Identification of a signalling molecule involved in bacterial intergeneric communication

Hua Xie,1 Xinghua Lin,1 Bing-Yan Wang,2 Jie Wu1 and Richard J. Lamont3

1School of Dentistry, Meharry Medical College, Nashville, TN 37208, USA
2Department of Periodontics and Endodontics, State University of New York at Buffalo, Buffalo, NY 14214, USA
3Department of Oral Biology, University of Florida, Gainesville, FL 32610-0424, USA

The development of complex multispecies communities such as biofilms is controlled by interbacterial communication systems. We have previously reported an intergeneric communication between two oral bacteria, Streptococcus cristatus and Porphyromonas gingivalis, that results in inhibition of fimA expression. Here, we demonstrate that a surface protein, arginine deiminase (ArcA), of S. cristatus serves as a signal that initiates intergeneric communication. An ArcA-deficient mutant of S. cristatus is unable to communicate with P. gingivalis. Furthermore, arginase activity is not essential for the communication, and ArcA retains the ability to repress expression of fimA in the presence of arginine deiminase inhibitors. These results present a novel mechanism by which intergeneric communication in dental biofilms is accomplished.

INTRODUCTION

Human dental plaque is a multispecies microbial biofilm that is associated with two common oral diseases, dental caries and periodontal disease. More than 700 bacterial species have been detected in the oral cavity, over 50% of which are identified by culture-independent molecular techniques (Aas et al., 2005). Formation of dental plaque is a highly organized developmental process involving a specific sequence of colonization that results in spatially and temporally organized structures (Kolenbrander et al., 2006). Formation of dental plaque is initiated by Gram-positive species, including streptococci and Actinomyces spp., which recognize salivary receptors exposed on the tooth surfaces (Gibbons et al., 1991; Li et al., 2000; Scannapieco et al., 1995). These early colonizers in turn provide new surfaces that attract and recruit succeeding organisms including Gram-negative potential pathogens, such as Porphyromonas gingivalis and Aggregatibacter (Actinobacillus) actinomycetemcomitans (Kolenbrander et al., 2002). Therefore, the early colonizers play a key role in the development of the dental plaque biofilm.

It is recognized that cell–cell communication occurs between bacterial strains, species and genera. A universal language for interspecies bacterial communication is autoinducer-2 (AI-2). LuxS, the AI-2 synthase, has been discovered in many oral bacteria, including Streptococcus mutans, S. oralis, S. gordoni, P. gingivalis and A. actinomycetemcomitans (Chung et al., 2001; James et al., 2006; Merritt et al., 2005; Rickard et al., 2006). LuxS-dependent intercellular communication appears to play an important role in biofilm formation in the oral cavity. McNab et al. (2003) found that a S. gordoni luxS mutant was unable to form normal biofilms with a LuxS-deficient strain of P. gingivalis, and complementation of the luxS mutation in S. gordoni restored normal biofilm formation with the luxS-deficient P. gingivalis. In addition to communication mediated through soluble extracellular signalling molecules, interspecies crosstalk can occur through direct cell-to-cell contact (Aoki et al., 2005). We reported earlier that expression of the P. gingivalis fimA gene, encoding the long fimbrial major subunit protein, is repressed by surface extracts of Streptococcus cristatus (Xie et al., 2000). As the long fimbriae of P. gingivalis are required to initiate heterotypic biofilm formation with oral streptococci, substrata of S. cristatus do not support the development of a mixed biofilm with P. gingivalis (Xie et al., 2000). We show here that arginine deiminase (ArcA) is the inhibitory molecule of S. cristatus. The ability of S. cristatus to communicate with P. gingivalis is diminished in an arcA mutant. We also provide evidence that the ability of ArcA to repress expression of the fimA in P. gingivalis is not correlated with its enzymic activity. This work presents a novel inter-species contact-dependent communication system between P. gingivalis and S. cristatus.

The GenBank/EMBL/DDBJ accession number for the arcA gene sequence of S. cristatus is EF435044.
Bacterial strains and growth conditions. The bacterial strains and plasmids are listed in Table 1. *Streptococcus* strains were grown in Trypticase peptone broth (TPB) supplemented with 0.5% glucose at 37 °C under aerobic conditions. *S. cristatus* CC5A was used as the parental strain for mutant construction. *P. gingivalis* ATCC 33277 was grown from frozen stocks in Trypticase soy broth (TSB) or on TSB blood agar plates, supplemented with 1 mg yeast extract ml−1, 5 μg haemin ml−1 and 1 μg menadione ml−1, at 37 °C in an anaerobic chamber (85% N2, 10% H2, 5% CO2). *Escherichia coli* DH5α was used as the host for plasmids. *E. coli* strains were grown in L broth at 37 °C. Antibiotics were used when appropriate, at the following concentrations: 100 μg gentamicin ml−1 for *P. gingivalis*, 200 μg erythromycin ml−1 for *E. coli* and 10 μg erythromycin ml−1 for *S. cristatus*, 2 μg tetracyline ml−1 for *E. coli* and *S. cristatus*, 50 μg ampicillin ml−1 and 50 μg kanamycin ml−1 for *E. coli*.

Partial purification of the *S. cristatus* inhibitory protein. Surface extracts of *S. cristatus* CC5A 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 (Xie et al., 2004). The fractions precipitated with 33, 42, 50, 55, 60 and 66% saturated ammonium sulfate were designated AS1, AS2, AS3, AS4, AS5 and AS6, respectively. For further purification, the AS6 fraction (1 ml) was dialysed overnight against Tris buffer (50 mM, pH 7.3). The dialysed sample was then applied to a Blue Sepharose column (GE Healthcare), dialysed overnight against Tris buffer (50 mM, pH 7.3). The non-bound proteins were collected from the column. Bound proteins were eluted with Tris buffer supplemented with 1 mM NAD+.

Proteomic analysis. Samples were separated by SDS-PAGE (12% gel) along with prestained size standards (Bio-Rad). Coomassie-stained protein bands of interest were excised and reduced with 10 μl 45 mM dithiothreitol for 20 min at 37 °C. The gel pieces were then digested with trypsin overnight. The peptides were extracted and reconstituted in 20 μl 0.1% trifluoroacetic acid. Approximately 0.4 μl of the peptides were spotted onto a MALDI plate. For each individual sample, the MALDI-TOF mass spectrum and the corresponding MS/MS fragmentation spectra were collectively searched against the SWISS-PROT database using GPS Explorer software (Applied Biosystems) running the MASCOT database search engine (Matrix Science). MALDI-TOF peptide mass maps were internally calibrated to within 20 p.p.m. mass accuracy using trypsin autolytic peptides (m/z 842.51 and 2211.10).

Sequencing of the *S. cristatus arcA* gene. The entire arcA gene of *S. cristatus* CC5A was amplified by the primers 5′-GTACC-GATGCGTCCTTTTGA-3′ and 5′-AGTTATCTACTGTCAGCAG-3′, which were designed based on the completely conserved regions among *Streptococcus suis* flps (AF546864), *Streptococcus equi* subsp. *zooepidemicus* arcA (AB210842) and the *Streptococcus gordonii* DL1 arc operon (AF34569). The PCR product was cloned into pCRII-TOPO vector (Invitrogen) and sequenced by an ABI capillary sequencer (Perkin-Elmer). The sequence is deposited in GenBank (accession number EF435044).

Construction of the *S. cristatus arcA* mutant and arcA-complemented strains. An insertional arcA mutant was generated by using ligation-independent cloning of PCR-mediated mutagenesis (LIC-PCR) (Aslanidis & de Jong, 1996). This procedure involved three steps of PCR to introduce a 2.1 kb ermF-ermAM cassette (Fletcher et al., 1995) into the arcA gene. First, the upstream DNA fragment (549 bp) of the arcA gene was amplified by using Taq DNA polymerase (1 U, Invitrogen) and chromosomal DNA of *S. cristatus* CC5A (0.1 μg) as template with specific primers (5′-ATGTCTACATCATAATTC-3′ and 5′-GATGTTGCAAATACCGATGAGCATC-TGCATACATGTGGTTGA-3′) containing the sequence (underlined) to within 20 p.p.m. mass accuracy using trypsin autolytic peptides (m/z 842.51 and 2211.10).

**Table 1.** Strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics*</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. cristatus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC5A</td>
<td>Low-passage plaque isolate</td>
<td>Lab collection</td>
</tr>
<tr>
<td>ArcAE</td>
<td>Derivative of CC5A containing an insertional mutation in the arcA gene; Em'</td>
<td>This study</td>
</tr>
<tr>
<td>cArcAE</td>
<td>A complemented strain of ARCE harbouring pT-ARCA</td>
<td>This study</td>
</tr>
<tr>
<td><strong>P. gingivalis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 33277</td>
<td>Type strain from ATCC</td>
<td>Lab collection</td>
</tr>
<tr>
<td>UPF</td>
<td>Derivative of ATCC 33277 containing fimA-lacZ gene fusion in its chromosomal DNA; Em’</td>
<td>Xie et al. (1997)</td>
</tr>
<tr>
<td>Mflac</td>
<td>Derivative of ATCC 33277 containing mfa1-lacZ gene fusion in its chromosomal DNA; Em’</td>
<td>This study</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DH5α</td>
<td>F’ 80DlacZA(lacZYA-argF)U169 endA1 supE44 recA1 relA1</td>
<td>BRL</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pVA3000</td>
<td>A suicide vector for <em>Bacteroides</em>; Em’, 5.3 kb</td>
<td>Lee et al. (1996)</td>
</tr>
<tr>
<td>pDN19lac</td>
<td>Contains a promoterless lacZ gene</td>
<td>Xie et al. (1997)</td>
</tr>
<tr>
<td>pRDR215</td>
<td>A wide-host-range plasmid</td>
<td>Xie et al. (1997)</td>
</tr>
<tr>
<td>pPGS749</td>
<td><em>E. coli</em>-Streptococcus shuttle plasmid with Em’</td>
<td>Kuramitsu &amp; Wang (2006)</td>
</tr>
<tr>
<td>pSF143</td>
<td>Suicide vector for streptococci with Tet'; replicates only in <em>E. coli</em></td>
<td>Tao et al. (1992)</td>
</tr>
<tr>
<td>pTet</td>
<td>Shuttle plasmid derived from both pPGS749 and pSF143 with Tet'; replicates in both <em>E. coli</em> and streptococci</td>
<td>This study</td>
</tr>
<tr>
<td>pT-ARCA</td>
<td>pTet plasmid carrying the arcA gene of <em>S. cristatus</em> CC5A</td>
<td>This study</td>
</tr>
<tr>
<td>pCRII-TOTO</td>
<td>A linearized plasmid with single 3’ dT residues; Km’ Am’</td>
<td>Invitrogen</td>
</tr>
</tbody>
</table>

*Km’, Tet’, Em’, Am’, resistance to kanamycin, tetracycline, erythromycin and ampicillin, respectively.
corresponding to the 5’ end of the *ermF-ermAM* cassette. The downstream DNA fragment (549 bp) of the *arcA* gene was amplified with specific primers (5’-ACACGGAGTTCCACACG-3’ and 5’-CCTCTTAGGTCGACCTGACATCCAAGGTGGAGTAGATT-3’) containing the sequence (underlined) corresponding to the 3’ end of the *ermF-ermAM* cassette. Primers 5’-GGTCATCGGTTATTGTTGAC-ACA-3’ and 5’-CTCGAGGTCGACTTAGGAAGG-3’ were used to amplify the *ermF-ermAM* cassette. Each PCR product of the *arcA* gene was then ligated with the *ermF-ermAM* cassette by the second PCR step with primers arcAF and ermR or primers ermF and arcAR, respectively. The second-step PCR products (100 ng) were then mixed and used as template with arcAF and arcAR as primers in the third PCR step to create the fragment arcA-erm-arcA containing the *ermF-ermAM* cassette flanked with upstream and downstream fragments of *arcA*.

The *arcA-erm-arcA* fragment was introduced into *S. cristatus* CC5A cells by DNA transformation (Wang & Kuramitsu, 2005). *arcA*-deficient mutants were constructed via a double-crossover event that introduces the *arcA-erm-arcA* fragment into the CC5A chromosome. The mutants were selected on TPB plates supplemented with erythromycin (10 \( \mu \text{g ml}^{-1} \)) and tetracycline. The mutants were confirmed by PCR analysis, and the one selected for study was designated *S. cristatus* ArcAE.

An *E. coli*–*Streptococcus* shuttle vector was used to construct a complemented strain of ArcAE. To create the *E. coli*–*Streptococcus* shuttle vector, plasmid pSF143 (obtained from L. Tao, University of Illinois, Chicago, IL, USA), which replicates only in *E. coli*, was digested with *HindIII* and *BamHI* to obtain a 5.4 kbp fragment containing a tetracycline-resistance gene (Tobian et al., 1984). Plasmid pPGS749 (Kuramitsu & Wang, 2006) was digested with *Smal* and *BglII*, and a 2.2 kbp fragment that contains a *Rep* origin which replicates in streptococci was purified using a QIAEX II Gel Extraction kit (Qiagen). The two fragments were ligated using T4 ligase to generate *pTet*, a shuttle plasmid with tetracycline resistance that replicates in both *E. coli* and streptococci. *pTet* was then used for complementation of the *arcA* gene. The encoding region of CC5A *arcA* along with 330 bp of upstream sequence from the constant start codon was amplified by PCR with primers 5’-GGGTACCATGCTACACATCCAGGCTGACGACACACAG (KpnI site underlined), and 5’-GGCCATGGGACAAGGTTCCACACG (NcoI site underlined). The PCR product was cloned into *pTet* vector. The recombinant plasmid, *pT-ARCA*, was introduced by transformation into the *arcA*-deficient mutant, *S. cristatus* ArcAE, to create *S. cristatus* ArcAE. After transformation, erythromycin- and tetracycline-resistant transconjugants were selected, and plasmid identity was confirmed by PCR analysis.

**RESULTS**

**Identification of *S. cristatus* inhibitory protein**

We reported previously that the expression of the *fimA* gene is repressed in the presence of surface extracts of *S. cristatus*, but not in the culture medium, indicating the presence of a LuxS-independent intergeneric communication system (Xie et al., 2004). For further purification, the active fraction (AS6, 1 ml) was then applied to a Blue Sepharose column to remove glyceraldehyde-3-phosphate dehydrogenase, one of the major proteins in the AS6 fraction. The non-bound proteins were collected from the column and the fractions were analysed by SDS-PAGE. To test their ability to repress *fimA* expression in *P. gingivalis*, each fraction was mixed with *P. gingivalis* UPF, a strain carrying an *fimA* promoter–*lacZ* fusion. Expression of the *lacZ* gene under control of the *fimA* promoter was measured by the standard spectrophotometric β-galactosidase assay with ONPG as the substrate, as described by Xie et al. (1997).

**Cloning and expression of the *arcA* gene in *E. coli***

The *arcA* gene encoding arginine deiminase, was amplified by PCR with primers 5’-GGGTACCATCGCTACACATCCAGGCTGACGACACACAG (KpnI site underlined) and 5’-GGCCATGGGACAAGGTTCCACACG (NcoI site underlined), which produced a 1200 bp PCR product. The PCR product was then cloned into pCRII-TOPO (Invitrogen). Recombinant arginine deiminase (*rArcA*) was expressed in *E. coli* by using a pTHiophis protein expression system (Invitrogen). The *arcA* DNA fragment was subcloned into pTHiophis-Δ downstream of a His tag. The recombinant *ArcA* was expressed in *E. coli* DH5α cells carrying the pTHiophis-Δ/*arcA* plasmid in the presence of IPTG and kanamycin. His-tagged rArcA was purified with ProBond resin (Invitrogen). The His-tag on the recombinant protein was cleaved with enterokinase and removed by His-bind resin. Enterokinase was then removed by using EKapture agarose.

**Arginine deiminase assay.** The arginine deiminase assay was performed in 96-well microplates as described by Thirkill et al. (1983). *S. cristatus* CC5A protein samples were adjusted with PBS to a constant 100 µl volume in each well, and mixed with 50 µl 0.1 M l-arginine. The mixtures were allowed to react for 1 h at 37 °C and the reactions were then terminated by the addition of 50 µl 20 % sulfuric acid. Finally, 1 % 2,3-butanediol monoxime (Sigma) was added to each well, and the reaction was developed by incubation in the dark for 1 h at 56 °C. The peach colour was quantified with a Benchmark plus microplate spectrophotometer (Bio-Rad) at 492 nm.

**Construction of *P. gingivalis* Mfac strain.** A *P. gingivalis* strain carrying an *mfa1* promoter–*lacZ* fusion was generated by the method described before (Xie et al., 1997). Briefly, the *mfa1* promoter region was amplified by PCR with primers 5’-ACCCATCTCTCTGCT-TCTGC-3’ and 5’-CTCGTTATACATATCCGAAACC-3’, and cloned into pDNHlac to generate the *mfa1* promoter–*lacZ* fusion. The recombinant plasmid was introduced into *P. gingivalis* ATCC 33277 by conjugation. The *P. gingivalis* transconjugants (Mfarc) were selected on TSB plates containing 10 µg erythromycin ml⁻¹.

**β-Galactosidase assays.** *S. cristatus* protein fractions (25 µg) were mixed with 10⁵ cells of *P. gingivalis* UPF, which contains a chromosomal *fimA* promoter–*lacZ* reporter construct, 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 control of the *fimA* promoter was measured by the standard spectrophotometric β-galactosidase assay with ONPG as the substrate, as described by Xie et al. (1997).
eliminate the possibility that a polar effect plays a role in regulating fimA expression, we constructed an arcA mutant of S. cristatus. Insertional inactivation of the S. cristatus arcA gene resulted in a prolonged lag period under the standard growth conditions for streptococci (Fig. 2). This is not surprising since the arginine deiminase pathway is partly responsible for ATP regeneration in bacteria (Crow & Thomas, 1982). Comparison of the ammonium sulfate precipitation fractions AS6 between wild-type CC5A and the mutant strain ArcAE showed that a 47 kDa band was missing from the mutant (Fig. 3a). Furthermore, mutation of arcA abrogated the inhibitory activity toward P. gingivalis fimA expression (Fig. 3b), indicating that arginine deiminase is indeed an effector molecule mediating communication between S. cristatus and P. gingivalis.

The arginine deiminase operon has been extensively studied in S. gordonii DL1 (Caldelari et al., 2000; Dong et al., 2002; Zeng et al., 2006) and consists of five genes that encode enzymes involved in the conversion of arginine to ornithine, ammonia and CO₂ with the concomitant production of ATP (Dong et al., 2002). arcA is the first gene in this operon. To eliminate the possibility that a polar effect plays a role in abolishing inhibitory activity in the arcA mutant, we complemented the mutant with the wild-type allele in trans. As shown in Fig. 3(a), production of ArcA was restored in the complemented strain cArcAE, although the expression level was lower compared to the parental CC5A strain. Complementation of the arcA mutant with the arcA gene partially restored the wild-type phenotype, since surface extracts isolated from the complemented strain cArcAE inhibited 50% of fimA expression in P. gingivalis (Fig. 3b).

**Activity of the arcA mutant and complemented strains**

To confirm the role of ArcA in regulation of fimA expression, we constructed an arcA mutant of S. cristatus. Insertional inactivation of the S. cristatus arcA gene resulted in a prolonged lag period under the standard growth conditions for streptococci (Fig. 2). This is not surprising since the arginine deiminase pathway is partly responsible for ATP regeneration in bacteria (Crow & Thomas, 1982). Comparison of the ammonium sulfate precipitation fractions AS6 between wild-type CC5A and the mutant strain ArcAE showed that a 47 kDa band was missing from the mutant (Fig. 3a). Furthermore, mutation of arcA abrogated the inhibitory activity toward P. gingivalis fimA expression (Fig. 3b), indicating that arginine deiminase is indeed an effector molecule mediating communication between S. cristatus and P. gingivalis.

The arginine deiminase operon has been extensively studied in S. gordonii DL1 (Caldelari et al., 2000; Dong et al., 2002; Zeng et al., 2006) and consists of five genes that encode enzymes involved in the conversion of arginine to ornithine, ammonia and CO₂ with the concomitant production of ATP (Dong et al., 2002). arcA is the first gene in this operon. To eliminate the possibility that a polar effect plays a role in abolishing inhibitory activity in the arcA mutant, we complemented the mutant with the wild-type allele in trans. As shown in Fig. 3(a), production of ArcA was restored in the complemented strain cArcAE, although the expression level was lower compared to the parental CC5A strain. Complementation of the arcA mutant with the arcA gene partially restored the wild-type phenotype, since surface extracts isolated from the complemented strain cArcAE inhibited 50% of fimA expression in P. gingivalis (Fig. 3b).

**Activity of recombinant ArcA protein**

We further confirmed the role of arginine deiminase in the repression of fimA expression in P. gingivalis by cloning and expressing arcA in E. coli. The fimA expression was repressed 2.5- to 3-fold in the presence of the recombinant protein (rArcA) (Fig. 3b), although the inhibitory activity was not as high as that of the natural protein, which was able to inhibit 96% of the fimA expression (Fig. 1). This could be due to incorrect folding or post-translational modification in the heterologous host. The role of rArcA in expression of the short fimbriae (mfa1) was also examined by using a P. gingivalis strain carrying an mfa1-lacZ fusion. In the presence of rArcA, the promoter activity of mfa1 was not modulated in P. gingivalis (Fig. 3b), suggesting a specific role of S. cristatus ArcA in fimA expression. As a control, a major surface protein, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), of S. cristatus CC5A was also cloned and expressed in E. coli. The rGAPDH had no effect on fimA expression (data not shown).

**Dual function of arginine deiminase**

While the arginine deiminase system is found in many bacteria (Burne & Marquis, 2000), relatively few arginine deiminase...
Arginine deiminase-positive bacteria are found in oral biofilms (Zeng et al., 2006). Arginine deiminase catalyses the hydrolysis of L-arginine to L-citrulline and ammonia, and the latter is believed to be important for oral biofilm pH homeostasis and caries prevention (Burne & Marquis, 2000). Besides arginase activity, ArcA can also function as an inhibitor of angiogenesis and tumour growth, which may be due to the depletion of arginine (Gong et al., 2000; Kang et al., 2000; Park et al., 2003). In addition, arginine deiminase plays an important role in the regulation of the level of nitric oxide that is synthesized by NO synthase from arginine, a substrate of arginine deiminase (Gotoh & Mori, 1999). Since these two enzymes compete for the same substrate, antiangiogenic activity may result from the suppression of nitric oxide generation. To address whether the inhibitory activity of ArcA depends on enzyme activity, we examined each fraction for its arginase activity. Relatively high arginine hydrolytic activity was detected in the surface extract of S. cristatus (Table 2). Arginine hydrolytic activity was abolished in the arcA mutant, but was partially restored in the surface extracts of the arcA-complemented strain, which is consistent with production of arginine deiminase. Surprisingly, the purified fraction of arginine deiminase (the unbound fraction of the Blue Sepharose column) did not show an increased hydrolytic activity, despite the fact that at least 10 times more inhibitory activity was found in the purified fraction than in the surface extracts (Table 2). We speculated that the arginase activity is not required for intergeneric communication between S. cristatus and P. gingivalis. To test this hypothesis, communication was tested in the presence of o-aminoguanidine (20 mM) and l-lysine (5 mM), both of which are arginine deiminase inhibitors (Ulisse et al., 2001). These agents completely inhibited the arginase activity in CC5A fractions, but had little effect on the inhibitory activity of the fractions on fimA expression in P. gingivalis (Table 2). These data suggest that the catalytic activity of ArcA is not required for the mechanism of inhibition of fimA expression. It appears that ArcA now joins a growing list of bacterial proteins that can have multiple functions, possibly depending on their location (Jeffery, 1999).

**DISCUSSION**

*P. gingivalis* is a secondary colonizer of dental plaque, and is significantly more prevalent in both supra- and subgingival plaque samples from periodontitis subjects in comparison with healthy subjects (Ximenez-Fyvie et al., 2000). The surface attachment of *P. gingivalis* is promoted by adhesive molecules including fimbriae. The long fimbriae, composed of the FimA subunit, mediate adherence of *P. gingivalis* to a variety of oral substrates and molecules, including proline-rich proteins and glycoproteins, statherin, fibrinogen, fibronectin and lactoferrin (Lamont & Jenkinson, 1998). The fimbriae are also important effector molecules in coaggregation interaction with various early plaque-forming bacteria, such as *Actinomyces viscosus* (Goulbourne & Ellen, 1991), *Streptococcus gordonii* (Lamont et al., 1993) and *Streptococcus oralis*. Amano et al. (1997) also demonstrated that the FimA C-terminal region is involved in coaggregation with *S. oralis*, with functional domains located in regions spanning amino acids 266–286 and 287–337. FimA is also a specific adhesin mediating coaggregation of *P. gingivalis* and *Treponema denticola*, another secondary colonizer (Hashimoto et al., 2003). This specific coaggregation ability with other oral bacteria suggests that the *P. gingivalis* long fimbriae contribute to bacterial integration into dental plaque by interacting with the early and secondary colonizers of...


**ACKNOWLEDGEMENTS**

This work was supported by Public Health Service grants DE014699 (H.X.) and DE12505 (R.J.L.) from the National Institute of Dental and Craniofacial Research.

---

**REFERENCES**


Edited by: M. A. Curtis