Identification of a signalling molecule involved in bacterial intergeneric communication

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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. gordonii*, *P. gingivalis* and *A. actinomyces* (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. gordonii* luxS mutant was unable to form normal biofilms with a LuxS-deficient strain of *P. gingivalis*, and complementation of the luxS mutation in *S. gordonii* 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.
**METHODS**

**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 tetracycline 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 sulfates 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), which was pre-washed with the same Tris buffer (Nelson et al., 2001). 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 autolysic 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-GATGGTCCTGTTTGA-3' and 5'-AGGTATTCTAATCTCGAGCGC-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 using 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, 1990). 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’-ATGTTCTACATACTTCC-3’ and 5’-GATGTTGGAATACCGGAGCATC-3’) containing the sequence (underlined)

### 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’ 80d lacZΔ(lacZΔY---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 in both <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’</td>
<td>Invitrogen</td>
</tr>
</tbody>
</table>

*Km’, Tet’, Em’, Am’, resistance to kanamycin, tetracyline, erythromycin and ampicillin, respectively.*
corresponding to the 5’ end of the *ermF-ermAM* cassette. The downstream DNA fragment (549 bp) of the argA gene was amplified with specific primers (5’-AACAGGTTCCACCCAGC-3’ and 5’-CCTCTAGATTGTAAGAGCCTCCATGAGCAGTGTG-3’) containing the sequence (underlined) corresponding to the 3’ end of the *ermF-ermAM* cassette. Primers 5’-GCTCATCGTTATTTGCAACA-3’ and 5’-CTCGAGCTGACTTACAGG-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 \( \mu \)g). The mutants were then grown overnight in Luria Broth (LB) containing constant 100 \( \mu \)g/ml erythromycin.

**Construction of *P. gingivalis* Mflac strain.** *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’-ACCACCTCTTGCTTTGC-3’ and 5’-CTGTTATCACATATCGGAACCTC-3’, and cloned into pDN19lac to generate the *mfa1* promoter–lacZ fusion. The recombinant plasmid was introduced into *P. gingivalis* ATCC 33277 by conjugation. The *P. gingivalis* transconjugants (Mflac) were selected on TSB plates containing 10 \( \mu \)g erythromycin ml\(^{-1}\).

**β-Galactosidase assays.** *S. cristatus* protein fractions (25 \( \mu \)g) were mixed with 10\(^5\) 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).

**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., 2000). To purify the signal molecule, we first fractionated *S. cristatus* CC5A surface extracts by ammonium sulfate precipitation (Xie et al., 2004). For further purification, the active fraction (AS6, 1 ml) was then applied to a Blue Sepharose column to remove gyceraldehyde-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 a *fimA* promoter–lacZ fusion. Expression of the *lacZ* gene under control of the *fimA* promoter was measured by β-galactosidase assay (Xie et al., 1997). The results shown in Fig. 1 reveal that a protein band of approximately 47 kDa had a high correlation with the inhibitory activity. The ability to repress *fimA* expression was enhanced as the purity of this 47 kDa protein increased. The expression of *fimA* in *P. gingivalis* was inhibited by as much as 96% by the unbound fraction after Blue Sepharose column chromatography (Fig. 1). These data suggested the involvement of the 47 kDa protein in intergeneric communication between *S. cristatus* and *P. gingivalis*.

To identify the 47 kDa protein, we performed in-gel digestion followed by MALDI-TOF mass spectrometry, as...
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 inhibitory activity 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
...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 aminoguanidine (20 μM) 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 surface attachment of *P. gingivalis* is a secondary colonizer of dental plaque, and has been shown to contribute to bacterial integration into dental plaque by interacting with the early and secondary colonizers of 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 aminoguanidine (20 μM) 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).

![Fig. 3. Inhibition of fimA expression in P. gingivalis by ArcA. (a) S. cristatus surface proteins were subjected to SDS-PAGE (12%) and stained with Coomassie blue. Lane 1, molecular mass markers; lane 2, ammonium sulfate fraction AS6 of CC5A; lane 3, ammonium sulfate fraction AS6 of ArcAE (arcA mutant); lane 4, ammonium sulfate fraction AS6 of cArcAE (arcA mutant complemented with the wild-type allele); lane 5, recombinant ArcA purified from *E. coli*. (b) P. gingivalis UPF carrying a fimA promoter-lacZ fusion and P. gingivalis Mlac carrying an mfa1 promoter-lacZ fusion were tested for LacZ activity in the presence or absence of surface extracts (50 μg) isolated from the *S. cristatus* strains indicated, or rArcA. The results are means ± SEM (n=3). Means with different letters are significantly different (P<0.05; Bonferroni test); means with the same letter are not significantly different.]
Table 2. Comparison of arginine activity and the inhibitory activity in protein fractions of *S. cristatus*

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Arginine deiminase activity*</th>
<th>LacZ activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>0.04 ± 0.00</td>
<td>278 ± 21</td>
</tr>
<tr>
<td><em>S. cristatus</em> CC5A surface extract (50 μg)</td>
<td>2.12 ± 0.06</td>
<td>78 ± 10</td>
</tr>
<tr>
<td><em>S. cristatus</em> ArcAE surface extract (50 μg)</td>
<td>0.18 ± 0.01</td>
<td>237 ± 15</td>
</tr>
<tr>
<td><em>S. cristatus</em> cArcAE surface extract (50 μg)</td>
<td>1.46 ± 0.10</td>
<td>152 ± 18</td>
</tr>
<tr>
<td>CC5A surface extract (50 μg) + 10 mM aminoguanidine</td>
<td>0.32 ± 0.04</td>
<td>ND</td>
</tr>
<tr>
<td>CC5A surface extract (50 μg) + 5 mM lysine</td>
<td>0.14 ± 0.01</td>
<td>ND</td>
</tr>
<tr>
<td>Purified fraction AS6 (25 μg)</td>
<td>0.8 ± 0.10</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Purified fraction AS6 (25 μg) + 10 mM aminoguanidine</td>
<td>0.15 ± 0.02</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Purified fraction AS6 (25 μg) + 5 mM lysine</td>
<td>0.15 ± 0.01</td>
<td>21 ± 4</td>
</tr>
</tbody>
</table>

*Arginine deiminase levels are means ± SD (n=3).
†Expression of the fimA gene was determined by measuring LacZ activity, expressed in Miller units. Results are means ± SEM (n=3). ND, Not determined.

dental plaque. Evidently, dental plaque colonization is beneficial to *P. gingivalis* survival in their optimum ecological niche, periodontal pockets. Our earlier finding demonstrated that *S. cristatus* is able to repress expression of the fimA gene in *P. gingivalis* and thus prevent subsequent heterotypic biofilm formation (Xie et al., 2000). The present results provide evidence for the first time that the surface protein arginine deiminase of *S. cristatus* is a signal molecule responsible for cell–cell communication between *S. cristatus* and *P. gingivalis*. As a result of this signal, *P. gingivalis* shuts down expression of the fimA gene. Communication between Gram-positive and Gram-negative bacteria as observed here may be fundamental to bacteria in multispecies biofilms. Interspecies cooperation and competition play important roles in biofilm development and organization. The identification of the molecular basis for an intergeneric contact-dependent communication system provides a molecular basis to begin to understand the differentiation of oral microbial communities from commensal to pathogenic. The study presented here could ultimately lead to the development of novel means to inhibit oral colonization of periodontal pathogens. Oral streptococci are some of the predominant early colonizers of oral plaque (Li et al., 2004), and this unique communication system of sensing foreign species via a surface protein may have been developed to uphold a dominant position in this specialized niche. The consequent inhibition of *P. gingivalis* biofilm formation suggests that susceptibility to periodontal disease may depend to some extent on the microbial composition of the early plaque biofilm and, moreover, that production of arginine deiminase by the oral streptococci may be significant in protection against periodontitis.

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.
formation with autoinducer 2 controls carbohydrate metabolism and biofilm in cancer cells.


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The hemagglutinin genes hagB and hagC of Porphyromonas gingivalis are transcribed in vivo as shown by use of a new expression vector. Infect Immun 64, 4802–4810.


