A protein secretion system linked to bacteroidete gliding motility and pathogenesis

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Abstract

Porphyromonas gingivalis secretes strong proteases called gingipains which are implicated in periodontal pathogenesis. Protein secretion systems common to other Gram-negative bacteria are lacking in *P. gingivalis* but several proteins, including PorT, have been linked to gingipain secretion. Comparative genome analysis and genetic experiments revealed 11 additional proteins involved in gingipain secretion. Six of these (PorK, PorL, PorM, PorN, PorW, Sov) were similar in sequence to *Flavobacterium johnsoniae* gliding motility proteins and two others (PorX, PorY) were putative two-component system regulatory proteins. Real-time RT-PCR analysis revealed that *porK, porL, porM, porN, porP, porT,* and *sov* were downregulated in *P. gingivalis porX* and *porY* mutants. Disruption of the *F. johnsoniae porT* ortholog resulted in defects in motility, chitinase secretion, and translocation of a gliding motility protein, SprB adhesin, to the cell surface, providing a link between a protein translocation system and a motility apparatus in members of the *Bacteroidetes* phylum.

Introduction

Periodontal disease, the major cause of tooth loss in industrial nations (1, 2), is one of the most frequently occurring infectious diseases in humans (3), and is a chronic inflammatory disease that results in destruction of periodontal tissue and alveolar bone (4). The Gram-negative anaerobic bacterium *Porphyromonas gingivalis* is a major periodontal pathogen (5). *P. gingivalis* secretes extracellular and cell-surface gingipain proteases that are major virulence factors involved in periodontal pathogenesis (6, 7). Gingipains consist of Arg-specific cysteine proteinases (Rgp) encoded by *rgpA* and *rgpB,* and the Lys-specific cysteine proteinase (Kgp) encoded by *kgp* (8-10). Gingipains have signal peptides to allow transit of the cytoplasmic membrane via the Sec machinery, but the mechanism of secretion across the outer membrane is not known. Studies of Gram-negative bacteria belonging to the phylum *Proteobacteria* have identified at least eight different protein secretion systems (11). Four of these (type I, III, IV and VI secretion systems) transport proteins across the entire Gram-negative cell envelope and thus do not typically transport proteins with N-terminal signal peptides. Other secretion systems (type II and type V machineries, the two-partner secretion system, and the chaperone/usher system) mediate only the final step (transit across the outer membrane) and rely on the Sec or twin arginine transport systems to escort proteins across the cytoplasmic membrane. *P. gingivalis* is a member of the *Bacteroidetes* phylum, and is thus not closely related to the *Proteobacteria*. Analysis of the *P. gingivalis* genome suggested that critical components of known bacterial protein secretion systems were lacking in *P. gingivalis*, suggesting that some other machinery may be involved in gingipain secretion.

Genetic and biochemical analyses indicate that the membrane protein PorT is involved in gingipain secretion (12). Genes related to *porT* are found in many members of the large and diverse *Bacteroidetes* phylum, including gliding bacteria such as *Flavobacterium johnsoniae* and *Cytophaga hutchinsonii*, and nonmotile anaerobes such as *Prevotella intermedia*. *porT* orthologs have not been detected outside of the *Bacteroidetes* phylum, and they are also lacking from some members of the *Bacteroidetes*, such as the intestinal anaerobes *Bacteroides thetaiotaomicron* and *Bacteroides fragilis*. In this study, eleven additional genes that appear to be involved in gingipain secretion and activation were identified by comparative genome analysis and genetic experiments.

Results

Identification of Genes Involved in Gingipain Secretion by Comparative Genome Analysis and Genetic Experiments. Bacterial protein secretion systems typically require multiple proteins that form a complex in the cell envelope. PorT is required for *P. gingivalis* gingipain secretion, and may interact with other proteins to form the translocation machinery. *porT* homologs are found in some other members of the phylum *Bacteroidetes*, such as *C. hutchinsonii* ATCC 33406, but not in others, such as *B. thetaiotaomicron* VPI-5482 (13-15). To identify possible additional components of the gingipain secretion system we identified 55 genes in addition to *porT* that were present in *P. gingivalis* and *C. hutchinsonii* but were absent in *B. thetaiotaomicron* (Fig. S1 and Table S1). *P. gingivalis* strains with deletion mutations in 46 of these genes were constructed and gingipain activities were determined. Mutation of *sov*

(PGN_0832), which was recently reported to be involved in gingipain secretion (16), or of any of 9 other genes which we designated *porK* (PGN_1676), *porL* (PGN_1675), *porM* (PGN_1674), *porP* (PGN_1677), *porQ* (PGN_0645), *porU* (PGN_0022), *porW* (PGN_1877), *porX* (PGN_1019) and *porY* (PGN_2001) resulted in decreased Rgp or Kgp activity in cells and culture supernatants (Fig. 1*A*). The mutants displayed cell-associated gingipain proproteins with high molecular masses as revealed by immunoblot analysis with anti-Hgp44 and anti-Kgp antibodies (Fig. 1*B*). These proproteins were inactive, as previously described (12). Introduction of wild-type copies of *por* genes on plasmids into the appropriate mutants resulted in complementation of the extracellular and cell surface gingipain defects, confirming the roles of the Por proteins in secretion (Fig. 1*C*).

Five of the genes described above, *porK*, *porL*, *porM*, *sov* and *porW*, are similar to *F. johnsoniae* gliding motility genes *gldK, gldL, gldM, sprA* and *sprE*, respectively (17, 18). In *F. johnsoniae*, *gldN*, which is also involved in gliding motility, is located immediately downstream of *gldK, gldL* and *gldM.* The *P. gingivalis gldN* ortholog (PGN_1673), which we refer to as *porN,* lies immediately downstream of *porK*, *porL*, and *porM*. A *porN* defective mutant of *P. gingivalis* was constructed, and was found to exhibit almost no extracellular or cell surface gingipain activities (Fig. 1*A*). Gingipain activity was restored by complementation with plasmid encoded wild-type PorN (Fig. 1*C*). *porN* mutant cells accumulated unprocessed gingipain proproteins intracellularly (Fig. 1*B*), further confirming a role for PorN in protein secretion.

Subcellular Localization of PorP-PorN Proteins in *P. gingivalis*. PorK has a hydrophobic amino terminal sequence terminated by a cysteine, which is characteristic of bacterial lipoproteins, whereas PorM, PorN and PorP have typical signal peptides at their amino termini and PorL is predicted to have two membrane-spanning helices near its amino terminus. Subcellular fractionation and immunoblot analyses were performed to experimentally determine the locations of these proteins. PorL and PorM were detected in the cytoplasmic membrane fraction, whereas PorK, PorN and PorP were in the outer membrane fraction (Fig. 2*A*). Blue native polyacrylamide gel electrophoresis revealed that anti-PorK antibody reacted to a protein band with a molecular mass of more than 1,200 kDa, which disappeared or decreased in intensity in the *porM* and *porN* mutants (Fig. 2*B*). In addition, anti-Myc antibody reacted to a protein band with a molecular mass of more than 1,200 kDa in strains expressing *porL'-'myc*, *porM'-'myc* or *porN'-'myc*. Thus, these proteins may form part of a protein complex.

***P. gingivalis* Genes Regulated by the Putative Response Regulator PorX and the Putative Histidine Kinase PorY.** PorX and PorY are similar to response regulatory proteins and histidine sensor kinases respectively of two-component signal transduction systems, and may have roles in regulation of expression of genes of the transport system. PorX and PorY are 'orphan' signal transduction proteins since the expected cognate partners do not appear to be encoded by nearby genes in either case. Given the similar phenotypes of the mutants it is possible that the two proteins function together as a two-component signal transduction system. To determine what genes are regulated by PorX, microarray analysis using a custom tiling DNA array chip with the genome

sequence of *P. gingivalis* ATCC 33277 was performed. The tiling DNA array analysis revealed that 20 genes were down-regulated in the *porX* deletion mutant to less than 60% of the wild type parent strain (Table S2). *porT*, *sov*, *porK*, *porL*, *porM*, *porN* and *porP*, which were among the 20 down-regulated genes, are each involved in gingipain secretion (Fig. S2), and the decreased expression of these genes was confirmed by RT-PCR analysis (Fig. 3*A*). RT-PCR analysis revealed that these genes were also down-regulated in the *porY* mutant (Fig. 3*B*).

Disruption of the *F. johnsoniae porT* Ortholog *sprT* Results in Motility Defects. *P. gingivalis* is nonmotile but many other members of the phylum *Bacteroidetes*, such as the cellulolytic bacterium *C. hutchinsonii*, the chitin digesting *F. johnsoniae*, and the fish pathogen *Flavobacterium psychrophilum* exhibit rapid gliding motility over surfaces. Bacteroidete gliding is not closely related to other well-studied forms of bacterial movement such as flagellar motility, type IV pilus-mediated twitching motility, myxobacterial gliding motility, and mycoplasma gliding motility (19). Fifteen genes (*gldA, gldB, gldD, gldF, gldG, gldH, gldI, gldJ, gldK, gldL, gldM, gldN*, *sprA, sprB,* and *sprE*) that play important roles in bacteroidete gliding have been identified (17-20). *F. johnsoniae*, *F. psychrophilum* and *C. hutchinsonii* each have a *porT* ortholog. Since PorT, PorK, PorL, PorM, and PorN appear to function together in *P. gingivalis*, we hypothesized that the *F. johnsoniae* PorT ortholog may function with GldK, GldL, GldM, and GldN and thus have a role in gliding. A *F. johnsoniae porT* ortholog mutant was constructed and was found to be deficient in gliding motility (Fig. 4*A*). The mutant formed nonspreading colonies on agar, and individual cells were severely

but not completely deficient in gliding in wet mounts. Whereas wild-type cells attached readily to the glass slide and displayed rapid motility, the mutant cells exhibited very limited motility. Most mutant cells failed to attach to the glass, and most of the cells that did attach failed to move. However, extended observation revealed a few cells (typically less than 1 out of 1000) that exhibited occasional slight movements. Because this phenotype is similar to that exhibited by nonspreading *sprA* mutants (17) we named the gene *sprT*. Complementation of the *sprT* mutant with a wild-type copy of the gene on a plasmid restored colony spreading and single cell motility.

***sprT* Mutant Cells are Defective in Translocation of the Gliding Motility Adhesin, SprB, to the Cell Surface.** There are several possible roles of protein translocation in bacteroidete gliding motility. Mounting evidence suggests that the gliding machinery propels adhesive proteins, such as SprB, along the cell surface (20). The secretion system may be needed for assembly of SprB or other cell-surface components of the motility apparatus on the cell surface. Latex spheres carrying antibodies against SprB attach specifically to wild-type *F. johnsoniae* cells, and are rapidly propelled along their surfaces (20). The antibody coated spheres failed to bind to cells of *sprB* mutants. We used antibody-coated spheres to determine whether SprB was present on the surface of cells of *sprT* mutants. Wild-type and *sprT* mutant cells produced SprB protein as determined by Western blot analysis (Fig. S3) but spheres carrying antibodies against SprB failed to bind to cells of the *sprT* mutant (Movies S1, S2, S3). Under the conditions tested 65 out of 100 randomly selected wild-type cells bound and propelled

spheres during a 30 s incubation, whereas none of 100 *sprT* mutant cells bound or propelled spheres. Complementation of the *sprT* mutant with the wild-type gene on a plasmid restored the ability to bind and propel antibody-coated spheres to wild-type levels (Movie S4). This indicates that SprB is not properly exposed on the cell surface of the *sprT* mutant, and suggests that SprT may be involved in secretion of this adhesin to the cell surface. Detection of SprB by immunofluorescence confirmed that *sprT* mutant cells are defective in surface localization of SprB protein (Fig. 4*B*). Cells with mutations in *gldK*, *gldL*, *gldM*, *gldN* and *sprA* behaved similary to those of the *sprT* mutant. They produced SprB protein (20) but were deficient in surface localization of SprB as determined by failure to bind spheres coated with anti-SprB antibodies, and by decreased staining by anti-SprB immunofluorescence microscopy.

***sprT* Mutant Cells are Deficient in Extracellular Chitinase Activity.** Previous studies demonstrated that many *F. johnsoniae* mutants defective in gliding also exhibit deficiencies in chitin utilization, but the reason for the lack of chitin utilization has not been elucidated (17). Chitinolytic activity was determined using three synthetic substrates, 4-methylumbelliferyl N-acetyl-β-D-glucosaminide (4-MU-GlcNAc), 4-methylumbelliferyl N, N'-diacetyl-β-D-chitobioside (4-MU-$(GlcNAc)_2$) and 4-methylumbelliferyl β-D-N, N', N''-triacetylchitotrioside (4-MU-$(GlcNAc)_3$). The wild-type strain and a *sprT*/*sprT*$^+$ plasmid-mediated complementation strain exhibited strong hydrolytic activities against 4-MU-$(GlcNAc)_2$ and 4-MU-$(GlcNAc)_3$ in their culture supernatants, whereas those of the *sprT* and *gldJ* mutants did not (Fig. 4*C*). The culture supernatants of the wild type and *sprT*/*sprT*$^+$ plasmid-mediated

complementation strain contained a putative chitinase encoded by Fjoh_4555 that appeared to be lacking in the culture fluids of *sprT* mutant cells (Fig. S4). Lysates of *Escherichia coli* cells expressing recombinant Fjoh_4555 protein also displayed chitinase activity (Fig. S5) supporting the role of Fjoh_4555 in chitin digestion. These results suggest that SprT and GldJ are involved in chitinase secretion by *F. johnsoniae*, and that part of the bacteroidete gliding motility machinery is also a protein translocation apparatus.

Discussion

Results in this study indicate a link between a protein translocation system and a motility apparatus in members of the *Bacteroidetes* phylum. Other well-studied bacterial motility machines also function in protein translocation. The flagellar basal body is a rotary motor, but also functions as a type III secretion system involved in flagellar assembly and has served as a paradigm for studies of type III protein secretion in general (11). Similarly, pilus mediated twitching motility involves movement of proteins across the cell envelope, and is closely related to type II secretion systems.

The protein secretion system identified in this study, which we refer to as the Por secretion system (PorSS), is because the proteins involved (PorK, PorL, PorM, PorN, PorP, PorT, PorW, and Sov) do not exhibit sequence similarity to components of known secretion systems. Protein products from *rgpA, rgpB, kgp* and *hagA* appear to be secreted via the *P. gingivalis* PorSS (12). These proteins have a conserved C-terminal domain (CTD) (21). CTD structures were also found in predicted proteins

of other bacteria in the phylum *Bacteroidetes*, including *P. intermedia*, *Tannerella forsythia*, *Parabacteroides distasonis, C. hutchinsonii*, *F. johnsoniae* and *F. psychrophilum,* each of which also contain *porT* orthologs (14, 15, 22). Genes encoding such CTDs were not found in *B. thetaiotaomicron* or *B. fragilis,* which both also lack *porT* orthologs, supporting the suggestion that the CTD proteins are secreted using the PorSS. The C-terminal regions of *F. johnsoniae* SprB (Fjoh_0979) and chitinase (Fjoh_4555) are not closely similar to the *P. gingivalis* CTD. If the CTD sequences are involved in interaction with the secretion apparatus they may have diverged considerably within the *Bacteroidetes* phylum, as have the individual components of the putative secretion systems of distantly related members of this phylum.

The gliding bacteria *F. johnsoniae, F. psychrophilum* and *C. hutchinsonii*, possess complete sets of *gld, spr* and *por* genes (Table S3). In contrast, the nonmotile *P. gingivalis, P. intermedia* and *P. distasonis* contain orthologs of some but not all of the *gld* and *spr* genes. Some of the missing orthologs, such as *gldD*, *gldF*, and *gldG* may have essential roles in motility, but not in protein export. Many of the *gld, spr* and *por* genes are absent from *B. fragilis* and *B. thetaiotaomicron,* which are both nonmotile and appear to lack PorSS.

Bacterial protein secretion has been most intensively studied in members of the phylum *Proteobacteria* and in Gram-positive bacteria. Few studies of protein secretion have been conducted for the members of other bacterial phyla. Our studies of members of the phylum *Bacteroidetes* identified what appears to be a protein secretion system that may have evolved independently from the previously known

systems. The PorSS is involved in pathogenesis of *P. gingivalis* and in gliding motility and chitin utilization of *F. johnsoniae*. PorSSs appear to be present in many other bacteroidetes where they may have diverse functions. These include important periodontal pathogens such as *P. intermedia* and *T. forsythia*, and common fish pathogens such as *F. psychrophilum* and *Flavobacterium columnare*. An improved understanding of the PorSS may aid in control of the diseases associated with these bacteria. The addition of the PorSS to the already extensive list of bacterial protein secretion systems suggests that machinery to transport proteins across the bacterial outer membrane may have evolved independently many times. Analysis of protein secretion in the many other under-studied phyla of bacteria may uncover additional secretion machines.

Materials and Methods

Bacterial Strains and Culture Conditions. Bacterial strains and plasmids used in this study are listed in Table S4. *P. gingivalis* cells were grown anaerobically (10% CO_2, 10% H_2, 80% N_2) in enriched brain heart infusion medium and on enriched tryptic soy agar (8). For blood agar plates, defibrinated laked sheep blood was added to enriched tryptic soy agar at 5%. For selection and maintenance of antibiotic-resistant *P. gingivalis* strains, antibiotics were added to the medium at the following concentrations: erythromycin (Em), 10 μg/ml; tetracycline (Tc), 0.7 μg/ml. *F. johnsoniae* ATCC 17061 (UW101) was the wild-type strain used in this study. *F. johnsoniae* cells were grown in Casitone-yeast extract medium at 30°C (23). To observe colony spreading as a result of gliding motility, *F. johnsoniae* cells were grown

on PY2 agar at 25°C (24). To observe gliding of individual cells, *F. johnsoniae* was grown in motility medium (MM) (25) overnight at 25°C without shaking. For selection and maintenance of the antibiotic-resistant *F. johnsoniae* strains, antibiotics were added to the medium at the following concentrations: Em, 100 μg/ml; Tc, 15 μg/ml.

Venn Diagram Analysis. The pairwise reciprocal best-hits were calculated among *P. gingivalis* ATCC 33277, *B. thetaiotaomicron* VPI-5482, and *C. hutchinsonii* ATCC 33406. Homologous genes were identified by BLASTP searches using E-value threshold $\leq 10^{-10}$. Orthologous genes were defined as reciprocal best-hits.

Construction of Bacterial Strains. Construction and complemetantion of *P. gingivalis* deletion mutants and of a *F. johnsoniae sprT* mutant were performed as previously described (8, 24) and are detailed in *SI Text*. Construction of an *E. coli* strain expressing Fjoh_4555 is described in *SI Text*. Primers used in this study are listed in Table S5.

Enzymatic Assays. For Kgp and Rgp assays, *P. gingivalis* cells were grown anaerobically in enriched brain heart infusion medium at 35°C overnight. Bacterial cells and culture supernatants were separated by centrifugation at 10,000 x g for 10 min. Cells were suspended in the original volume of PBS. Kgp and Rgp activities were determined using the synthetic substrates

t-butyl-oxycarbonyl-L-valyl-L-leucyl-L-lysine-4-methyl-7-coumarylamide (Boc-Val-Leu-Lys-MCA) and carbobenzoxy-L-phenyl-L-arginine-4-methyl-7-coumarylamide (Z-Phe-Arg-MCA) in 20 mM sodium phosphate buffer (pH 7.5) containing 5 mM cysteine in a total volume of 1 ml. After incubation at 40°C for 10 min, the reaction was terminated by adding 1 ml of 10 mM iodoacetamide (pH 5.0), and the released 7-amino-4-methylcoumarin was measured at 460 nm (excitation at 380 nm). One unit of enzyme activity was defined as the amount of enzyme required to release 1 nmol of 7-amino-4-methylcoumarin under the conditions. Kgp and Rgp activities are indicated at units per ml of cell suspensions or culture supernatants. All cultures had similar cell densities of $OD_{600\ nm}$ of ~1.0.

For chitinase activity assay, *F. johnsoniae* cells were incubated in MM (25) at 25°C overnight with shaking at 170 rpm. Bacterial cells and culture supernatants were separated by centrifugation at 10,000 x *g* for 10 min at 4°C. Cells were suspended in the original volume of a buffer consisting of 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2PO_4, 2mM KH_2PO_4, pH 7.4 (PBS). Chitinase activity was determined with the synthetic substrates 4-methylumbelliferyl N-acetyl-β-D-glucosaminide (4-MU-GlcNAc, Sigma); 4-methylumbelliferyl N, N'-diacetyl-β-D-chitobioside (4-MU-$(GlcNAc)_2$, Sigma); 4-methylumbelliferyl β-D-N, N', N''-triacetylchitotrioside (4-MU-$(GlcNAc)_3$, Sigma) using a chitinase assay kit (Chitinase Assay Kit, Fluorimetric, Sigma) according to manufacturer's instructions. Enzyme assays were performed in triplicate. One unit of the enzyme activity was defined as the amount of enzyme able to release 1 μmol of 4-methylumbelliferone per min. Chitinase activity is indicated as units per ml of cell suspensions or culture supernatants. For measurement of recombinant chitinase

activity in *E. coli* BL21(DE3) harboring pKF003, cells were grown in LB broth at 37°C until $OD_{600\ nm}$ of 0.6. Cells were placed on ice for 10 min, supplemented with 0.1 mM isopropyl-β-D-thiogalactopyranoside and incubated at 16°C for 15 h. Cells were harvested by centrifugation, resuspended in PBS, disrupted by sonication, and chitinase activity was determined as described above. The recombinant chitinase activity is indicated as units per microgram of protein.

Quantification of Gene Expression by Real-Time RT-PCR. Total RNA was isolated from *P. gingivalis* cells grown to mid-exponential phase (OD_{600nm} of ~1.0) by using an RNeasy Mini kit (Qiagen Science). DNA was removed with RNase-Free DNase. cDNA was generated in a reaction mixture containing a random primer (Promega), dNTP mixture, RNase inhibitor, dithiothreitol, Superscript III Reverse Transcriptase (Invitrogen) and DEPC-treated water. Real-time quantitative PCR (qPCR) was performed using Brilliant SYBR Green II QPCR Master Mix (Stratagene) with Mx3005PTM Real-Time PCR System (Stratagene) according to the manufacturer's instruction. Primers for the real-time qPCR are listed in table S6 and were designed using Primer3 program. Real-time qPCR conditions were as follows: 1 cycle at 95°C for 10 min, and 35 cycles of 95°C for 30 sec and 60°C for 1 min. At each cycle, accumulation of PCR products was detected by the reporter dye from the dsDNA-binding SYBR Green. To confirm that a single PCR product was amplified, after the PCR, a dissociation curve (melting curve) was constructed in the range of 55°C to 95°C. All data were analyzed using Mx3005P software. The expression level of each targeted gene was normalized to that of the 16S rRNA gene, which was used as a

reference. All PCR reactions were carried out in triplicate. The efficiency of primers binding was determined by linear regression by plotting the cycle threshold (C_T) value versus the log of the cDNA dilution. Relative quantification of transcript was determined using the comparative C_T method ($2^{-\Delta\Delta CT}$) calibrated to 16S rRNA. qPCR experiments were performed multiple times independently with comparable results.

Subcellular Fractionation. Subcellular fractionation of *P. gingivalis* cells was performed as previously described (12). Briefly, *P. gingivalis* cells from a 3,000 ml culture were harvested by centrifugation at 10,000 x *g* for 30 min at 4°C, and resuspended with 100 ml of PBS containing 0.1 mM N^{a}-*p*-tosyl-L-lysine chloromethyl ketone (TLCK), 0.1 mM leupeptin and 0.5 mM EDTA. The cells were disrupted by 2 passes through a French pressure cell at 100 MPa. The remaining intact bacterial cells were removed by centrifugation at 2,400 x *g* for 10 min, and the supernatant was subjected to ultracentrifugation at 100,000 x *g* for 60 min. The pellets were treated with 1% Triton X-100 in PBS containing 20 mM $MgCl_2$ for 30 min at 20°C to solubilize the cytoplasmic membrane. The outer membrane fraction was recovered as a precipitate by ultracentrifugation at 100,000 x *g* for 60 min at 4°C. The supernatant was obtained as the cytoplasmic membrane fraction.

Protein Preparation and Western Blot Analysis. Western blots were performed to detect SprB in extracts of wild-type and mutant cells of *F. johnsoniae*. Overnight cultures were grown in MM at 25°C without shaking. Cells were pelleted by

centrifugation at 3,800 x *g* for 10 min and suspended in a buffer consisting of 10 mM Tris, 8 mM $MgSO_4$, pH 7.5 (TM). Cells were lysed by boiling for 5 min in SDS-PAGE loading buffer. Protein (25 μg), as determined by BCA assay (Pierce), was loaded per lane and proteins were separated on 3 to 8% Criterion XT Tris-acetate acrylamide gels (Bio-Rad, Hercules, CA) and transferred to nitrocellulose membranes. SprB was detected with SuperSignal West Pico (Pierce) using a FOTO/Analyst LuminaryFx Workstation (Fotodyne).

For preparation of protein samples of culture supernatants of *F. johnsoniae,* cells incubated in MM at 25°C overnight with shaking at 170 rpm were centrifuged at 10,000 x *g* for 10 min at 4°C. Solid ammonium sulfate was added to the culture supernatant to 50% (w/v) saturation. After centrifugation for 15 min, the pellets were dissolved in 10 mM Hepes (pH 7.4) and dialyzed overnight against 10 mM Hepes (pH 7.4) in a Slide-A-Lyzer ® Dialysis Cassette (3,500 MWCO) (PIERCE) and subjected to SDS-polyacrylamide gel electrophoresis (PAGE).

Blue Native PAGE. *P. gingivalis* cells from a 0.5 ml culture were harvested by centrifugation at 10,000 x *g* for 10 min at 4°C, and suspended with 200 μl of sample buffer containing 1% *n*-dodecyl-β-D-maltoside (DDM). After sonication, the samples were centrifuged at 20,000 x *g* for 30 min at 4°C to clarify. The samples were supplemented with Coomassie blue G250 at a final concentration of 0.25% and electrophoresed on a non-denaturing polyacrylamide gel (NativePAGE™ Novex® 3-12% Bis-Tris Gels, Invitrogen).

Mass Spectrometry. A gel plug containing proteins was subjected to the following procedures: washing with 50% (v/v) acetonitrile, washing with 100% acetonitrile, reduction with 10 mM DTT, alkylation with 55 mM iodoacetamide, washing/dehydration with 50% (v/v) acetonitrile, and digestion for 10 h with 10 μg/ml trypsin. The resulting peptides were extracted from the gel plug with 0.1% (v/v) trifluoroacetic acid/50% (v/v) acetonitrile and concentrated using C-18 Zip-tips (Millipore, Bedford, MA, USA). Digests were spotted on a MALDI target using α–cyano-4-hydroxycinnamic acid as a matrix. Spectra were acquired on a 4800 MALDI TOF/TOF^{TM} Analyzer (Applied Biosystems, Foster City, CA, USA). MS/MS spectra were acquired automatically.

Preparation of Antisera. Rabbit antiserum against a peptide derived from the amino acid sequence F^{387}GLYDMAGNVAEWT^{400} of PGN_1676 (PorK) in which a cysteine residue was synthesized at the N-terminus of the peptide and conjugated to keyhole limpet hemocyanin was purchased from Sigma Genosys. Preparation of anti-Kgp and anti-Hgp44 antisera has been described previously (12, 26). c-Myc antibody was obtained from Sigma.

Tiling Microarray Analysis. Custom tiling microarrays spanning the whole genome of *P. gingivalis* ATCC 33277 with 25-mer probes each of which was 8-bases shifted on the genome sequence were purchased from Affymetrix. Anti-sense biotinylated cDNA was prepared from 10 μg of total RNA according to the Affymetrix GeneChip prokaryotic one-cycle target preparation protocol (Affymetrix). In short, reverse

transcriptase (Superscript II, Invitrogen, Carlsbad, CA, USA) and random hexamer primers were used to produce DNA complementary to the RNA. The cDNA products were then fragmented by DNase I and labeled at the 3' terminal with terminal transferase and biotinylated Gene Chip DNA labeling reagent (Affymetrix). The fragmented and labeled cDNA was hybridized to the GeneChip at 45^{o}C for 16 h. Staining, washing, and scanning procedures were carried out according to the GeneChip Expression Analysis Technical manual (Affymetrix). Hybridization was performed three times with the labeled cDNAs independently prepared. Signal intensities were quantified with the GeneChip Operating Software (Affymetrix), and further data analyses were performed with a microarray genomic analysis program (Insilico Molecular Cloning Array edition, In Silico Biology) and Microsoft Excel software (Microsoft). The normalization constant from the 16S rRNA gene was used to calculate the calibrated ratio for every CDS within the *P. gingivalis* ATCC 33277 genome.

Binding and Movement of Protein G-Coated Polystyrene Spheres on *F. johnsoniae* Cells. Movement of surface localized SprB was detected as previously described (20). Cells were grown overnight at 25^{o}C in MM without shaking. Purified anti-SprB (1 μl of a 1:10 dilution of 300 mg/liter stock), 0.5-μm-diameter protein G-coated polystyrene spheres (1 μl of a 0.1% stock preparation; Spherotech Inc., Libertyville, IL), and bovine serum albumin (1 μl of a 1% solution) were added to 7 μl of cells (approximately 5 x 10^{8} cells per ml) in MM. The cells were spotted on a glass slide, covered with a glass cover slip, and examined using an Olympus BH2 phase-contrast microscope with a

heated stage at 25°C. Images were recorded with a Photometrics Cool-SNAP$_{cf}^2$ camera and analyzed using MetaMorph software. Samples were examined 1 min after spotting, and images were captured for 30 s.

Detection of Surface Localized SprB on *F. johnsoniae* Cells by Immunofluorescence Microscopy. Wild-type and mutant cells were examined by immunofluorescence microscopy to identify cell-surface localized SprB. Cells were grown overnight in MM medium at 25°C. 10 μl of cells were diluted in 140 μl of MM and fixed with 1% formaldehyde for 15 min. Cells were collected on 0.4 μm Isopore membrane filters (Millipore, Billerica, MA) by filtration. Cells were washed three times with 200 μl of PBS and were blocked with 0.1% BSA in PBS for 30 min. After removal of the blocking solution by filtration cells were exposed to 200 μl of a 1:200 dilution of purified anti-SprB (20) in PBS with 0.1% BSA for 90 min. Cells were washed five times with 200 μl of PBS, and exposed to 200 μl of a 1:5000 dilution of secondary antibody conjugated to Alexa-488 (Invitrogen) in PBS + 0.1% BSA. Cells were incubated 60 min in the dark, the liquid was removed, and cells were washed five times with PBS. During the final PBS wash, 5 μl of InSpeck 0.3% relative intensity fluorescence beads (Invitrogen) were added as controls. The final wash was removed by filtration, the filters were mounted on glass slides with 6 μl of VectaShield with DAPI (Vector Laboratories Inc., Burlingame, CA), cover slips were applied, and samples were observed using a Nikon Eclipse 50i microscope. Images were captured with a Photometrics CoolSNAP$_{ES}$ camera with exposure times of 1500 ms (DAPI) and 500 ms (Alexa-488).

Distribution of *gld-*, *spr-* and *por*-Homologous Genes among Bacterial Species in Bacteroidetes Phylum. The genome sequences (accession numbers in parentheses) of *F. johnsoniae* UW101 (NC_009441), *Flavobacterium psychrophilium* JIP02/86 (NC_009613), *C. hutchinsonii* ATCC 33406 (NC_008255), *P. gingivalis* ATCC 33277 (NC_010729), *Parabacteroides distasonis* ATCC 8503 (NC_009615), *B. fragilis* YCH46 (NC_006347), and *B. thetaiotaomicron* VPI-5482 (NC_004663) were obtained through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The genome sequence of *Prevotella intermedia* 17 was obtained through JCVI (J. Craig Venter Institute) (http://www.jcvi.org/). Predicted homologs of *F. johnsoniae* UW101 motility proteins were identified by BLASTP searches for each of the selected bacterial genomes using E-value threshold $\leq 10^{-4}$. Orthologous genes were defined as reciprocal best-hits.

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Figure legend

Fig. 1. Kgp and Rgp proteins and activities in wild-type and mutant *P. gingivalis*. (***A***) Rgp and Kgp activities in intact cells and in culture supernatants. *P. gingivalis* cells were grown anaerobically in enriched brain heart infusion medium at 37^{o}C for 24 h. (***B***) Accumulation of gingipain proproteins with high molecular masses in mutant cells. *P. gingivalis* cell lysates were subjected to SDS-PAGE and immunoblot analysis with anti-Kgp and anti-Hgp44. (***C***) Plasmid mediated complementation. Rgp and Kgp activities in intact cells and in culture supernatants were measured for wild-type, mutant, and complemented cells.

Fig. 2. Subcellular location of PorP, PorK, PorL, PorM and PorN proteins and formation of a large complex. (***A***) Subcellular location of the Por proteins. The cell lysates of *P. gingivalis* strains were subjected to detergent fractionation followed by SDS-PAGE and immunoblot analysis with anti-PorK antibody for the wild-type strain, and anti-Myc antibody for the *porP*/*porP'*-'*myc*, *porL*/*porL'*-'*myc*, *porM*/*porM'*-'*myc* and *porN*/*porN'*-'*myc* strains. As a control, a major outer membrane protein, RagA, which was identified by Mass spectrometry, is indicated by asterisk. CBB, Coomassie Brilliant Blue staining. (***B***) Detection of protein complexes by blue native PAGE. Cell lysates of *P. gingivalis* strains were subjected to 3-12% gradient blue native PAGE and immunoblot analysis with anti-Myc antibody and anti-PorK antibody.

Fig. 3. Response regulator PorX- and histidine kinase PorY-mediated regulation of the expression of *porT*, *sov*, *porP*, *porK*, *porL*, *porM*, and *porN*. (***A***) Real time RT-PCR analysis of gene expression of *porP*, *porK*, *porL*, *porM*, *porN*, *porT* and *sov* in the wild-type, *porX* and *porX porX*$^{+}$ (complemented) strains. (***B***) Real time RT-PCR analysis of gene expression of *porP*, *porK*, *porL*, *porM*, *porN*, *porT*, *sov* and *porX* in the wild-type, *porY* and *porY porY*$^{+}$ (complemented) strains.

Fig. 4. Effect of disruption of *F. johnsoniae sprT* on gliding motility, surface localization of SprB protein, and extracellular chitinase activity. (***A***) Colony morphology of wild-type, mutant, and complemented strains. Cells were incubated on PY2 agar at 25^{o}C for 45 h. Bars equal 0.5 mm. (***B***) Detection of surface localized SprB protein by immunofluorescence microscopy. Cells of wild-type and mutant *F. johnsoniae* were exposed to DAPI and to anti-SprB antibodies followed by secondary antibodies conjugated to Alexa-488. Arrows indicate InSpeck relative intensity fluorescence beads. Bar indicates 10 μm. (***C***) Chitinase activities of intact cells and culture supernatants of *F. johnsoniae* strains with three synthetic substrates for chitinase, 4-MU-GlcNAc, 4-MU-$(GlcNAc)_2$ and 4-MU-$(GlcNAc)_3$.

Fig. 1

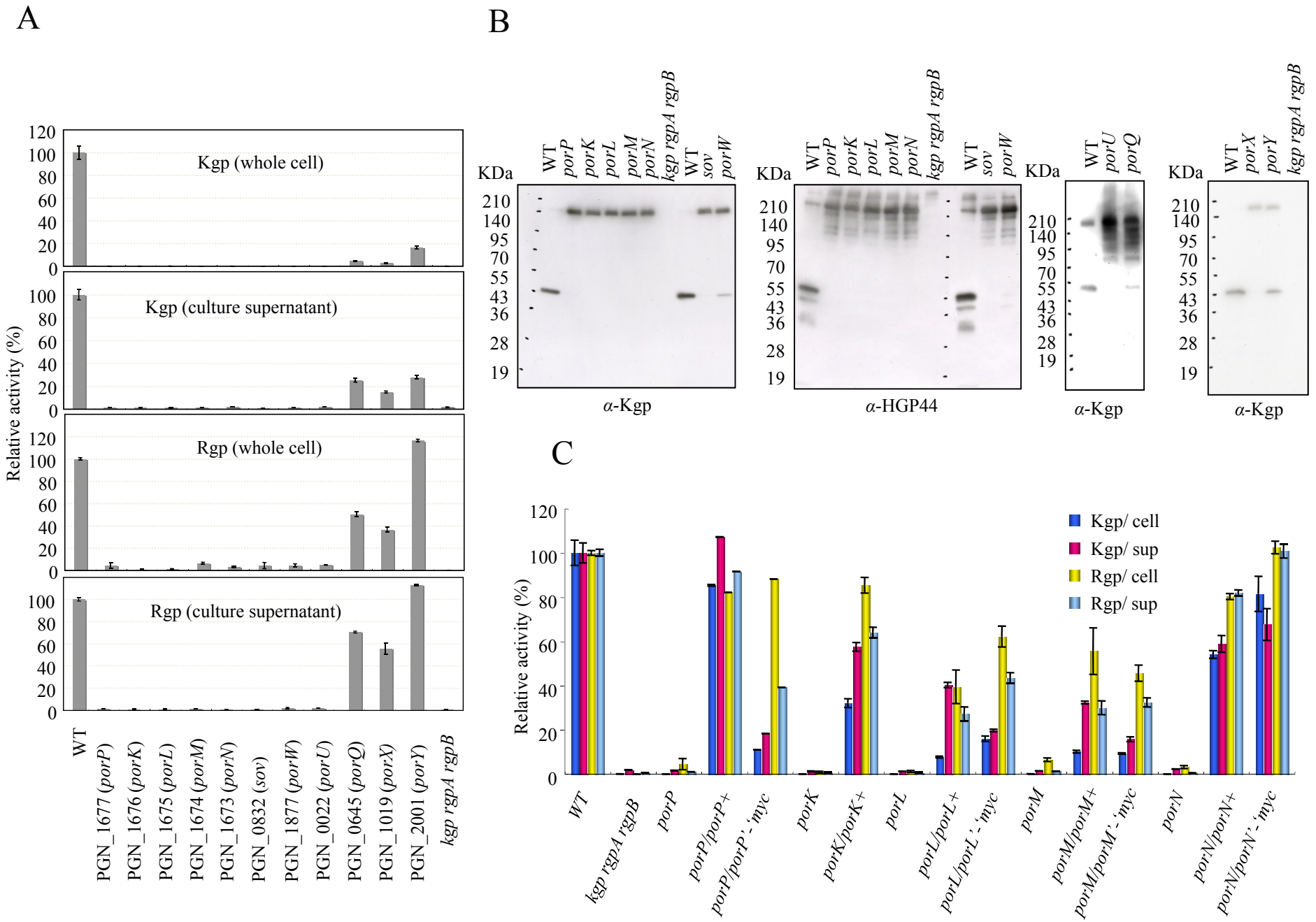

A
Kgp (whole cell)
Kgp (culture supernatant)
Rgp (whole cell)
Rgp (culture supernatant)
Relative activity (%)
WT
PGN_1677 (porP)
PGN_1676 (porK)
PGN_1675 (porL)
PGN_1674 (porM)
PGN_1673 (porN)
PGN_0832 (sov)
PGN_1877 (porW)
PGN_0022 (porU)
PGN_0645 (porQ)
PGN_1019 (porX)
PGN_2001 (porY)
kgp rgpA rgpB
B
KDa
α-Kgp
α-HGP44
C
Kgp/ cell
Kgp/ sup
Rgp/ cell
Rgp/ sup
Relative activity (%)
WT
kgp rgpA rgpB
porP
porP/porP+
porP/porP' - 'myc
porK
porK/porK+
porL
porL/porL+
porL/porL' - 'myc
porM
porM/porM+
porM/porM' - 'myc
porN
porN/porN+
porN/porN' - 'myc

Fig. 2

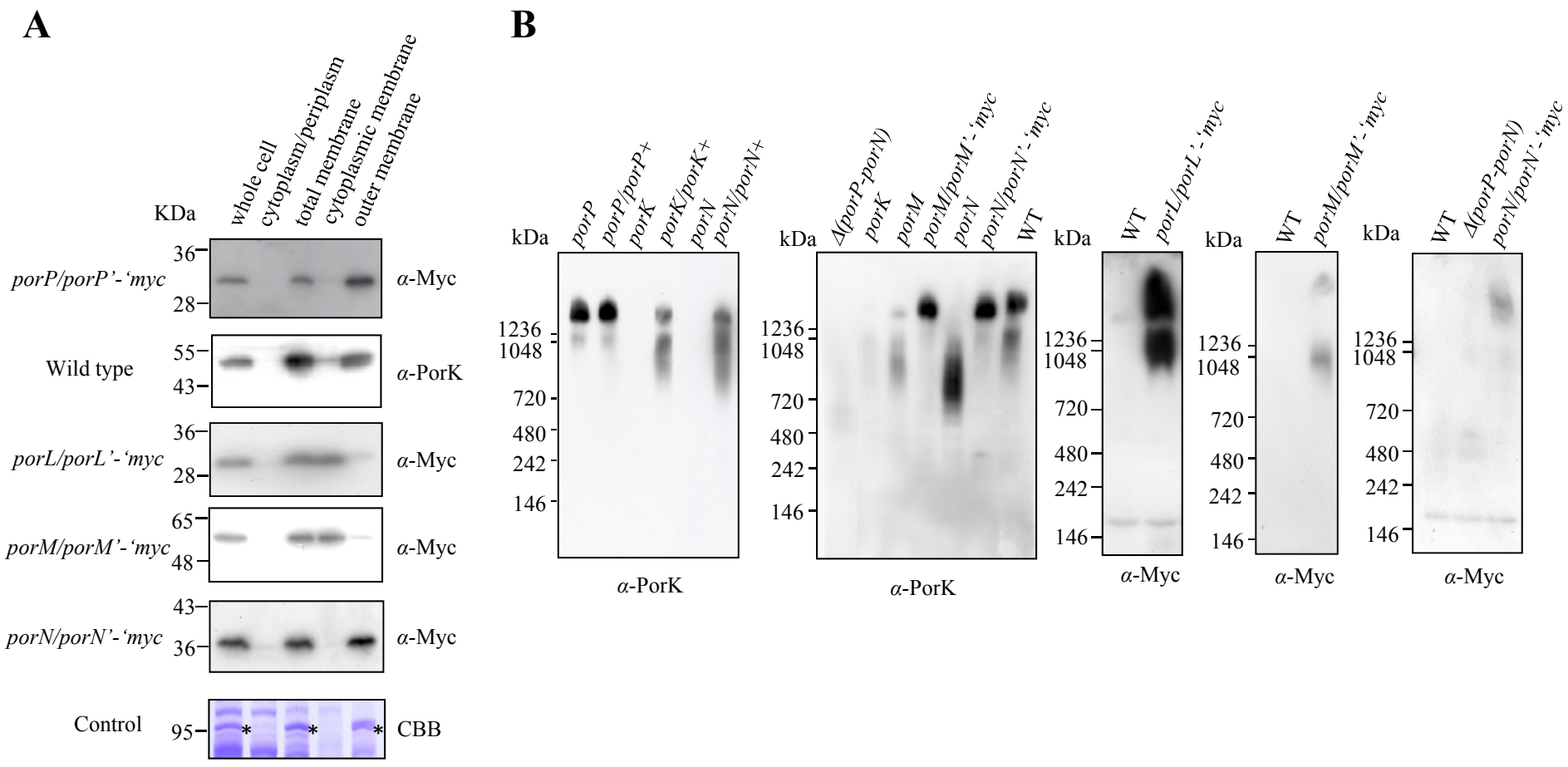

A
whole cell
cytoplasm/periplasm
total membrane
cytoplasmic membrane
outer membrane
KDa
porP/porP'-'myc
Wild type
porL/porL'-'myc
porM/porM'-'myc
porN/porN'-'myc
Control
36
28
55
43
65
48
95
α-Myc
α-PorK
CBB
B
kDa
porP
porP/porP+
porK
porK/porK+
porN
porN/porN+
Δ(porP-porN)
porM
porM/porM'-'myc
porN/porN'-'myc
WT
porL/porL'-'myc
1236
1048
720
480
242
146
α-PorK
α-Myc

Fig. 3

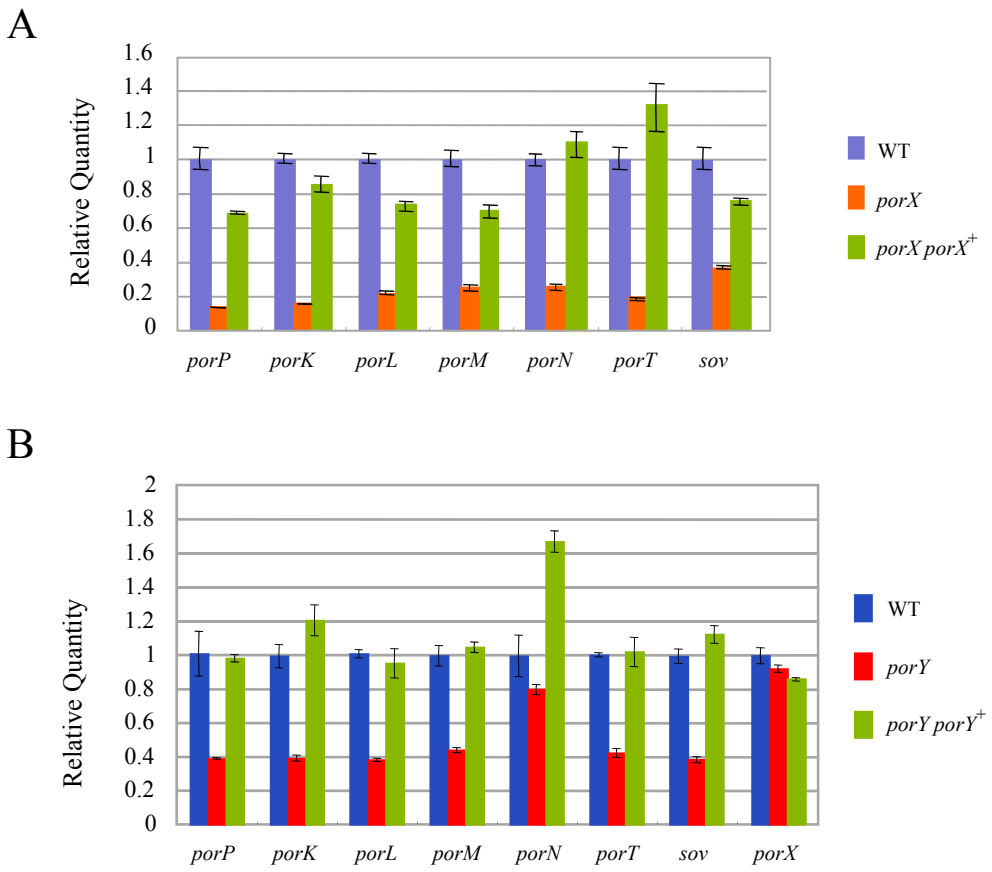
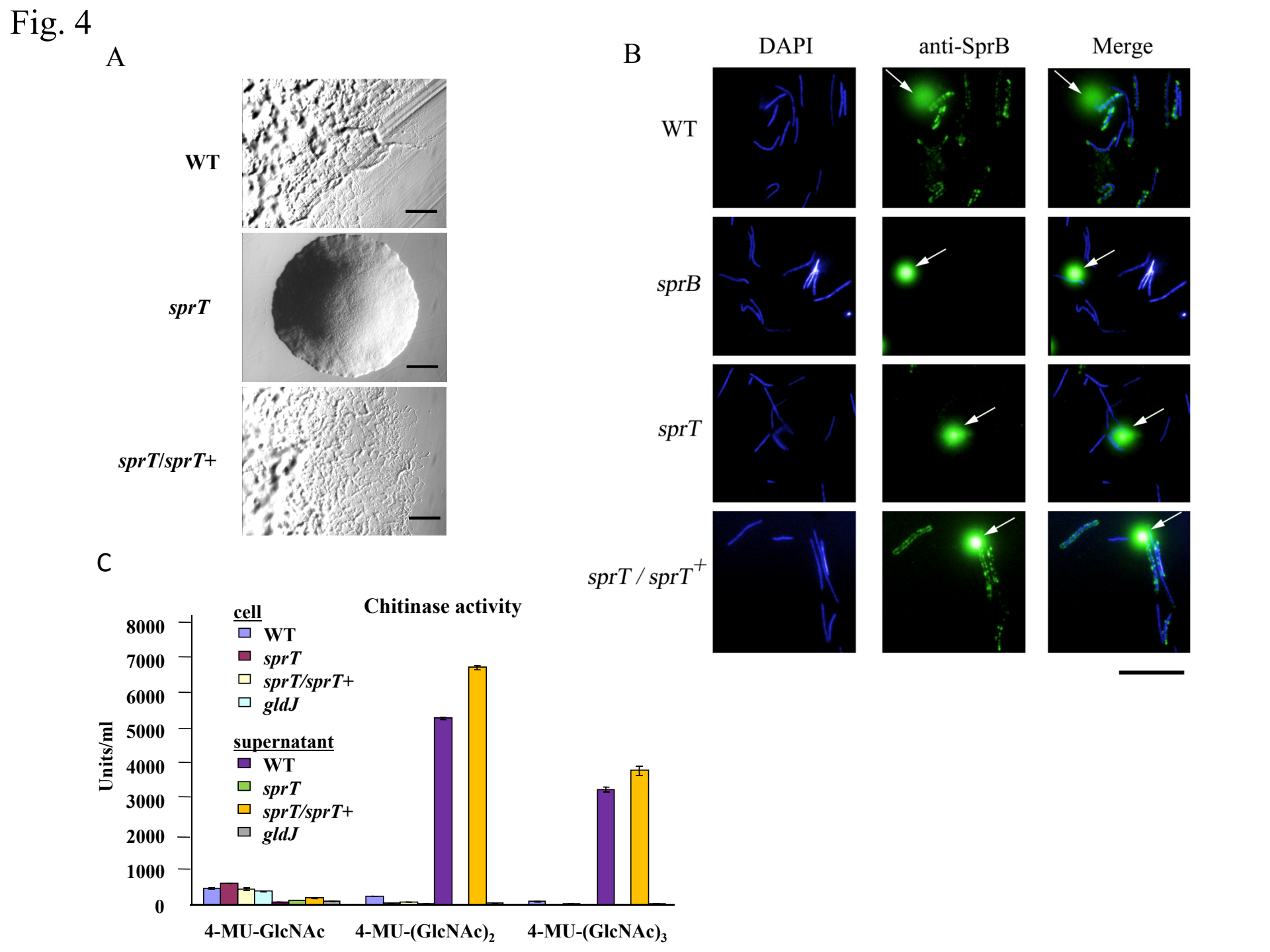

A
Relative Quantity
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
porP
porK
porL
porM
porN
porT
sov
WT
porX
porX porX+
B
Relative Quantity
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
porP
porK
porL
porM
porN
porT
sov
porX
WT
porY
porY porY+

Fig. 4


A
WT
sprT
sprT/sprT+
B
DAPI
anti-SprB
Merge
WT
sprB
sprT
sprT / sprT+
C
Chitinase activity
cell
WT
sprT
sprT/sprT+
gldJ
supernatant
WT
sprT
sprT/sprT+
gldJ
Units/ml
8000
7000
6000
5000
4000
3000
2000
1000
0
4-MU-GlcNAc
4-MU-(GlcNAc)2
4-MU-(GlcNAc)3

Supplemental Figure Legends

Figure S1. Venn diagram analysis of genes of *P. gingivalis, C. hutchinsonii* and *B. thetaiotaomicron*. Reciprocal BLASTP best-hits were calculated among *P. gingivalis* ATCC 33277, *B. thetaiotaomicron* VPI-5482, and *C. hutchinsonii* ATCC 33406.

Figure S2. Tiling microarray analysis of the *porX* mutant. (**A**) Tiling microarray analysis in the vicinity of *porT*. (**B**) Tiling microarray analysis in the vicinity of *sov*. (**C**) Tiling microarray analysis in the vicinity of *porP-porN*.

Figure S3. Western blot analysis of SprB in cells of wild-type and mutant *F. johnsoniae* strains. Cells were boiled in SDS-PAGE loading buffer, proteins were separated by electrophoresis, and detected using anti-SprB antibody. WT, wild type *F. johnsoniae*; *sprB, sprB* mutant FJ156; *sprT, sprT* mutant KDF001; *sprT*/*sprT*$^{+}$, KDF001 complemented with pKF002 which carries wild-type *sprT*.

Figure S4. SDS-PAGE protein profiles of culture supernatants of the wild-type, *sprT* and *sprT/sprT*$^{+}$ strains. Protein bands excised from the CBB-stained gel were subjected to MS/MS analysis after in-gel trypsin digestion.

Figure S5. Chitinase activity of recombinant *E. coli* expressing Fjoh_4555.

fig. S1

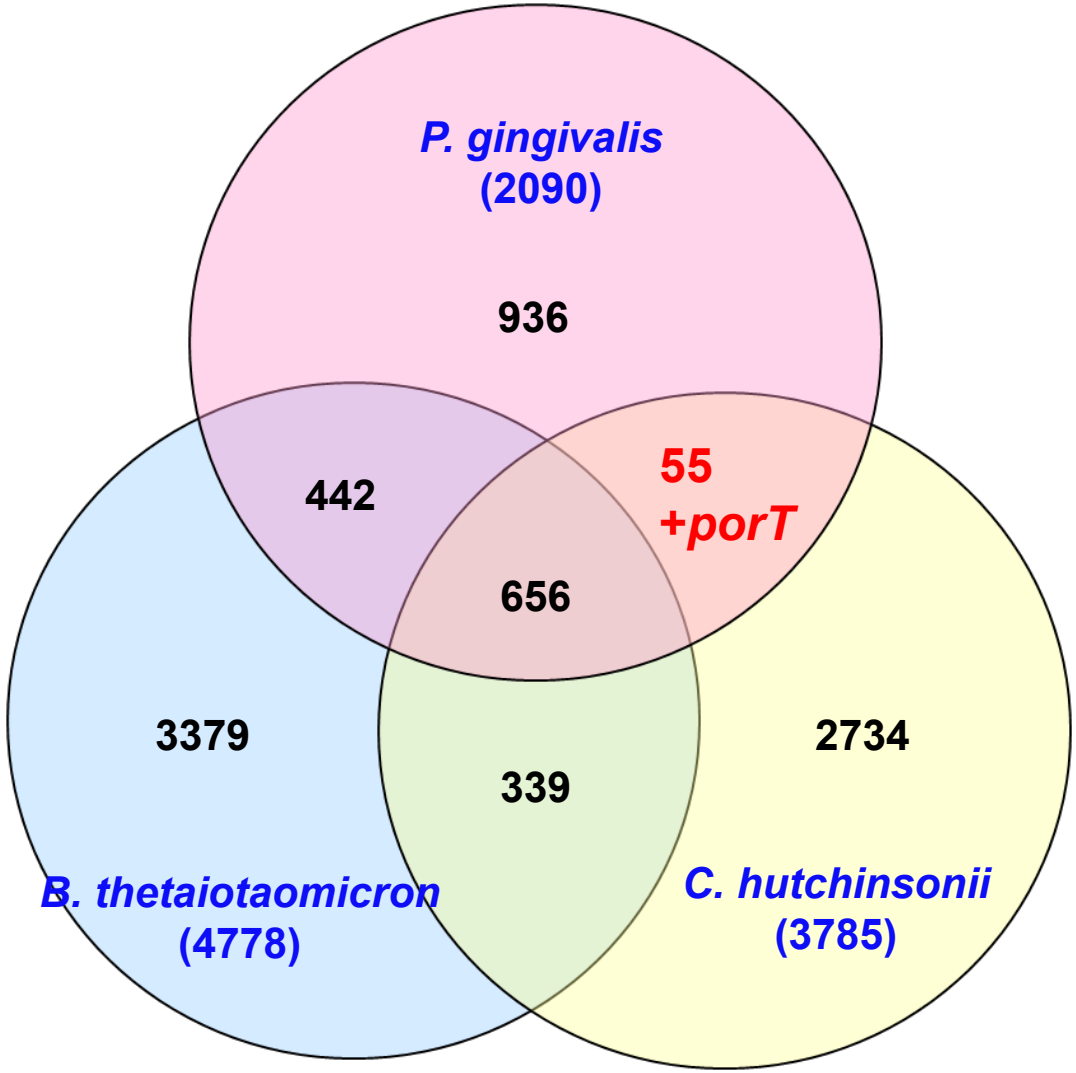

P. gingivalis
(2090)
936
442
55
+porT
656
3379
339
2734
B. thetaiotaomicron
(4778)
C. hutchinsonii
(3785)

fig. S2

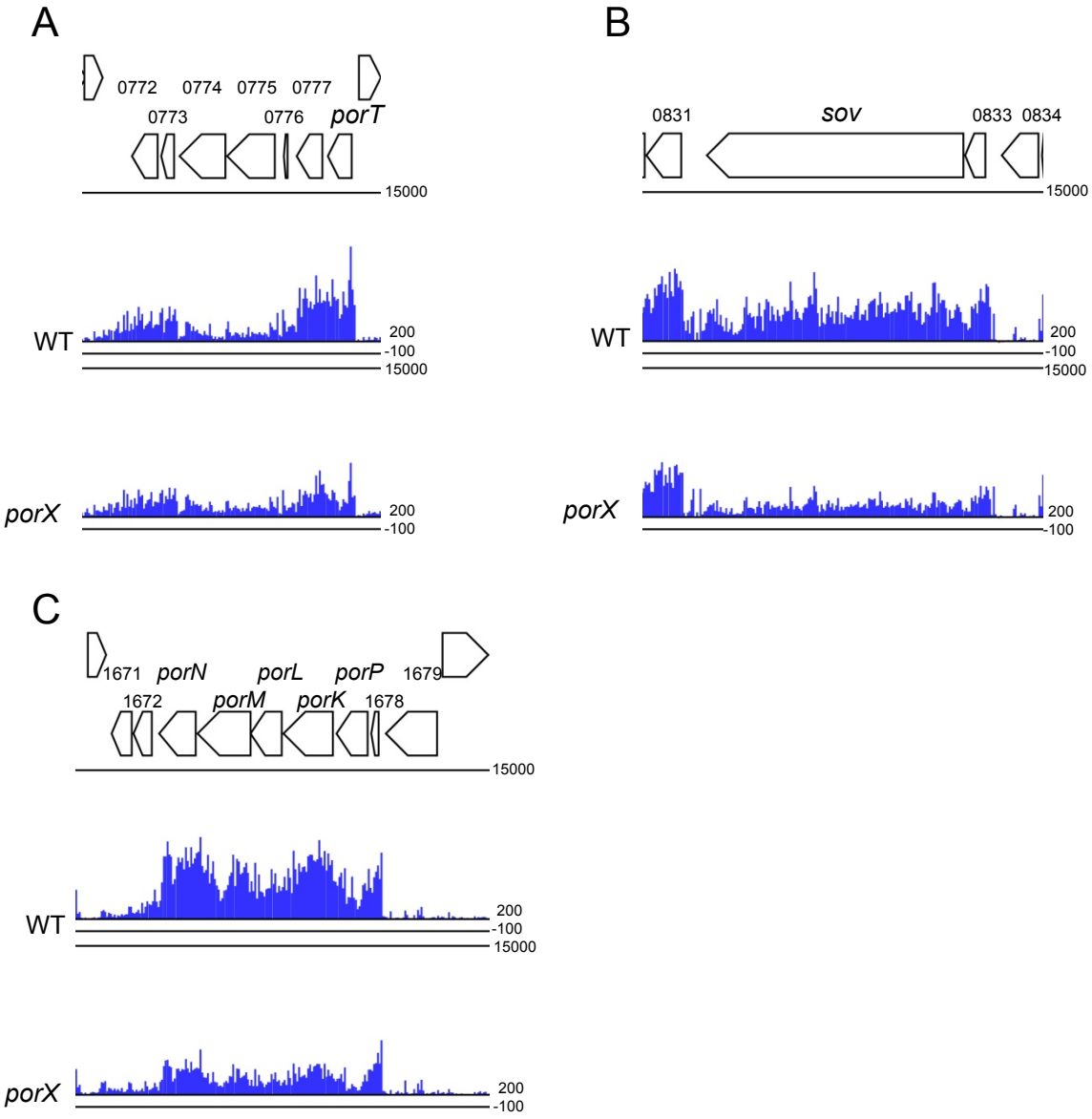

A
0772
0774
0775
0777
0773
0776
porT
15000
WT
200
-100
15000
porX
200
-100
B
0831
sov
0833
0834
15000
WT
200
-100
15000
porX
200
-100
C
1671
porN
porL
porP
1679
1672
porM
porK
1678
15000
WT
200
-100
15000
porX
200
-100

fig. S3

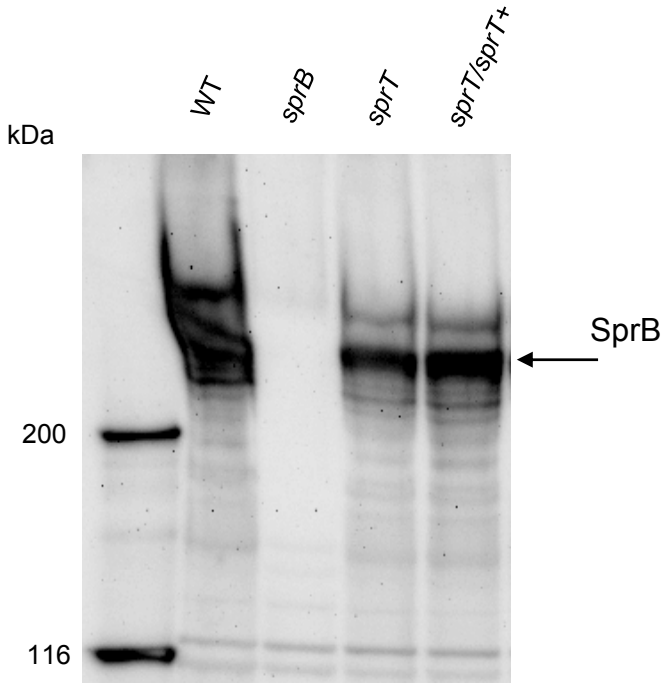
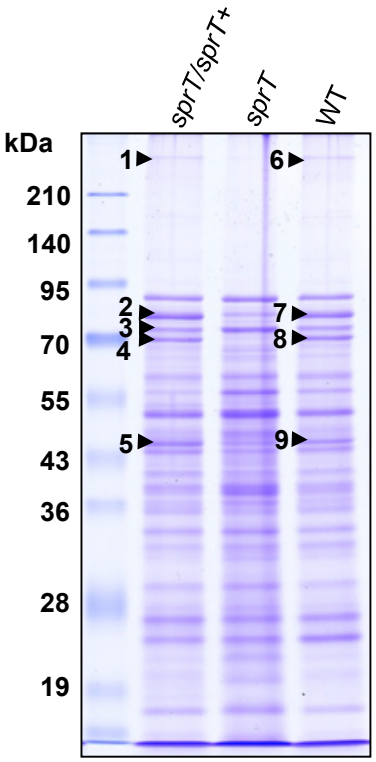


fig. S4



band	protein
1	Fjoh_0979 (SprB)
2	Fjoh_0403
3	Fjoh_0736
4	Fjoh_4555
5	Fjoh_0404
6	Fjoh_0979 (SprB)
7	Fjoh_0403
8	Fjoh_4555
9	Fjoh_0404

fig. S5

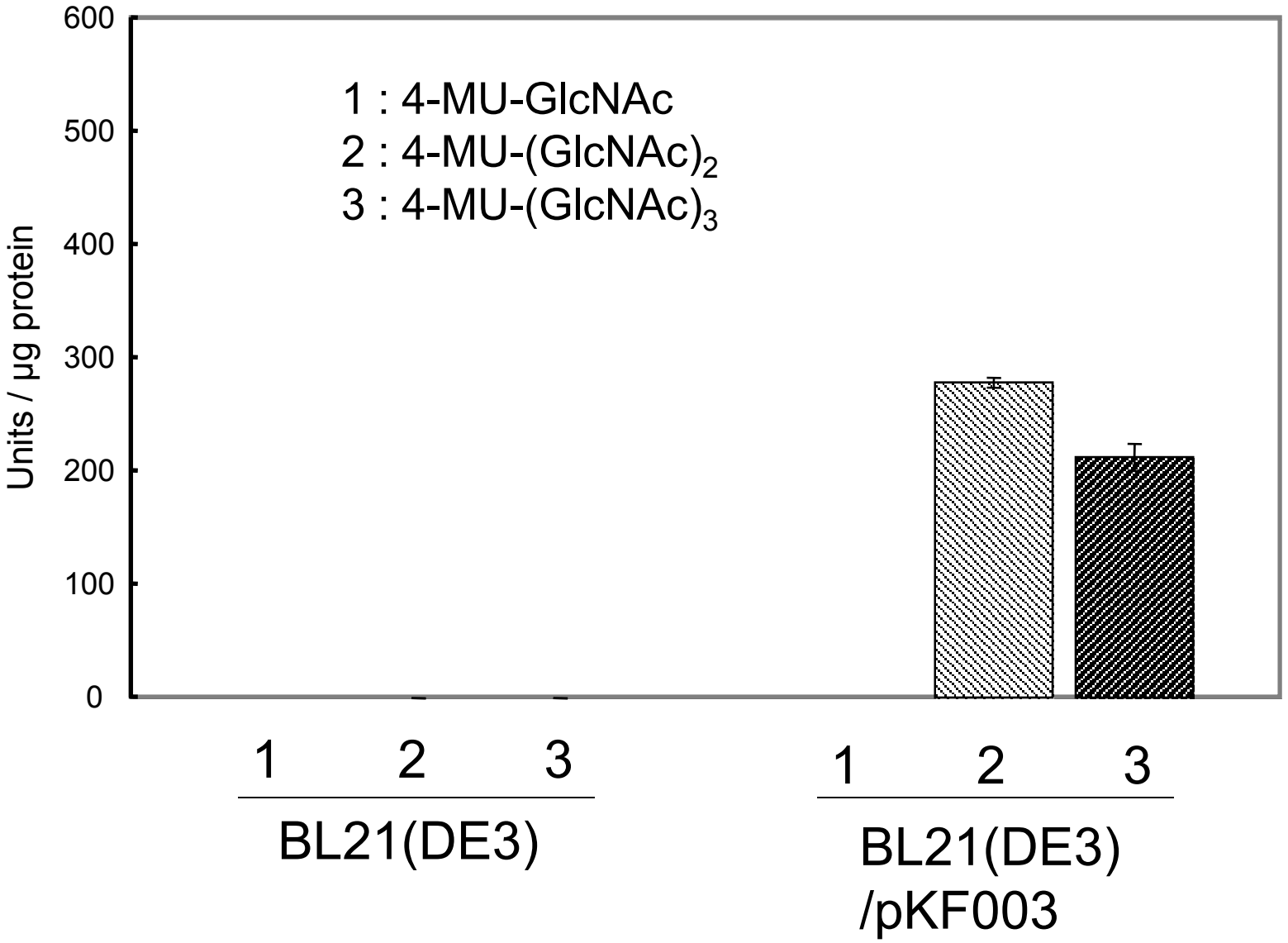

600
500
400
300
200
100
0
Units / μg protein
1 : 4-MU-GlcNAc
2 : 4-MU-(GlcNAc)2
3 : 4-MU-(GlcNAc)3
1
2
3
BL21(DE3)
1
2
3
BL21(DE3)
/pKF003

Table S1. *P. gingivalis* CDSs belonging to the *porT* sector depicted in Fig. 1

PGN#	length (aa)	gene name	product
PGN_0011	256		conserved hypothetical protein
PGN_0015	234		probable transcriptional regulator
PGN_0022	1158	porU	conserved hypothetical protein
PGN_0134	352	bioB	putative biotin synthetase
PGN_0144	419		hypothetical protein
PGN_0151	324		conserved hypothetical protein
PGN_0204	465		protoporphyrinogen oxidase
PGN_0227	352		probable glycosyl transferase family 1
PGN_0240	345	hemH	putative ferrochelatase
PGN_0242	382		probable glycosyl transferase family 1
PGN_0246	501		conserved hypothetical protein
PGN_0291	1228		conserved hypothetical protein
PGN_0306	280		conserved hypothetical protein
PGN_0316	614	cbiGF	cobalamin biosynthesis protein CbiG/precorrin-4 C11-methyltransferase
PGN_0335	821		conserved hypothetical protein with Zinc carboxypeptidase domain
PGN_0341	506		conserved hypothetical protein
PGN_0509	768		immunoreactive 84 kDa antigen (PG93)
PGN_0539	471		metallo-beta-lactamase superfamily protein
PGN_0580	1870		conserved hypothetical protein
PGN_0645	346	porQ	conserved hypothetical protein
PGN_0778	244	porT	PorT protein

PGN_0786	450		conserved hypothetical protein
PGN_0832	2499	sov	gliding motility protein SprA
PGN_0982	305	mrr	putative Mrr restriction system protein
PGN_1005	442		probable ABC transporter permease protein
PGN_1019	518	porX	response regulator
PGN_1124	326		Band 7 protein
PGN_1126	141		putative error-prone repair: SOS-response transcriptional repressor UmuD homolog
PGN_1176	281		putative 3-hydroxybutyryl-CoA dehydrogenase
PGN_1227	724		TPR domain protein
PGN_1308	310		probable iron dependent repressor
PGN_1317	496		conserved hypothetical protein
PGN_1324	300		putative inner membrane translocator
PGN_1325	294		putative inner membrane translocator
PGN_1353	138		conserved hypothetical protein
PGN_1411	424		putative N-ethylammeline chlorohydrolase
PGN_1454	241		probable abortive infection protein
PGN_1475	228		probable 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
PGN_1525	247		probable capsular polysaccharide biosynthesis protein
PGN_1533	242		putative carbonic anhydrase
PGN_1580	63	rpsU	putative 30S ribosomal protein S21
PGN_1627	504		probable 4-amino-4-deoxy-L-arabinose transferase
PGN_1652	179		probable nitroreductase
PGN_1668	298		conserved hypothetical protein

PGN_1674	516	porM	conserved hypothetical protein
PGN_1675	309	porL	conserved hypothetical protein
PGN_1676	491	porK	conserved hypothetical protein
PGN_1677	313	porP	conserved hypothetical protein
PGN_1695	293		putative fructose-bisphosphate aldolase, class I
PGN_1713	445		conserved hypothetical protein
PGN_1719	164		conserved hypothetical protein with appr-1-p processing enzyme domain
PGN_1783	302		conserved hypothetical protein
PGN_1796	1125		conserved hypothetical protein
PGN_1877	1160	porW	TPR domain protein
PGN_1920	801		conserved hypothetical protein
PGN_2001	395	porY	putative sensor histidine kinase

Yellow backgrounds indicate CDSs mutants of which were constructed in this study.

Table S2. Microarray analysis of total mRNA of the *porX* deletion mutant using a custom tiling array chip

	start	stop	CDS		*porX* vs. WT < 0.6	*porX*/*porX*+ vs. WT >0.6
conserved hypothetical protein	29901	31076	PGN_0023	+	0.443	0.687
putative 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	31083	31571	PGN_0024	+	0.438	0.752
conserved hypothetical protein	294206	294340	PGN_0273	−	0.513	0.946
PorT	847377	848111	PGN_0778	−	0.508	0.701
Sov	916648	924147	PGN_0832	−	0.548	0.620
ferredoxin 4Fe-4S	995192	995362	PGN_0895	−	0.559	0.840
PorX	1133660	1135216	PGN_1019	+	0.093	1.292
regulator PorR	1381563	1382717	PGN_1236	−	0.571	0.711
conserved hypothetical protein	1383104	1384240	PGN_1238	−	0.586	0.740
probable lipopolysaccharide biosynthesis glycosyltransferase	1384351	1385211	PGN_1239	−	0.509	0.718
conserved hypothetical protein	1508085	1509077	PGN_1350	−	0.573	0.846
conserved hypothetical protein	1509152	1509436	PGN_1351	−	0.351	0.832
conserved hypothetical protein with DUF1706 domain	1509570	1510106	PGN_1352	−	0.327	0.910
conserved hypothetical protein	1510110	1510526	PGN_1353	−	0.410	0.922
hypothetical protein	1718720	1719772	PGN_1535	+	0.253	0.626
PorN	1867949	1869028	PGN_1673	−	0.506	0.699
PorM	1869037	1870587	PGN_1674	−	0.514	0.647
PorL	1870591	1871520	PGN_1675	−	0.533	0.603
PorK	1871561	1873036	PGN_1676	−	0.493	0.708
PorP	1873093	1874034	PGN_1677	−	0.525	0.621

Table S3. Distribution of *gld*, *spr* and *por* genes in bacteria of the phylum *Bacteroidetes*

	F. joh	F. psy	C. hut	P. gin	P. int	P. dis	B. fra	B. the
gldA	Fjoh_1516	FP0252	CHU_1545	PGN_1004	PIN_A1093	BDI_1335	BF2629	BT_0562
gldB	Fjoh_1793	FP2069	CHU_3691	PGN_1061	PIN_A1414	BDI_1780	BF0973	BT_4189
gldC	Fjoh_1794	FP2068	CHU_0945					
gldD	Fjoh_1540	FP1663	CHU_3683			BDI_1991		
gldF	Fjoh_2722	FP1089	CHU_1546					
gldG	Fjoh_2721	FP1090	CHU_1547					
gldH	Fjoh_0890	FP0024	CHU_0291	PGN_1566		BDI_1879	BF4095	BT_3818
gldI	Fjoh_2369	FP1892	CHU_3665	PGN_0743				
gldJ	Fjoh_1557	FP1389	CHU_3494	PGN_1676	PIN_A0879	BDI_3324	BF2407	
gldK(porK)	Fjoh_1853	FP1973	CHU_0171	PGN_1676	PIN_A0879	BDI_3324	BF2407	
gldL(porL)	Fjoh_1854	FP1972	CHU_0172	PGN_1675	PIN_A0878	BDI_3323	BF2931	
gldM(porM)	Fjoh_1855	FP1971	CHU_0173	PGN_1674	PIN_A0877	BDI_3322	BF2932	
gldN(porN)	Fjoh_1856	FP1970	CHU_2610	PGN_1673	PIN_A0876	BDI_3321		
sprA(sov)	Fjoh_1653	FP2121	CHU_0029	PGN_0832	PIN_A1146	BDI_2659		
sprB	Fjoh_0979	FP0016	CHU_2225	PGN_1317	PIN_A1872			
porP	Fjoh_3477	FP2412	CHU_0170	PGN_1677	PIN_A0880	BDI_3325		
porQ	Fjoh_2755	FP1713	CHU_2991	PGN_0645	PIN_0248	BDI_3738		
porT(sprT)	Fjoh_1466	FP0326	CHU_2709	PGN_0778	PIN_A1079	BDI_1856		
porU	Fjoh_1556	FP1388	CHU_3237	PGN_0022	PIN_A0180	BDI_2576		
porW(sprE)	Fjoh_1051	FP2467	CHU_0177	PGN_1877	PIN_A2099	BDI_3149		
porX	Fjoh_2906	FP1066	CHU_1040	PGN_1019	PIN_A2097	BDI_3342	BF2968	BT_0818
porY	Fjoh_1592	FP2349	CHU_0334	PGN_2001	PIN_A0086	BDI_2438	BF0583	BT_1470

Homologous genes were identified by BLASTP searches for each of the selected bacterial genomes using E-value threshold $\leq 10^{-4}$ with a query on *F. johnsoniae* UW101. Orthologous genes were defined as reciprocal best-hits. Yellow, ortholog; Green, homolog; F. joh, *Flavobacterium johnsoniae*; F. psy, *Flavobacterium psychrophilum*; C. hut, *Cytophaga hutchinsonii*; P. gin, *Porphyromonas gingivalis*; P. dis, *Parabacteroides distasonis*; B. fra, *Bacteroides fragilis*; B. the, *Bacteroides thetaiotaomicron*.

Table S4. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Reference or source
E. coli strain		
BL21(DE3)	Host strain for expression vector pET-32b	Nippongene
S17-1	RP4-2-Tc::Mu *aph*::Tn7 *recA*, Sm^r	1
P. gingivalis strain		
ATCC 33277	wild type	ATCC
KDP136	*kgp-2*::*cat rgpA2*::[*ermF ermAM*] *rgpB2*::*tetQ*, Cm^r Em^r Tc^r	2
KDP354	*porP::ermF*, Em^r	this study
KDP355	*porK::ermF*, Em^r	this study
KDP356	*porL::ermF*, Em^r	this study
KDP357	*porM::ermF*, Em^r	this study
KDP358	*porN::ermF*, Em^r	this study
KDP359	*porW::ermF*, Em^r	this study
KDP360	*porU::ermF*, Em^r	this study
KDP362	*porQ::ermF*, Em^r	this study
KDP363	*porX::ermF*, Em^r	this study
KDP364	*porY::ermF*, Em^r	this study
KDP365	*sov::ermF*, Em^r	this study
KDP367	KDP354/pKD959, Em^r Tc^r	this study
KDP368	KDP355/pKD960, Em^r Tc^r	this study
KDP369	KDP356/pKD961, Em^r Tc^r	this study
KDP370	KDP357/pKD962, Em^r Tc^r	this study
KDP371	KDP358/pKD963, Em^r Tc^r	this study
KDP372	KDP363 *fimA::*[$porX^+$ *tetQ*], Em^r Tc^r	this study
KDP373	KDP354/pKD968, Em^r Tc^r	this study
KDP374	KDP356/pKD969, Em^r Tc^r	this study
KDP375	KDP357/pKD970, Em^r Tc^r	this study
KDP376	KDP358/pKD971, Em^r Tc^r	this study
KDP377	KDP364 *fimA::*[$porY^+$ *tetQ*], Em^r Tc^r	this study
F. johnsoniae strain		
ATCC 17061 (UW101)	wild type	ATCC
FJ156	*sprB*	3
KDF001	*sprT*::pKF001, Em^r	this study
KDF002	KDF001/pKF002, Em^r Tc^r	this study
UW102-48	*gldJ*	4
E. coli plasmid		
pBluescript II SK(-)	Ap^r, cloning vector	Stratagene

pET-32b	Ap^r, expression vector	Novagen
pKD713	Ap^r Tc^r, pBSSK-*fimA::tetQ*	5
pKD954	Ap^r, pBluescript II SK(-) (pBSSK)-p	This study
pKD955	Ap^r, pBSSK-p-*porK*$^+$-T	This study
pKD956	Ap^r, pBSSK-p-*porL*$^+$-T	This study
pKD957	Ap^r, pBSSK-p-*porM*$^+$-T	This study
pKD958	Ap^r, pBSSK-p-*porN*$^+$-T	This study
pKD964	Ap^r, pBSSK-p-*porP-myc*-T	This study
pKD965	Ap^r, pBSSK-p-*porL-myc*-T	This study
pKD966	Ap^r, pBSSK-p-*porM-myc*-T	This study
pKD967	Ap^r, pBSSK-p-*porN-myc*-T	This study
pKD972	Ap^r, pBSSK-*myc*-T	This study
pKD973	Ap^r Tc^r, pBSSK-*fimA*::[*porX*$^+$ *tetQ*]	This study
pKD974	Ap^r Tc^r, pBSSK-*fimA*::[*porY*$^+$ *tetQ*]	This study
pKF003	Ap^r, pET-32b containing *F. johnsoniae* Fjoh_4555 gene	This study
P. gingivalis plasmid		
pTCB	Ap^r Tc^r, *E. coli-P. gingivalis* shuttle plasmid	6
pKD959	Ap^r Tc^r, pTCB-p-*porP*$^+$-T	This study
pKD960	Ap^r Tc^r, pTCB-p-*porK*$^+$-T	This study
pKD961	Ap^r Tc^r, pTCB-p-*porL*$^+$-T	This study
pKD962	Ap^r Tc^r, pTCB-p-*porM*$^+$-T	This study
pKD963	Ap^r Tc^r, pTCB-p-*porN*$^+$-T	This study
pKD968	Ap^r Tc^r, pTCB-p-*porP-myc*-T	This study
pKD969	Ap^r Tc^r, pTCB-p-*porL-myc*-T	This study
pKD970	Ap^r Tc^r, pTCB-p-*porM-myc*-T	This study
pKD971	Ap^r Tc^r, pTCB-p-*porN-myc*-T	This study
pKD973	Ap^r Tc^r, pBSSK-*fimA::* [*porX*$^+$ *tetQ*]	This study
pKD974	Ap^r Tc^r, pBSSK-*fimA::* [*porY*$^+$ *tetQ*]	This study
F. johnsoniae plasmid		
pLYL03	Ap^r Em^r, suicide plasmid for *Bacteroides-Flavobacterium* , pUC ori	7
pCP23	Ap^r Tc^r, *E. coli-F. johnsoniae* shuttle plasmid	8
pKF001	Ap^r Em^r, pLYL03 containing *F. johnsoniae sprT* (internal region)	This study
pKF002	Ap^r Tc^r, pCP23 containing *F. johnsoniae sprT*$^+$	This study

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gene-deletion mutants. *J Med Microbiol.* 56:1536-1548.

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8. Agarwal S, Hunnicutt DW, & McBride MJ (1997) Cloning and characterization of the *Flavobacterium johnsoniae* (*Cytophaga johnsonae*) gliding motility gene, *gldA. Proc Natl Acad Sci U S A.* 94:12139-12144.

Table S5. Primer DNA used in this study

PGN0011-U-F-NotI: GCGGCCGCTCGTTTACGACCGAGGAAGTG

PGN0011-U-R-BamHI: GGATCCTGATCTGCAGCATCTGCTATTCT

PGN0011-D-F-BamHI: GGATCCCCATCCGAACAAATCTTGTCGGA

PGN0011-D-R-KpnI: GGTACCCAGTCAGGGCAATCGCATAGACA

PGN0015-U-F-NotI: GCGGCCGCTCTCTTATCGCGGAGCTTTCG

PGN0015-U-R-BglII: AGATCTTTACTCCGATTGAATAAATCGTC

PGN0015-D-F-BglII: AGATCTAATCAGCCGATCCTATCATCTTC

PGN0015-D-R-KpnI: GGTACCACAGCAAGATCATAGTAGACCTG

PGN0022-U-F-NotI: GCGGCCGCGTCTCCGAAGGCTGAACAATC

PGN0022-U-R-BamHI: GGATCCGGAATGCGACTATTGGAAGTATT

PGN0022-D-F-BamHI: GGATCCCCGAAGCAGTTCTGCCGTTTACG

PGN0022-D-R-KpnI: GGTACCACCTCCGTCATAGGAGATCTTCG

PGN0134-U-F-NotI: GCGGCCGCTATGGAGCCTTCCACCCCTAA

PGN0134-U-R-BamHI: GGATCCGTAGAAAGAAGCTCCGGCAATAG

PGN0134-D-F-BamHI: GGATCCCGAAATGCTGCGTTTGGATCGCA

PGN0134-D-R-KpnI: GGTACCTGGCAGGAAATACTGTACGGGCA

PGN0144-U-F-NotI: GCGGCCGCGATTTCGATGGCCGGCGACTT

PGN0144-U-R-BamHI: GGATCCACCTTATAATGCTAAGTCAAACA

PGN0144-D-F-BamHI: GGATCCCGGCTCGATCGGCGAACGTTACA

PGN0144-D-R-KpnI: GGTACCACATATAGTCATCCACGTCGAGC

PGN0151-U-F-NotI: GCGGCCGCATGAAATACAGAAGCATTATCC

PGN0151-U-R-BamHI: GGATCCTACCTGCTGCCGGATCGTGCCGCT

PGN0151-D-F-BamHI: GGATCCAGCGTAACGAATACGGCCAAAGGA

PGN0151-D-R-KpnI: GGTACCCTACTCCAGCTTCGTGAGCAGGGT

PGN0204-U-F-NotI: GCGGCCGCATGGATCATCTGACCGTCATCA

PGN0204-U-R-BamHI: GGATCCAGCCAAGGCTCCGACCGTCTCATT

PGN0204-D-F-BamHI: GGATCCATGAATCAGCCGTGTTCGAGCAGC

PGN0204-D-R-KpnI: GGTACCTCAGCCTCTGGCAGAGCGTTCGGC

PGN0227-U-F-NotI: GCGGCCGCATGAAACAAGGCGTTTTTGTA

PGN0227-U-R-BamHI: GGATCCCCTAGGATTAGTCTTATAGAAGTC

PGN0227-D-F-BamHI: GGATCCGAGCATTCTCCTCTTCTTAGGCTT

PGN0227-D-R-KpnI: GGTACCGATCTCATAATATATTCTGTTCAA

PGN0240-U-F-NotI: GCGGCCGCGCAAGAAGCAGAAACAGGATG

PGN0240-U-R-BamHI: GGATCCTTCTTCTACGATCTGCCATAATT

PGN0240-D-F-BamHI: GGATCCTACAGCATGCTCAGTTATCATAC

PGN0240-D-R-KpnI: GGTACCTTCTCGTCTGTCCGATACAGTAC

PGN0242-U-F-NotI: GCGGCCGCATGAATCGTATAGCTTTTGATG

PGN0242-U-R-BamHI: GGATCCCATCACATCGCGCTTGGTCTGTTC

PGN0242-D-F-BamHI: GGATCCGATGTCGTCTATCAGGGTTGTTCG

PGN0242-D-R-KpnI: GGTACCCCAGCATGGATGCCATCATTTCGG

PGN0246-U-F-NotI: GCGGCCGCCATCGGATAAGGTCTATATGC

PGN0246-U-R-BamHI: GGATCCGCGTTCGACAATCCCCATAGCTC

PGN0246-D-F-BamHI: GGATCCGCCTACTACGTCGATTCTCTTCT

PGN0246-D-R-KpnI: GGTACCTTCGACTCCTCATTTCGGAGGAG

PGN0291-U-F-NotI: GCGGCCGCGAGACAGTACGCTATGAAAGG

PGN0291-U-R-BamHI: GGATCCCGCAATCAAAAGCGCATGCCATC

PGN0291-D-F-BamHI: GGATCCGATGATGCTATTGCGACTATAGG

PGN0291-D-R-KpnI: GGTACCTCCTCGGAGGAGATAACTGTACC

PGN0306-U-F-NotI: GCGGCCGCGGATTTACAACTACTTTCACTC

PGN0306-U-R-BamHI: GGATCCACAGCAATAGGGATGATTACTGCA

PGN0306-D-F-BamHI: GGATCCCATTTATCGCCATACCATCGATAT

PGN0306-D-R-KpnI: GGTACCCTATTTCCCCGGCTCAGACTCCAT

PGN0316-U-F-NotI: GCGGCCGCATGTCGAAACCGAATCATACTG

PGN0316-U-R-BamHI: GGATCCGTATCCAATGCCCAGAGTCCTGTT

PGN0316-D-F-BamHI: GGATCCTGGCACTCTTCGACCACTACGGCA

PGN0316-D-R-KpnI: GGTACCAGGTGTTTGAATTCGTCGGCATAC

PGN0335-U-F-NotI: GCGGCCGCCTTGCAGAAGTTAGACCTGCA

PGN0335-U-R-BamHI: GGATCCGCCTGTTGTGCCTGCATGCTGCC

PGN0335-D-F-BamHI: GGATCCTCGTACGGAAAAGATCCATATCG

PGN0335-D-R-KpnI: GGTACCGCTGCCTATTCTTCATGGTCTGA

PGN0341-U-F-NotI: GCGGCCGCCCAACGGATACGGCCTTGTTCA

PGN0341-U-R-BamHI: GGATCCTACAAAGCTAACGTAACCGAGCG

PGN0341-D-F-BamHI: GGATCCTCGAAGTAAAGCCCCGTCCTTG

PGN0341-D-R-KpnI: GGTACCGCATCCGTAAAGGTCTCCCATC

PGN0539-U-F-NotI: GCGGCCGCTATTGAGCCCGGGCAGTTTTG

PGN0539-U-R-BamHI: GGATCCCATCGAAGTGATGTAGTATGCAC

PGN0539-D-F-BamHI: GGATCCTCCCAAAACGGACTATGTATGTC

PGN0539-D-R-KpnI: GGTACCGGTATAGAAAGCGGTAATATCGG

PGN0580-U-F-NotI: GCGGCCGCAACATTGTCAGACCGATTCGGG

PGN0580-U-R-BamHI: GGATCCCCTTCATGTGCTGCGTTTAGAC

PGN0580-D-F-BamHI: GGATCCCACGGCATAATGCTTTTGCACC

PGN0580-D-R-KpnI: GGTACCTCACAAGTTCGAGGCTCTCCTT

PGN0645-U-F-NotI: GCGGCCGCGTGATAACGACAGCAGCGAAC

PGN0645-U-R-BamHI: GGATCCCATTGAACATATAGAGACCTTTG

PGN0645-D-F-BamHI: GGATCCTACACCATTAACTATCAGCTTAC

PGN0645-D-R-KpnI: GGTACCTTGGCACGCAATTCGTCCAAGCG

PGN0786-U-F-NotI: GCGGCCGCATCAAAGCCCAACGATACACC

PGN0786-U-R-BamHI: GGATCCCATCAAGAAGCTGACACGAGGCA
PGN0786-D-F-BamHI: GGATCCAATTGGGGCATTTCAGTTCCTTC
PGN0786-D-R-KpnI: GGTACCGAAACAGCGTCATCGCAGATGCT
PGN0832-U-F-NotI: AGCGGCCGCTACACAACTGATCCGAGAAGA
PGN0832-U-R-BamHI: GGATCCTGGTCACAAGCAGATAAGTATTGG
PGN0832-D-F-BamHI: GGATCCGCAACCTGGGCTTTATCCAAGACC
PGN0832-D-R-KpnI: GGTACCCATAGTATCCTCTCATATGAGACC
PGN0982-U-F-NotI: GCGGCCGCTTATCACTGCCCAAACCGACC
PGN0982-U-R-BamHI: GGATCCTCAGGGTTTGGAAATCTGGTATC
PGN0982-D-F-BamHI: GGATCCAGTTGAAAAAGCTCGATAGTGAC
PGN0982-D-R-KpnI: GGTACCTGTCTCGGCTTTTATCGGAGAGG
PGN1019-U-F-NotI: GCGGCCGCTTGCTCAGCCTCCAATCATTG
PGN1019-U-R-BamHI: GGATCCATAGTACGGTATACGGTCTCATG
PGN1019-D-F-BamHI: GGATCCCCCGTAATTACGATGCAACCCAA
PGN1019-D-R-HindIII: AAGCTTTACGAAAATGAGGCTGAGCAGGG
PGN1176-U-F-NotI: GCGGCCGCTTGGCCATGGCTTGTGACATC
PGN1176-U-R-BamHI: GGATCCATTGAACGATACCGCTGCCCATG
PGN1176-D-F-BamHI: GGATCCAAGACCGGCAAAGGCTTCTACGA
PGN1176-D-R-HindIII: AAGCTTTCACTGAGGTTGTACCATGTCTC
PGN1227-U-F-NotI: GCGGCCGCGAATTGGAAGCAGAGGTCGTT
PGN1227-U-R-BamHI: GGATCCATCGGATCTGCTCATCGACTTCA
PGN1227-D-F-BamHI: GGATCCAAATACCCTGACAGTAGTCGAGC
PGN1227-D-R-HindIII: AAGCTTTCTGCACGAGAATATTATCGCGC
PGN1308-U-F-NotI: GCGGCCGCACGCTTCCATAGCCTCCTCAA
PGN1308-U-R-BamHI: GGATCCACGGCGTACATCGAAGCGAAACA
PGN1308-D-F-BamHI: GGATCCAAGACCTAAACGCTCAGACCAAC
PGN1308-D-R-KpnI: GGTACCAGTACAGCGATATATCCCTTGGG
PGN1317-U-F-NotI: GCGGCCGCAGCGTGAGCTGAAAATTCTGCG
PGN1317-U-R-BglII: AGATCTGTCTTGGCAAAGACGAAGTTCTTG
PGN1317-D-F-BglII: AGATCTTTTGGGGGGAAACCTCCCACGATA
PGN1317-D-R-KpnI: GGTACCGACAGTCGATCTCGGATTCCGA
PGN1353-U-F-NotI: GCGGCCGCACAGAAACATCAGCTGCTCAC
PGN1353-U-R-BamHI: GGATCCTTCAACGCCAGTTCGATCTAAGC
PGN1353-D-F-BamHI: GGATCCGAAATATGGCAAGAGCAACGACG
PGN1353-D-R-KpnI: GGTACCTCTTCATCGCCCAGTCGTAATGA
PGN1411-U-F-NotI: GCGGCCGCCTTGACATAGACACCGTATTC
PGN1411-U-R-BamHI: GGATCCACACCTTCCAGCAGTGCCTGTTT
PGN1411-D-F-BamHI: GGATCCACAAACTGATGGCAAAGGCCCAC
PGN1411-D-R-KpnI: GGTACCACCCTCTTTGACCGGGATATTCA

PGN1454-U-F-NotI: GCGGCCGCCGGCACAGTTACTCCAAGTGAG

PGN1454-U-R-BamHI: GGATCCCATTGCTTACAAAAATACGATGG

PGN1454-D-F-BamHI: GGATCCACAGATAGCTGCCCACTACTAC

PGN1454-D-R-KpnI: GGTACCTAAAGCGGGGACAATAGCTTCC

PGN1475-U-F-NotI: GCGGCCGCAATGGTAGGCAAAGTGGCGTC

PGN1475-U-R-BamHI: GGATCCTGGCTACTACCACACCGATAGTC

PGN1475-D-F-BamHI: GGATCCATTCTCGAAAGAGTATTCGATGC

PGN1475-D-R-KpnI: GGTACCAGAGATGCAGACGTGGGAAGTAG

PGN1525-U-F-NotI: GCGGCCGCGAGATAGAACGACTGTACACAG

PGN1525-U-R-BamHI: GGATCCCTTGTCCGGAGTGATAGCAGCAAG

PGN1525-D-F-BamHI: GGATCCAACGTTATCTATACATGGAGGAGA

PGN1525-D-R-KpnI: GGTACCTAAGACGACCGGCAGAAATCCACC

PGN1533-U-F-NotI: GCGGCCGCGCAGATGGAATCCTGCACCTG

PGN1533-U-R-BamHI: GGATCCAATGTTAAACCTTACCTGCATAC

PGN1533-D-F-BamHI: GGATCCACGTGGTACAAGTTCCCTCCGAT

PGN1533-D-R-KpnI: GGTACCCTTTTATAGTCGCTAAAGCTCCG

PGN1652-F: TAGTACAGCATCAGGCGCGTAAATACGGC

PGN1652-R: TTGATGCTATCAATCAACTGTGCAGTCAC

PGN1668-U-F-NotI: GCGGCCGCATGGGGACTGAACCGGAGCGA

PGN1668-U-R-BamHI: TGGATCCAGGTGGGAGACATCTTCGTCGT

PGN1668-D-F-BamHI: AGGATCCGCTGATCCTTAGAAAGATCAGA

PGN1668-D-R-KpnI: TGGTACCAGCCGTCTTGGCGGACAAAGGA

PGN1674-U-F-NotI: GCGGCCGCAGATGATCAACCTGATGTACCT

PGN1674-U-R-BamHI: GGATCCCGCCTTGTCGGTCATGAGTGTAGC

PGN1674-D-F-BamHI: GGATCCATCAACAATGGTACGCTCACTCGC

PGN1674-D-R-KpnI: GGTACCTTCTGTTACGTAGAAACGCTTGCC

PGN1675-U-F-NotI: GCGGCCGCATGGGTCATTATAGAAGATACA

PGN1675-U-R-BamHI: GGATCCTTGGCCTTTTCACGAAGATACTCC

PGN1675-D-F-BamHI: GGATCCCGCGGAAAGGCCATCTTCTGTGCG

PGN1675-D-R-KpnI: GGTACCATTGCCGGGCATACCGGGTAGACC

PGN1676-U-F-NotI: GCGGCCGCGGTGAACTGACCGGTGCCAAGC

PGN1676-U-R-BamHI: GGATCCCGCTTTGCGGCCTTGAGCTGATGC

PGN1676-D-F-BamHI: GGATCCCGGACATCAAGGTAGACCCCAACG

PGN1676-D-R-KpnI: GGTACCGTGAGCGCAGCTTTGTATTCCAGT

PGN1677-U-F-NotI: GCGGCCGCTCGATATACCCATGGAAGAGC

PGN1677-U-R-BamHI: GGATCCATGCCACGGTGGTAAAACGAAGC

PGN1677-D-F-BamHI: GGATCCAACTGCCTTAGCGCAGGATTCGG

PGN1677-D-R-KpnI: GGTACCTCCAAATGAGGAGTTACGGGTTC

PGN1713-U-F-NotI: GCGGCCGCATGAATCGAGAAAGCTTTTTAC

PGN1713-U-R-BamHI: GGATCCGGCATTGACCATTCCACCCATAAG

PGN1713-D-F-BamHI: GGATCCAGATACATCAACCGGCAGATGGAC

PGN1713-D-R-KpnI: GGTACCTCAGCGGAAAGGATAGAATTGTAG

PGN1719-U-F-NotI: GCGGCCGCATGTACCGCCTCTTCCGCAATC

PGN1719-U-R-BglII: AGATCTATACCCTGAACGGGCGTGAAGTCG

PGN1719-D-F-BglII: AGATCTCTATCGAAAAGCCTTGCAGTATAC

PGN1719-D-R-KpnI: GGTACCAGGGTAAATACCTGCGGCTTGTGC

PGN1783-U-F-NotI: GCGGCCGCCTGGAAAGATGGGCTTCTGCAT

PGN1783-U-R-BamHI: GGATCCTCTCCTTCTCATACCAATCCGAT

PGN1783-D-F-BamHI: GGATCCAGGAAACAAGCTGAATCCGCTCA

PGN1783-D-R-KpnI: GGTACCATACACCCGGTAAGCTTTGCTGT

PGN1796-U-F-NotI: GCGGCCGCAAAAGGCCCATTCCCGAGCTGA

PGN1796-U-R-BamHI: GGATCCAGCTCTTGCATCCATGCCCATAC

PGN1796-D-F-BamHI: GGATCCGGAAGAATGTAAGCGTCAGCAC

PGN1796-D-R-KpnI: GGTACCGAAGGAGTCTATGGCTATCACG

PGN1877-U-F-NotI: GCGGCCGCATGAGGAACTGGATCGCTATGC

PGN1877-U-R-BglII: AGATCTCAGCATACCGAATACGACAGCTAC

PGN1877-D-F-BglII: AGATCTAGAAAGCCGGACAGAACAAATCCG

PGN1877-D-R-HindIII: AAGCTTATAGTGCTGACGCAGATATGCGAG

PGN1920-U-F-NotI: GCGGCCGCAACTACTTCGAGCACAAGGACA

PGN1920-U-R-BamHI: GGATCCATGGCGAAACCACCTGTTAATGC

PGN1920-D-F-BamHI: GGATCCCGATTAGGCATATGCCTGTACA

PGN1920-D-R-KpnI: GGTACCGGCTGCCCATGTCATCATAGGT

PGN2001-U-F-NotI: GCGGCCGCCAATGTGTGGGGATTTCGTGCT

PGN2001-U-R-BglII: AGATCTAGCCCATACCCTGTAGTTTTCGCT

PGN2001-D-F-BglII: AGATCTGATCTGCCATAAGTATACGAACGA

PGN2001-D-R-KpnI: GGTACCGTTCCTCTGCGGTAAACGGCTCCA

p6-34-F-KpnI: GGTACCTTCGTCGTCAATCAGCATCCCAG

p6-34-R-SalI: GTCGACTGTTTTGTCTCTTATTTAAGTTA

506-TF-XbaI: TCTAGATTCACACTGCAATTCTCTAATAAG

506-TF-Pst1: ACTGCAGTTCACACTGCAATTCTCTAAT

506-TR-NotI: GCGGCCGCCCTACCCGACCATAAACCGCCA

PorP-CF: GGATCCATGGAAGAGCTATAGGGACAAAGG

PorP-CR: GGTACCTAGACCATTTGATCCGGATTGAGC

PorK-CF: GTCGACATGTGTAGAAAGAACAGGTTTTTC

PorK-CR: TCTAGATTATTTCTTTGTGCTGCGACGCGA

PorL-CF: GTCGACGTGTACGTACTTCCATTGCCTTCT

PorL-CR: TCTAGATTATAAGGGTGAGCTGCCGGATGA

PorM-CF: GTCGACATGGCAGTAGGTTCTAATGGGAAT

PorM-CR: TCTAGATTAGTTCACAATTACTTCAATGGC

PorN-CF: GTCGACGTGTTTATGAAAGTATTCAAAGCA

PorN-CR: TCTAGATTATTTGCGGCGACGAACCGAGCG

PorX-CF: GGATCCACCGTAGAACAGCTTCCCACTCAT

PorX-CR: GGATCCTGAGATAGAAGTCCCGAATGACGA

PorY-CF: AGATCTTTCCGCGCGTAAACTCATCCGACA

PorY-CR: AGATCTAATGCTGTAAATCATTTATGCACA

myc-F-XhoI: CTCGAGCTTGGGCCCGAACAAAAACTCATC

myc -F-HindIII: GAAGCTTGGGCCCGAACAAAAACTCATC

pBAD-R-Pst1: GCTGCAGTTAATGATGATGATGATGATG

PorP-MF-SalI: GTCGACTTGCATAAATCTTTCCGTTCGCTC

PorP-MR-HindIII: AAGCTTGAGGAAACGAATGCTTTTATACT

PorL-MR-HindIII: AAGCTTTAAGGGTGAGCTGCCGGATGATGA

PorM-MR-HindIII: AAGCTTGTTCACAATTACTTCAATGGCCGG

PorN-MR-XhoI: CTCGAGTTTGCGGCGACGAACCGAGCGAGT

F1466-DF: ATCGTCATTTTATTGGTCTTAACGACA

F1466-DR: TTACTTGATAAATTTAAAGTAGTAGAC

F1466-CF: GGTACCTACTTTGATGCCATTACGGTAGG

F1466-CR: TCTAGACACAATGGGATCTATTGCCAGAG

REALpg0778F2: TTGTACCCGAAGGGAGTACG

REALpg0778R2: TGGATCGAACGGAGAAAGAG

REALpg0832F1: CGCAAGAACTAAGCGGAATC

REALpg0832R1: CTGATAAACCTGCCCGTTGT

REALpg1677F1: TCTTTGCCATCAATGAGCTG

REALpg1677R1: TCACTTTGGTAAGGGGGATG

REALpg1676F1: GTCCGCTTAGCAGCGAATAC

REALpg1676R1: GATCTGTCCTTCAGGCAAGC

REALpg1675F1: CGTCGCAGCACAAAGAAATA

REALpg1675R1: AAAGCATCTCATTGCCCATC

REALpg1674F1: AGTAGGCAGCGAAGCCATTA

REALpg1674R1: ATTTCACGCTTACCCAAACG

REALpg1673F2: TCGCTCGTGAACGAGTAATG

REALpg1673R2: GAATCGGGCGTAGGACAGTA

REALpg1019F1:GATCGGGGACAGAAGTACCA

REALpg1019R1:ATTCGGGTAGGCGAAGAAGT

pg16SrF1: CTTGACTTCAGTGGCGGCA

pg16SrR1: AGGGAAGACGGTTTTCACCA

Supplemental Movie Legends

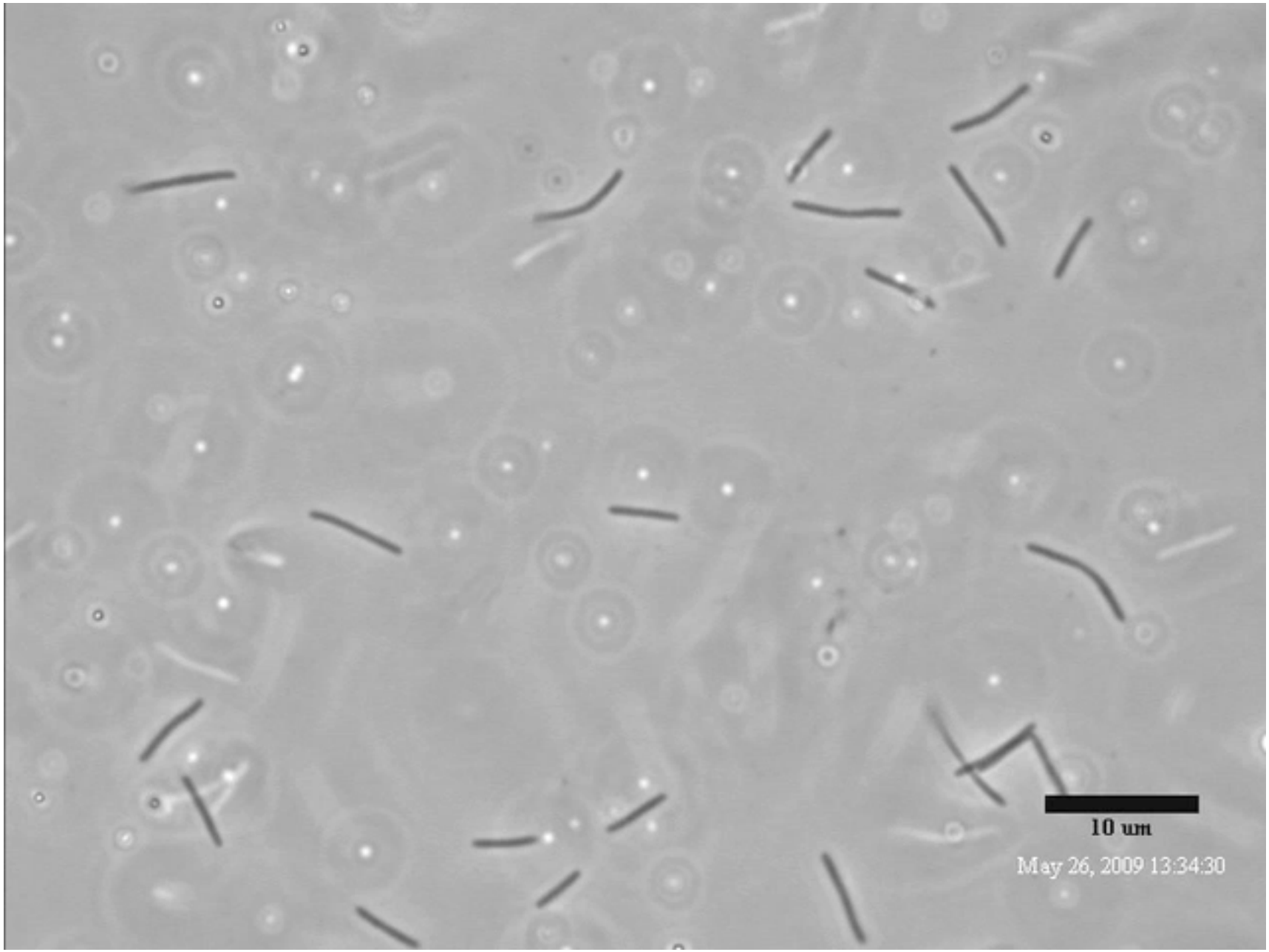
Movie S1. Wild-type cells of *F. johnsoniae* fail to bind or propel Protein G-coated polystyrene spheres. Protein G-coated 0.5 μm polystyrene spheres were added to cells of *F. johnsoniae* in MM plus 0.1% BSA and images were recorded using a Photometrics CoolSNAP$_{cf}^2$ camera mounted on an Olympus BH-2 phase-contrast microscope. Bar indicates 10 μm.

Movie S2. Wild-type cells of *F. johnsoniae* bind and propel Protein G-coated polystyrene spheres when anti-SprB is present. Protein G-coated 0.5 μm polystyrene spheres with anti-SprB antibodies were added to cells of *F. johnsoniae* in MM plus 0.1% BSA and images were recorded using a Photometrics CoolSNAP$_{cf}^2$ camera mounted on an Olympus BH-2 phase-contrast microscope. Bar indicates 10 μm.

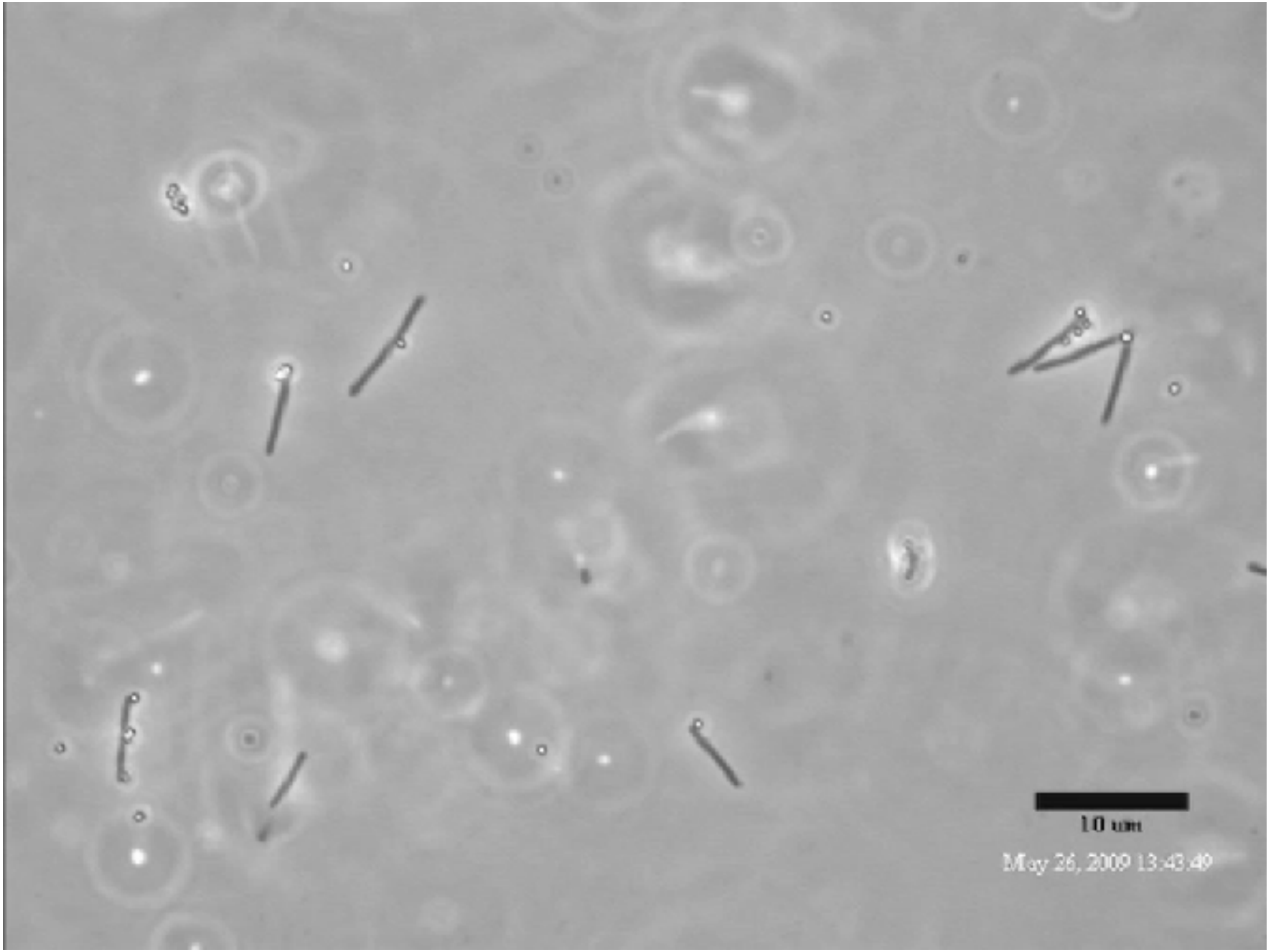
Movie S3. Cells of the *F. johnsoniae sprT* mutant KDF001 fail to bind or propel Protein G-coated polystyrene spheres carrying anti-SprB. Protein G-coated 0.5 μm polystyrene spheres and anti-SprB antibodies were added to cells of the *F. johnsoniae sprT* mutant KDF001 in MM plus 0.1% BSA and images were recorded using a Photometrics CoolSNAP$_{cf}^2$ camera mounted on an Olympus BH-2 phase-contrast microscope. Bar indicates 10 μm.

Movie S4. Complementation of the *F. johnsoniae sprT* mutant KDF001 with pKF002, which carries the wild-type *sprT* gene, restores the ability to bind and propel Protein G-coated polystyrene spheres carrying anti-SprB. Protein G-coated 0.5 μm polystyrene spheres and anti-SprB antibodies were added to cells of the complemented *F. johnsoniae sprT* mutant in MM plus 0.1% BSA and images were recorded using a Photometrics CoolSNAP$_{cf}^{2}$ camera mounted on an Olympus BH-2 phase-contrast microscope. Bar indicates 10 μm.

Still image of movie S1



Still image of movie S2



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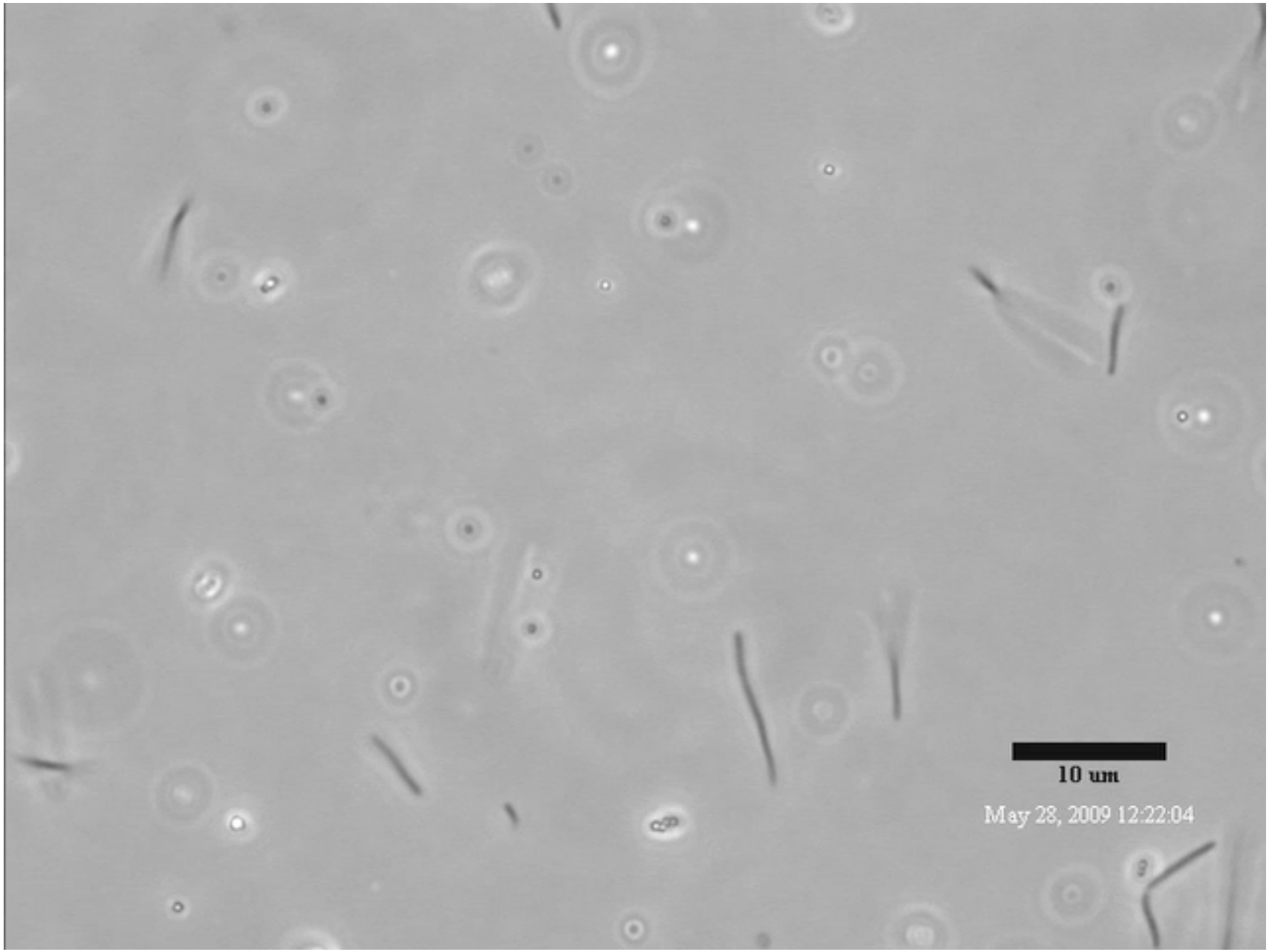
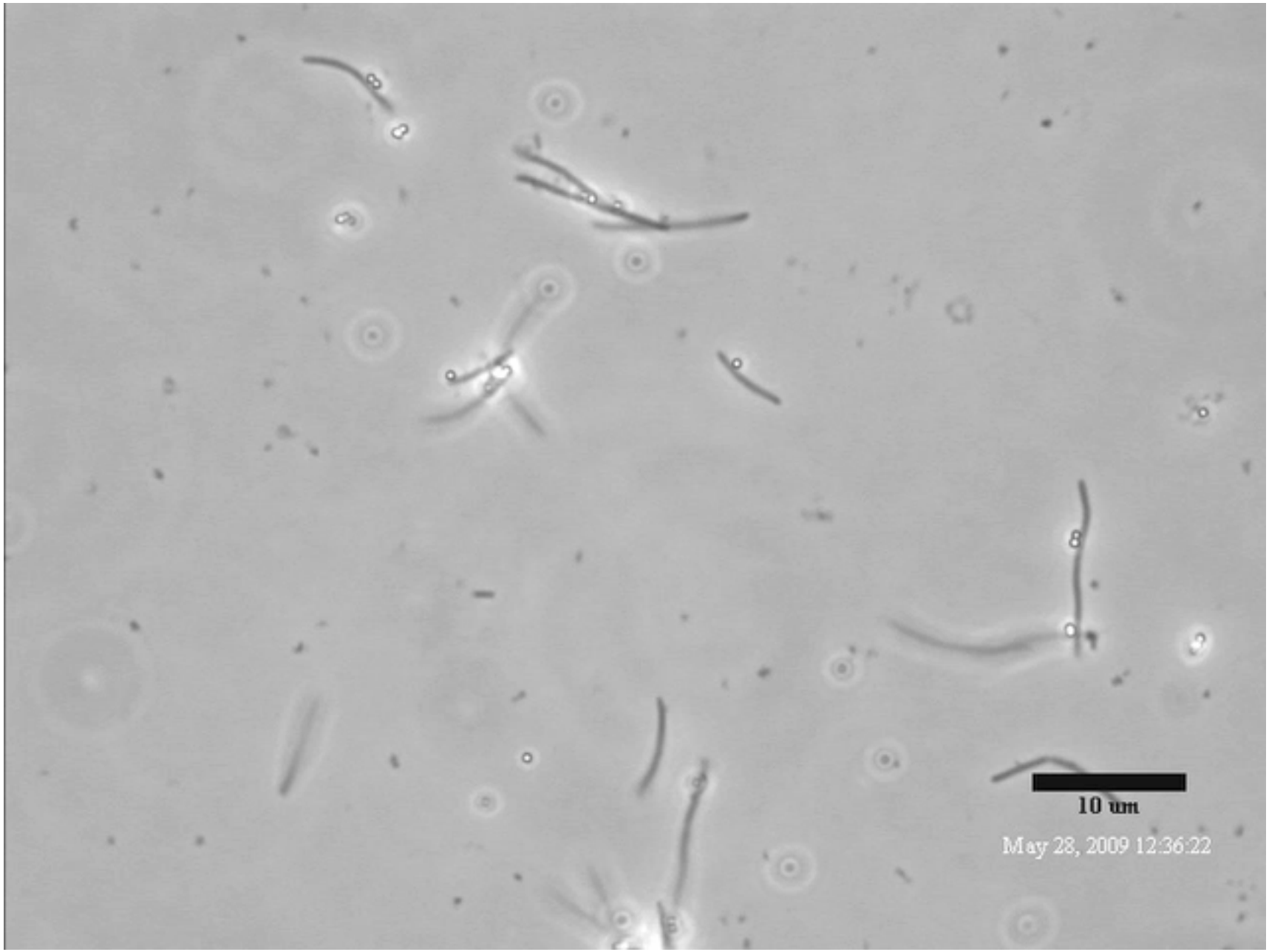


Still image of movie S4



SI Text

Construction of Bacterial Strains

P. gingivalis deletion mutants were constructed as follows. DNA regions upstream and downstream of a gene were PCR-amplified from the chromosomal DNA of *P. gingivalis* ATCC 33277 using pairs of primers (PGN gene number-U-F plus PGN gene number-U-R and PGN gene number-D-F plus PGN gene number-D-R), respectively, where 'U' indicates upstream, 'F' indicates forward, 'D' indicates downstream, and 'R' indicates reverse. Primers used in this study are listed in Table S5. The amplified DNAs upstream of each gene were double-digested with NotI plus a corresponding restriction enzyme (BamHI or BglII). DNA downstream of each gene was digested with KpnI plus a corresponding restriction enzyme (BamHI or BglII). Both digested products were ligated together with pBluescript II SK(-) which had been digested with NotI and KpnI. The 1.5-kb BamHI *ermF* DNA fragment was inserted into the BamHI or BglII site of the resulting plasmids to yield plasmids for mutagenesis. The plasmids were digested with NotI and KpnI and introduced into *P. gingivalis* ATCC 33277 by transformation. Double recombination resulted in deletion of the targeted gene and acquisition of erythromycin resistance.

A complemented strain of the *porP* mutant KDP354 was constructed as follows. The entire *porP* gene containing its promoter was PCR-amplified from the chromosomal DNA using the pair of primers PPCF and PPCR, digested with BamHI and KpnI, and then inserted into the BamHI-KpnI site of pTCB plasmid, resulting in

pKD959. After mating of *E. coli* S17-1 containing pKD959 and *P. gingivalis* KDP354, an Em^r Tc^r transconjugant (KDP367) was obtained.

The promoter region of *Porphyromonas gulae* catalase gene (accession number AB083039) was PCR-amplified from *P. gulae* VPB3492 chromosomal DNA using the pair of primers PRO-U and PRO-R, digested with KpnI plus SalI, and inserted into the KpnI-SalI region of pBSSK to yield pKD954. The entire *porK* gene was PCR-amplified from *P. gingivalis* ATCC 33277 chromosomal DNA using the pair of primers PorK-CF and PorK-CR, and digested with SalI plus NotI. The transcriptional terminator DNA region of the *P. gingivalis rgpB* gene was PCR-amplified from the *P. gingivalis* ATCC 33277 chromosomal DNA using the pair of primers TE-1F and TE-1R, and then digested with XbaI plus NotI. The *porK* and terminator DNA fragments were ligated with the larger SalI-NotI fragment of pKD954 to construct pKD955. The smaller KpnI-NotI fragment of pKD955 was then ligated with the larger KpnI-NotI fragment of pTCB to construct pKD960. After mating of *E. coli* S17-1 containing pKD960 with the *P. gingivalis porK* mutant KDP355, an Em^r Tc^r transconjugant (KDP368) was obtained. Construction of complemented strains of KDP356 (*porL* mutant), KDP357 (*porM* mutant) and KDP358 (*porN* mutant) were performed essentially as described for KDP368 except that the primer pairs used for plasmid construction were: PorL-CF and PorL-CR for *porL* (resulting in pKD961 in complemented strain KDP369), PorM-CF and PorM-CR for *porM* (resulting in pKD962 in complemented strain KDP370), and PorN-CF and PorN-CR for *porN* (resulting in pKD963 in complemented strain KDP371).

For construction of a *porP'-'myc* chimera gene, a myc-tag DNA was PCR-amplified from the pBAD/Myc-His plasmid (Invitrogen) using the pair of primers MYC-F-HindIII and BAD-R-Pst1, and digested with HindIII and PstI. The *rgpB* transcriptional terminator DNA region was PCR-amplified from *P. gingivalis* ATCC 33277 chromosomal DNA using the pair of primers TE-2F and TE-2R, and then digested with PstI and NotI. The *porP* and *myc* fragments were ligated with the larger HindIII-PstI fragment of pBSSK to construct pKD972. pKD972 was digested with HindIII and NotI to obtain a DNA fragment carrying the myc-tag and the transcriptional terminator DNA region. The entire *porP* region was PCR-amplified from *P. gingivalis* ATCC 33277 chromosomal DNA using the primer pair PorP-MF-SalI and PorP-MR-HindIII. The resulting DNA was digested with SalI and HindIII to obtain a DNA fragment carrying *porP*. The two fragments were ligated with the larger SalI-NotI fragment of pKD954 to construct pKD964. The smaller KpnI-NotI fragment of pKD964 was then ligated with the larger KpnI-NotI fragment of pTCB to construct pTCB-p-*porP-myc*-T (pKD968). After mating of *E. coli* S17-1 containing pKD968 with *P. gingivalis* KDP354 (*porP*), an Em^r Tc^r transconjugant (KDP373) was obtained. Construction of *porL'-'myc*, *porM'-'myc* and *porN'-'myc* chimera genes and corresponding mutant strains complemented by the corresponding chimera genes were performed essentially as described for *porP*.

An *F. johnsoniae sprT* mutant was constructed as follows. An internal DNA region of Fjoh_1466 (*sprT*) was PCR-amplified from chromosomal DNA of *F. johnsoniae* ATCC 17061 using the primer pair F1466-DF and F1466-DR. The resulting DNA fragment, which was cloned into pGEM-T EASY vector plasmid and

digested with EcoRI, was inserted into the EcoRI site of pLYL03 to yield pKF001. After mating of *E. coli* S17-1 containing pKF001 with *F. johnsoniae* ATCC 17061, an Em^r transconjugant (KDF001) was obtained.

An *sprT*$^+$-complemented strain of KDF001 was constructed as follows. The *sprT* gene was PCR-amplified from the chromosomal DNA of *F. johnsoniae* ATCC 17061 using the primer pair F1466-CF and F1466-CR. The amplified DNA was digested with KpnI and XbaI and then inserted into the KpnI-XbaI site of pCP23 to yield pKF002. The plasmid was transferred to *F. johnsoniae* KDF001 by conjugation as described above, resulting in KDF002.

For construction of an *E. coli* strain expressing Fjoh_4555, the gene was PCR-amplified from *F. johnsoniae* ATCC 17061 chromosomal DNA using the pair of primers F4555-HF and F4555-HR. The amplified DNA was digested with BamHI and XhoI, and inserted into BamHI-XhoI digested pET-32b (Novagen), resulting in pKF003. *E. coli* BL21 (DE3) harboring pKF003 was grown on LB broth at 30^{o}C and expression of the Fjoh_4555 gene was induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside.