

## Mutations in *Flavobacterium johnsoniae* *gldF* and *gldG* Disrupt Gliding Motility and Interfere with Membrane Localization of GldA

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*Flavobacterium johnsoniae* moves rapidly over surfaces by a process known as gliding motility. The mechanism of this form of motility is not known. Four genes that are required for *F. johnsoniae* gliding motility, *gldA*, *gldB*, *gldD*, and *ftsX*, have recently been described. GldA is similar to the ATP-hydrolyzing components of ATP binding cassette (ABC) transporters. Tn4351 mutagenesis was used to identify two additional genes, *gldF* and *gldG*, that are required for cell movement. *gldF* and *gldG* appear to constitute an operon, and a Tn4351 insertion in *gldF* was polar on *gldG*. pMK314, which carries the entire *gldFG* region, restored motility to each of the *gldF* and *gldG* mutants. pMK321, which expresses GldG but not GldF, restored motility to each of the *gldG* mutants but did not complement the *gldF* mutant. GldF has six putative membrane-spanning segments and is similar in sequence to channel-forming components of ABC transporters. GldG is similar to putative accessory proteins of ABC transporters. It has two apparent membrane-spanning helices, one near the amino terminus and one near the carboxy terminus, and a large intervening loop that is predicted to reside in the periplasm. GldF and GldG are involved in membrane localization of GldA, suggesting that GldA, GldF, and GldG may interact to form a transporter. *F. johnsoniae* *gldA* is not closely linked to *gldFG*, but the *gldA*, *gldF*, and *gldG* homologs of the distantly related gliding bacterium *Cytophaga hutchinsonii* are arranged in what appears to be an operon. The exact roles of *F. johnsoniae* GldA, GldF, and GldG in gliding are not known. Sequence similarities of GldA to components of other ABC transporters suggest that the Gld transporter may be involved in export of some material to the periplasm, outer membrane, or beyond.

Gliding motility, the movement of cells over surfaces without the aid of flagella, is a common form of movement exhibited by diverse bacteria. Bacterial gliding motility was first observed nearly 200 years ago (54). Since then a number of mechanisms have been proposed to explain this form of cell propulsion (6, 28, 35, 50). Recently it has become clear that different types of “motors” are used by different groups of gliding bacteria (28). *Mycoplasma* gliding is thought to rely on the cell cytoskeleton (53), whereas gliding of filamentous cyanobacteria may be powered by polysaccharide secretion (18). *Myxococcus xanthus* has two independent motility systems. “S” or social gliding motility requires type IV pili and is probably related to the twitching motility of *Pseudomonas aeruginosa* and other bacteria (23, 45, 55, 58). Extension and retraction of pili appear to be responsible for this type of cell movement (30, 52). The other form of motility employed by *M. xanthus* is “A” or adventurous gliding motility (16, 50). The mechanism of *M. xanthus* A motility is not known, but it does not appear to involve pili (50).

Numerous bacteria which belong to the *Cytophaga-Flavobacterium-Bacteroides* (CFB) branch of the eubacterial phylogenetic tree exhibit rapid gliding motility (4, 42). This form of gliding does not appear to involve pili (15, 42). Lapidus and Berg proposed that cells of *Cytophaga* U67 have a system of

tracks anchored to the peptidoglycan. In their model, outer membrane components are driven along these tracks by periplasmic and cell membrane proteins that obtain energy from the proton motive force (PMF) (27). Other models to explain gliding of members of the CFB group include contraction or expansion of fibrils in the periplasm or cytoplasm (5), the functioning of rotary motors (36), the generation of waves in the outer membrane (8), or the movement of “conveyor belts” made of polysaccharide or protein along the cell surface (28).

*Flavobacterium johnsoniae* (formerly *Cytophaga johnsonae*) (4) is a common gliding bacterium that belongs to the CFB group. Cells of *F. johnsoniae* glide at rates of 2 to 10  $\mu\text{m/s}$  (35). Cells also occasionally attach to a surface by one pole and rotate in place at frequencies of about 2 revolutions per s. Techniques to genetically manipulate *F. johnsoniae* were recently developed (29) and have been used to identify four genes, *gldA*, *gldB*, *gldD*, and *ftsX*, that are required for gliding motility (2, 21, 22, 24).

GldA exhibits sequence similarity to ATP binding cassette (ABC) transport proteins (2). ABC transporters are common in bacteria, archaea, and eukarya. They utilize ATP hydrolysis as a source of energy to transport substances across one or more membranes into or out of cells (9, 19, 60). Molecules that are transported by different ABC transporters include amino acids, peptides, proteins, polysaccharides, lipids, inorganic ions, and various drugs. Bacterial ABC transporters often consist of several subunits. GldA is similar to the ATP-hydrolyzing subunits. It does not have any obvious hydrophobic regions and probably requires the presence of integral membrane proteins to form a functional transporter. The genes that code for these membrane proteins do not appear to be closely linked to *gldA* (2). In this report, we describe the identification and charac-

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TABLE 1. Plasmids and primers used in this study<sup>a</sup>

Plasmid or primer	Description and primer sequence	Source or reference
<b>Plasmids</b>		
pBC SK(+)	ColE1; Cm <sup>r</sup>	Stratagene
pCR-Script Cam SK(+)	ColE1 <i>ori</i> ; Cm <sup>r</sup>	Stratagene
pSTBlue-1	ColE1 <i>ori</i> ; Ap <sup>r</sup>	Novagen
pT7Blue	ColE1 <i>ori</i> ; Ap <sup>r</sup>	Novagen
pSPORT1	ColE1 <i>ori</i> ; Ap <sup>r</sup>	Gibco BRL
pMAL-c2	MalE fusion protein expression vector; Ap <sup>r</sup>	New England Biolabs
pCP29	<i>E. coli-F. johnsoniae</i> shuttle plasmid; Ap <sup>r</sup> (Cf <sup>r</sup> Em <sup>r</sup> )	24
pSA21	<i>gldA</i> in <i>E. coli-F. johnsoniae</i> shuttle plasmid pCP23; Ap <sup>r</sup> (Tc <sup>r</sup> )	2
pMK312	3.1-kb <i>gldFG</i> PCR product cloned into the <i>SrfI</i> site of pCR-Script Cam SK(+); Cm <sup>r</sup>	This study
pMK313	3.1-kb <i>gldFG</i> fragment of pMK312 cloned as a <i>NotI-SalI</i> fragment into pSPORT1; Ap <sup>r</sup>	This study
pMK314	3.1-kb <i>gldFG</i> fragment of pMK313 cloned as a <i>KpnI-SphI</i> fragment into pCP29; Ap <sup>r</sup> (Cf <sup>r</sup> Em <sup>r</sup> )	This study
pMK315	1.3-kb <i>PstI</i> fragment of pMK312 containing <i>gldF</i> cloned into pCP29; Ap <sup>r</sup> (Cf <sup>r</sup> Em <sup>r</sup> )	This study
pMK316	1.3-kb <i>PstI</i> fragment of pMK312 containing <i>gldF</i> cloned into pCP29 (opposite orientation of pMK315); Ap <sup>r</sup> (Cf <sup>r</sup> Em <sup>r</sup> )	This study
pMK317	2.5-kb <i>EagI</i> fragment of pMK312 containing <i>gldG</i> , cloned into pBC SK(+); Cm <sup>r</sup>	This study
pMK321	2.6-kb fragment spanning <i>gldFG</i> , with an in-frame deletion of 540 bp in <i>gldF</i> , cloned into pCP29; Ap <sup>r</sup> (Cf <sup>r</sup> Em <sup>r</sup> )	This study
pMM274	2.5-kb fragment of pMK317 containing <i>gldG</i> cloned into pCP29; Ap <sup>r</sup> (Cf <sup>r</sup> Em <sup>r</sup> )	This study
pDH254	1.3-kb fragment containing 3' end of <i>gldG</i> in <i>EcoRV</i> site of pSTBlue-1; Ap <sup>r</sup>	This study
pDH289	<i>gldG</i> expression plasmid, 1.3-kb <i>HindIII-PstI</i> fragment of pDH254 in pMAL-c2; Ap <sup>r</sup>	This study
pDH264	923-bp fragment containing <i>gldA</i> in <i>EcoRV</i> site of pT7Blue; Ap <sup>r</sup>	This study
pDH265	<i>gldA</i> expression plasmid, 905-bp <i>EcoRI-XbaI</i> fragment of pDH264 in pMAL-c2; Ap <sup>r</sup>	This study
<b>Primers</b>		
340	5' GACTTGGATACCTCACGCC 3'; primer in IS 4351	
341	5' TTGGAAATTTCTGGGAGG 3'; primer in <i>ermF</i> of Tn 4351	
396	5' AAAATACGAAATGCAGACGG 3'; primer in <i>fjo15</i>	
405	5' AGTTTCAAGGTCTGAAGCCG 3'; primer in <i>dnaN</i>	
410	5' AGACGTTTACTGCAGGAAACCAAACA 3'; primer for amplification of 3' end of <i>gldG</i>	
411	5' TATTAACATAAGCTTACTGTACTTTCT 3'; primer for amplification of 3' end of <i>gldG</i>	
430	5' ACCGTGATTCAATATATTGTAGTCTCC 3'; primer for construction of in-frame deletion of <i>gldF</i>	
431	5' GGCGTTATTGATACCCGC 3'; primer for construction of in-frame deletion of <i>gldF</i>	
444	5' AAATAAAAAGAATTCTCGATAGAAGTA 3'; primer for amplification of <i>gldA</i>	
445	5' AACTGAGGCTCTAGATTATTAGTAAT 3'; primer for amplification of <i>gldA</i>	
491	5' CCGCGTTGTGCAATCAACC 3'; primer in <i>C. hutchinsonii gldG</i>	
493	5' TTGTGTCTATACATCTGCCGG 3'; primer in <i>C. hutchinsonii gldF</i>	

<sup>a</sup> Antibiotic resistance phenotypes: Ap<sup>r</sup>, ampicillin; Cf<sup>r</sup>, cefoxitin; Cm<sup>r</sup>, chloramphenicol; Em<sup>r</sup>, erythromycin; and Tc<sup>r</sup>, tetracycline. Unless indicated otherwise, antibiotic resistance phenotypes are those expressed in *E. coli*. Antibiotic resistance phenotypes listed in parentheses are those expressed in *F. johnsoniae* but not in *E. coli*.

terization of two genes, *gldF* and *gldG*, that are required for *F. johnsoniae* gliding motility. GldF and GldG are membrane proteins that appear to interact with GldA to form an ABC transporter that is required for gliding.

#### MATERIALS AND METHODS

**Bacterial and bacteriophage strains, plasmids, and growth conditions.** *F. johnsoniae* UW101 (ATCC 17061) was the wild-type strain used in these studies, and all mutants were derived from this strain. The 50 nonmotile mutants of *F. johnsoniae* (obtained from J. Pate) were previously described (7, 22, 57). The bacteriophages active against *F. johnsoniae* that were used in this study were  $\phi$ Cj1,  $\phi$ Cj7,  $\phi$ Cj13,  $\phi$ Cj23,  $\phi$ Cj29,  $\phi$ Cj42,  $\phi$ Cj48, and  $\phi$ Cj54 (7, 38, 57). The *Escherichia coli* strains used were DH5 $\alpha$ MCR (Gibco BRL Life Technologies), S17-1 (46), and BW19851 (31). *E. coli* strains were grown in Luria-Bertani medium at 37°C, and *F. johnsoniae* strains were grown in Casitone-yeast extract medium at 30°C, as previously described (29). To observe colony spreading, *F. johnsoniae* was grown on PY2 agar medium (2) at 25°C. Antibiotics were used at the following concentrations when needed: ampicillin, 100  $\mu$ g/ml; cefoxitin, 100  $\mu$ g/ml; chloramphenicol, 30  $\mu$ g/ml; erythromycin, 100  $\mu$ g/ml; and tetracycline, 20  $\mu$ g/ml. Plasmids and primers used in this study are listed in Table 1.

**Tn4351 mutagenesis of *F. johnsoniae* and identification of sites of insertion in**

***gldF* and *gldG*.** Tn4351 was introduced into wild-type *F. johnsoniae*, and nonmotile mutants were isolated as described previously (22). The sites of transposon insertion in the nonmotile mutants CJ776, CJ785, CJ787, and CJ788 were determined by cloning the Tn4351-disrupted genes essentially as previously described (24) and by inverse PCR (33). For inverse PCR, chromosomal DNA was digested with *HindIII* and ligated, resulting in the formation of closed circles. Tn4351-specific primers 340 and 341 were used to amplify the DNA adjacent to the site of the insertion using *Taq* polymerase.

**Nucleic acid sequencing.** Nucleic acid sequencing was performed by the dideoxy nucleotide procedure using an automated (Applied Biosystems) sequencing system. Sequences were analyzed with the MacVector and AssemblyLign software (Oxford Molecular Group Inc., Campbell, Calif.), and comparisons to database sequences were made using the BLAST (3) and FASTA (40) algorithms. Predictions regarding cellular localization and topology of membrane proteins were made using PSORT (<http://PSORT.NIBB.AC.JP> [32]) and TMpredict (17).

**Amplification and analysis of the *Cytophaga hutchinsonii gldF* and *gldG* genes.** Preliminary sequence data for *C. hutchinsonii* were obtained from The DOE Joint Genome Institute at <http://jgi.doe.gov/>. The region spanning the end of *gldF* and the beginning of *gldG* was amplified with primers 491 and 493 to allow completion of the sequence.

**Construction of plasmids for complementation of *gldF* and *gldG* mutants.** A 3.1-kb region of *F. johnsoniae* DNA which spans *gldFG* was amplified by PCR

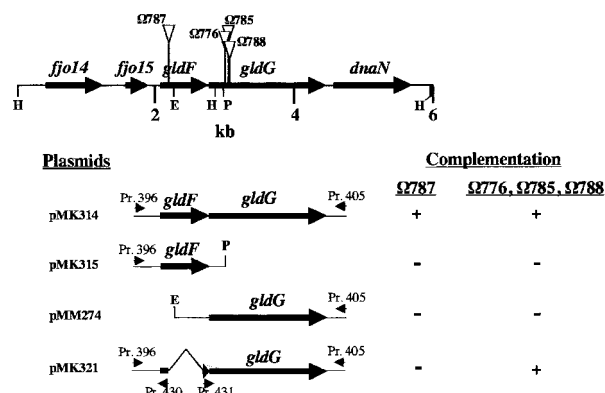


FIG. 1. Map of the *gldFG* region of *F. johnsoniae*. The sites of the Tn4351 insertions in CJ776, CJ785, CJ787, and CJ788 are indicated by triangles. Complementation of *gldF* and *gldG* mutants of *F. johnsoniae* by fragments cloned into pCP29 is indicated beneath the map. Primers (Pr.) used to amplify fragments for plasmid construction are shown. Restriction sites: E, *EagI*; H, *HindIII*; and P, *PstI*.

using *Elongase* (Gibco BRL Life Technologies) and primers 396 and 405. This PCR product was cloned into the *SrfI* site of pCR-Script Cam SK(+) to generate pMK312 and was transferred to pSPORT1 as a *NotI-SalI* fragment to form pMK313. pMK313 was digested with *KpnI* and *SphI*, and the fragment containing *gldFG* was introduced into pCP29 to generate pMK314 (Fig. 1). pMK315, which carries just *gldF*, was constructed by cloning the 1.3-kb *PstI* fragment from pMK312 into pCP29 (Fig. 1). pMK316 is identical to pMK315, except that the *gldF* fragment is inserted in pCP29 in the opposite orientation. pMK317, which contains *gldG* but not *gldF*, was constructed by transferring the 2.5-kb *EagI* fragment from pMK312 into pBC SK(+). The 2.5-kb fragment of pMK317 was cloned as a *NotI-BamHI* fragment into pSPORT1 and was then transferred as a *KpnI-SphI* fragment into pCP29 to generate pMM274 (Fig. 1).

pMK321, which contains a 540-bp in-frame deletion within the 723-bp coding region of *gldF* (Fig. 1), was constructed to allow production of GldG in the absence of functional GldF. pMK313 was digested with *PvuII* to release a 3.1-kb *F. johnsoniae* fragment containing *gldF* and *gldG* flanked by vector sequences. The *gldFG* fragment was treated with T4 DNA ligase to form closed circles, and the ends of *gldF* and the entire *gldG* gene were amplified using primers 430 and 431 and Vent polymerase. The resulting PCR product was incubated with T4 DNA ligase to form closed circles, was digested with *KpnI* and *SphI*, which cut in the polycloning sites flanking *gldFG*, and was introduced into pCP29 to generate pMK321.

For complementation analyses, plasmids were introduced into the *F. johnsoniae* mutants by conjugation or electroporation as previously described (22, 29).

**Microscopic observations of cell movement.** Wild-type and mutant cells of *F. johnsoniae* were examined for movement over glass and agar surfaces and for their ability to propel polystyrene latex spheres by phase-contrast microscopy as previously described (21, 22).

**Measurements of phage sensitivity.** Sensitivity to *F. johnsoniae* phage was determined essentially as previously described by spotting 2.5 to 10  $\mu$ l of phage lysates ( $2 \times 10^7$  PFU/ml) onto lawns of cells in overlay agar (22). The plate contents were incubated for 24 h at 25°C to observe lysis.

**Protein expression and antibody production.** A 923-bp fragment coding for GldA was amplified by PCR using Vent polymerase (New England Biolabs) with primers 444 and 445. The amplified fragment was cloned into pT7Blue (Novagen) to produce pDH264. The *gldA* fragment was isolated as an *EcoRI-XbaI* fragment and ligated into pMAL-c2 to produce pDH265. A fragment which codes for the C-terminal 439 amino acids of GldG was amplified as described above, using primers 410 and 411, and cloned into pSTBlue-1 (Novagen) to generate pDH254. The *gldG* fragment was isolated from pDH254 as a *HindIII-PstI* fragment and was ligated into pMAL-c2 to produce pDH289. Both fusion constructs were verified by DNA sequencing. Expression of fusion proteins was induced in *E. coli* DH5 $\alpha$ MCR by the addition of 0.3 mM isopropyl- $\beta$ -D-thiogalactopyranoside. Fusion proteins were purified by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and antibodies were generated as previously described (22). Anti-GldA antiserum was affinity purified essentially

as previously described (22). Anti-GldG antiserum was purified by selective removal of antibodies that reacted with antigens in extracts of the *gldG* mutant CJ776. Cells of CJ776 were disrupted with a French press, and the cell extract was boiled for 5 min. The extract was applied to a polyvinylidene difluoride membrane and was incubated for 16 h at 4°C. The membrane was blocked with 5% skim milk in Tris-buffered saline (20 mM Tris hydrochloride [pH 7.5] and 0.15 M NaCl) for 4 h and washed with Tris-buffered saline, and crude antiserum was added and incubated for 16 h at 4°C.

**Cell fractionation and Western blot analyses.** *F. johnsoniae* cells were disrupted with a French press and fractionated into soluble and membrane fractions as described (22) except that 0.6 mM phenylmethylsulfonyl fluoride was added to cell extracts. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and Western blot analyses were performed as previously described (22), except that GldG was separated on gels containing 10% acrylamide, and antigens were detected using goat anti-rabbit immunoglobulin G-horseradish peroxidase conjugate and Opti-4CN (Bio-Rad, Hercules, Calif.).

**Genetic nomenclature.** Genes required for gliding motility were given the name *gld* followed by a letter. Open reading frames (ORFs) that exhibited strong sequence similarity to genes of known function were named after the corresponding genes. *F. johnsoniae* ORFs of unknown function that did not exhibit strong similarity to previously described genes were given the provisional name *fjo* (for *F. johnsoniae* ORF) followed by a number. *C. hutchinsonii* ORFs of unknown function that did not exhibit similarity to previously described genes were named *cho* followed by a number.

**Nucleotide sequence accession number.** The sequence reported in this paper has been deposited in the GenBank database under accession no. AY036615.

## RESULTS

**Tn4351 mutagenesis of *F. johnsoniae*.** *F. johnsoniae* was mutagenized with Tn4351, and 29 nonmotile mutants were isolated. Eleven of these had defects in cell division and were not considered further in this study. Complementation analysis revealed that five of the remaining mutants had Tn4351 insertions in previously identified gliding motility genes (one in *gldA* and four in *gldB*). Southern blot analyses revealed that five other mutants had multiple Tn4351 insertions. The remaining eight mutants were analyzed further.

**Identification of *gldF* and *gldG*.** To identify the regions disrupted by Tn4351 in each of the mutants, the DNA flanking the sites of insertion was cloned or amplified as described in Materials and Methods. Sequence analysis revealed that four of the eight nonmotile mutants contained insertions in a 1-kb region of the genome. A 5.9-kb fragment of DNA spanning this region was sequenced. Analysis of this sequence identified five ORFs (Fig. 1). CJ787 contained a Tn4351 insertion in *gldF*, and CJ776, CJ785, and CJ788 contained insertions in *gldG*. The stop codon of *gldF* and the start codon of *gldG* overlap by 1 nucleotide, which suggests that *gldF* and *gldG* are organized as an operon.

The predicted protein product of *gldF* is 241 amino acids long and has a molecular weight of 27 kDa. Hydrophilicity analyses suggest that GldF has six transmembrane domains. GldF exhibits sequence similarity to a hypothetical protein that we refer to as *C. hutchinsonii* GldF (46% identity over 241 amino acids). *F. johnsoniae* GldF also exhibits sequence similarity (27 to 36% amino acid identity) to several transmembrane channel-forming proteins of ABC transporters, including *Bradyrhizobium japonicum* NosY (27% identity over 208 amino acids) (GenBank accession no. AJ002531) and *Sinorhizobium meliloti* NosY (30% identity over 153 amino acids) (20).

GldG is 561 amino acids long and has a predicted molecular weight of 63.7 kDa. Hydrophilicity analyses suggest that GldG

has two transmembrane regions, one at the N terminus and one at the C terminus. The large hydrophilic region between these transmembrane domains is predicted to reside in the periplasm based on sequence analysis. GldG exhibits similarity to a protein that we refer to as *C. hutchinsonii* GldG (32% amino acid identity over 549 residues) and to several putative membrane-anchored accessory proteins of ABC transporters, such as *Borrelia burgdorferi* BB0752 (23% identity over 561 amino acids) (10) and *P. aeruginosa* PA3670 (23% identity over 259 amino acids) (51).

*fjo15* lies upstream of *gldF*. The stop codon of *fjo15* and the start codon of *gldF* are separated by 179 nucleotides. The predicted protein product of *fjo15* does not exhibit amino acid sequence similarity to any proteins of known function in the databases. An 18-nucleotide inverted repeat starts 3 nucleotides after the predicted *fjo15* stop codon (5' GTTTCAAGT TTCAGGTTT...//107 nucleotides//...AAACCTGAAACTT GAAAC 3'). In the 107 nucleotides between the inverted repeats, there are two copies of a 9-nucleotide direct repeat (5' CGCAAAGAA 3'). The functions of the direct and inverted repeats are not known. The transcriptional start site for *gldF* is likely to reside in the region between *fjo15* and *gldF*. The region of DNA between the inverted repeat and the *gldF* start codon is AT rich (83% AT over 33 nucleotides), as is often observed in promoter regions of other bacteria (44). *fjo14* lies upstream of *fjo15*. The predicted product of *fjo14* is not similar to any proteins of known function in the databases. *F. johnsoniae* *dnaN* is predicted to start 107 nucleotides downstream of the *gldG* stop codon. *F. johnsoniae* DnaN is 25% identical to *E. coli* DnaN (34) and 27% identical to *Vibrio cholerae* DnaN (14). DnaN is the  $\beta$  subunit of bacterial DNA polymerase III. There is no evidence indicating that *fjo14*, *fjo15*, or *dnaN* is involved in gliding motility.

**Complementation of Tn4351-induced *gldF* and *gldG* mutants of *F. johnsoniae*.** Introduction of pMK314, which carries *gldF* and *gldG*, into the *gldF* mutant CJ787 and into the *gldG* mutants CJ776, CJ785, and CJ788 resulted in complementation of each (Fig. 1 and 2C and G). The resulting colonies spread over agar, and cells exhibited rapid gliding motility. Plasmids containing just *gldF* (pMK315 and pMK316) failed to complement any of the mutants (Fig. 1 and 2D). Similarly, pMM274, which contains *gldG* and 527 bp of upstream DNA but lacks the promoter and translational start site of *gldF*, failed to complement any of the mutants (Fig. 1 and 2H). pMK321, which contains the putative *gldF* promoter region and *gldG* but has an in-frame deletion within *gldF*, was constructed to allow production of GldG in the absence of functional GldF. Introduction of pMK321 restored motility to each of the Tn4351-induced *gldG* mutants but failed to complement the *gldF* mutant CJ787 (Fig. 1 and 2E and I). pMK321 is a derivative of pCP1 and has a copy number of approximately 10 in *F. johnsoniae*. *gldG* mutants complemented with pMK321 did not spread as well as wild-type colonies. This may be due to an imbalance in the ratio of GldF to GldG in these strains. It is also possible that GldF and GldG have difficulty assembling to form a functional complex unless they are transcribed and translated together. The results of the complementation analyses described above indicate that GldF and GldG are both needed for gliding motility. They also support the idea that

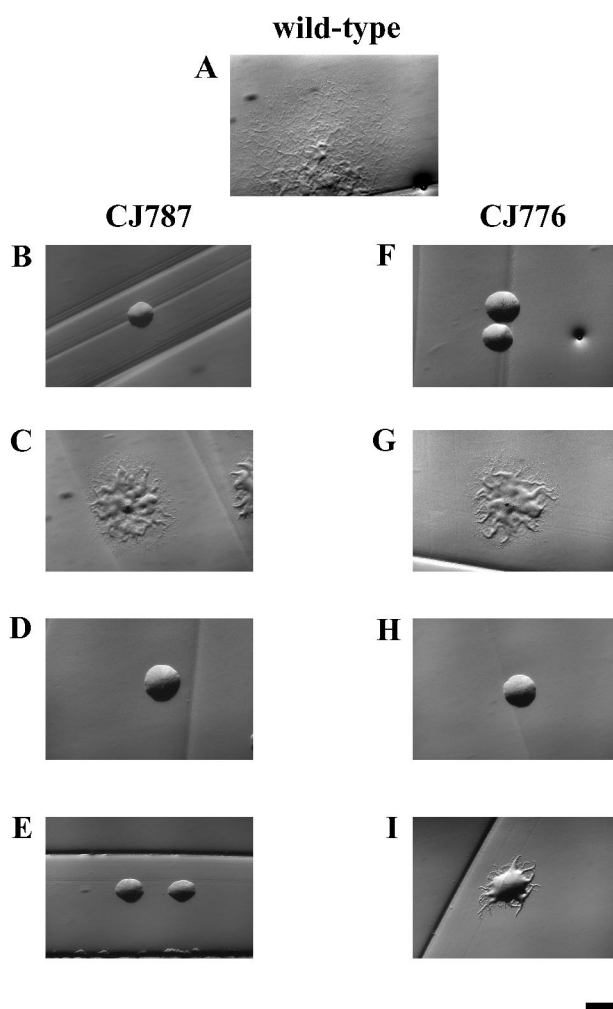


FIG. 2. Photomicrographs of *F. johnsoniae* colonies. Colonies were grown for 2 days at 25°C on PY2 agar media. Photomicrographs were taken with a Diagnostic Instruments RT color digital camera mounted on a Nikon Diaphot inverted phase-contrast microscope. Bar indicates 1 mm. (A) Wild-type *F. johnsoniae* UW101. (B) *gldF* mutant CJ787. (C) CJ787 complemented with pMK314, which carries *gldF* and *gldG*. (D) CJ787 with pMK315, which carries *gldF*. (E) CJ787 carrying pMK321, which expresses *gldG* but not *gldF*. (F) *gldG* mutant CJ776. (G) CJ776 complemented with pMK314, which carries *gldF* and *gldG*. (H) CJ776 with pMM274, which carries *gldG* but lacks the transcriptional start site of *gldF*. (I) CJ776 carrying pMK321, which expresses *gldG* but not *gldF*.

*gldF* and *gldG* are transcriptionally coupled, since the transposon insertion in *gldF* was polar on *gldG*.

**Identification of *gldF* and *gldG* point mutants.** Pate and colleagues isolated a large number of spontaneous and chemically induced nonmotile mutants (7, 57). We introduced pMK314 into 50 of these mutants to determine if any has defects in *gldF* or *gldG*. pMK314 restored motility to 5 of the 50 mutants (UW102-25, UW102-34, UW102-39, UW102-75, and UW102-77). Colonies of the complemented mutants spread over agar, and cells exhibited wild-type motility in wet mounts. pMK321 (which expresses *gldG* but not *gldF*) restored motility to UW102-25, UW102-34, and UW102-75 but failed to complement the other two mutants. pMK315 (which express

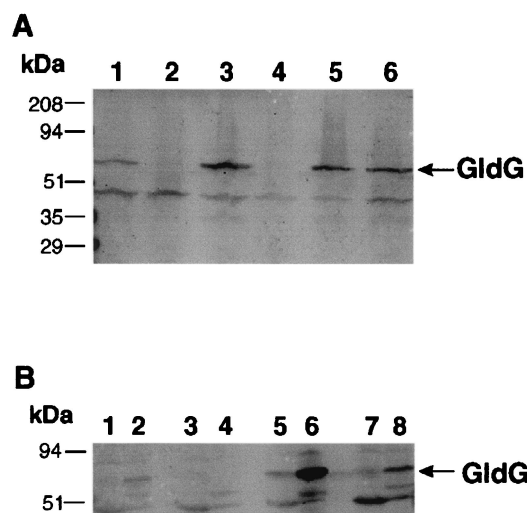


FIG. 3. Expression and localization of GldG. (A) Cell extracts were examined for GldG by Western blot analysis. Lane 1, wild-type cells; lane 2, CJ776 (Tn4351 insertion in *gldG*); lane 3, CJ776 with pMK314, which carries *gldFG*; lane 4, CJ787 (Tn4351 insertion in *gldF*); lane 5, CJ787 with pMK314, which carries *gldFG*; and lane 6, CJ787 with pMK321, which carries the *gldFG* region with an in-frame deletion in *gldF*. (B) Cell fractions were examined for GldG by Western blot analysis. Odd-numbered lanes contain soluble fractions, while even-numbered lanes contain membrane fractions. Lanes 1 and 2, wild-type cells; lanes 3 and 4, CJ787 (Tn4351 insertion in *gldF*); lanes 5 and 6, CJ787 with pMK321, which carries the *gldFG* region with an in-frame deletion in *gldF*; and lanes 7 and 8, CJ101-288 (insertion mutation in *gldA*). kDa, kilodaltons.

*gldF* but not *gldG*) failed to complement any of the mutants. These results suggest that UW102-25, UW102-34, and UW102-75 have mutations in *gldG* and that UW102-39 and UW102-77 have mutations in *gldF* that are polar on *gldG*. The exact sites of the mutations in each mutant were determined by amplification and sequencing of the *gldFG* region. Each mutation was the result of insertion or deletion of 1 or 2 bases. As expected, UW102-39 and UW102-77 had mutations in *gldF*, and UW102-25, UW102-34, and UW102-75 had mutations in *gldG*. UW102-39 had a T inserted at position 91 (numbered from the A of the *gldF* start codon), while UW102-77 had a deletion of nucleotides 17 and 18 (TA). UW102-25 had a G deleted at position 951 (numbered from the A of the *gldG* start codon), and UW102-34 and UW102-75 each had a single base deletion within a run of A's starting at position 162.

**Expression and localization of GldG.** Antisera were used to detect GldG in extracts of wild-type and mutant cells by Western blot analyses (Fig. 3A). GldG was detected in wild-type cells and migrated with an apparent molecular weight of approximately 62 kDa (Fig. 3A, lane 1) but was absent from the *gldG* mutant CJ776 (Fig. 3A, lane 2). Introduction of pMK314, which carries *gldF* and *gldG*, into CJ776 restored production of GldG (lane 3). Expression of GldG in this strain was higher than seen in wild-type cells, as expected, since pMK314 has a copy number of approximately 10 in *F. johnsoniae*. The *gldF* mutant CJ787 failed to produce GldG protein (Fig. 3A, lane 4), indicating that the transposon insertion in *gldF* was polar on *gldG*. Strains with frameshift mutations in *gldF* (UW102-39

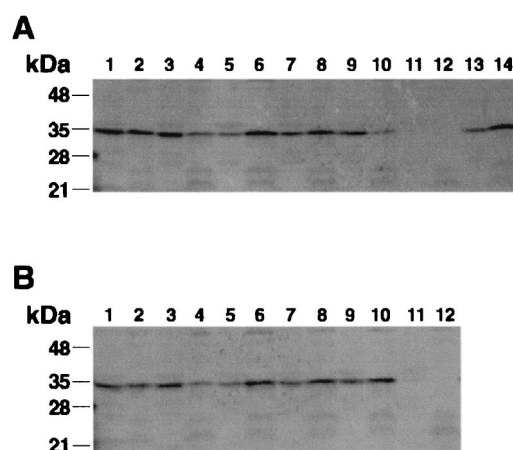


FIG. 4. Localization of GldA in wild-type cells and in *gldF* and *gldG* mutants. Cell fractions were examined for GldA by Western blot analysis. Odd-numbered lanes contain soluble fractions, while even-numbered lanes contain membrane fractions. An equal amount, based on total cell protein measurements prior to fractionation, was loaded in each lane. (A) Lanes 1 and 2, wild-type cells; lanes 3 and 4, CJ787 (Tn4351 insertion in *gldF*); lanes 5 and 6, CJ787 with pMK314, which carries *gldFG*; lanes 7 and 8, CJ787 with pMK315, which carries *gldF*; lanes 9 and 10, CJ787 with pMK321, which carries the *gldFG* region with an in-frame deletion in *gldF*; lanes 11 and 12, CJ288 (*gldA* mutant); and lanes 13 and 14, wild-type cells with pMK314. (B) Lanes 1 and 2, wild-type cells; lanes 3 and 4, CJ776 (Tn4351 insertion in *gldG*); lanes 5 and 6, CJ776 with pMK314; lanes 7 and 8, CJ776 with pMK315; lanes 9 and 10, CJ776 with pMK321; and lanes 11 and 12, CJ288 (*gldA* mutant).

and UW102-77) also failed to produce GldG (data not shown). Introduction of pMK314 into CJ787 restored production of GldG protein (Fig. 3A, lane 5). Introduction of pMK321, which carries *gldF* with an in-frame deletion within *gldF*, resulted in production of GldG in the absence of GldF (Fig. 3A, lane 6). GldG was found primarily in membrane fractions of wild-type cells (Fig. 3B, lanes 1 and 2) or of cells which expressed GldG in the absence of GldF (Fig. 3B, lanes 5 and 6) or GldA (Fig. 3B lanes 7 and 8).

**GldF and GldG are involved in membrane localization of GldA.** Sequence analyses of GldA did not identify any putative transmembrane domains, but it was suggested that GldA is probably associated with membrane proteins to form an ABC transporter (2). Western blot analyses demonstrated that GldA is found in both the membrane and soluble fractions of wild-type cells (Fig. 4A and B, lanes 1 and 2). In contrast, GldA was found primarily in the soluble fraction of extracts of the *gldF* mutant CJ787 (Fig. 4A, lanes 3 and 4) or the *gldG* mutant CJ776 (Fig. 4B, lanes 3 and 4), suggesting that GldA may interact with GldF or GldG. Introduction of pMK314, which expresses *gldF* and *gldG*, into cells of the wild type, *gldF* mutant CJ787, or *gldG* mutant CJ776 resulted in most of the GldA being sequestered to the membrane fraction (Fig. 4A, lanes 5, 6, 13, and 14, and B, lanes 5 and 6). Introduction of pMK315 (which expresses *gldF*) to CJ787 or CJ776 resulted in partial localization of GldA to the membrane fraction (Fig. 4A and B, lanes 7 and 8). Introduction of pMK321 (which expresses GldG) restored membrane localization of GldA to the *gldG* mutant CJ776, (Fig. 4B, lanes 9 and 10) but not to the *gldF*

mutant CJ787 (Fig. 4A, lanes 9 and 10). Apparently GldF and GldG participate either directly or indirectly in localization of GldA to the membrane.

**Phage resistance of mutants.** It has previously been reported that many nonmotile mutants of *F. johnsoniae* are resistant to infection by a number of *F. johnsoniae* bacteriophages (56). The reason for this pleiotropy is not known. We tested the sensitivity of *F. johnsoniae* strains UW101, CJ787, CJ776, CJ880 (CJ787 carrying pMK314), and CJ878 (CJ776 carrying pMK314) to the *F. johnsoniae* bacteriophages  $\phi$ Cj1,  $\phi$ Cj7,  $\phi$ Cj13,  $\phi$ Cj23,  $\phi$ Cj29,  $\phi$ Cj42,  $\phi$ Cj48, and  $\phi$ Cj54. *F. johnsoniae* UW101 was readily lysed by these phages, whereas CJ787 and CJ776 were resistant to infection by each of them. Introduction of pMK314 into CJ787 and CJ776 restored sensitivity to each of these phages in addition to restoring gliding motility.

***gldF* and *gldG* mutants fail to move latex spheres.** Wild-type cells of *F. johnsoniae* and related bacteria bind latex spheres and propel them along their surfaces (27, 36). We examined the ability of cells of the *F. johnsoniae* strains UW101, CJ787, CJ776, CJ880 (CJ787 carrying pMK314), and CJ878 (CJ776 carrying pMK314) to bind and propel latex spheres. Wild-type cells propelled the spheres, whereas cells of the *gldF* or *gldG* mutants CJ787 and CJ776 did not. Introduction of pMK314 into CJ787 and CJ776 restored the ability to propel spheres, in addition to restoring gliding motility.

***C. hutchinsonii gldF* and *gldG* are adjacent to *gldA*.** As noted above, *F. johnsoniae gldF* and *gldG* have homologs in the *C. hutchinsonii* genome. We used PCR amplification and DNA sequencing to determine the arrangement of the *C. hutchinsonii* genes. *C. hutchinsonii gldF* and *gldG* appear to be arranged in an operon with *gldA* and one additional gene. The order of the *C. hutchinsonii* genes is *gldA*, *gldF*, *gldG*, and *cho1*. The protein product of *C. hutchinsonii gldA* exhibits 49% identity with *F. johnsoniae GldA* over 297 amino acids. *Cho1*, which is predicted to be a periplasmic or outer membrane protein, exhibits no significant similarity to protein sequences in the databases.

## DISCUSSION

The mechanism responsible for *F. johnsoniae* gliding motility is not known. Previously, we identified four genes, *gldA*, *gldB*, *gldD*, and *ftsX*, that are required for gliding (2, 21, 22, 24). *gldA* codes for a protein that is similar in sequence to the ATP-hydrolyzing components of ABC transporter complexes. GldB and GldD are anchored in the cytoplasmic membrane. The exact functions of these proteins in gliding motility are not known. *FtsX* is needed for both cell division and gliding motility. The inability of *ftsX* mutants to glide may be an indirect effect of the defects in cell division. The results presented in this paper identify two additional genes, *gldF* and *gldG*, that are required for gliding. Transposon insertions in *gldF* or *gldG* eliminated gliding motility, and introduction of a wild-type copy of *gldFG* on a plasmid restored motility to the mutants.

*gldF* and *gldG* appear to be organized in an operon, and both genes are required for motility. Insertional inactivation of *gldF* by Tn4351 was polar on *gldG*. The *gldF* mutant failed to produce detectable levels of GldG protein, and complementation of gliding required the introduction of both *gldF* and *gldG*. Expression of either gene alone did not restore motility. The

polarity of the Tn4351 insertion in *gldF* on *gldG* was unexpected. Tn4351 has outward-reading promoters at each end (47). These promoters appear to function in *F. johnsoniae*, since insertion of Tn4351 in either orientation in the first gene of the *gldBC* operon did not eliminate expression of the downstream gene (22). The polarity of the Tn4351 insertion in *gldF* may be the result of disruption of translational coupling. The overlapping stop and start codons of *gldF* and *gldG*, and lack of an obvious ribosome binding site upstream of *gldG* are consistent with this explanation.

*F. johnsoniae GldF* is predicted to be a cytoplasmic membrane protein with six membrane-spanning domains. It exhibits amino acid sequence similarity to proteins that are thought to be cytoplasmic membrane channel-forming components of ABC transporters. *F. johnsoniae GldF* is most similar to the putative *C. hutchinsonii GldF*. It also exhibits similarity to *S. meliloti* NosY, which is involved in maturation of periplasmic nitrous oxide reductase NosZ (20). NosY is thought to form an ABC transporter with NosF (the ATP binding protein of the transporter) and NosD (a periplasmic protein which is thought to interact with NosZ) (20, 61). Mutations in *nosD*, *nosF*, or *nosY* prevent assembly of the Cu center of the reductase. It is not known whether these proteins function in Cu import or export or in some other process required for NosZ maturation.

*F. johnsoniae GldG* is a cytoplasmic membrane protein that exhibits similarity to proteins that are thought to be accessory components of ABC transporters. The functions of these transporters are not known. GldG has two predicted transmembrane domains, one near its amino terminus and the other near its carboxy terminus, and a large hydrophilic loop which is predicted to reside in the periplasm. Some polysaccharide exporters of the ABC-2 family employ membrane proteins, such as *E. coli KpsE*, that have similar topology and may perform similar roles (39, 60).

ABC transporters typically have two ATP binding domains located in the cytoplasm. Neither GldF or GldG has an obvious ATP binding domain. GldA, another protein that is required for gliding, is similar to ATP binding components of ABC transporters. GldA was predicted to be a component of an ABC transporter, but the membrane-spanning components of this transporter were not identified. The results reported in this paper suggest that GldF and GldG are the membrane proteins that interact with GldA to form an ABC transporter. *F. johnsoniae GldA* was found in the membrane and soluble fractions of wild-type cells. In contrast, GldA was found primarily in the soluble fraction in extracts of cells with mutations in *gldF* or *gldG*. Introduction of *gldFG* on a plasmid into the mutants restored membrane localization to GldA. Increased expression of *gldF* and *gldG* (approximately 10-fold) resulted in increased localization of GldA to the membrane fraction. Both GldF and GldG were needed to obtain maximal sequestration of GldA in the membrane fraction. This suggests that GldA interacts with GldF and GldG to form an ABC transporter that is required for gliding motility.

The genes encoding components of ABC transporters are usually arranged in operons, but *F. johnsoniae gldA* is not closely linked to *gldFG*. However, the homologous genes from *C. hutchinsonii* appear to be organized as an operon. *F. johnsoniae GldA*, GldF, and GldG exhibit 49, 46, and 32% amino acid identity, respectively, with their *C. hutchinsonii*

homologs. *C. hutchinsonii* exhibits rapid gliding motility and is distantly related to *F. johnsoniae*. Although it has not been demonstrated, it is likely that the *C. hutchinsonii* genes are involved in gliding motility. *C. hutchinsonii* *gldA*, *gldF*, and *gldG* may be cotranscribed with one additional gene, *cho1*. *cho1*, which lies downstream of *gldG*, is predicted to encode a periplasmic or outer membrane protein. Cho1 may be an additional component of the ABC transporter and may be required for gliding motility. (Cho1 is not likely to be the cargo of the transporter, since it appears to have a cleavable signal peptide and is probably transported by the general secretory machinery.) We do not know whether *F. johnsoniae* has a homolog to Cho1 or if additional proteins are part of the *F. johnsoniae* Gld transporter.

GldA exhibits sequence similarity to a large number of ATP-hydrolyzing components of ABC transporters (2). Homologs of known function include *Bacillus subtilis* SpaF (40% identity over 220 amino acids), *Bacillus licheniformis* BcrA (33% identity over 295 amino acids), *S. meliloti* NodI (33% identity over 299 amino acids), *S. meliloti* NosF (38% identity over 216 amino acids), and *M. xanthus* PilH (38% identity over 213 amino acids), among others. SpaF (25) and BcrA (41) are involved in resistance to the peptide antibiotics subtilin and bacitracin, respectively, presumably by exporting them. NodI is thought to be involved in lipochitin oligosaccharide export (48, 49). NosF is thought to interact with NosD and NosY (GldF homolog) to form a transporter that is involved in maturation of the periplasmic nitrous oxide reductase NosZ (20, 61). *M. xanthus* PilH is the ATP binding component of a transporter involved in pilus biogenesis and social gliding motility (59). Pili have not been observed in *F. johnsoniae* and related gliding bacteria (15, 42; M. J. McBride, unpublished data), and genes similar to those required for pilus assembly and function (other than *pilH*) have not been identified. While it appears unlikely that pili are responsible for *F. johnsoniae* motility, GldA and *M. xanthus* PilH may perform analogous roles in the assembly, modification, or functioning of their respective motility machineries.

It is not known whether GldA, GldF, and GldG function in import or export, but the similarities listed above suggest that GldA may be involved in export of some material to the periplasm, cell surface, or beyond. Several cell surface molecules have been implicated in *F. johnsoniae* gliding. Sulfonolipids are found in the outer membrane of *F. johnsoniae*, and several mutants that fail to make sulfonolipids fail to glide (1, 11, 12). Sulfonolipids may be directly involved in gliding or may provide an environment that allows proper assembly and functioning of the motility apparatus. Cell surface polysaccharides and glycoproteins have also been implicated in gliding (11, 13, 35, 37). GldA, GldF, and GldG may be involved in export of sulfonolipids or polysaccharides or in formation of cell surface glycoproteins. Alternatively they might be involved in export of protein components of the gliding machinery to the periplasm or outer membrane. The *gld* ABC transporter may be needed for assembly or modification of the motility apparatus, as suggested above, or may have a direct role in gliding by importing or exporting conveyor belts that run along the cell surface. It is unlikely that ATP hydrolysis by GldA is the sole driving force for cell movement, however, because others have demonstrated that PMF is required for gliding of *F. johnsoniae* and

related bacteria (36, 43). Gliding could require the coordinated activity of two transporters with different energy requirements or a single transporter which requires both ATP and PMF. The HlyA hemolysin exporter of *E. coli* is an example of an ABC transporter that may require both ATP hydrolysis and PMF for transport (26, 60).

Gliding motility is thought to be the result of movement of cell surface components. The movement of these components can be observed by adding latex spheres which bind to cells and are propelled along their surfaces. Spheres move at approximately the same speed as gliding cells, and metabolic poisons and uncouplers that reduce PMF inhibit motility and block sphere movement (36). Nonmotile mutants fail to propel spheres (2, 7, 21, 22, 24). Our results confirm and extend this finding. Cells of *gldF* or *gldG* mutants failed to propel latex spheres, but complementation of the motility defect by introduction of *gldFG* on a plasmid restored this ability. These results support the suggestion that the machinery that propels latex spheres along the cell surface is also responsible for cell movement.

Many nonmotile mutants of *F. johnsoniae* are resistant to bacteriophage that infect wild-type cells (56). In addition, chemicals that dissipate PMF block gliding motility of wild-type cells (36) and also block adsorption of phage (56). Cells with mutations in *gldF* or *gldG* were resistant to bacteriophage infection. Restoration of motility by introduction of *gldFG* on a plasmid restored sensitivity to bacteriophage. These results are similar to those obtained for strains with mutations in *gldA*, *gldB*, *gldD*, or *ftsX* (2, 21, 22, 24) and indicate that mutations that disrupt gliding motility generally result in resistance to diverse *F. johnsoniae* bacteriophages. Bacteriophage usually attach to specific receptors on the cell surface, such as outer membrane proteins, polysaccharides, lipopolysaccharides, pili, or flagella. The receptors for *F. johnsoniae* bacteriophages have not been identified. If the Gld ABC transporter has an outer membrane component, it could provide a phage attachment site and the transporter could function as a conduit for entry of phage nucleic acid into the cytoplasm. Alternatively, the transporter may be needed to assemble or modify cell surface components which function as phage receptors and as components of the motility apparatus. Finally, phage resistance may be a nonspecific result of the defect in motility. In the absence of cell surface movement, phage receptors may be inaccessible.

Pate and colleagues isolated a bank of 50 independent spontaneous or chemically induced mutants that are completely deficient in gliding motility (7, 22, 57). Complementation and sequence analyses indicate that four of these mutants are nonmotile because of mutations in *gldA* (2), four fail to glide because of mutations in *gldB* (22), three others have mutations in *gldD* (21), and five are nonmotile because of mutations in *gldF* or *gldG* (this study). Complementation of 16 of 50 nonmotile mutants by just five genes suggests that a relatively small number of genes, perhaps less than 20, are required specifically for gliding motility.

The mechanism of *F. johnsoniae* gliding motility remains a mystery. Genetic analyses have uncovered six genes (*gldA*, *gldB*, *gldD*, *gldF*, *gldG*, and *ftsX*) that are required for *F. johnsoniae* gliding motility. The results presented in this paper suggest that the products of *gldA*, *gldF*, and *gldG* form a com-

plex that functions as an ATP-dependent transporter that is required for cell movement. Further analysis of GldA, GldF, and GldG should elucidate the role of this transporter in *F. johnsoniae* gliding motility.

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#### REFERENCES

- Abbanat, D. R., E. R. Leadbetter, W. Godchaux III, and A. Escher. 1986. Sulphonolipids are molecular determinants of gliding motility. *Nature* **324**:367–369.
- Agarwal, S., D. W. Hunnicutt, and M. J. McBride. 1997. Cloning and characterization of the *Flavobacterium johnsoniae* (*Cytophaga johnsonae*) gliding motility gene, *gldA*. *Proc. Natl. Acad. Sci. USA* **94**:12139–12144.
- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**:403–410.
- Bernardet, J.-F., P. Segers, M. Vancannet, F. Berthe, K. Kersters, and P. Vandamme. 1996. Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (basionym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int. J. Syst. Bacteriol.* **46**:128–148.
- Burchard, R. P. 1984. Gliding motility and taxes, p. 301. In E. Rosenberg (ed.), *Myxobacteria, development and cell interactions*. Springer-Verlag, New York, N.Y.
- Burchard, R. P. 1981. Gliding motility of prokaryotes: ultrastructure, physiology, and genetics. *Annu. Rev. Microbiol.* **35**:497–529.
- Chang, L. Y. E., J. L. Pate, and R. J. Betzig. 1984. Isolation and characterization of nonspreading mutants of the gliding bacterium *Cytophaga johnsonae*. *J. Bacteriol.* **159**:26–35.
- Duxbury, T., B. A. Humphrey, and K. C. Marshall. 1980. Continuous observations of bacterial gliding motility in a dialysis microchamber: the effects of inhibitors. *Arch. Microbiol.* **124**:169–175.
- Fath, M. J., and R. Kolter. 1993. ABC transporters: bacterial exporters. *Microbiol. Rev.* **57**:995–1017.
- Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Lathigra, O. White, K. A. Ketchum, R. Dodson, E. K. Hickey, M. Gwinn, B. Dougherty, J. F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M. D. Adams, J. Gocayne, and J. C. Venter. 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* **390**:580–586.
- Godchaux, W., III, L. Gorski, and E. R. Leadbetter. 1990. Outer membrane polysaccharide deficiency in two nongliding mutants of *Cytophaga johnsonae*. *J. Bacteriol.* **172**:1250–1255.
- Godchaux, W., III, and E. R. Leadbetter. 1988. Sulfonolipids are localized in the outer membrane of the gliding bacterium *Cytophaga johnsonae*. *Arch. Microbiol.* **150**:42–47.
- Godchaux, W., III, M. A. Lynes, and E. R. Leadbetter. 1991. Defects in gliding motility in mutants of *Cytophaga johnsonae* lacking a high-molecular-weight cell surface polysaccharide. *J. Bacteriol.* **173**:7607–7614.
- Heidelberg, J. F., J. A. Eisen, W. C. Nelson, R. A. Clayton, M. L. Gwinn, R. J. Dodson, D. H. Haft, E. K. Hickey, J. D. Peterson, L. A. Umayam, S. R. Gill, K. E. Nelson, T. D. Read, H. Tettelin, D. Richardson, M. D. Ermolaeva, J. Vamathevan, S. Bass, H. Qin, I. Dragoi, P. Sellers, L. McDonald, T. Utterback, R. D. Fleischmann, W. C. Nierman, O. White, S. L. Salzberg, H. O. Smith, R. R. Colwell, J. J. Mekalanos, J. C. Venter, and C. M. Fraser. 2000. DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature* **406**:477–483.
- Henrichsen, J., and J. Blom. 1975. Examination of fimbriation of some Gram-negative rods with and without twitching and gliding motility. *Acta Pathol. Microbiol. Scand. Sect. B* **83**:161–170.
- Hodgkin, J., and D. Kaiser. 1979. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacteriales): two gene systems control movement. *Mol. Gen. Genet.* **171**:177–191.
- Hofmann, K., and W. Stoffel. 1993. TMbase—a database of membrane spanning proteins segments. *Biol. Chem. Hoppe-Seyler* **374**:166.
- Hoicyk, E., and W. Baumeister. 1998. The junctional pore complex, a prokaryotic secretion organelle, is the molecular motor underlying gliding motility in cyanobacteria. *Curr. Biol.* **8**:1161–1168.
- Holland, I. B., and M. A. Blight. 1999. ABC-ATPases, adaptable energy generators fuelling transmembrane movement of a variety of molecules in organisms from bacteria to humans. *J. Mol. Biol.* **293**:381–399.
- Holloway, P., W. McCormick, R. J. Watson, and Y. K. Chan. 1996. Identification and analysis of the dissimilatory nitrous oxide reduction genes, *nosRZDFY*, of *Rhizobium meliloti*. *J. Bacteriol.* **178**:1505–1514.
- Hunnicutt, D. W., and M. J. McBride. 2001. Cloning and characterization of the *Flavobacterium johnsoniae* gliding motility genes *gldD* and *gldE*. *J. Bacteriol.* **183**:4167–4175.
- Hunnicutt, D. W., and M. J. McBride. 2000. Cloning and characterization of the *Flavobacterium johnsoniae* gliding motility genes, *gldB* and *gldC*. *J. Bacteriol.* **182**:911–918.
- Kaiser, D. 1979. Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. *Proc. Natl. Acad. Sci. USA* **76**:5952–5956.
- Kempf, M. J., and M. J. McBride. 2000. Transposon insertions in the *Flavobacterium johnsoniae* *ftsX* gene disrupt gliding motility and cell division. *J. Bacteriol.* **182**:1671–1679.
- Klein, C., and K. D. Entian. 1994. Genes involved in self-protection against the antibiotic subtilin produced by *Bacillus subtilis* ATCC 6633. *Appl. Environ. Microbiol.* **60**:2793–2801.
- Koronakis, V., C. Hughes, and E. Koronakis. 1991. Energetically distinct early and late stages of HlyB/HlyD-dependent secretion across both *Escherichia coli* membranes. *EMBO J.* **10**:3263–3272.
- Lapidus, I. R., and H. C. Berg. 1982. Gliding motility of *Cytophaga* sp. strain U67. *J. Bacteriol.* **151**:384–398.
- McBride, M. J. 2001. Bacterial gliding motility: multiple mechanisms for cell movement over surfaces. *Annu. Rev. Microbiol.* **55**:49–75.
- McBride, M. J., and M. J. Kempf. 1996. Development of techniques for the genetic manipulation of the gliding bacterium *Cytophaga johnsonae*. *J. Bacteriol.* **178**:583–590.
- Merz, A. J., M. So, and M. P. Sheetz. 2000. Pilus retraction powers bacterial twitching motility. *Nature* **407**:98–102.
- Metcalf, W. W., W. Jiang, L. L. Daniels, S.-K. Kim, A. Haldimann, and B. L. Wanner. 1996. Conditionally replicative and conjugative plasmids carrying *lacZα* for cloning, mutagenesis, and allele replacement in bacteria. *Plasmid* **35**:1–13.
- Nakai, K., and M. Kanehisa. 1991. Expert system for predicting protein localization sites in gram-negative bacteria. *Proteins* **11**:95–110.
- Ochman, H., A. S. Gerber, and D. L. Hartl. 1988. Genetic applications of an inverse polymerase chain reaction. *Genetics* **120**:621–623.
- Ohmori, H., M. Kimura, T. Nagata, and Y. Sakakibara. 1984. Structural analysis of the *dnaA* and *dnaN* genes of *Escherichia coli*. *Gene* **28**:159–170.
- Pate, J. L. 1988. Gliding motility. *Can. J. Microbiol.* **34**:459–465.
- Pate, J. L., and L.-Y. E. Chang. 1979. Evidence that gliding motility in prokaryotic cells is driven by rotary assemblies in the cell envelopes. *Curr. Microbiol.* **2**:59–64.
- Pate, J. L., and D. M. De Jong. 1990. Use of nonmotile mutants to identify a set of membrane proteins related to gliding motility in *Cytophaga johnsonae*. *J. Bacteriol.* **172**:3117–3124.
- Pate, J. L., S. J. Petzold, and L.-Y. E. Chang. 1979. Phages for the gliding bacterium *Cytophaga johnsonae* that infect only motile cells. *Curr. Microbiol.* **2**:257–262.
- Pazzani, C., C. Rosenow, G. J. Boulnois, D. Bronner, K. Jann, and I. S. Roberts. 1993. Molecular analysis of region 1 of the *Escherichia coli* K5 antigen gene cluster: a region encoding proteins involved in cell surface expression of capsular polysaccharide. *J. Bacteriol.* **175**:5978–5983.
- Pearson, W. R. 1990. Rapid and sensitive sequence comparison with FASTP and FASTA. *Methods Enzymol.* **183**:63–98.
- Podlesek, Z., A. Comino, B. Herzog-Velikonja, D. Zgur-Bertok, R. Komel, and M. Grabnar. 1995. *Bacillus licheniformis* bacitracin-resistance ABC transporter: relationship to mammalian multidrug resistance. *Mol. Microbiol.* **16**:969–976.
- Reichenbach, H. 1992. The order Cytophagales, p. 3631–3675. In A. Balows, H. G. Truper, M. Dworkin, W. Harder, and K. M. Schleifer (ed.), *The prokaryotes*. Springer-Verlag, New York, N.Y.
- Ridgway, H. F. 1977. Source of energy for gliding motility in *Flexibacter polymorphus*: effects of metabolic and respiratory inhibitors on gliding movement. *J. Bacteriol.* **131**:544–556.
- Ross, W., K. K. Gosink, J. Salomon, K. Igarashi, C. Zou, A. Ishahama, K. Severinov, and R. L. Gourse. 1993. A third recognition element in bacterial promoters: DNA binding by the  $\alpha$  subunit of RNA polymerase. *Science* **262**:1407–1413.
- Semmler, A. B. T., C. B. Whitchurch, and J. S. Mattick. 1999. A re-examination of twitching motility in *Pseudomonas aeruginosa*. *Microbiology* **145**:2863–2873.
- Simon, R., U. Priefer, and A. Puhler. 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in Gram negative bacteria. *Bio/Technology* **2**:784–791.
- Smith, C. J., M. B. Rogers, and M. L. McKee. 1992. Heterologous gene expression in *Bacteroides fragilis*. *Plasmid* **27**:141–154.
- Spaink, H. P. 2000. Root nodulation and infection factors produced by rhizobial bacteria. *Annu. Rev. Microbiol.* **54**:257–288.



49. **Spaink, H. P., A. H. M. Wijffjes, and B. J. J. Lugtenberg.** 1995. *Rhizobium* NodI and NodJ proteins play a role in the efficiency of secretion of lipochitin oligosaccharides. *J. Bacteriol.* **177**:6276–6281.
50. **Spormann, A. M.** 1999. Gliding motility in bacteria: insights from studies of *Myxococcus xanthus*. *Microbiol. Mol. Biol. Rev.* **63**:621–641.
51. **Stover, C. K., X. Q. Pham, A. L. Erwin, S. D. Mizoguchi, P. Warrenner, M. J. Hickey, F. S. L. Brinkman, W. O. Hufnagle, D. J. Kowalik, M. Lagrou, R. L. Garber, L. Goltry, E. Tolentino, S. Westbrook-Wadman, Y. Yuan, L. L. Brody, S. N. Coulter, K. R. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G. K.-S. Wong, Z. Wu, I. T. Paulsen, J. Reizer, M. H. Saier, R. E. W. Hancock, S. Lory, and M. V. Olson.** 2000. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* **406**:959–964.
52. **Sun, H., D. R. Zusman, and W. Shi.** 2000. Type IV pilus of *Myxococcus xanthus* is a motility apparatus controlled by the *frz* chemosensory system. *Curr. Biol.* **10**:1143–1146.
53. **Trachtenberg, S.** 1998. Mollicutes: wall-less bacteria with internal cytoskeletons. *J. Struct. Biol.* **124**:244–256.
54. **Vaucher, J. P.** 1803. Histoire des conferves d'eau douce, contenant leurs differens modes de reproduction, et la description de leurs principales especes, suivie de l'histoire des tremelles et des ulves d'eau douce. J. J. Paschoud, Geneva, Switzerland.
55. **Wall, D., and D. Kaiser.** 1999. Type IV pili and cell motility. *Mol. Microbiol.* **32**:1–10.
56. **Wolkin, R. H., and J. L. Pate.** 1986. Phage adsorption and cell adherence are motility-dependent characteristics of the gliding bacterium *Cytophaga johnsonae*. *J. Gen. Microbiol.* **132**:355–367.
57. **Wolkin, R. H., and J. L. Pate.** 1985. Selection for nonadherent or nonhydrophobic mutants co-selects for nonspreading mutants of *Cytophaga johnsonae* and other gliding bacteria. *J. Gen. Microbiol.* **131**:737–750.
58. **Wu, S. S., and D. Kaiser.** 1995. Genetic and functional evidence that type IV pili are required for social gliding motility in *Myxococcus xanthus*. *Mol. Microbiol.* **18**:547–558.
59. **Wu, S. S., J. Wu, Y. L. Cheng, and D. Kaiser.** 1998. The *pilH* gene encodes an ABC transporter homologue required for type IV pilus biogenesis and social gliding motility in *Myxococcus xanthus*. *Mol. Microbiol.* **29**:1249–1261.
60. **Young, J., and I. B. Holland.** 1999. ABC transporters: bacterial exporters—revisited five years on. *Biochim. Biophys. Acta* **1461**:177–200.
61. **Zumft, W. G.** 1997. Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.* **61**:533–616.