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Gliding Motility and Por Secretion System Genes Are Widespread among Members of the Phylum *Bacteroidetes*

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The phylum *Bacteroidetes* is large and diverse, with rapid gliding motility and the ability to digest macromolecules associated with many genera and species. Recently, a novel protein secretion system, the Por secretion system (PorSS), was identified in two members of the phylum, the gliding bacterium *Flavobacterium johnsoniae* and the nonmotile oral pathogen *Porphyromonas gingivalis*. The components of the PorSS are not similar in sequence to those of other well-studied bacterial secretion systems. The *F. johnsoniae* PorSS genes are a subset of the gliding motility genes, suggesting a role for the secretion system in motility. The *F. johnsoniae* PorSS is needed for assembly of the gliding motility apparatus and for secretion of a chitinase, and the *P. gingivalis* PorSS is involved in secretion of gingipain protease virulence factors. Comparative analysis of 37 genomes of members of the phylum *Bacteroidetes* revealed the widespread occurrence of gliding motility genes and PorSS genes. Genes associated with other bacterial protein secretion systems were less common. The results suggest that gliding motility is more common than previously reported. Microscopic observations confirmed that organisms previously described as nonmotile, including *Croceibacter atlanticus*, "*Gramella forsetii*," *Paludibacter propionicigenes*, *Riemerella anatipestifer*, and *Robiginitalea biformata*, exhibit gliding motility. Three genes (*gldA*, *gldF*, and *gldG*) that encode an apparent ATP-binding cassette transporter required for *F. johnsoniae* gliding were absent from two related gliding bacteria, suggesting that the transporter may not be central to gliding motility.

Cells of *Flavobacterium johnsoniae*, and of many other members of the phylum *Bacteroidetes*, crawl rapidly over surfaces by a process known as gliding motility (1). Cells of *F. johnsoniae* move smoothly in the direction of their long axes at approximately 2 μm per second on wet glass surfaces. They occasionally reverse their direction of movement, and they also exhibit flipping and pivoting movements that are characteristic of bacteroidete gliding motility. Cell movement appears to be powered by the proton gradient across the cytoplasmic membrane (2–4).

GldA, GldB, GldD, GldF, GldG, GldH, GldI, GldJ, GldK, GldL, GldM, and GldN (or its paralog GldO) are essential for gliding of *F. johnsoniae* (Fig. 1) (5, 6). Cells with mutations in the genes encoding these proteins grow as well as wild-type cells but are completely nonmotile. GldA, GldF, and GldG comprise an apparent ATP-binding cassette (ABC) transporter whose exact function in gliding is not known (7, 8). GldI is a predicted peptidylprolyl isomerase that probably assists folding of other motility proteins in the periplasm (9). GldJ and GldK are lipoproteins that exhibit similarity to each other and, to a more limited extent, to sulfatase-modifying enzymes such as the human formylglycine-generating enzyme (FGE) (5, 10, 11). FGE activates sulfatases by catalyzing the conversion of active-site cysteine to formylglycine, but GldJ and GldK are not thought to have this activity since they lack the critical active-site residues of FGE. The remaining Gld proteins (GldB, -D, -H, -L, -M, and -N) are novel, and homologs have not been found outside the phylum *Bacteroidetes*. SprA, SprE, and SprT are also important for gliding. Cells with mutations in the genes encoding these proteins are severely crippled, but a few cells exhibit occasional slight movements (12–14).

Mobile cell surface adhesins, such as SprB and RemA, are also involved in gliding (15, 16). SprB is involved in movement over agar, and RemA is involved in movement over surfaces such as glass coated with polysaccharide produced by *F. johnsoniae*. Cells with mutations in either *sprB* or *remA* still glide rapidly, but cells with mutations in both genes are more severely affected. Genome analysis identified genes predicted to encode additional adhesins that may allow movement over other surfaces (16, 17). Gliding involves the rapid movement of the semiredundant motility adhesins SprB and RemA along the cell surface (15, 16). The adhesins are thought to be propelled by the poorly understood gliding "motor," which may be composed of some of the Gld proteins.

A subset of the motility proteins constitute a novel protein secretion system, the Por secretion system (PorSS) (6, 13, 14, 16). The *F. johnsoniae* PorSS, which consists of GldK, GldL, GldM, GldN, SprA, SprE, and SprT, is needed for secretion of SprB and RemA to the cell surface and for secretion of an extracellular chitinase. *Porphyromonas gingivalis*, a nonmotile member of the phylum *Bacteroidetes* and a human pathogen, also contains a PorSS, comprised of proteins that are homologous to the *F. johnsoniae* proteins (14, 18, 19). The *P. gingivalis* PorSS is needed for secretion of gingipain protease virulence factors.

The components of the *F. johnsoniae* and *P. gingivalis* PorSSs are not related to those of the well-defined type I to type VI secretion systems (T1SS to T6SS) (20–24). They are also not similar to the components of the mycobacterial ESX pathway, which has been referred to as the type VII secretion system (25, 26); the extracellular nucleation-precipitation (ENP) pathway involved in secretion and assembly of curly amyloid fibers, which has been

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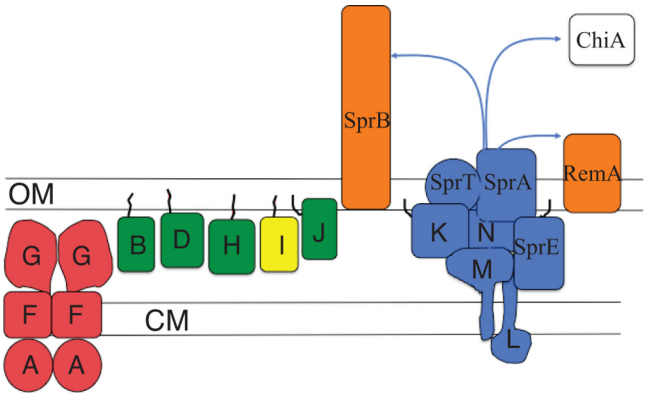

FIG 1 Proteins involved in *F. johnsoniae* gliding motility and protein secretion. SprB and RemA (orange) are thought to function as adhesins that are propelled along the cell surface by the some of the other proteins shown. GldA, GldF, and GldG (red) comprise an ATP-binding cassette transporter whose exact role in gliding is not known. GldI (yellow) is a peptidylprolyl isomerase involved in protein folding. Proteins in blue (GldK, GldL, GldM, GldN, SprA, SprE, SprT) constitute the PorSS and are required for secretion of SprB and RemA and for motility. They also secrete the chitinase ChiA (white), which is not involved in motility. Proteins in green (GldB, GldD, GldH, and GldJ) are also required for gliding. Black lines indicate lipid tails on lipoproteins. Proteins are not drawn to scale, stoichiometry of components is not known, and it is not yet known whether any of the lipoproteins localize to the cytoplasmic membrane (CM) instead of the outer membrane (OM) as shown.

proposed as the type VIII secretion system (27, 28); or the components of the chaperone-usher pathway (29).

Recently, numerous genomes of members of the phylum *Bacteroidetes* have been sequenced. In this study, we analyzed the first 37 completely sequenced genomes of species of the phylum *Bacteroidetes* and found that gliding motility genes and PorSS genes were widespread. Microscopic examination of organisms that contained gliding motility genes but that were previously described as nonmotile revealed gliding motility in additional members of the phylum. Genome analyses revealed that PorSS genes were found in all gliding members of the phylum, suggesting an important role in gliding. They also revealed that GldA, GldF, GldG, and GldI, which are required for *F. johnsoniae* gliding, are not essential for gliding of some other members of the phylum *Bacteroidetes*.

MATERIALS AND METHODS

Genome analyses. The genomes of 37 members of the phylum *Bacteroidetes* were analyzed in this study (see Table S1 in the supplemental material). This included all members with completely sequenced genomes that were available as of September 2011, with the following exceptions: (i) completed genome sequences were available for multiple strains of *Bacteroides fragilis*, *P. gingivalis*, and *Riemerella anatipestifer*, but only the type strains were analyzed; and (ii) intracellular symbionts or parasites with reduced genome sizes (members of the genus *Blattabacterium* and uncultured bacteria that have thus been given "*Candidatus*" status) were not included. Genomes were accessed via the Integrated Microbial Genomes (IMG version 3.5) website at http://img.jgi.doe.gov/. Gliding motility and PorSS genes of *F. johnsoniae* were used to identify homologs in the genomes by BLAST analyses using the IMG Gene Profile tool. These genes were verified as reciprocal best hits. The presence of genes encoding components of other secretion systems was determined with appropriate COG clusters, PFAM terms, and TIGRFAM terms, using the IMG Function Profile tool and appropriate Function Carts to probe each genome.

Motility analyses. Strains of marine origin (*Cellulophaga algicola*, *Croceibacter atlanticus*, *Robiginitalea biformata*, *Zunongwangia profunda*, "*Gramella forsetii*," and *Maribacter* sp. strain HTCC2170) were grown on Difco marine broth 2216 (Becton, Dickinson and Co., Sparks, MD) solidified with 1.5% agar and on Cytophaga medium (DSMZ medium 172) and were incubated at 25°C, except for *R. biformata*, which was incubated at 30°C, and *C. algicola*, which was incubated at 15°C. "*G. forsetii*" is written within quotation marks because it does not yet have official standing in the bacterial nomenclature. *Dyadobacter fermentans* was cultured using R2A (Teknova, Hollister, CA), Casitone-yeast extract (CYE) (30), and PY2 (7) agar media at 25°C. *Paludibacter propionicigenes* was incubated anaerobically on peptone, yeast extract, glucose (PYG) agar (DSMZ medium 104) at 25°C. *Riemerella anatipestifer* was grown with reduced oxygen and elevated CO_2 in a candle jar on Trypticase soy (TS) agar (Becton, Dickinson and Co.) at 37°C. *Weeksella virosa* was grown on CASO (DSMZ medium 220), LB (31), CYE, and PY2 agar and LB broth media at 30°C. *Leadbetterella byssophila* was grown on TS, CYE, and PY2 agar and TS broth media at 30°C. *Spirosoma linguale* was grown on CYE, PY2, and *Spirosoma* agar and broth media (ATCC medium 507) at 25°C.

Motility on glass was analyzed using liquid-filled tunnel slides. Tunnel slides were prepared by attaching a glass coverslip to a glass slide with strips of double-stick tape as previously described (16) to make a chamber to which cells suspended in growth media were added. Cells were examined near the edge of the coverslip (within 1 mm) or near the tape (within 1 mm) to avoid loss of motility as a result of depletion of O_2. *P. propionicigenes* is an anaerobe, so movement of this organism on glass was measured in standard wet mount instead of in a tunnel slide.

Movement on agar was examined by spotting cells on a pad of 1% agar medium on a glass slide, allowing the spot to dry, and covering it with an O_2-permeable Teflon membrane (Yellow Springs Instrument Co., Yellow Springs, OH) that prevented dehydration and served as a coverslip. In all cases cells were observed using an Olympus BH-2 phase-contrast microscope, and images were recorded using a Photometrics Cool-SNAP$_{cf}^2$ camera and analyzed using MetaMorph software (Molecular Devices, Downingtown, PA).

RESULTS AND DISCUSSION

Defining a core set of genes required for bacteroidete gliding motility. The 12 known gliding members of the phylum *Bacteroidetes* with completed genome sequences were examined for the presence of orthologs to the 15 genes (*gldA*, *gldB*, *gldD*, *gldF*, *gldG*, *gldH*, *gldI*, *gldJ*, *gldK*, *gldL*, *gldM*, *gldN*, *sprA*, *sprE*, and *sprT*) that are essential or nearly essential for *F. johnsoniae* gliding. These bacteria included *Capnocytophaga canimorsus*, *Capnocytophaga ochracea*, *C. algicola*, *Cellulophaga lytica*, *Chitinophaga pinensis*, *Cytophaga hutchinsonii*, *F. johnsoniae*, *Flavobacterium psychrophilum*, *Maribacter* sp. HTCC2170, *Marivirga tractuosa*, *Pedobacter heparinus*, and *Pedobacter saltans* (17, 32–41). Each of these bacteria had orthologs for 11 of the motility genes, *gldB*, *gldD*, *gldH*, *gldJ*, *gldK*, *gldL*, *gldM*, *gldN*, *sprA*, *sprE*, and *sprT* (Fig. 2; see also Table S2 in the supplemental material). We defined these genes, which are (i) required for *F. johnsoniae* gliding and (ii) present in each of the gliding members of the phylum *Bacteroidetes* analyzed, as the core bacteroidete gliding motility genes.

A *gldI* ortholog is not required for movement of all gliding members of the phylum *Bacteroidetes*. *gldI*, which encodes a predicted peptidylprolyl *cis*/*trans* isomerase, is required for *F. johnsoniae* gliding (9). *gldI* orthologs were identified in all known gliding members of the class *Flavobacteriia* but were not found in gliding bacteria belonging to the classes *Cytophagia* and *Sphingobacteriia*. GldI is a predicted periplasmic lipoprotein thought to function in protein folding. In *F. johnsoniae* it may assist folding of Gld or Spr proteins involved in motility. Although members of the

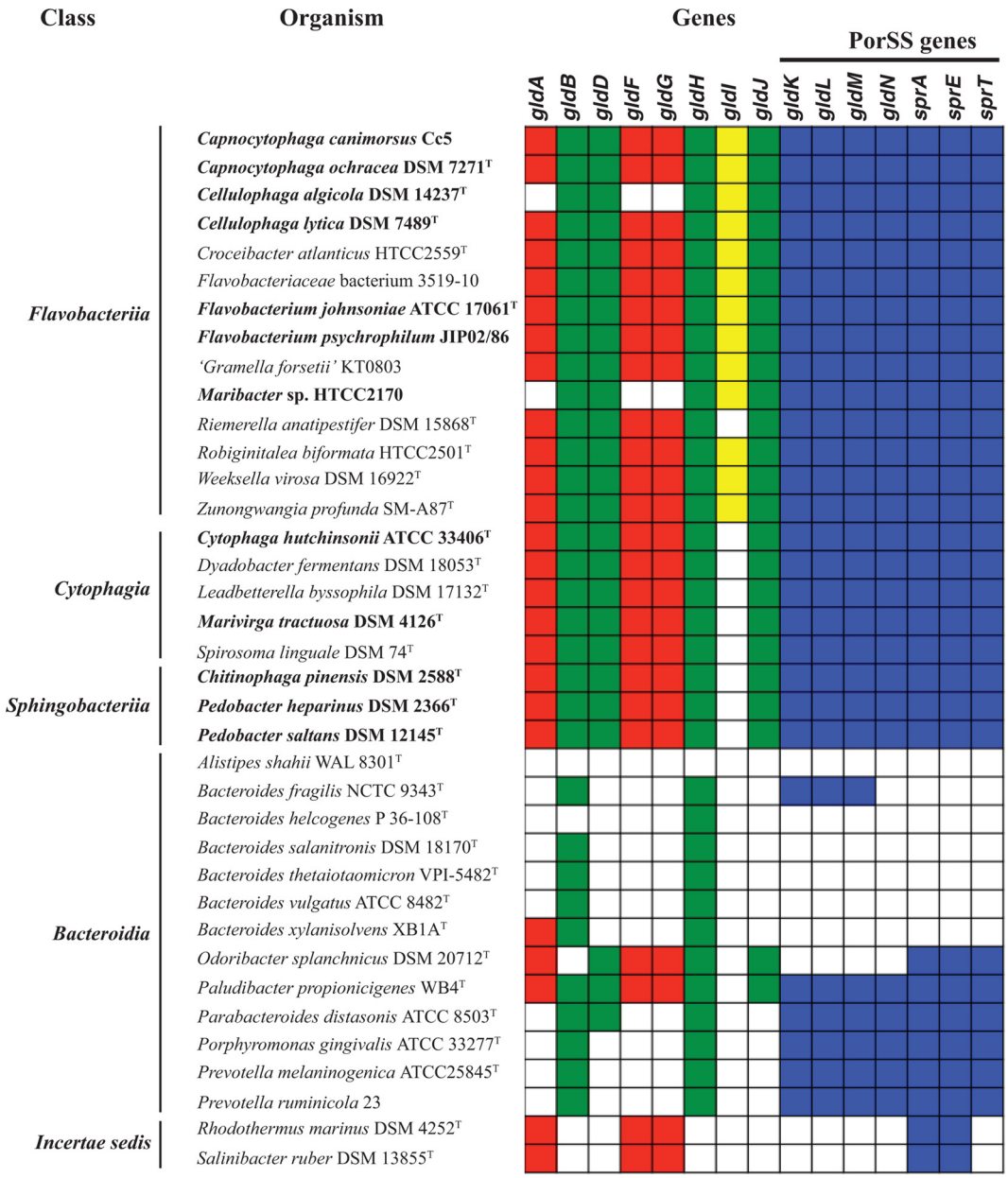

FIG 2 Distribution of *gld* and *spr* orthologs in completed *Bacteroidetes* genomes. Orthologs were identified by reciprocal BLASTP analysis using the *F. johnsoniae* motility protein sequences. Maximum E values were set at 1e−50 for GldA and 1e−5 for the other proteins. In order to confirm weak hits for bacteria distantly related to *F. johnsoniae* (class *Flavobacteriia*), genomes were also searched with protein sequences from *C. hutchinsonii* (class *Cytophagia*), *P. heparinus* (class *Sphingobacteriia*), and *P. gingivalis* (class *Bacteroidia*). A colored square indicates the presence of an ortholog, and a white square indicates the absence of an ortholog. Colors of squares correspond to those assigned to individual proteins in Fig. 1 based on predicted or verified functional groups: red, ABC transporter components; yellow, peptidylprolyl isomerase; blue, PorSS components; green, additional proteins required for gliding. The core gliding motility genes as defined here correspond to the green and blue columns. Species are listed alphabetically within each class. Species previously known to exhibit gliding motility are in bold.

classes *Cytophagia*, *Sphingobacteriia*, and *Bacteroidia* lacked obvious *gldI* orthologs, each of them encoded predicted periplasmic peptidylprolyl isomerases that might function similarly to *F. johnsoniae* GldI. Additional studies will be required to determine whether any of these proteins have roles in motility.

***gldA*, *gldF*, and *gldG* are not present in the gliding bacteria *C. algicola* and *Maribacter* sp. HTCC2170.** *gldA*, *gldF*, and *gldG* are required for *F. johnsoniae* gliding but were not found in all gliding bacteroidetes. GldA, GldF, and GldG are components of a predicted ABC transporter whose exact role in gliding is not known (7, 8). GldA is the soluble, cytoplasmic, ATP-binding component of the transporter and shares sequence similarity with many other ATP-binding components of ABC transporters. GldF and GldG are cytoplasmic membrane protein components of the complex. Homologs of GldF and GldG are common in members of the phylum *Bacteroidetes*, but they are not common outside this phylum (8). Most of the known gliding members of the phylum had orthologs for *gldA*, *gldF*, and *gldG*, but *C. algicola* and *Maribacter*

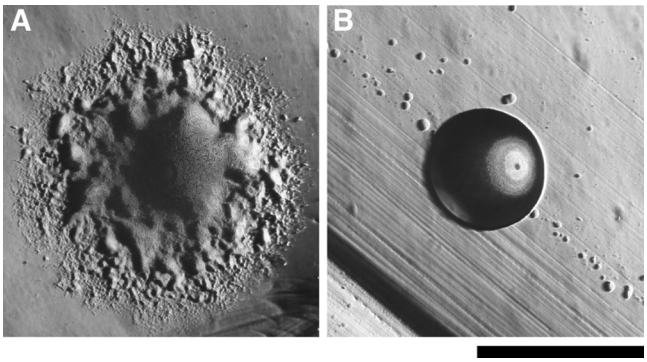

FIG 3 Colony morphologies of the gliding bacteria *C. algicola* and *Maribacter* sp. HTCC2170, which lack homologs of *gldA*, *gldF*, and *gldG*. (A) Spreading colony of *C. algicola* on Cytophaga agar after incubation for 5 days at 15°C; (B) nonspreading colony of *Maribacter* sp. HTCC2170 on marine agar after incubation for 9 days at 25°C. Photomicrographs were taken with a Photometrics CoolSNAP$_{cf}^{2}$ camera mounted on an Olympus IMT-2 phase-contrast microscope. The bar indicates 1 mm and applies to both panels.

sp. HTCC2170, which exhibit active gliding motility, did not (Fig. 2; see also Movie S1 in the supplemental material). Gliding of *C. algicola* cells resulted in the formation of spreading colonies, whereas gliding of *Maribacter* sp. HTCC2170 did not (Fig. 3). Formation of nonspreading colonies is not unique to *Maribacter* sp. HTCC2170, since many other gliding bacteria also fail to form spreading colonies on agar (42), so examination of colony morphology is not sufficient to demonstrate absence of gliding motility.

It was possible that the strains used for genome sequencing had recently lost *gldA*, *gldF*, and *gldG* and that the sequenced strains were nonmotile mutants. In *C. lytica*, and in many other members of the class *Flavobacteriia*, the region containing *gldF* and *gldG* is flanked by a gene encoding a predicted *S*-adenosyl-L-methionine hydroxide adenosyltransferase and by *dnaN*. These genes are also adjacent to each other in the *C. algicola* and *Maribacter* sp. HTCC2170 genomes, but *gldF* and *gldG* are missing. We amplified and sequenced the regions between these genes from our motile strains of *C. algicola* and *Maribacter* sp. HTCC2170 and demonstrated that *gldF* and *gldG* were absent. The absence of *gldA*, *gldF*, and *gldG* in *C. algicola* and *Maribacter* sp. HTCC2170 suggests that the ABC transporter required for *F. johnsoniae* gliding may not have a central and irreplaceable role in bacteroidete gliding motility. ABC transporters are common in most organisms, and not surprisingly, *C. algicola* and *Maribacter* sp. HTCC2170 have other ABC transporters. In each case, one of these may replace the GldA-GldF-GldG transporter, thus allowing gliding in its absence. Alternatively, *C. algicola* and *Maribacter* sp. HTCC2170 gliding may not require the assistance of an ABC transporter.

Gliding motility genes, and gliding motility, are widespread among members of the phylum *Bacteroidetes*. Of the remaining 25 completed genome sequences of members of the phylum *Bacteroidetes*, all but one belong to bacteria that had been described as nongliding. The one exception was *Flavobacteriaceae* bacterium 3519-10, for which no information regarding motility was available. These 25 genomes were examined for the presence of gliding motility genes (Fig. 2; see also Table S2 in the supplemental material). Eleven species (*C. atlanticus*, *D. fermentans*, *Flavobacteriaceae* bacterium 3519-10, "*G. forsetii*," *L. byssophila*, *P. propionicigenes*, *R. anatipestifer*, *R. biformata*, *S. linguale*, *W. virosa*, and *Z. profunda*) had each of the core gliding motility genes.

We examined each of the 11 bacteria listed above, with the exception of *Flavobacteriaceae* bacterium 3519-10, which was not available for study, by phase-contrast microscopy and demonstrated that at least 5 of them (*C. atlanticus*, "*G. forsetii*," *P. propionicigenes*, *R. anatipestifer*, and *R. biformata*) exhibited gliding motility (Fig. 4; see also Movies S2 to S6 in the supplemental material). *C. atlanticus*, *R. biformata*, and *P. propionicigenes* exhibited rapid gliding on glass that was easily observed in real time in tunnel slides or wet mounts (see Movies S2, S3, and S6 in the supplemental material). The movements exhibited by these bacteria were similar to those observed for *F. johnsoniae* and other gliding members of the phylum and included pivoting and flipping movements in addition to smooth translocation over the surface. Cells of *R. biformata* and *P. propionicigenes* also moved on agar (see Movies S3 and S6 in the supplemental material), whereas cells of *C. atlanticus* did not. Cells of "*G. forsetii*" and *R. anatipestifer* moved less rapidly, requiring time-lapse studies for detection. Cells of *R. anatipestifer* moved slowly on agar or glass, whereas cells of "*G. forsetii*" failed to move on glass but exhibited slow movements on agar (see Movies S4 and S5 in the supplemental material). Note that none of these five bacteria formed spreading colonies on agar growth media, so they would not have been recognized as gliding bacteria by simple examination of colony morphology. Despite extensive efforts, we did not observe motility of the other five species (*D. fermentans*, *L. byssophila*, *S. linguale*, *W. virosa*, and *Z. profunda*) that had each of the core gliding motility genes. These bacteria may be nonmotile, or they may glide under conditions that we did not examine.

C. atlanticus, "*G. forsetii*," and *R. biformata* are marine bacteria that are thought to play important roles in the digestion of macromolecules (43–45). As with other marine bacteroidetes, these bacteria are probably enriched on surfaces such as algal cells or organic detritus particles known as "marine snow," and the ability to glide over these surfaces may be important for their survival (46). *R. anatipestifer* is an important poultry pathogen (47), and motility may play a role in pathogenesis. *P. propionicigenes*, which was isolated from an anoxic rice field (48), has not been studied extensively, and the importance of gliding motility in its lifestyle is unclear.

In addition to the 22 finished genome sequences for members of the classes *Flavobacteriia*, *Cytophagia*, and *Sphingobacteriia* described above, draft genome sequences were available for another 27 members of these classes (see Table S3 in the supplemental material). We analyzed these and determined that all contained the 11 core gliding motility genes (*gldB*, *gldD*, *gldH*, *gldJ*, *gldK*, *gldL*, *gldM*, *gldN*, *sprA*, *sprE*, and *sprT*). Included in this list were *Algoriphagus* sp. strain PR1, *Chryseobacterium gleum* F93^{T}, *Kordia algicida* OT-1^{T}, *Krokinobacter* sp. strain 4H-3-7-5, *Lacinutrix* sp. strain 5H-3-7-4, *Mucilaginibacter paludis* TPT56^{T}, *Polaribacter irgensii* 23-P^{T}, *Sphingobacterium spiritivorum* ATCC 33861^{T}, and *Ulvibacter* sp. strain SCB49, which have each been described as nonmotile. Given the results presented above, it is likely that some of these have the ability to glide.

Of the members of the phylum *Bacteroidetes* for which finished and draft genomes are available, only *F. johnsoniae* appears to have been selected for sequencing because of its gliding motility (17). With this in mind, the presence of gliding motility genes in each of the 49 free-living members of the classes *Flavobacteriia*, *Cytopha-*

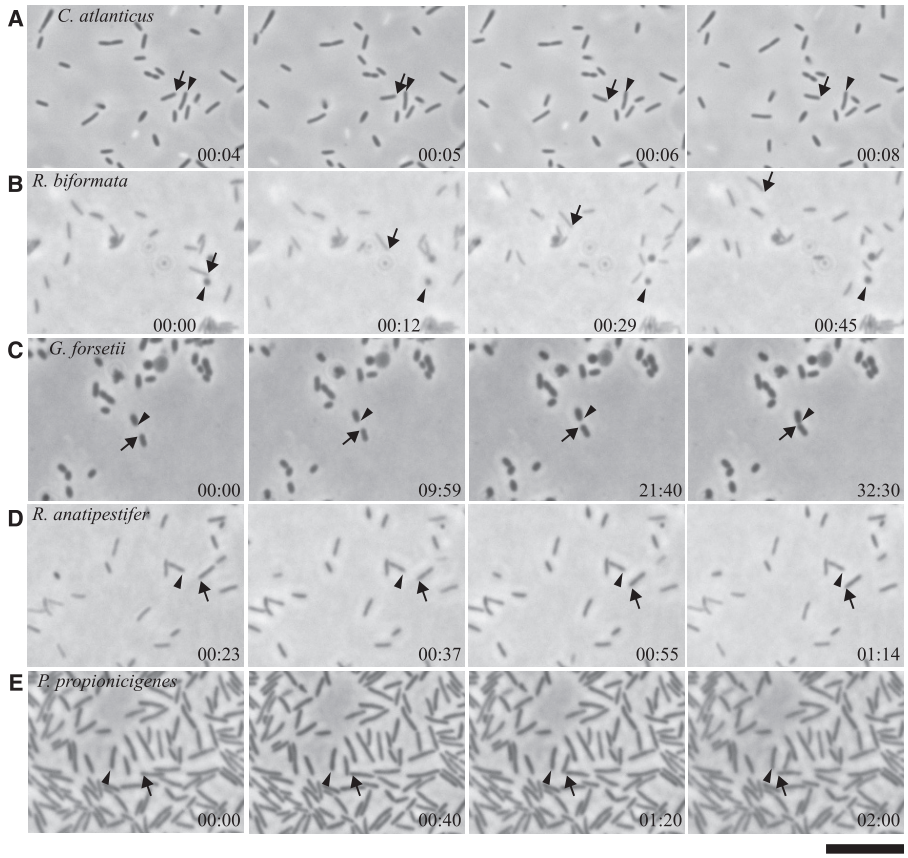

FIG 4 *C. atlanticus*, *R. biformata*, "*G. forsetii*," *R. anatipestifer*, and *P. propionicigenes* exhibit gliding motility. Cells gliding on agar or glass were recorded using a Photometrics Cool-SNAP$_{cf}^2$ camera mounted on an Olympus BH-2 phase-contrast microscope. The images were taken from Movies S2 to S6 in the supplemental material. (A) Cells of *C. atlanticus* in marine broth gliding on glass in a tunnel slide. The images come from Movie S2 between 4 and 8 s and correspond to the middle portion of the field. (B) Cells of *R. biformata* on marine agar. The images come from the second part of Movie S3 and correspond to the top middle portion of the field. (C) Cells of "*G. forsetii*" on modified Cytophaga agar (yeast extract and tryptone omitted). The images come from Movie S4 and correspond to the top middle portion of the field. (D) Cells of *R. anatipestifer* on TS agar. The images come from the second part of Movie S5 and correspond to the lower right portion of the field. (E) Cells of *P. propionicigenes* on PYG agar. The images come from the second part of Movie S6 and correspond to the lower left portion of the field. All cells were incubated at 25°C during analysis. The numbers indicate time (minutes and seconds). Arrows indicate moving cells, and arrowheads indicate stationary reference points. The bar at the lower right indicates 10 μm and applies to all panels. The movies in the supplemental material contain additional footage demonstrating gliding.

gia, and *Sphingobacteriia* with sequenced genomes suggests that gliding motility is more common among these large and diverse groups of bacteria than was previously suspected. Gliding motility is much less common among members of the final class of the phylum, *Bacteroidia*, but as demonstrated above for *P. propionicigenes*, it also occurs in some members of that group.

Bernardet and Bowman recently reported that gliding was underreported for members of the genus *Flavobacterium*. They demonstrated that four species of the genus *Flavobacterium* (*Flavobacterium antarcticum*, *Flavobacterium degerlachei*, *Flavobacterium frigoris*, and *Flavobacterium indicum*) that had been described as nonmotile exhibit gliding motility and that the type strain of *Flavobacterium gelidilacus*, which was reported to be nonmotile, also glided (49). We also examined *F. indicum* and observed rapid gliding motility. Our results confirm and extend those of Bernardet and Bowman and suggest that gliding is underreported not only for members of the genus *Flavobacterium* but also for many genera and species within the phylum *Bacteroidetes*.

Gliding motility is found in members of many bacterial phyla, but genes similar in sequence to the core bacteroidete gliding motility genes are uncommon outside the phylum *Bacteroidetes*. BLASTP searches (cutoffs at 10% identity and 0.01 E value) were performed with each of the core gliding motility proteins against translated products from the genomes of gliding bacteria from five different phyla, including *Myxococcus xanthus* DK1622, *Mycoplasma mobile* 163K, *Nostoc punctiforme* PCC 73102, *Chloroflexus aurantiacus* J-10-fl, and *Chloroherpeton thalassium* ATCC 35110. *C. aurantiacus*, *M. mobile*, and *N. punctiforme* had no orthologs for the *F. johnsoniae* core gliding motility proteins. *C. aurantiacus* had an ortholog for SprA (Ctha_1407) but lacked orthologs to GldB, GldD, GldH, GldJ, GldK, GldL, GldM, GldN, SprE, and SprT. *M. xanthus* had an ortholog for GldK (MXAN_3455) but lacked orthologs to the other 10 proteins. Neither Ctha_1407 nor MXAN_3455 has been linked to motility, and we do not know the functions of these proteins in their respective organisms. The scarcity of orthologs to *F. johnsoniae* motility proteins among these diverse gliding bacteria suggests that they employ gliding machineries that are not closely related to the *F. johnsoniae* gliding apparatus. Components of the *M. xanthus* and *M. mobile* gliding motility machineries have been identified (50–55), and most of these

do not have orthologs in *F. johnsoniae* (5), further supporting this suggestion.

PorSS genes are found in most genera and species of the phylum *Bacteroidetes*. PorSSs have been studied in the motile *F. johnsoniae* and the nonmotile *P. gingivalis*. A core set of PorSS genes was defined as the genes common to both of these organisms that have been demonstrated to be required for secretion. The core PorSS genes (*gldK*, *gldL*, *gldM*, *gldN*, *sprA*, *sprE*, and *sprT*) are a subset of the *F. johnsoniae* gliding motility genes (6, 13, 14). The *F. johnsoniae* PorSS is required for secretion of the cell surface motility proteins SprB and RemA and for secretion of a soluble extracellular chitinase.

Orthologs of the PorSS genes were identified in 21 of 26 genera and 27 of the 37 species of the phylum *Bacteroidetes* that were analyzed (Fig. 2; see also Table S2 in the supplemental material). The PorSS genes were found in all members of the classes *Flavobacteriia*, *Cytophagia*, and *Sphingobacteriia* and in 5 of the 13 members of the class *Bacteroidia* that were analyzed (Fig. 2). In contrast, the core PorSS genes were not found in members of other phyla, suggesting that, as with bacteroidete gliding motility, PorSSs may be confined to the phylum *Bacteroidetes*.

The core PorSS genes were present in each of the gliding bacteria of the phylum *Bacteroidetes*. This is perhaps not surprising, since the *F. johnsoniae* PorSS is necessary for assembly of the outermost components of the motility apparatus. The PorSS may be an integral part of the gliding motility machinery, analogous to the T3SS that is built into the flagellar apparatus and is involved in its assembly (56). The PorSS may also have roles in motility beyond assembly of the apparatus. The PorSS proteins GldL and GldM are the only known core components of the motility machinery that span the cytoplasmic membrane. Such transmembrane proteins are expected to play critical roles in conversion of chemical energy into movement. They are likely to be involved in powering protein secretion, but they may also constitute the gliding motor that propels SprB and RemA along the cell surface, resulting in cell movement. Alternatively, some other transmembrane proteins not yet identified may constitute the gliding motor. Numerous spontaneous and transposon-induced nonmotile mutants have been analyzed, but genes encoding such components could have been missed because of built-in redundancy or because they are essential for viability. Further study of the PorSS and its relationship to gliding should help determine whether it has evolved to function as a motor that can propel cells in addition to secreting proteins across the outer membrane.

The Sec and Tat protein export pathways are common among members of the phylum *Bacteroidetes*. In Gram-negative bacteria, "protein export" describes the movement of proteins into or across the cytoplasmic membrane. In contrast, "protein secretion," discussed above, refers to movement across the outer membrane (22). Some protein secretion systems transport proteins across the cytoplasmic membrane and the outer membrane, whereas others only facilitate transport across the outer membrane. The PorSS is thought to secrete proteins from the periplasm across the outer membrane (14) and thus requires a protein export system to deliver the proteins across the cytoplasmic membrane. In other bacteria, the Sec and Tat systems perform this function, and genes associated with both systems were present in most of the bacteroidete genomes analyzed (see Table S4 in the supplemental material).

Proteins secreted by the PorSSs of *F. johnsoniae* and *P. gingivalis* have N-terminal signal peptides that are typical of those exported by the Sec system, suggesting that the Sec system exports the PorSS cargo proteins across the cytoplasmic membrane prior to their secretion by the PorSS. Targeting of these proteins to the PorSS may involve conserved C-terminal domains (CTDs) that are found on proteins known to be secreted by PorSSs (14, 57–61). Consistent with this, each of the genomes of the organisms predicted to have PorSSs encoded many proteins with conserved PorSS-type CTDs identified by the TIGRFAMs, TIGR04131 and TIGR04183, whereas all but two of the remaining bacteria had few, if any, genes encoding proteins with these conserved domains. The two exceptions were *Rhodothermus marinus* and *Salinibacter ruber*, which each had numerous genes matching TIGR04183. *R. marinus* and *S. ruber* lack many of the PorSS genes, but they have *sprA* and *sprE* homologs, suggesting the possibility that the CTD proteins in these bacteria may interact with the outer membrane protein SprA and/or the lipoprotein SprE.

Other genes predicted to be involved in protein secretion. Secretion of proteins across Gram-negative bacterial outer membranes is mediated by a variety of different secretion systems (20–24). T1SSs, T3SSs, T4SSs, and T6SSs transport proteins from the cytoplasm across both membranes of the cell, whereas T2SSs, T5SSs, the ENP pathway involved in biogenesis of curli (T8SS), and the chaperone-usher pathway involved in pilus assembly only facilitate secretion across the outer membrane. The 37 bacteroidete genomes were analyzed for the presence of key genes of each system. Only 5 of the 37 genomes encoded the core components of T2SSs, and the other secretion systems also appeared to be rare in this phylum (see Table S4 in the supplemental material). The only T3SS genes identified were in the flagellar operons of *R. marinus* and *S. ruber*. Bacterial flagella have dedicated T3SSs involved in their assembly, and this is the likely role of the *R. marinus* and *S. ruber* T3SSs. Genes encoding VirB4-like and VirD4-like components associated with T4SSs were identified in 13 of the genomes, but other components typically associated with T4SSs were absent. Several of the T4SS genes were associated with known conjugative transposons (62, 63), and the others may also be involved in conjugative transfer of DNA. Genes associated with possible T1SSs, T5SSs, and T8SSs were detected in a few species, whereas T6SSs, T7SSs, and chaperone-usher pathway genes were not detected in any. It should be noted that the COGs, PFAMs, and TIGRFAMs used to detect genes involved in T1SSs, T4SSs, T5SSs, T6SSs, T8SSs, and chaperone-usher secretion systems were based primarily on secretion system genes from proteobacteria and thus might have missed divergent secretion systems distantly related to these. For example, predicted outer membrane efflux proteins with some similarity to TolC were found in each genome. These could be components of novel T1SSs, or they could be involved in efflux of small molecules.

The analyses described above suggest that most bacterial protein secretion systems are uncommon in members of the phylum *Bacteroidetes*, whereas the *Bacteroidetes*-specific PorSS is found in members of most genera and species of this phylum for which genome sequences are available. The known components of the PorSS are not similar in sequence to components of the type I to type VIII protein secretion systems, and we thus suggest that the PorSS could be referred to as the type IX secretion system (T9SS) to highlight this distinction.

Near-universal presence of *gldB* and *gldH* in members of the phylum *Bacteroidetes*. Two gliding motility genes, *gldB* and *gldH*,

were unusual in that they were found in almost all members of the phylum *Bacteroidetes*, even those that lacked other gliding motility and PorSS genes (Fig. 2). The only exceptions were *Bacteroides helcogenes* and *Odoribacter splanchnicus*, which lacked *gldB*, and *Alistipes shahii*, *R. marinus*, and *S. ruber*, which lacked *gldB* and *gldH*. Disruption of either *gldB* or *gldH* of *F. johnsoniae* eliminates gliding motility, but the cells do not exhibit growth defects, so these genes are not essential. However, their presence in nearly all members suggests that they may perform some important function, and it is possible that the motility defects are a secondary effect of loss of this function. Disruption of the *P. gingivalis gldB* ortholog had no effect on secretion of gingipains (K. Sato and K. Nakayama, unpublished data), so a central role in the PorSS is unlikely. GldB and GldH are essential for *F. johnsoniae* gliding, but their exact roles in *F. johnsoniae*, and the functions of the orthologs in nongliding members of the phylum, remain unknown.

The phylum *Bacteroidetes* is large, and its members are diverse. This study reveals the high prevalence of gliding motility and PorSS (T9SS) genes among members of the phylum, and it necessitates modification of the descriptions of several genera and species that were previously described as nongliding. Some of the bacteria described here are pathogens of animals (33, 47) or humans (64, 65), and motility and/or secretion may be important in interaction with their respective hosts. Many members of the phylum use novel enzymes to digest recalcitrant polysaccharides such as cellulose, hemicelluloses, chitin, or algal polysaccharides (17, 41, 66–68). The *F. johnsoniae* PorSS secretes a chitinase that is required for chitin digestion. It is likely that other polysaccharide-digesting enzymes produced by *F. johnsoniae* and by other members of the phylum are secreted by the same route. Proteins required for bacteroidete gliding motility and protein secretion have been identified, but we are just beginning to ask questions regarding the mechanisms underlying these processes. With many genetic tools now available, the answers to these questions are within reach.

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