/10 (I)	3.1 ± 0.2 (bc)
OMZ409 (II)	5.2 ± 1.5 (b)
ATCC 49417 (II)	2.3 ± 0.5 (c)
HG564 (IV)	1.4 ± 0.1 (c)
W83 (IV)	1.3 ± 0.2 (c)

[a] Levels of *fimA* transcripts were measured by real-time PCR, and the change in expression levels was calculated by dividing the copy number of the gene transcript in the *P. gingivalis* strains grown in the absence of *S. cristatus* CC5A ArcA by that in the strains grown in the presence of ArcA. Results are means and standard deviations from three independent experiments. Two-way analysis of variance revealed a significant difference in changes among the groups ($P < 0.001$). Means with different letters are significantly different by the post hoc test (Student-Newman-Keuls method, $P < 0.05$).

TABLE 3. Quantification of *arcA* expression in *S. cristatus* strains[a]

Strain	*arcA* expression
S. cristatus CC5A	1.00 ± 0.06
S. cristatus PSH1a	0.04 ± 0.05
S. cristatus PSH1b	0.05 ± 0.01
S. cristatus CR3	1.73 ± 0.01
S. cristatus CR311	3.70 ± 0.40
S. cristatus CH34110	0.09 ± 0.01
S. cristatus ARCE (*arcA* mutant)	0.00 ± 0.00
S. gordonii DL1	0.09 ± 0.01

[a] Transcript levels were measured by real-time PCR, and the relative expression of *arcA* was normalized by 23S rRNA to the expression level in *S. cristatus* CC5A. Results are means and standard deviations from four independent experiments.

RESULTS

Inhibitory spectrum of *S. cristatus* ArcA on *fimA* expression in *P. gingivalis*. We have previously demonstrated the ability of *S. cristatus* CC5A ArcA to repress expression of the *fimA* gene in *P. gingivalis* 33277 (34). Since several variants of FimA have been identified according to their deduced amino acid sequences (21), we tested the inhibitory activity of *S. cristatus* ArcA in a group of *P. gingivalis* strains representing different ATCC type strains and clinical isolates, including strains displaying type I, II, and IV fimbriae. As shown in Table 2, although the greatest ArcA action on *fimA* expression was detected in *P. gingivalis* 33277, expression of *fimA* was inhibited at least twofold in *P. gingivalis* strains carrying type I and II fimbriae (33277, BH18/10, OMZ409, and ATCC 49417). However, *S. cristatus* ArcA had a modest effect on *fimA* expression in type IV FimA strains (W83 and HG567). These results suggest that the inhibitory activity of *S. cristatus* ArcA on *fimA* expression is fimbrial-type specific and that the inhibitory activity of ArcA on *fimA* expression is greater in type I and II *fimA* genotypes of *P. gingivalis*.

Expression and inhibitory function of *arcA* in *S. cristatus*. Since we observed both a higher expression of ArcA and *fimA* inhibitory activity in *S. cristatus* CC5A than in *S. gordonii* DL1, we sought to determine if all *S. cristatus* strains produced the same level of ArcA. Using PCR analysis, we showed that all six strains of our *S. cristatus* collection carried the *arcA* gene (data not shown). However, differential expression of *arcA* in these strains was observed by using real-time PCR. The expression level of *arcA* in CC5A, CR3, and CR311 was at least 10-fold higher than that in PSH1a, PSH1b, and CH34110 (Table 3). As much as a 75-fold difference in expression of *arcA* was observed between *S. cristatus* strains CR311 and PSH1a. To determine differential expression of ArcA in *S. cristatus* at the protein level, we performed Western blot analysis with anti-CC5A antibodies as a probe. The expression pattern of mRNA corresponded with the observed ArcA protein levels. Higher expression of ArcA was found in CC5A, CR3, and CR311 than in PSH1a, PSH1b, and CH34110 (Fig. 1). These results suggest a differential expression of *arcA* among *S. cristatus* strains that may result in a difference in their ability to repress *fimA* expression in *P. gingivalis*.

Our recent study indicated that ArcA is a dual-function protein (34). Besides the ability to repress *fimA* expression in *P. gingivalis*, the enzymatic activity of ArcA catalyzes the hydrolysis of L-arginine to L-citrulline and ammonia. To determine whether *S. cristatus* strains producing more ArcA have higher enzymatic and inhibitory activities, we compared both activities in six strains of *S. cristatus*. The ability to repress *fimA* expression in *P. gingivalis* was measured by a β-galactosidase assay. As shown in Fig. 2A, higher *arcA* expression levels in CC5A, CR3, and CR311 corresponded to a significantly stronger inhibition of *fimA* expression. Expression of *fimA* in *P. gingivalis* was decreased more than 10-fold in the presence of surface extracts isolated from CC5A, CR3, and CR311. However, only 30 to 50% inhibitory activity was observed in PSH1a, PSH1b, and CH34110, similar to that found in *S. gordonii* DL1. We next examined arginine hydrolytic activity in *S. cristatus* strains. As shown in Fig. 2B, high hydrolytic activity was found in CC5A, CR3, and CR311, while much lower hydrolytic activity was observed in PSH1a, PSH1b, and CH34110.

Distribution of *S. cristatus* strains expressing higher levels of ArcA and *P. gingivalis* in dental plaque. Our previous studies indicated that *S. cristatus* CC5A prevents *P. gingivalis* colonization in in vitro studies (32). Thus, we postulated that the distribution of *P. gingivalis* in subgingival bacterial communities is negatively correlated with the distribution of *S. cristatus*, especially strains carrying an *arcA* gene with a high degree of homology with the CC5A *arcA* gene. To test this hypothesis, we collected dental plaques from the deepest periodontal pocket in 20 periodontitis patients prior to periodontal treatment. *P. gingivalis* cells and *S. cristatus* cells in the plaque were enumerated by quantitative PCR with *P. gingivalis* species-specific 16S rRNA gene primers (29) or strain-specific primers for the *arcA* gene for CC5A, CR3, and CR311. The *P. gingivalis* 16S rRNA gene was detected in 13 samples (65%), while the *S.*

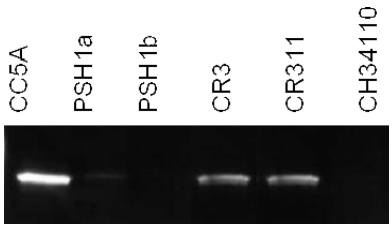

FIG. 1. Western blot analysis of ArcA production in *S. cristatus*. Streptococcal strains were grown in Trypticase peptone broth for 18 h. Cell extracts (5 μg) from streptococcal cells were used.

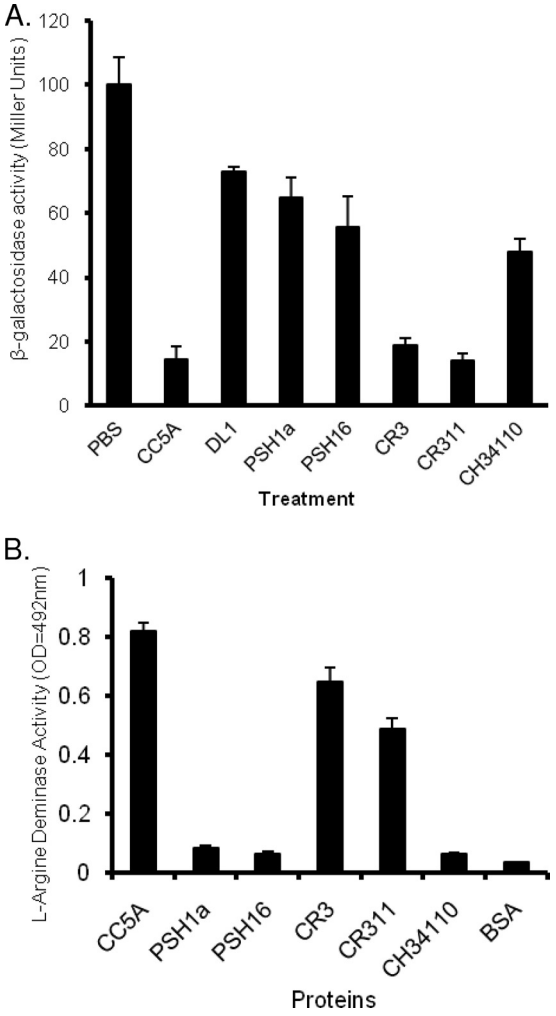

FIG. 2. Evaluation of ArcA function among *S. cristatus* strains. (A) Expression of *fimA* in *P. gingivalis* in response to streptococcal strains. *P. gingivalis* UPF carrying a *fimA* promoter-*lacZ* fusion gene was tested for β-galactosidase activity in the presence and absence of surface extracts (50 μg) isolated from *S. cristatus* or *S. gordonii* strains. The β-galactosidase activity of *P. gingivalis* grown in the presence of *S. cristatus* is indicated relative to the activity level in *P. gingivalis* grown in the absence of *S. cristatus* surface extract (set at 100). Standard errors are indicated ($n = 3$). (B) Comparison of arginase activity in protein fractions of *S. cristatus* strains. Arginine deiminase levels are means ($n = 3$) ± standard deviations.

cristatus arcA gene was detected in 12 samples (55%). Seven samples were negative for both *S. cristatus arcA* and *P. gingivalis* 16S rRNA; therefore, they were not used for further correlation analysis due to the possibility of a false-negative result. As shown in Table 4, there was a statistically significant negative correlation between *P. gingivalis* and *S. cristatus* in the 13 positive samples, by the Spearman correlation analysis ($r = -0.57222$, $P < 0.05$).

DISCUSSION

Periodontal diseases such as chronic periodontitis are among the most common chronic infectious diseases occurring in humans. Because of the chronic nature of periodontitis and concerns of emerging bacterial resistance to antibiotics, development of novel therapeutic agents for the management of periodontitis is warranted. Therapeutic agents that can locally interfere with the colonization of periodontal pathogens would be desirable, since the virulence of the periodontal pathogens is dependent on their initial colonization in the oral cavity. FimA is one of major surface adhesions of *P. gingivalis* and is responsible for bacterial attachment to a number of host and bacterial substrates (13, 30, 31). *P. gingivalis* is classified into at least five types based on the variation of the *fimA* nucleotide sequence (2, 21). We have demonstrated here that ArcA from *S. cristatus* inhibits expression of *fimA* in type I and II genotypes of *P. gingivalis*. Studies of FimA genotypes of *P. gingivalis* showed that the majority of periodontitis patients carried suggested type II FimA genotype, suggesting a strong association with adult periodontitis (1, 5, 19). Although the function and mechanism of FimA variants are not clear, it appears that recombinant type II FimA has higher adhesive activity to human epithelial cells than other types of FimA (22). Therefore, the ability of ArcA to repress type II FimA production may make ArcA a good candidate for a novel therapeutic agent for periodontitis prevention.

Our data demonstrate that not all *S. cristatus* strains are able to inhibit FimA production in *P. gingivalis*. Only strains producing elevated levels of ArcA may be considered antagonistic to *P. gingivalis*. Therefore, based on the expression level of *arcA* and the ability to repress *fimA* expression in *P. gingivalis*, *S. cristatus* strains may be divided into two groups. The promoter regions of *arcA* of CC5A, CR3, and CR311 were sequenced, and DNA alignments show 98% homology among these strains (data not shown). It appears, however, that there is a low degree of homology between the *arcA* promoter regions for the two groups, since amplification of the promoter regions of PSH1a, PSH1b, and CH34110 by PCR using several sets of CC5A *arcA* primers was unsuccessful. It is likely that differential expression of arcA in *S. cristatus* strains is due to variation of their promoter structures.

Several recent studies indicated that the prevalence of *P. gingivalis* as detected with PCR ranges from 61% to 79% in patients without any periodontal treatment (15, 24, 25). We

TABLE 4. Distribution of *P. gingivalis* and *S. cristatus* in subgingival plaques

Patient	No. of bacterial cells/100 ng DNA/sample[a]	
	S. cristatus	*P. gingivalis*
1	53	2,626
2	462	1
3	207	7
4	0	1,862
5	660	2
6	12	220
7	518	12
8	105	1,302
9	707	160
10	1,239	7
11	103	20
12	3,157	100
13	289	1,220

[a] The numbers of *P. gingivalis* and *S. cristatus* cells were determined by using quantitative PCR with 16S rRNA and *arcA*-specific primers, respectively.

report here a 65% detection rate of *P. gingivalis* in previously untreated periodontitis patients. Using quantitative PCR analysis, the numbers of *P. gingivalis* organisms in dental plaque samples were determined and compared with the numbers of *S. cristatus* organisms detected in the same samples. Interestingly, more *P. gingivalis* cells were detected in the plaque samples where the number of *S. cristatus* cells was low. Conversely, fewer *P. gingivalis* cells were discovered in the samples harboring more *S. cristatus*. This negative correlation between the *P. gingivalis* and *S. cristatus* distributions provides evidence of an antagonistic relationship between these oral bacteria. It is likely that lower numbers of *P. gingivalis* detected in dental plaque are due to repression of FimA production induced by the presence of *S. cristatus*. Further studies are required to investigate if prevalence of *S. cristatus* in subgingival plaque is associated with the severity and prognosis of periodontitis. Apparently, the numbers of *S. cristatus* organisms in the subgingival dental plaques are not sufficient to eliminate *P. gingivalis*. It is well known that the oral streptococci are major colonizers in supragingival plaque and can constitute up to 80% of the plaque (26). However, the numbers of oral streptococci and gram-positive bacteria decrease in subgingival plaque in which gram-negative anaerobic bacteria such as *P. gingivalis* are proportionally increased (11, 26). Our observation of the negative correlation between *P. gingivalis* and *S. cristatus* distribution provides opportunities to develop therapeutics specifically against *P. gingivalis* colonization in subgingival plaque.

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