Supporting Information

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SI Materials and Methods

Construction of Recombinant Plasmids. To construct pCafA, a plasmid expressing coaggregation factor A, primers were designed to amplify the *cafA* promoter, 5′ UTR region, and *cafA* ORF (Table S4). The resulting PCR product was digested with BamHI and PstI and ligated with pJRD215 precut with BamHI/XbaI to generate pCafA. For deletions and truncations of CnaB-type domains, inverse PCR was used, utilizing pCafA as a template accordingly (1). PCR products were purified, phosphorylated at the 5′ ends, and treated with ligase to reform the circular plasmid. Inverse PCR was also used to generate cysteine mutations within the Cna1 region. The resulting plasmids were confirmed by sequencing and electroporated into the appropriate deletion mutant strains.

To generate the vector for CafA antibodies, primers (Table S4) and MG1 chromosomal DNA were used to PCR-amplify the coding region of *cafA* that lacks the N-terminal signal sequence and C-terminal cell wall sorting signal. The resulting DNA fragment was cloned into the expression vector pMCGS7 via ligation-independent cloning (2). The generated plasmid was confirmed by DNA sequencing and transformed into *Escherichia coli* BL21 (DE3). Recombinant CafA was purified and used for antibody production as previously described (3).

Generation of *Actinomyces oris* Mutant Strains. A nonpolar, in-frame deletion of *cafA* was obtained by homologous recombination using mCherry as a counterselectable marker (4). A gene deletion cassette composed of regions flanking the *cafA* ORF was generated by cross-over PCR using specific primers (Table S4) according to a previous protocol (4). Following restriction digest, the DNA fragment was cloned into the nonreplicating vector pCWU3 (4), which harbors *mCherry*, and electroporated into *Actinomyces oris* MG1. Homologous recombination was selected by growth at 37 °C in the presence of kanamycin. To induce plasmid loss, antibiotic-resistant colonies were grown overnight in nonselective media and subsequently plated onto HI agar. Deletion mutants were selected using loss of mCherry fluorescence and PCR analysis. Nonpolar, in-frame deletions of other *aca* genes were obtained using *galK* as a counterselectable marker (5).

Purification of FimB and CafA Proteins from *A. oris*. Recombinant vectors $pFimB_{His6}$ and $pCafA_{His6}$ expressing FimB and CafA, respectively, with a 6×-His tag inserted upstream of the LPXTG motif were generated. *A. oris* Δ*fimB* or Δ*cafA* was transformed with the resulting vectors to generate strains Wu61 and Wu62, respectively. The generated strains were grown in heart infusion (HI) broth until midlog phase, and cells were harvested by centrifugation. Cells were washed twice with sterile water, resuspended in SMM buffer [0.5 M sucrose, 10 mM $MgCl_2$, 10 mM maleate (pH 6.8)], and treated with 5 kilounits of muramidase and 100 mg of lysozyme at 37 °C for 12 h. The soluble cell wall fractions containing His-tagged FimB and CafA were collected by centrifugation, and the proteins were purified by nickel-affinity chromatography.

Cell Fractionation and Western Blot Analysis. This procedure was followed according to a published protocol (6). Briefly, overnight cultures of *Actinomyces* were diluted (1:50) in HI broth and grown at 37 °C until midlog phase. Equivalent aliquots of cells, which were normalized based on OD, were collected and separated from the culture medium by centrifugation. Washed cell pellets were treated with muramidase in SMM buffer at 37 °C for 3 h to obtain cell wall fractions. Culture medium and cell wall fractions were precipitated with trichloroacetic acid, washed with acetone, and resuspended in SDS sample buffer. Samples were separated using 3–12% (mass/vol) Tris·glycine gradient gels; immunoblotted with α-CafA (1:2,500), α-FimA (1:5,000), α-FimB (1:1,000), or α-FimP (1:10,000); and detected by chemiluminescence.

Immunoelectron Microscopy. *Actinomyces* cells grown overnight on HI agar plates were suspended in 0.1 M NaCl and washed twice with PBS. A drop of bacterial suspension in PBS was placed onto nickel-coated carbon grids for 1 min before being washed in PBS containing 2% BSA. Following this step, samples were blocked with PBS containing 0.1% gelatin for 1 h and were then washed and stained with α-CafA (1:100), α-FimA (1:100), α-FimB (1:50), or α-FimP (1:100) for another hour. Grids were subsequently washed and blocked once more before staining with 12-nm or 18-nm gold-conjugated goat anti-rabbit IgG (1:20) in PBS with 2% BSA for 1 h. Finally, samples were washed five times with water, stained with 1% uranyl-acetate, and viewed with a JEOL JEM-1400 electron microscope. For double labeling, after labeling with primary antibodies and IgG-gold particles, samples were reblocked with PBS containing 0.1% gelatin and stained with secondary antibodies, followed by washing with PBS/2% BSA and blocking with gelatin. Samples were then treated with IgG conjugated to a different gold particle diameter than previously used, followed by washing with water and staining with uranyl-acetate.

Biofilm Formation. Overnight cultures of *Actinomyces* were diluted (1:100) into 3 mL of HI broth supplemented with 1% sucrose and 50 μg/mL kanamycin when appropriate. Cultures were placed in a 24-well plate and grown stationary at 37 °C with 5% CO_2 for 48 h. Following incubation, the culture medium was removed by aspiration, cells in wells were washed gently three times with PBS, and the plate was inverted and dried overnight at room temperature. Biofilms were then stained with 1% crystal violet for 10 min, washed three times with water, and dried for 4 h at 37 °C. For quantification of biofilm growth, each well was treated with 95% ethanol for 1 h, and aliquots of 300 μL were transferred to a 96-well plate for absorbance measurement at 580 nm using a Tecan Infinite M1000 plate reader. The experiments were repeated three times in triplicate. Control wells were not inoculated with bacteria.

Bacterial Coaggregation and Hemagglutination Assays. Overnight cultures of *Actinomyces* grown in HI broth and streptococci cultivated in HI broth supplemented with 0.4% glucose were used to perform coaggregation assays as previously reported (5). Cells, harvested by centrifugation, were washed in coaggregation buffer [Tris-buffered saline (TBS) containing 0.1 M $CaCl_2$ (pH 7.5)] and suspended to equal cell densities equivalent to an OD_{600} of 1.0. One-milliliter aliquots of *Actinomyces* and *Streptococcus oralis* cells were mixed in test tubes for 1 min and then transferred to a 24-well plate for imaging using a FluorChem Q Imager (Alpha Innotech). For blocking of coaggregation using recombinant CafA and FimA proteins, *S. oralis* cells were incubated with various amounts of recombinant proteins for 20 min at room temperature before mixing with *A. oris* cells.

For blocking of coaggregation using polyclonal antibodies, overnight cultures of *A. oris* were normalized to an OD_{600} of 2.0 and cell pellets were collected by centrifugation. Cells were washed twice in sterile water and then resuspended in 1 mL of

blocking buffer (TBS, 1% BSA) for 2 h with gentle shaking at room temperature. After incubation, cells were washed with TBS; resuspended in blocking buffer containing various dilutions of FimB, FimA, or CafA antisera; and incubated for another 2 h at room temperature. Of note, the concentrations of α-FimB, α-FimA, and α-CafA were ∼48 mg/mL, ∼47 mg/mL, and ∼68 mg/mL, respectively. Cells were then centrifuged, resuspended in coaggregation buffer, and combined with an equal cell density of *S. oralis* S34 or OC1 to test for coaggregation.

For hemagglutination, the assay was performed as previously described with minor modifications (7). Briefly, O^+-type RBCs (4% packed cells per volume) were treated or mock-untreated with 0.6 units of sialidase (Sigma type VIII *Clostridium perfringens* neuramidase) in hemagglutination buffer [0.2 M PBS (pH 7.4), 0.1 mM $CaCl_2$, 2 mg/mL BSA] for 2 h at 37 °C with gentle agitation. Following the incubation, sialidase was removed by washing the RBCs three times with hemagglutination buffer. Stationary phase cultures of *A. oris* were normalized to an OD_{600} of 1.3 and were also washed three times with hemagglutination buffer. RBCs were combined with *A. oris* in a 12-well plate to form a final concentration of 1% packed cells per volume. The cells were left at room temperature, with gentle agitation before imaging.

1. Wu C, et al. (2012) Structural determinants of *Actinomyces* sortase SrtC2 required for membrane localization and assembly of type 2 fimbriae for interbacterial coaggregation and oral biofilm formation. *J Bacteriol* 194(10):2531–2539.
2. Stols L, et al. (2002) A new vector for high-throughput, ligation-independent cloning encoding a tobacco etch virus protease cleavage site. *Protein Expr Purif* 25(1):8–15.
3. Mishra A, Das A, Cisar JO, Ton-That H (2007) Sortase-catalyzed assembly of distinct heteromeric fimbriae in *Actinomyces naeslundii*. *J Bacteriol* 189(8):3156–3165.
4. Wu C, Ton-That H (2010) Allelic exchange in *Actinomyces oris* with mCherry fluorescence counterselection. *Appl Environ Microbiol* 76(17):5987–5989.
5. Mishra A, et al. (2010) The *Actinomyces oris* type 2 fimbrial shaft FimA mediates co-aggregation with oral streptococci, adherence to red blood cells and biofilm development. *Mol Microbiol* 77(4):841–854.
6. Mishra A, et al. (2011) Two autonomous structural modules in the fimbrial shaft adhesin FimA mediate *Actinomyces* interactions with streptococci and host cells during oral biofilm development. *Mol Microbiol* 81(5):1205–1220.
7. Costello AH, Cisar JO, Kolenbrander PE, Gabriel O (1979) Neuraminidase-dependent hamagglutination of human erythrocytes by human strains of *Actinomyces viscosus* and *Actinomyces naeslundii*. *Infect Immun* 26(2):563–572.

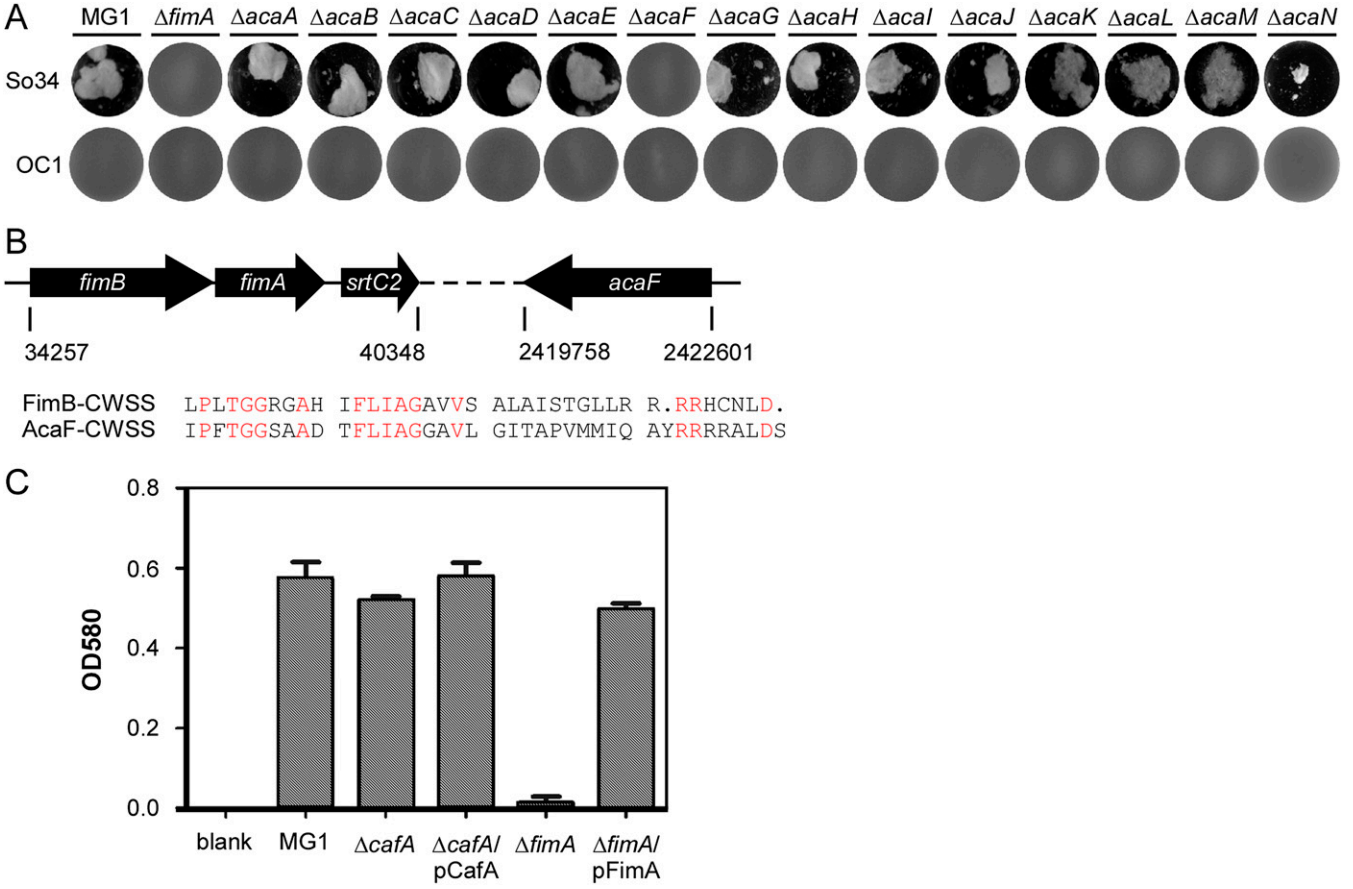

Fig. S1. Identification of coaggregation factor CafA. (*A*) *A. oris* MG1 and its isogenic mutants lacking *fimA* or individual *aca* genes were examined for their coaggregation with receptor polysaccharid (RPS)-positive [*S. oralis* 34 (So34)] and RPS-negative (OC1) *S. oralis*. (*B*) Shown are the arrangement of the type 2 fimbrial gene cluster and *acaF* and the alignment of the cell wall sorting signal (CWSS) of FimB and CafA. Numbers indicate nucleotide positions in the MG1 genome sequence. Conserved residues are highlighted in red. (*C*) *A. oris* cells were grown without agitation in 24-well plates at 37 °C with 5% CO_2 for 48 h. The resulting biofilms were washed with PBS, stained with crystal violet, and quantified by measuring the OD at 580 nm. The values shown are representative of three independent experiments performed in triplicate, with error bars denoting SDs.

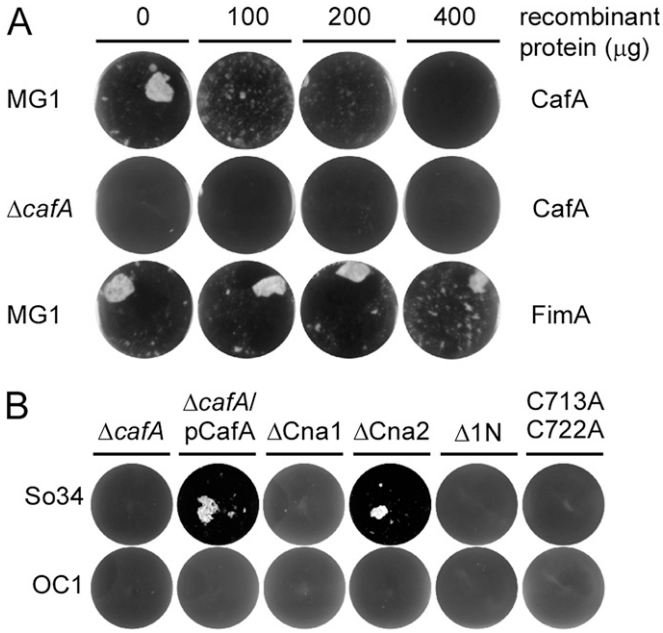

Fig. S2. Requirement of CafA for *A. oris* coaggregation with *S. oralis*. (*A*) *S. oralis* cells were incubated with increasing concentrations of the recombinant CafA or FimA protein before examination for coaggregation with *A. oris* cells. (*B*) *A. oris* strains were examined for coaggregation with RPS-positive (So34) and RPS-negative (OC1) *S. oralis*.

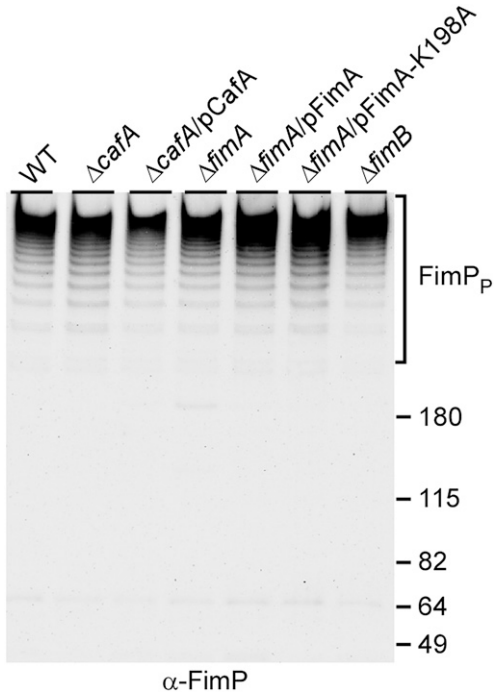

Fig. S3. Cell wall fractions of *A. oris* MG1 (WT) and its isogenic derivatives grown to midlog phase were isolated by muramidase treatment. Equivalent protein samples were separated on 3–12% Tris·glycine gradient gels and detected by immunoblotting with antibodies against α-FimP. Protein polymers (P) and molecular mass markers (kilodaltons) are indicated.

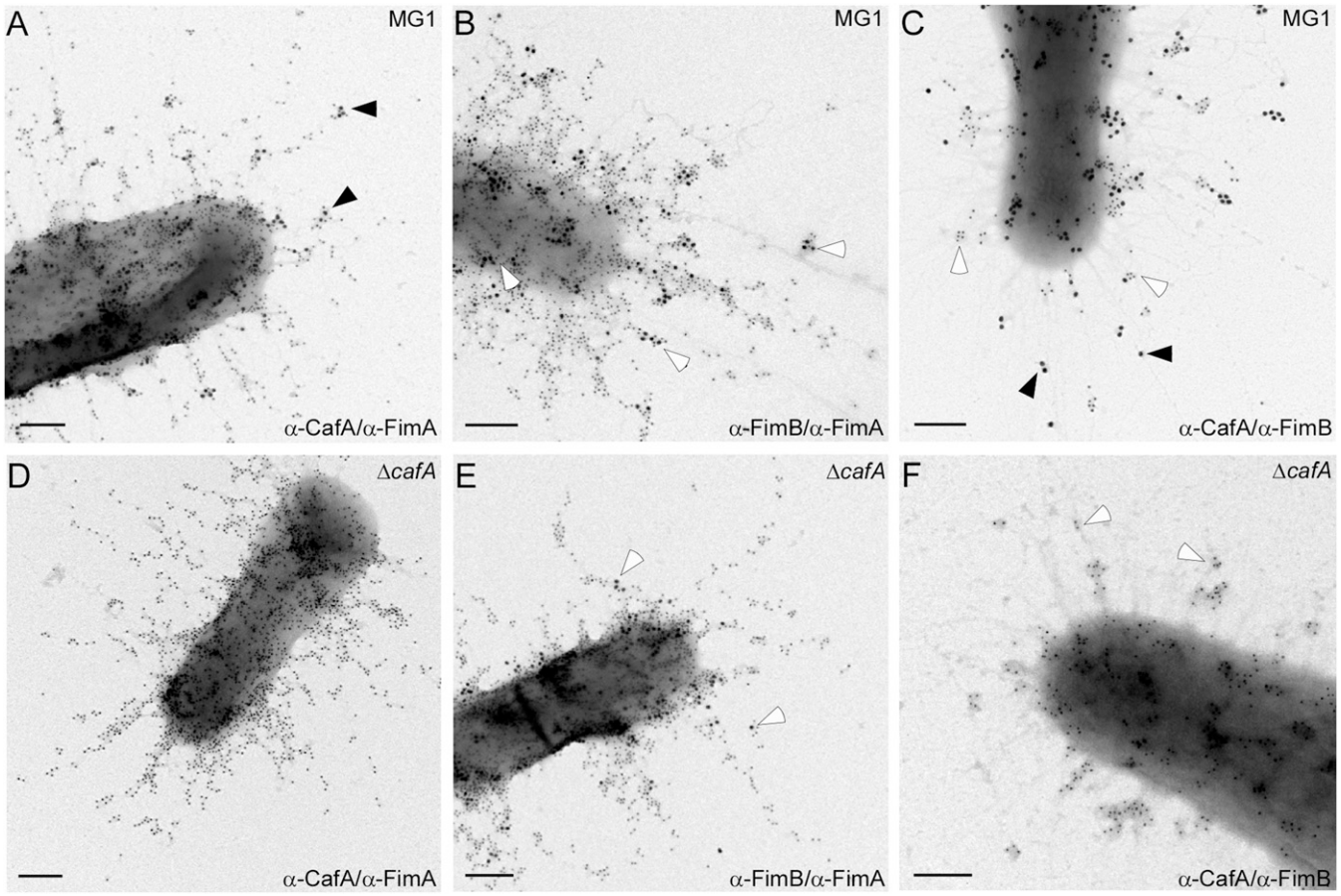

Fig. S4. Colocalization of CafA by immunoelectron microscopy. Bacterial cells were immobilized on carbon-coated nickel grids and stained with α-FimA (*A*, *B*, *D*, and *E*) or α-FimB (*C* and *F*), followed by goat anti-rabbit IgG conjugated to 12-nm gold particles. For double labeling, cells were additionally stained with α-CafA (black arrowheads) or α-FimB (white arrowheadss), followed by goat anti-rabbit IgG conjugated to 18-nm gold particles. (Scale bars: 0.2 μm.)

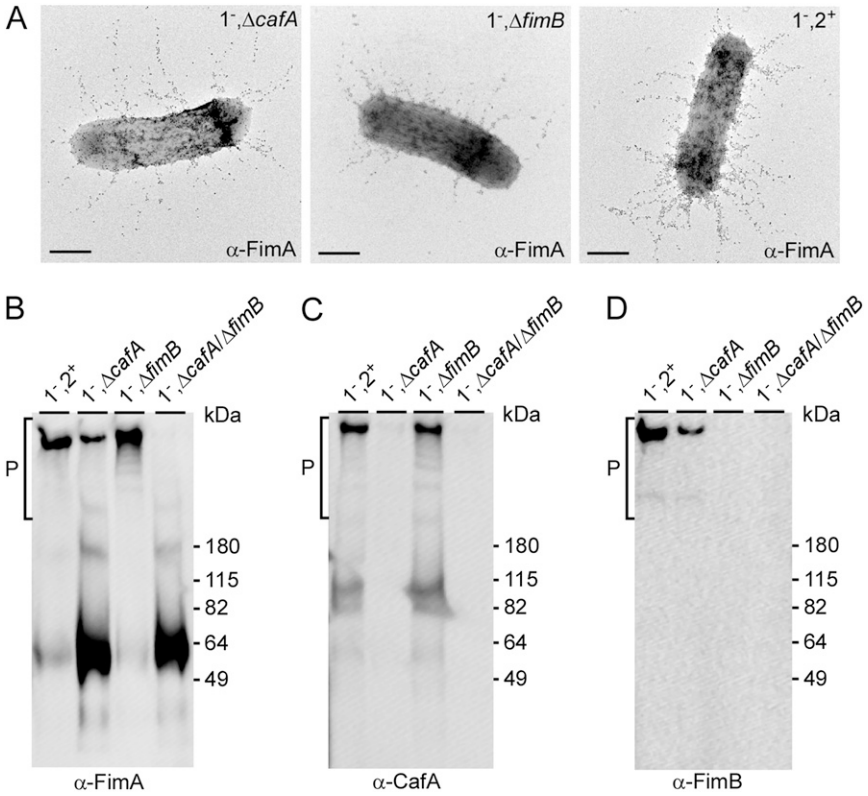

Fig. S5. Requirement of CafA and FimB for FimA polymerization. (*A*) Bacterial variants lacking type 1 fimbriae (1^-) were immobilized on nickel-coated carbon grids and stained with α-FimA, followed by goat anti-rabbit IgG conjugated to 12-nm gold particles. (Scale bars: 0.2 μm.) (*B–D*) Cell wall fractions of *A. oris* strain expressing type 2 fimbriae only ($1^-,2^+$) and its isogenic derivatives lacking *cafA*, *fimB*, or both grown to midlog phase were isolated by muramidase treatment. Equivalent protein samples were separated on 3–12% Tris·glycine gradient gels and detected by immunoblotting with α-FimA (*B*), α-CafA (*C*), or α-FimB (*D*). Protein polymers and molecular mass markers are indicated.

Table S1. Putative cell wall-anchored proteins of *A. oris* MG1

Locus tag*	Homology	Sorting signal†	Amino acid length
AcaA (ANA_0196)	Hemagglutinin-related protein	**LPLTG**TQALFLGGIALLLLAGGGVALMLRRRRLTGSEAG	397
AcaB (ANA_1080)	Copper-binding protein	**LPLTG**ASLTGIVLAVAAIVMGGGFILVRRRMAS	813
AcaC (ANA_1291)	Hypothetical protein	**LAQTG**ANGLLFGGIAAALVAIGGGALVVRRRKA	320
AcaD ANA_1294	Hypothetical protein	**LAHTG**ANGLLFGGIAAFLVVAGGAALVLRRRNKA	565
AcaE (ANA_1409)	Matrix protein 1	**LAHTG**ANAVALVLLAGAGLGGGALLVARRRNAS	643
AcaF (ANA_2235)	CnaB protein	**IPFTG**GSAADTFLIAGGAVLGITAPVMMIQAYRRRRALDS	948
AcaG (ANA_2282)	Matrix protein 2	**LASTG**VRIGLPLGIAVVAVMGGALLVSRRRA	983
AcaH (ANA_2653)	5′-nucleotidase	**LARTG**ASLGAGVLALALLVVGGYLILRRRQQVA	828
AcaI (ANA_2709)	Sialidase	**LSRTG**TNALLVLGLAGVAVVGGYLLLRARRSKN	901
AcaJ (ANA_2720)	PE-PGRS family protein	**LPVTG**VAIGVLSVAAILLSAGLIVLRLRRA	443
AcaK (ANA_1671)	GDSL-like	**LPNTG**INASRLTALAILGLLTGAAVLHYRRKVTS	754
AcaL (ANA_0730)	SCP-like	**LATTG**PSIAVAVVAAGLLGSGAFLVMRRRQAS	319
AcaM (ANA_2013)	Levanase	**LARTG**TSLTLGALGAAAAATGAYALRLRRRRS	943
AcaN (ANA_2328)	Hypothetical protein	**LAPTG**DSQRDGAIAGISSKLDKLRQNNQQRQPTQAPTRTHHPRR	679

GDSL, GDSL lipase; PE-PGRS, PE-PGRS motif; SCP, sperm coating glycoprotein.
*Based on the *A. oris* MG1 genome sequence (http://genome.brop.org/).
†The LPXTG motif is shown in bold.

Table S2. Bacterial strains used in this work

Strain	Genotype and description	Source
A. oris MG1	WT	1
A. oris CW1	Δ*galK*, an isogenic derivative of MG1	1
A. oris CW2	Δ*fimB*, an isogenic derivative of CW1	2
A. oris AR4	Δ*fimA*, an isogenic derivative of CW1	2
A. oris WU50	Δ*acaA*, an isogenic derivative of CW1	3
A. oris AR6	Δ*acaB*, an isogenic derivative of MG1	This study
A. oris WU51	Δ*acaC*, an isogenic derivative of CW1	This study
A. oris WU52	Δ*acaD*, an isogenic derivative of CW1	This study
A. oris AR8	Δ*acaE*, an isogenic derivative of MG1	This study
A. oris AR5	ΔacaF, an isogenic derivative of MG1	This study
A. oris WU53	ΔacaG, an isogenic derivative of CW1	This study
A. oris AR10	Δ*acaH*, an isogenic derivative of MG1	This study
A. oris AR7	Δ*acaI*, an isogenic derivative of MG1	This study
A. oris WU54	Δ*acaJ,* an isogenic derivative of CW1	This study
A. oris WU55	Δ*acaK,* an isogenic derivative of CW1	This study
A. oris WU56	Δ*acaL,* an isogenic derivative of CW1	This study
A. oris WU57	Δ*acaM,* an isogenic derivative of CW1	This study
A. oris WU58	Δ*acaN,* an isogenic derivative of CW1	This study
A. oris WU59	Δ*fimB*/Δ*acaF*, an isogenic derivative of CW1	This study
A. oris AR5c	AR5 containing pAcaF	This study
A. oris AR5c1	AR5 containing $pAcaF_{His6}$	This study
A. oris CW2c	CW2 containing $pFimB_{His6}$	This study
A. oris AR5c2	AR5 containing $pAcaF_{\Delta 691–781}$	This study
A. oris AR5c3	AR5 containing $pAcaF_{\Delta 691–731}$	This study
A. oris AR5c4	AR5 containing $pAcaF_{\Delta 732–781}$	This study
A. oris AR5c5	AR5 containing $pAcaF_{(C713A/C722A)}$	This study
A. oris AR5c6	AR5 containing $pAcaF_{\Delta 810–894}$	This study
A. oris WU65	Δ*fimPQ*, an isogenic derivative of CW1 and type 1-negative	This study
A. oris WU66	Δ*fimPQ*/Δ*srtC1*, an isogenic derivative of WU65	This study
A. oris WU67	Δ*fimPQ*/Δ*srtC1*/Δ*fimB*, an isogenic derivative of WU66	This study
A. oris WU68	Δ*fimPQ*/Δ*srtC1*/Δ*acaF*, an isogenic derivative of WU66	This study
A. oris WU69	Δ*fimPQ*/Δ*srtC1*/Δ*acaF*/Δ*fimB*, an isogenic derivative of WU68	This study
S. oralis 34	WT strain (type 1 Gn receptor polysaccharide)	4
S. oralis OC1	Δ*wchA*, an isogenic derivative of 34	5

1. Mishra A, Das A, Cisar JO, Ton-That H (2007) Sortase-catalyzed assembly of distinct heteromeric fimbriae in *Actinomyces naeslundii*. *J Bacteriol* 189(8):3156–3165.
2. Mishra A, et al. (2010) The *Actinomyces oris* type 2 fimbrial shaft FimA mediates co-aggregation with oral streptococci, adherence to red blood cells and biofilm development. *Mol Microbiol* 77(4):841–854.
3. Wu C, Ton-That H (2010) Allelic exchange in *Actinomyces oris* with mCherry fluorescence counterselection. *Appl Environ Microbiol* 76(17):5987–5989.
4. Abeygunawardana C, Bush CA, Tjoa SS, Fennessey PV, McNeil MR (1989) The complete structure of the capsular polysaccharide from *Streptococcus sanguis* 34. *Carbohydr Res* 191(2): 279–293.
5. Yoshida Y, Ganguly S, Bush CA, Cisar JO (2006) Molecular basis of L-rhamnose branch formation in streptococcal coaggregation receptor polysaccharides. *J Bacteriol* 188(11):4125–4130.

Table S3. Plasmids used in this work

Plasmid	Genotype and description	Source
pARU82	Expressing *acaF* (residues 44–913)	This study
pAcaF	pJRD215 expressing WT AcaF from MG1	This study
$pAcaF_{His6}$	pJRD215 expressing His-tagged AcaF	This study
$pAcaF_{\Delta 691–781}$	pJRD215 expressing AcaF lacking the Cna1 domain	This study
$pAcaF_{\Delta 691–731}$	pJRD215 expressing AcaF lacking residues 691–731	This study
$pAcaF_{\Delta 732–781}$	pJRD215 expressing AcaF lacking residues 632–781	This study
$pAcaF_{(C713A/C722A)}$	pJRD215 expressing AcaF with C713A/C722A mutations	This study
$pAcaF_{\Delta 810–894}$	pJRD215 expressing AcaF lacking the Cna2 domain	This study
$pFimB_{His6}$	pJRD215 expressing His-tagged FimB	This study
pCWU2	Derivative of pHTT177, expressing GalK under the control of the *rpsJ* promoter	1
pCWU3	Derivative of pHTT177, expressing mCherry under the control of the *rpsJ* promoter	2
pJRD215	*Actinomyces/E. coli* shuttle vector, kanamycin-resistant	2
pCWU2-AcaA	pCWU2 allelic replacement of *acaA*	This study
pCWU3-AcaB	pCWU3 allelic replacement of *acaB*	This study
pCWU2-AcaC	pCWU2 allelic replacement of *acaC*	This study
pCWU2-AcaD	pCWU2 allelic replacement of *acaD*	This study
pCWU3-AcaE	pCWU2 allelic replacement of *acaE*	This study
pCWU3-AcaF	pCWU3 allelic replacement of *acaF*	This study
pCWU2-AcaF	pCWU2 allelic replacement of *acaF*	This study
pCWU2-AcaG	pCWU2 allelic replacement of *acaG*	This study
pCWU2-AcaH	pCWU3 allelic replacement of *acaH*	This study
pCWU2-AcaI	pCWU3 allelic replacement of *acaI*	This study
pCWU2-AcaJ	pCWU2 allelic replacement of *acaJ*	This study
pCWU2-AcaK	pCWU2 allelic replacement of *acaK*	This study
pCWU2-AcaL	pCWU2 allelic replacement of *acaL*	This study
pCWU2-AcaN	pCWU2 allelic replacement of *acaN*	This study
pCWU2-fimQ	pCWU2 allelic replacement of *fimQ*	3
pCWU2-fimB	pCWU2 allelic replacement of fimB	1

1. Mishra A, et al. (2010) The *Actinomyces oris* type 2 fimbrial shaft FimA mediates co-aggregation with oral streptococci, adherence to red blood cells and biofilm development. *Mol Microbiol* 77(4):841–854.
2. Wu C, Ton-That H (2010) Allelic exchange in *Actinomyces oris* with mCherry fluorescence counterselection. *Appl Environ Microbiol* 76(17):5987–5989.
3. Wu C, et al. (2011) Dual function of a tip fimbrillin of *Actinomyces* in fimbrial assembly and receptor binding. *J Bacteriol* 193(13):3197–3206.

Table S4. Primers used in this study

Primer	Sequence*	Used for
AcaA-up-F	GGCGGAATTCGCCGGAGGCGCCGTCGGGGAAG	pCWU2-AcaA
AcaA-up-R	GGCGGGTACCAGGATCTCCGTTAGACACGG	pCWU2-AcaA
AcaA-dn-F	GGCGGGTACCCAGCGAGACTGCGACCAGCAG	pCWU2-AcaA
AcaA-dn-R	GGCGTCTAGAGGTGGGCGTACTTCTGGTCCAT	pCWU2-AcaA
AcaB-A	AAAGAATTCTTCACGGTCGTGGCCGAG	pCWU3-AcaB
AcaB-B	CCCATCCACTAAACTTAAACAT	pCWU3-AcaB
AcaB-C	TGTTTAAGTTTAGTGGATGGG	pCWU3-AcaB
AcaB-D	AAAGAATTCAACTCGGAGGGG	pCWU3-AcaB
AcaC-up-F	GGCGGAATTCCCTGGGACTGCCGGACGAGTCCTA	pCWU2-AcaC
AcaC-up-R	GGCGGGTACCTCCAAGACGCATGAGTGCTCCTAG	pCWU2-AcaC
AcaC-dn-F	GGCGGGTACCACCGACGCCACCCCGGCGGCCTCCA	pCWU2-AcaC
AcaC-dn-R	GGCGTCTAGATTCTCCTGGGTGATCTTGAAGCTG	pCWU2-AcaC
AcaD-up-F	GGCGGGTACCCGCGCCAGCTCCAGTAGCCGGTCG	pCWU2-AcaD
AcaD-up-R	GGCGTCTAGAGCGCGCCCTCATGGGCTGCGCATCC	pCWU2-AcaD
AcaD-dn-F	GGCGTCTAGAGAGTAGATCTGCGTCTGCTGCTGCT	pCWU2-AcaD
AcaD-dn-R	GGCGGAATTCGGACTCCACCTCCCACGACGAGCA	pCWU2-AcaD
AcaE-A	AAAGAATTCTCCCCACCGTCT	pCWU3-AcaE
AcaE-B	CCCATCCACTAAACTTAAACA	pCWU3-AcaE
AcaE-C	TGTTTAAGTTTAGTGGATGGG	pCWU3-AcaE
AcaE-D	AAAGAATTCAAAGAATTCAGCTCAACGACC	pCWU3-AcaE
AcaF-A	AAATCTAGAGAGTCTTGTTGACG	pCWU3-AcaF
AcaF-B	CCCATCCACTAAACTTAAACA	pCWU3-AcaF
AcaF-C	TGTTTAAGTTTAGTGGATGGG	pCWU3-AcaF
AcaF-D	AAATCTAGATGTCACCGACTA	pCWU3-AcaF
AcaG-up-F	GGCGTCTAGAGATCGTGCGTCGGTCGGCCCGCG	pCWU2-AcaG
AcaG-up-R	GGCGGGTACCTCTCATGCGACGGTCACGAATCGG	pCWU2-AcaG
AcaG-dn-F	GGCGGGTACCTGACGCCGGCCGCTAGTTCCCAC	pCWU2-AcaG
AcaG-dn-R	GGCGGAATTCCATCCTGCCAGTCATTGACCCAT	pCWU2-AcaG
AcaH-A	AAAGAATTCGCGATCTACGCC	pCWU2-AcaH
AcaH-B	CCCATCCACTAAACTTAAACA	pCWU2-AcaH
AcaH-C	TGTTAAAGTTTAGTGGATGGG	pCWU2-AcaH
AcaH-D	AAAGAATTCCAGGACGGAGTT	pCWU2-AcaH
AcaI-A	AAAGAATTCGTGCCACCTGCA	pCWU2-AcaI
AcaI-B	CCCATCCACTAAACTTAAACA	pCWU2-AcaI
AcaI-C	TGTTTAAGTTTAGTGGATGGG	pCWU2-AcaI
AcaI-D	AAAGAATTCGCCATCGCTAAA	pCWU2-AcaI
AcaJ-up-F	GGCGTCTAGAGGCGCCGGCTTGGGTCGCCAGG	pCWU2-AcaJ
AcaJ-up-R	GGCGGAATTCACGCACAGCCTTCTTCACTGTTAC	pCWU2-AcaJ
AcaJ-dn-F	GGCGGAATTCTGAAGCAGGGGCGGCGGCGCGG	pCWU2-AcaJ
AcaJ-dn-R	GGCGGGTACCCGTTCGTGCGCCTTATGGCACG	pCWU2-AcaJ
AcaK-up-F	GGCGTCTAGACGACTTCATCCTGTCTGTGAGCG	pCWU2-AcaK
AcaK-up-R	GGCGGGTACCGTTCATGTGTCGTTCTCTCTGATC	pCWU2-AcaK
AcaK-dn-F	GGCGGGTACCAGCTAACTGAGCTGAAGGCTCTC	pCWU2-AcaK
AcaK-dn-R	GGCGGAATTCGTCCGTGCGCTGGGACGGCAGCC	pCWU2-AcaK
AcaL-up-F	GGCGGGTACCCACGTGGTTTCAGTGGAAGTAC	pCWU2-AcaL
AcaL-up-R	GGCGGAATTCGCGATTCACTTCGGTTTCCTTTCG	pCWU2-AcaL
AcaL-dn-F	GGCGGAATTCACCTCATAGCCGGTGATACCCG	pCWU2-AcaL
AcaL-dn-R	GGCGTCTAGATCGAGATCGCCATGGAGCTGGAG	pCWU2-AcaL
AcaM-up-F	GGCGGGTACCGCGGCCAGGTCGGCGGCGCTGATC	pCWU2-AcaM
AcaM-up-R	GGCGGAATTCGGAGTGTAGTGGCTCTGCGGGCGC	pCWU2-AcaM
AcaM-dn-F	GGCGGAATTCTGAGCGGCAGCGCCTCCCTCACG	pCWU2-AcaM
AcaM-dn-R	GGCGTCTAGAGATTTGCACGACGTGGATCCCGT	pCWU2-AcaM
AcaN-up-F	GGCGGAATTCGACTATCAGGGCATGCCTCCTTTG	pCWU2-AcaN
AcaN-up-R	GGCGCATATGACTCATCAGGCTCAGAGCCGTGAAC	pCWU2-AcaN
AcaN-dn-F	GGCGCATATGCAGGACCCGATCAAGGCCCGCCT	pCWU2-AcaN
AcaN-dn-R	GGCGGGTACCCTTCTTGGTCTCTCCAGCGCCCTG	pCWU2-AcaN
Com-acaF-F	GGCGGGTACCCAAGAAGCGCGTCGTAGATCTCCCAGG	pAcaF, $AcaF_{His6}$
Com-acaF-R	GGCGCATATGGTCATGAGTCCAGGGCCCGCCGGCGT	pAcaF, $pAcaF_{His6}$
AcaF-(6his)-F	CACCACCACCACCACCACGTGCCCGCCATCCCCTTCACCGGAG	$pAcaF_{His6}$
AcaF-(TEV)-R	TCCTTGGAAGTAGAGGTTCTCCGCCGACTTGCGGTTGGAGATCTTGCCCAG	$pAcaF_{His6}$
AcaF(cna1)-F	CCCACCCTCGACCTGGGTCAGGTCACCAAC	$pAcaF_{\Delta691–781}$
AcaF(cna1)-R	CGGGTTGCCGGCGTCGTCGACTTG	$pAcaF_{\Delta691–781}$
AcaF(cna1)-2F	CGTGCCGGGTTCTTCAGGGTCTCCGG	$pAcaF_{\Delta691–731}$

Table S4. Cont.

Primer	Sequence*	Used for
AcaF(nca1)-2R	AGGGTCTTGGTCCACCGAGCCATTGGG	pAcaF$_{\Delta 732–781}$
AcaF(cna2)-F	CGGGTTGCCGGCGTCGTCGAC	pAcaF$_{\Delta 810–894}$
AcaF(cna2)-R	TCGACGACGCCGGCAACCCGC	pAcaF$_{\Delta 810–894}$
FimB-(6his)-F	CACCACCACCACCACCACACCCCCCGCTTGCCCCTGACAGGCGG	pFimB$_{His6}$
FimB-(TEV)-R	TCCTTGGAAGTAGAGGTTCTCGGCCGCCTTCTCGTTGGTGAAGGCCTTCG	pFimB$_{His6}$
Com-fimB-F	GGCGGAATTCAGGGCCTCGGCTTCGCCATCCGTG	pFimB$_{His6}$
Com-fimB-R	GGCGCATATGCGTCTTGTCCGACGGCGTCACCGG	pFimB$_{His6}$
AcaF(C713/722A)-F	GGGAAGGCCACGGCACCCAATGGCTCGGTGGACCAAGACCCTCG	pAcaF$_{(C713/722A)}$
AcaF(C713/722A)-R	ACCGGTGACGACTGCGTCGGTCACTTCCTTCTGACCGCCGTTGAG	pAcaF$_{(C713/722A)}$
AcaF-Bam-5	AAAGGATCCCTGGGCACAGCGACGCTC	pARU82
AcaF-Pst-3	AAACTGCAGTCATCCGGTGAAGGGGATG	pARU82

dn, downstream; F, forward; R, reverse; TEV, tobacco etch virus; up, upstream.
*Restriction sites in the primers are underlined.