Flavobacterium johnsoniae Gliding Motility Genes Identified by *mariner* Mutagenesis

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Cells of *Flavobacterium johnsoniae* glide rapidly over surfaces. The mechanism of *F. johnsoniae* gliding motility is not known. Eight *gld* genes required for gliding motility have been described. Disruption of any of these genes results in complete loss of gliding motility, deficiency in chitin utilization, and resistance to bacteriophages that infect wild-type cells. Two modified *mariner* transposons, *HimarEm1* and *HimarEm2*, were constructed to allow the identification of additional motility genes. *HimarEm1* and *HimarEm2* each transposed in *F. johnsoniae*, and nonmotile mutants were identified and analyzed. Four novel motility genes, *gldK*, *gldL*, *gldM*, and *gldN*, were identified. *GldK* is similar in sequence to the lipoprotein *GldJ*, which is required for gliding. *GldL*, *GldM*, and *GldN* are not similar in sequence to proteins of known function. Cells with mutations in *gldK*, *gldL*, *gldM*, and *gldN* were defective in motility and chitin utilization and were resistant to bacteriophages that infect wild-type cells. Introduction of *gldA*, *gldB*, *gldD*, *gldFG*, *gldH*, *gldI*, and *gldJ* and the region spanning *gldK*, *gldL*, *gldM*, and *gldN* individually into 50 spontaneous and chemically induced nonmotile mutants restored motility to each of them, suggesting that few additional *F. johnsoniae* *gld* genes remain to be identified.

Cells of *Flavobacterium johnsoniae* move rapidly over surfaces in a process called gliding motility. Gliding motility is common in the phylum Bacteroidetes (also known as the *Cytophaga-Flavobacterium-Bacteroides* group), of which *F. johnsoniae* is a member. Cells of *F. johnsoniae* move at speeds of up to 10 $\mu\text{m/s}$, and colonies have thin spreading edges. Cells of *F. johnsoniae* also adsorb latex spheres and propel these rapidly around the cell in multiple paths. Several models have been proposed to explain gliding motility of *F. johnsoniae* and related bacteria, but the mechanism of cell movement remains unknown (22, 25, 26).

Development of genetic techniques for *F. johnsoniae* led to the identification of genes required for motility (29). *gldA*, *gldF*, and *gldG* are thought to encode components of an ATP-binding cassette (ABC) transporter that is required for motility (1, 15). Genes encoding lipoproteins that are required for gliding (*GldB*, *GldD*, *GldH*, *GldI*, and *GldJ*) have also been identified (5, 16, 17, 27, 28). Cells with mutations in any of these genes are completely nonmotile. They do not exhibit movement on agar or glass surfaces, fail to propel latex spheres, and form nonspreading colonies. These mutants are deficient in utilization of the insoluble polysaccharide chitin and are resistant to infection by bacteriophages that infect wild-type cells. Some of the *Gld* proteins are thought to function as transporters, and it has been suggested that gliding motility, chitin utilization, and sensitivity to bacteriophages may each rely on these transporters. The lipoprotein *GldJ* forms helical bands in the cell envelope, but the organization of the other *Gld* proteins is not known (5).

Tn4351 mutagenesis was used to identify many of the *gld*

genes described above. A drawback of Tn4351 mutagenesis is that it is not completely random. Nineteen of 37 independent Tn4351-induced nonmotile mutants carried insertions in *gldB* (reference 17 and unpublished data). Multiple insertions were also obtained in *gldA* (five insertions), *gldD* (three insertions), *gldF* (two insertions), *gldG* (four insertions), and *gldH* (four insertions), but insertions in *gldI* and *gldJ* were not isolated (references 1, 15, 16, and 28 and unpublished data). *gldI* and *gldJ* were identified by cosmid complementation of nonmotile point mutants (5, 27). Complementation analyses using a bank of 50 spontaneous and chemically induced nonmotile mutants suggested that several additional *gld* genes remained to be identified. Introduction of *gldA*, *gldB*, *gldD*, *gldFG*, *gldH*, *gldI*, and *gldJ* individually into the 50 mutants restored motility to 33 of them (5). Repeated attempts to identify additional *gld* genes by cosmid complementation of the remaining 17 mutants were not successful. The nonrandomness of Tn4351 insertion and the difficulties associated with cosmid complementation of some nonmotile mutants suggested that additional tools were needed to identify the remaining *gld* genes. This paper describes the development of *mariner*-based transposons, *HimarEm1* and *HimarEm2*, that function in *F. johnsoniae* and the use of these transposons to identify four additional motility genes.

MATERIALS AND METHODS

Bacterial strains and bacteriophages, plasmids, and growth conditions. *F. johnsoniae* strains UW101, MM101, and FJ1, which are each direct descendants of the *F. johnsoniae* type strain ATCC 17061 but have diverged slightly through handling in the laboratory, were the wild-type strains used in this study. MM101 differs from UW101 and FJ1 in that it has a partial defect in chitin utilization (27). *F. johnsoniae* strains MM101 and FJ1 were used for *HimarEm* mutagenesis. Mutants derived from MM101 were named “CJ” followed by a number, and mutants derived from FJ1 were given the designation “FJ” followed by a number. *HimarEm* insertions in MM101 were referred to as “HMM” followed by the mutant strain number, and insertions in FJ1 were designated “HFJ” followed by

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the mutant strain number. The 50 spontaneous and chemically induced motile-nonspreading mutants of *F. johnsoniae* UW101 were previously described (7, 17, 52) and were designated "UW102-" followed by a number. The bacteriophages active against *F. johnsoniae* that were used in this study were ϕ Cj1, ϕ Cj13, ϕ Cj23, ϕ Cj28, ϕ Cj29, ϕ Cj42, ϕ Cj48, and ϕ Cj54 (7, 35, 52). The *Escherichia coli* strains used were EC100D *pir*⁺ (Epicenter), Top10 (Invitrogen), S17-1 (42), and S17-1 λ *pir* (48). *E. coli* strains were grown in Luria-Bertani medium at 37°C, and *F. johnsoniae* strains were grown in Casitone-yeast extract (CYE) medium at 30°C, as previously described (29). Growth rates were determined by monitoring optical density at 600 nm of duplicate cultures. To observe colony spreading, *F. johnsoniae* was grown on PY2 agar medium (1) at 25°C. Chitin utilization was detected as previously described except that strains derived from *F. johnsoniae* FJ1 were incubated for 3 days whereas strains derived from *F. johnsoniae* MM101 were incubated for 12 days because of the partial deficiency in chitin utilization (27, 28). Antibiotics were used at the indicated concentrations when needed: ampicillin, 100 μ g/ml; cefoxitin, 100 μ g/ml; chloramphenicol, 30 μ g/ml; erythromycin, 100 μ g/ml; kanamycin, 30 μ g/ml; tetracycline, 20 μ g/ml. Plasmids and primers used in this study are listed in Table 1.

Construction of pHimarEm1 and pHimarEm2. The 2.4-kbp EcoRI-EcoNI fragment of pLYL03 spanning the *ermF* gene was made blunt using end conversion mix (Novagen) and inserted into the SalI site of pMiniHimarRB1, which had also been treated with end conversion mix. The resulting plasmids, pHimarEm1 and pHimarEm2, are identical except that they have the *ermF* gene inserted in opposite orientations (Fig. 1).

HimarEm mutagenesis of *F. johnsoniae* and identification of sites of insertion. pHimarEm1 and pHimarEm2 were introduced into wild-type *F. johnsoniae* by conjugation from *E. coli* S17-1 λ *pir* essentially as previously described (17). HimarEm mutants were selected by plating cells on PY2 agar containing erythromycin. Mutants that formed nonspreading colonies and were defective in gliding motility as determined by microscopic analysis (28) were chosen for further study. Chromosomal DNA was isolated from mutants and digested with XbaI or NsiI. The resulting fragments were diluted and ligated to form closed circles and introduced into *E. coli* EC100D *pir*⁺ by electroporation. Cells were plated on LB medium containing kanamycin to select clones carrying HimarEm1 or HimarEm2 and adjacent chromosomal DNA. Plasmids were sequenced using primer 609 and primer 615, which hybridize near inverted repeats 1 and 2, respectively. Nucleic acid sequencing was performed by the University of Chicago Cancer Research Center DNA Sequencing Facility. Sequences were analyzed with MacVector and AssemblyLign software (Accelrys, San Diego, CA), and comparisons to database sequences were made using the BLAST (2) and FASTA (36) algorithms. Predictions regarding cellular localization were made using PSORT (32) and TMPredict (14), and potential coiled-coil structures were identified using COILS (24) and Multicoil (49).

Cloning of the region spanning *gldK* through *gldO*. The region of DNA spanning *gldK* through *gldO* was assembled from two plasmids, pMM306 and pMU5, which carry HimarEm2 insertions HMM1300 and HMM1304, respectively (Fig. 2 and Table 1). The 3.0-kbp EcoRV-EcoRI fragment of pMU5 spanning *gldK* and *gldL* was inserted into pAED4, which had been digested with EcoRV and EcoRI, to create pTB46. The 3.8-kbp EcoRI fragment of pMM306 spanning *gldM*, *gldN*, and *gldO* was inserted into the EcoRI site of pTB46 to generate pTB74. To facilitate transfer of the region spanning *gldK* through *gldO* into the shuttle vector pCP29, the 2.1-kbp BamHI fragment of pHP450kan, which confers resistance to kanamycin, was inserted into the BamHI site within the poly-cloning region of pTB74 to generate pTB84. The 9.0-kb SalI fragment of pTB84 which includes the kanamycin resistance cassette and spans *gldK*, *gldL*, *gldM*, *gldN*, and *gldO* was inserted into the SalI site of the shuttle vector pCP29 to generate pTB90 (Fig. 2 and Table 1). pTB98, (which carries *gldL*, *gldM*, *gldN*, and *gldO*) was constructed by deleting the 798-bp NsiI fragment from pTB90, resulting in an in-frame deletion within *gldK*.

For complementation analyses, a series of plasmids were constructed that carried portions of the *gldK* through *gldO* region (Fig. 2 and Table 1). The following restriction fragments were used: the 3.0-kbp EcoRV-EcoRI fragment spanning *gldK* and *gldL* (pTB88), the 1.3-kbp PstI fragment spanning *gldL* (pTB81a), the 2.7-kbp HindIII fragment spanning *gldM* and the first 781 bp of *gldN* (pTB94a), the 1.8-kbp PstI fragment spanning *gldN* (pTB79), and the 3.7-kbp XbaI fragment of pTB90 spanning *gldO* and the kanamycin resistance cassette (pTB97a). To generate pTB99, which carries just *gldK*, an 1,868-bp fragment was amplified using primers 685 and 696 and ligated into the SmaI site of pCP23. Plasmids were introduced into the *F. johnsoniae* mutants by conjugation or electroporation as previously described (17, 29).

RNA analysis. Probes for Northern blot analyses were made using the DIG RNA labeling kit (Roche Diagnostics Corp.). Internal fragments of *gldK*, *gldL*, *gldM*, and *gldN* were amplified using the primer pairs 690/617, 616/691, 692/693,

and 694/695, respectively. Products were gel purified and ligated into pBCSK⁺. The ligation products were used as templates in a second amplification using the T7 primer and primers 690, 616, 692, and 694 for the *gldK*, *gldL*, *gldM*, and *gldN* products, respectively. The products were used for in vitro transcription to produce digoxigenin-labeled probes. Total RNA for Northern blots was isolated from overnight cultures of *F. johnsoniae* using RNeasy and RNA Protect bacterial reagent (QIAGEN). Northern blotting was performed essentially as described previously (40).

RNA for identification of the *gldK* and *gldL* transcription start sites was isolated from overnight cultures of *F. johnsoniae* using RiboPure-Bacteria (Ambion). The transcription start sites were identified by 5' rapid amplification of cDNA ends (RACE) using the BD SMART RACE cDNA amplification kit (BD Biosciences). The *gldK* specific nested primers 611, 617, and 699 were used to amplify and sequence the 5' end of the *gldK* transcript region, and the nested primers 693, 682, and 691, which hybridize within *gldM* and *gldL*, were used to amplify and sequence the 5' end of the *gldLMN* transcript region.

Western blot analysis. *F. johnsoniae* cells were grown to late log phase, pelleted, and suspended in 100 μ l 10 mM Tris, pH 7.5. Two microliters was used to determine protein concentration with the bicinchoninic acid reagent (Pierce). Ninety-eight microliters of 2 $\times$ sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer was added to the remainder, and samples were boiled for 3 min and sonicated briefly. Proteins (2 μ g) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and Western blot analyses were performed as previously described (17), except that Opti-4CN (Bio-Rad) was used for detection.

Measurements of bacteriophage sensitivity. Sensitivity to *F. johnsoniae* bacteriophages was determined essentially as previously described by spotting 3 μ l of phage lysates (10⁹ PFU/ml) onto lawns of cells in CYE overlay agar (17). The plates were incubated for 24 h at 25°C to observe lysis.

Nucleotide sequence accession number. The sequence reported in this paper has been deposited in the GenBank database (accession no. AY882986).

RESULTS

HimarEm mutagenesis and identification of *gldK*, *gldL*, *gldM*, and *gldN*. pHimarEm1 and pHimarEm2 were constructed to facilitate identification of novel *F. johnsoniae* motility genes. *F. johnsoniae* was mutagenized with HimarEm1 and HimarEm2, and 140 mutants that formed nonspreading colonies were isolated. Twenty of the mutants were completely deficient in gliding motility as determined by microscopic analysis of individual cells in wet mounts. Five of these exhibited filamentous morphology and were not considered further in this study. The sites of insertion in the remaining 15 nonmotile mutants were identified by cloning HimarEm and adjacent *F. johnsoniae* chromosomal DNA as plasmids in *E. coli* and determining the DNA sequences. FJ109 had an insertion in *gldI*, and FJ123 and FJ129 had insertions in *gldI*. *gldI* and *gldJ* have previously been described (5, 27). Eleven insertions mapped to a 5-kb region of DNA spanning four genes which we designated *gldK*, *gldL*, *gldM*, and *gldN* (Fig. 2). Cells with insertions in any of these genes formed nonspreading colonies (Fig. 3F, I, K, and M). Cells of the *gldK* mutants (CJ1309, CJ1361, CJ1372, CJ1373, FJ131, and FJ150), *gldL* mutants (CJ1253 and CJ1300), and *gldM* mutant (FJ113) were completely deficient in gliding motility. Unlike wild-type cells, the mutant cells failed to move in wet mounts and failed to propel polystyrene latex spheres over their surfaces. Cells of the *gldN* mutants (CJ1304 and CJ1382) were almost completely nonmotile, but a small percentage of cells (less than 1%) exhibited slight movements when examined in wet mounts.

Analysis of the *gld* genes and proteins. The *gldK* coding region is 1,395 nucleotides in length. A sequence that matches a *Bacteroides* consensus promoter region (TAXXTTGT) (3) ends 97 bp upstream of the *gldK* start codon. The amino-

TABLE 1. Plasmids and primers used in this study^a

Plasmid or primer	Description and sequence	Reference or source
Plasmids		
pAED4	pUC; Ap ^r	11
pBCSK ⁺	ColE1; Cm ^r	Stratagene
pHP45Ωkan	Plasmid carrying Km ^r cassette with transcriptional terminators at each end; Ap ^r Km ^r	13
pLYL03	Plasmid carrying <i>ermF</i> gene; Ap ^r (Em ^r)	23
pCP23	<i>E. coli-F. johnsoniae</i> shuttle plasmid; Ap ^r (Tc ^r)	1
pCP29	<i>E. coli-F. johnsoniae</i> shuttle plasmid; Ap ^r (Cf ^r Em ^r)	18
pMiniHimarRB1	Plasmid carrying mini- <i>himarRB1</i> ; Km ^r	4
pHimarEm1	Plasmid carrying <i>HimarEm1</i> ; Km ^r (Em ^r)	This study
pHimarEm2	Plasmid carrying <i>HimarEm2</i> ; Km ^r (Em ^r)	This study
pMM306	Plasmid carrying <i>HimarEm1</i> insertion HMM1300 in <i>gldL</i> and adjacent chromosomal DNA to NsiI site; Km ^r (Em ^r)	This study
pMU5	Plasmid carrying <i>HimarEm2</i> insertion HMM1304 in <i>gldN</i> and adjacent chromosomal DNA to XbaI sites; Km ^r (Em ^r)	This study
pTB46	3.0-kbp EcoRV-EcoRI fragment spanning <i>gldK</i> and <i>gldL</i> in pAED4; Ap ^r	This study
pTB74	6.8-kbp region spanning <i>gldK</i> , <i>gldL</i> , <i>gldM</i> , <i>gldN</i> , and <i>gldO</i> in pAED4; Ap ^r	This study
pTB79	1.8-kbp PstI fragment of pTB74 spanning <i>gldN</i> in pCP23; Ap ^r (Tc ^r)	This study
pTB81a	1.3-kbp PstI fragment of pTB74 spanning <i>gldL</i> in pCP23. <i>gldL</i> expressed from the pCP23 <i>orfI</i> promoter; Ap ^r (Tc ^r)	This study
pTB84	pTB74 with kanamycin resistance cassette from pHP45Ωkan inserted in the BamHI site in the polycloning region; Ap ^r Km ^r	This study
pTB88	3.1-kbp SalI fragment of pTB46 spanning <i>gldK</i> and <i>gldL</i> in pCP29; Ap ^r (Cf ^r Em ^r)	This study
pTB90	9.0-kbp SalI fragment of pTB84 spanning <i>gldK</i> , <i>gldL</i> , <i>gldM</i> , <i>gldN</i> , and <i>gldO</i> inserted in the SalI site of pCP29; Ap ^r Km ^r (Cf ^r Em ^r)	This study
pTB91	2.7-kbp HindIII fragment of pTB74 spanning <i>gldM</i> and the first 781 bp of <i>gldN</i> in pAED4; Ap ^r	This study
pTB94a	2.86-kbp XbaI fragment of pTB91 spanning <i>gldM</i> and the first 781 bp of <i>gldN</i> in pCP23. <i>gldM</i> expressed from the pCP23 <i>orfI</i> promoter; Ap ^r (Tc ^r)	This study
pTB97a	3.7-kbp XbaI fragment of pTB90 spanning <i>gldO</i> and the kanamycin resistance cassette in pCP23; Ap ^r Km ^r (Tc ^r)	This study
pTB98	<i>gldL</i> , <i>gldM</i> , <i>gldN</i> , and <i>gldO</i> in pCP29; Ap ^r Km ^r (Cf ^r Em ^r)	This study
pTB99	<i>gldK</i> in pCP23; Ap ^r (Tc ^r)	This study
Primers		
609	5' TGGGAATCATTGAAGGTTGG 3'; used for sequencing chromosomal DNA adjacent to IR1 of <i>HimarEm</i>	
611	5' TCTTGTACAGTTCTGAAACCAATG 3'; used for <i>gldK</i> 5'-RACE	
615	5' TCGGTATCGCTCTTGAAGGG 3'; used for sequencing chromosomal DNA adjacent to IR2 of <i>HimarEm</i>	
616	5' CAGTTGAGGATGAATTAGATTGG 3'; used to construct <i>gldL</i> probe for Northern blot analyses	
617	5' TGTTTCCAAGTCACACCTACTACTG 3'; used to construct <i>gldK</i> probe for Northern blot analyses and for <i>gldK</i> 5'-RACE	
682	5' TGTAACCGTCTCCTGTAAACC 3'; primer within <i>gldM</i> used for <i>gldLMN</i> 5'-RACE	
685	5' TCCGCTGGAAGAAGAGGAAC 3'; used to construct pTB99	
690	5' GATGAGTATTATGCTGAAGTAATGG 3'; used to construct <i>gldK</i> probe for Northern blot analyses	
691	5' GAAATTGCTTTTGAGCTCC 3'; used to construct <i>gldL</i> probe for Northern blot analyses and to sequence the <i>gldLMN</i> 5'-RACE product	
692	5' AAGGATTTCCTGCGATCGC 3'; used to construct <i>gldM</i> probe for Northern blot analyses	
693	5' GTTCTCCTACTCCACCGGC 3'; used to construct <i>gldM</i> probe for Northern blot analyses and for <i>gldLMN</i> 5'-RACE	
694	5' CAATGTATTTTCTGTAGATACAGC 3'; used to construct <i>gldN</i> probe for Northern blot analyses	
695	5' TTAAGATATACTCAGCAGGAACCG 3'; used to construct <i>gldN</i> probe for Northern blot analyses	
696	5' AGTTATTATTATTTCTCCGCTCC 3'; used to construct pTB99	
699	5' TACCACTCTACAAATTGACGG 3'; used to sequence <i>gldK</i> 5'-RACE product	

^a Antibiotic resistance phenotypes: ampicillin, Ap^r; cefoxitin, Cf^r; chloramphenicol, Cm^r; erythromycin, Em^r; kanamycin, Km^r; tetracycline, Tc^r. 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*.

terminal sequence of GldK (MKKFIAFAAMLTLVIGCGKS GDKGE) contains a hydrophobic stretch terminated by a cysteine (underlined), which is characteristic of lipoproteins. GldK is similar to a protein of unknown function from the

distantly related gliding bacterium *Cytophaga hutchinsonii* (GenBank accession no. ZP00309779). The *C. hutchinsonii* protein is 120 amino acids shorter than *F. johnsoniae* GldK but exhibits 50.1% identity with GldK over 305 amino acids if a

FIG. 1. Map of pHimarEm1. pHimarEm2 is identical to pHimarEm1 except that *ermF* is inserted in the opposite orientation.

large gap is allowed. *F. johnsoniae* GldK displays more limited similarity to proteins that exhibit sequence similarity to the domain of unknown function DUF323. These include *F. johnsoniae* GldJ (30% identity with GldK over 382 amino acids), human SUMF1 (31% identity over 195 amino acids), *Geobacillus stearothermophilus* XaiF (33% identity over 152 amino acids), and *Pectobacterium carotovorum* CarF (39% amino acid identity over 84 amino acids). GldJ is required for gliding motility (5); SUMF1 is an enzyme that activates sulfa-

tases by converting a cysteine residue into the active-site formylglycine (9, 10); XaiF is involved in the production, export, or activation of extracellular xylanase (8); and CarF is involved in resistance to the β -lactam antibiotic carbapenem (30). Given the diverse functions of these proteins, the exact function of GldK remains uncertain.

gldL, *gldM*, and *gldN* lie downstream of *gldK*. The predicted untranslated regions between *gldK* and *gldL*, *gldL* and *gldM*, and *gldM* and *gldN* are 84, 54, and 47 nucleotides, respectively. An inverted repeat (AAAAAACTCTTACTACCTTTGTAGT AAGAGTTTTTT) that may function as a transcription terminator begins 22 bp downstream of the *gldN* stop codon. GldL, GldM, and GldN do not exhibit significant amino acid sequence similarity to proteins of known functions. Amino acid sequence analyses suggest that GldM and GldN are likely to be periplasmic proteins. TMpredict analysis suggests that GldL has two membrane-spanning helices near its amino terminus and a domain of about 150 amino acids that may reside in the periplasm. The carboxy-terminal 60 amino acids are predicted to form an α -helical coiled-coil structure, which may indicate that this region is involved in multimerization or interaction with other proteins.

The *gldO* start codon begins 144 nucleotides after the putative *gldN* transcription terminator. Two potential promoter sequences (TACCTTTG and TAATTTTG) begin 166 and 76 bp upstream of the start codon, respectively. A potential transcription terminator (AAAAAACTCTTGCTACCTTTGCAG TAAGAGTTTTTT) lies 22 nucleotides downstream of the *gldO* stop codon. GldO exhibits 85% identity over 329 amino

FIG. 2. Map of the *gldKLMNO* region of *F. johnsoniae*. Restriction sites are indicated as follows: E, EcoRI; N, NsiI; V, EcoRV; X, XbaI. Numbers below the map refer to kilobase pairs of sequence. Fragments of DNA present in plasmids are indicated beneath the map. The sites of *HimarEm* insertions are indicated by inverted triangles.

FIG. 3. Photomicrographs of *F. johnsoniae* colonies. Colonies were incubated at 25°C on PY2 agar medium for 30 h except for those in panels C and D, which were incubated for 40 h, and those in panel I, which were incubated for 32 h. Photomicrographs were taken with a Kodak DC290 digital camera mounted on an Olympus IMT-2 inverted microscope. Bar, 1 mm. (A) Wild-type *F. johnsoniae* FJ1. (B) Wild-type *F. johnsoniae* MM101 with shuttle vector pCP29. (C) Wild-type *F. johnsoniae* MM101 with pTB90 which carries *gldK*, *gldL*, *gldM*, *gldN*, and *gldO*. (D) Wild-type *F. johnsoniae* MM101 with pTB88 which carries *gldK* and *gldL*. (E) Wild-type *F. johnsoniae* MM101 with pTB98 which carries *gldL*, *gldM*, *gldN*, and *gldO*. (F) *gldK* mutant CJ1372 with pCP29. (G) CJ1372 complemented with pTB90. (H) CJ1372 with pTB98. (I) *gldL* mutant CJ1300 with pCP29. (J) CJ1300 complemented with pTB98. (K) *gldM* mutant FJ113. (L) FJ113 complemented with pTB98. (M) *gldN* mutant CJ1304. (N) CJ1304 complemented with pTB98.

acids with GldN. *gldO* mutants have not been isolated, and it is not known whether *gldO* is required for motility. *fjo30* (which encodes a protein of unknown function) lies downstream of *gldO*, and *fjo29* (which encodes a putative arginase family protein) lies upstream of *gldK*. There is no evidence that these genes are involved in gliding.

The sequence analyses described above suggest that several of the *gld* genes may constitute an operon. Northern blot analyses were performed to determine the nature of the predominant transcripts in this region. Internal probes for *gldL*, *gldM*, and *gldN* each hybridized to a transcript of 3.4 kb, suggesting that these genes are cotranscribed (Fig. 4). We were unable to detect a *gldK* transcript by Northern blot analysis. *gldK* may have been expressed at a low level under the conditions employed in these experiments. The *gldK* transcript was detected by 5'-RACE analysis with a start site at the "G" 92 bases upstream of the *gldK* start codon. The transcription start site of the *gldLMN* operon was also identified by 5'-RACE analysis at the "U" 70 bases upstream of the *gldL* start codon.

***gldK*, *gldL*, *gldM*, and *gldN* are each required for efficient motility.** Complementation analyses revealed that *gldK*, *gldL*, *gldM*, and *gldN* were each required for efficient motility. Introduction of pTB90, which spans the entire region, resulted in complementation of mutations in each gene. Individual cells glided in wet mounts and propelled latex spheres over their

surfaces. The complemented strains also formed spreading colonies on agar, but colonies did not spread as well as those formed by wild-type cells (Fig. 3B and G). Introduction of pTB98, which spans the same region but has an in-frame deletion in *gldK*, restored motility to the *gldL*, *gldM*, and *gldN* mutants (Fig. 3J, L, and N) but failed to restore motility to the

FIG. 4. Northern blot analysis. Wild-type RNA was separated on an agarose gel, transferred to a nylon membrane, and probed with digoxigenin-labeled RNA internal to *gldK* (lane 1), *gldL* (lane 2), *gldM* (lane 3), or *gldN* (lane 4). Numbers correspond to the sizes in kilobases of RNA molecular size markers.

TABLE 2. Complementation of spontaneous and chemically induced *gld* mutants by plasmids^a

Strain	pTB90 (<i>gldKLMNO</i>)	pTB98 (<i>gldLMNO</i>)	pTB88 (<i>gldKL</i>)	pTB99 (<i>gldK</i>)	pTB81a (<i>gldL</i>)	pTB94a (<i>gldMN</i> ^b)	pTB79 (<i>gldN</i>)	Predicted site of mutation
UW102-57	— ^c	—	+	+	—	—	—	<i>gldK</i>
UW102-61	+	—	+	+	— ^c	—	—	<i>gldK</i>
UW102-68	+	—	+	+	—	—	—	<i>gldK</i>
UW102-94	+	—	+	+	—	—	—	<i>gldK</i>
UW102-98	+	—	+	+	—	—	—	<i>gldK</i>
UW102-108	— ^d	—	+	+	—	—	—	<i>gldK</i>
UW102-141	+	— ^d	+	+	— ^e	—	—	<i>gldK</i>
UW102-78	+	+	— ^c	—	— ^c	—	—	?
UW102-40	+	+	—	—	—	+	—	<i>gldM</i>
UW102-53	+	ND	—	ND	—	+	—	<i>gldM</i>
UW102-56	+	ND	—	ND	—	+	—	<i>gldM</i>
UW102-69	+	ND	—	ND	—	+	—	<i>gldM</i>
UW102-101	+	ND	—	ND	— ^c	+	—	<i>gldM</i>
UW102-140	+	ND	—	ND	—	+	—	<i>gldM</i>
UW102-300	+	ND	—	ND	—	+	— ^c	<i>gldM</i>
UW102-302	+	ND	— ^c	ND	—	+	—	<i>gldM</i>
UW102-33	+	ND	—	ND	—	+	+	<i>gldN</i>

^a +, complementation; —, lack of complementation; ND, not determined; ?, site of mutation uncertain.

^b pTB94 contains all of *gldM* and the first 781 bp of *gldN*.

^c Although free plasmid DNA did not restore motility, rare recombination of the plasmids into the chromosome resulted in restoration of gliding motility.

^d Colonies did not spread, but some cells moved in wet mount.

^e Colonies initially failed to spread, but after 3 or 4 days limited spreading was observed.

gldK mutants CJ1309 and CJ1372 (Fig. 3H and data not shown). pTB81a, which carries just *gldL*, failed to complement any of the *HimarEm* mutants (data not shown). Lack of complementation of the *gldL* mutants by pTB81a suggests that the insertions in *gldL* exhibit polar effects on downstream genes as expected from the results of the Northern blot analyses. Plasmid pTB94a, which carries *gldM* and most of *gldN*, partially restored motility to the *gldM* mutant FJ113, and to the *gldN* mutant CJ1304, but did not restore motility to *gldK* or *gldL* mutants (data not shown). pTB94a expresses *gldM* and *gldN* from a vector promoter. The weak complementation may be explained by polarity of the *gldM* insertion on *gldN* and the inability of truncated GldN (expressed from pTB94a) to restore motility to wild-type levels. These complementation data are consistent with the suggestion that *gldL*, *gldM*, and *gldN* are cotranscribed. pTB79, which spans *gldN*, restored motility to the *gldN* mutant CJ1304 but not to the *gldM* mutant FJ113 (data not shown). The inability of pTB79 to restore motility to FJ113 supports the idea that the motility defect of the *gldM* mutant is not simply due to polarity on *gldN* but that *gldM* is required for gliding. Because of the sequence similarities between GldN and GldO, the ability of pTB97a, which carries *gldO*, to suppress mutations in *gldN* was tested. Introduction of pTB97a restored motility to CJ1304, suggesting that GldO can replace GldN.

Overexpression of *gldK* inhibits gliding. To determine the reason for the poor complementation of mutants with pTB90, which carries *gldK*, *gldL*, *gldM*, *gldN*, and *gldO*, we introduced various plasmids into wild-type cells. Introduction of pTB90 or pTB88 (which spans *gldK* and *gldL*) into wild-type cells resulted in a dramatic reduction of colony spreading (Fig. 3C and D). In contrast, introduction of pTB98, which is identical to pTB90 except for the deletion of 798 bp within *gldK*, did not inhibit motility or colony spreading of wild-type cells (Fig. 3E). Introduction of pTB90 and pTB88, and to a lesser extent pTB98, also resulted in a reduction in growth rate. Wild-type

F. johnsoniae MM101 carrying pCP29 growing at 30°C in CYE with cefoxitin had a doubling time of 69 min. In contrast, MM101 carrying pTB88 or pTB90 had doubling times of 135 min and MM101 carrying pTB98 had a doubling time of 104 min. Strains carrying pTB88 or pTB90 (Fig. 3C, D, and G) and to a lesser extent strains carrying pTB98 (Fig. 3E, H, J, L, and N) also exhibited slow colony growth on PY2 agar. Surprisingly, cells with pTB99, which carries *gldK*, or pTB81a, which carries *gldL*, were fully motile and grew as fast as cells with control vector. All of the plasmids used in these studies have copy numbers of about 10 in *F. johnsoniae*. It appears that moderate overexpression of *gldK* and *gldL* together interferes with normal motility and adversely affects cell growth.

Complementation of spontaneous and chemically induced mutants. Pate and colleagues isolated 50 spontaneous and chemically induced nonmotile mutants of *F. johnsoniae* UW101 (7, 52). The mutations responsible for the motility defects of 33 of these mutants have previously been identified (1, 5, 15–17, 27, 28). Introduction of pTB90 or subclones of pTB90 into the remaining 17 mutants restored motility to each of them (Table 2). Colonies of the complemented mutants spread over agar, and cells exhibited motility in wet mounts. Complementation analyses allowed tentative identification of the locations of the mutations (Table 2). The complementation results did not allow us to unambiguously assign the sites of the mutations for UW102-78 and UW102-141. Introduction of pTB98, which spans *gldL*, *gldM*, *gldN*, and *gldO*, restored motility to UW102-78, but introduction of plasmids carrying *gldL*, *gldM*, and *gldN* individually did not (Table 1). UW102-78 may have multiple mutations or a deletion spanning several of the *gld* genes. UW102-141 appears to have a mutation in *gldK* since introduction of pTB99 restored motility to near-wild-type levels. Surprisingly, introduction of pTB81a, which spans *gldL*, also restored some motility to this mutant. Colonies of wild-type cells or of UW102-141 complemented with pTB99 spread extensively in the first 2 days of incubation, whereas colonies of

FIG. 5. Effect of mutation in *gldK*, *gldL*, *gldM*, and *gldN* on bacteriophage resistance. Bacteriophages (3 μ l of lysates containing approximately 10^9 phage/ml) were spotted onto lawns of cells in CYE overlay agar. The plates were incubated at 25°C for 24 h to observe lysis. Bacteriophages were spotted in the following order from left to right: top row, ϕ Cj1, ϕ Cj13, and ϕ Cj23; middle row, ϕ Cj28, ϕ Cj29, and ϕ Cj42; bottom row, ϕ Cj48 and ϕ Cj54. (A) Wild-type *F. johnsoniae* MM101 with shuttle vector pCP29. (B) Wild-type *F. johnsoniae* FJ1 with pCP29. (C) *gldK* mutant CJ1372 with pCP29. (D) CJ1372 complemented with pTB90. (E) *gldL* mutant CJ1300 with pCP29. (F) CJ1300 complemented with pTB98. (G) *gldM* mutant FJ113. (H) FJ113 complemented with pTB98. (I) *gldN* mutant CJ1304 with pCP23. (J) CJ1304 complemented with pTB79. The diameter of the petri dish is 9 cm.

UW102-141 carrying pTB81a failed to spread until 3 or 4 days of incubation, at which time a thin fringe of spreading was typically observed. The complementation results suggest that UW102-141 has a mutation in *gldK* that can be partially suppressed by overexpression of *gldL*. To test this prediction, we amplified the region spanning *gldK* and *gldL* from UW102-141 using primers 682 and 685 and determined the nucleotide sequence. A single base change was detected in this region, a “G”-to-“A” substitution at position 1158 numbered from the “A” of the *gldK* start codon. This change converts the codon for W₃₈₆ into a stop codon. Apparently the C-terminal 79 amino acids of GldK are not absolutely essential for function, since the mutation is partially suppressed by overexpression of *gldL*.

Bacteriophage resistance of *gld* mutants. Many nonmotile mutants of *F. johnsoniae* are resistant to infection by *F. johnsoniae* bacteriophages (51). The sensitivity of wild-type and *gld* mutant cells to bacteriophages was tested. Wild-type cells of *F. johnsoniae* were readily lysed (Fig. 5A and B) whereas cells with mutations in *gldK*, *gldL*, or *gldM* were resistant to infection by each of the bacteriophages (Fig. 5C, E, and G and data not shown). Complementation restored sensitivity to each of the phages in addition to restoring gliding motility (Fig. 5D, F, and H). The presence of turbid plaques in Fig. 5D may be a result of the partial inhibition of motility or growth caused by pTB90 as described above. GldN mutants, which as described above retain some ability to glide, were resistant to infection by most of the bacteriophages but remained partially susceptible to infection by ϕ 28, ϕ 42, and ϕ 54, resulting in turbid zones of clearing (Fig. 5I). Complementation with pTB79 restored gliding motility and full susceptibility to all of the bacteriophages (Fig. 5J).

FIG. 6. Effect of mutations in *gld* genes on ability to utilize chitin. Approximately 4×10^7 cells of *F. johnsoniae* were spotted on PY2-chitin medium and incubated at 25°C for 3 days (A and C) or 12 days (B and D). (A) 1, wild-type *F. johnsoniae* FJ1 with shuttle vector pCP29; 2, *gldK* mutant FJ131 with pCP29; 3, FJ131 complemented with pTB90. (B) 1, wild-type *F. johnsoniae* MM101 with pCP29; 2, *gldL* mutant CJ1300 with pCP29; 3, CJ1300 complemented with pTB98. (C) 1, wild-type *F. johnsoniae* FJ1 with shuttle vector pCP23; 2, *gldM* mutant FJ113 with pCP23; 3, FJ113 complemented with pTB94a. (D) 1, wild-type *F. johnsoniae* MM101 with pCP23; 2, *gldN* mutant CJ1304 with pCP23; 3, CJ1304 complemented with pTB79.

Effect of mutations in *gldK*, *gldL*, *gldM*, and *gldN* on chitin utilization. Wild-type cells of *F. johnsoniae* digest chitin (43) whereas many nonmotile mutants fail to utilize this insoluble polysaccharide (5, 7, 27, 28). The effects of mutations in *gldK*, *gldL*, *gldM*, and *gldN* on chitin utilization were determined in PY2 medium supplemented with chitin. Cells with mutations in *gldK*, *gldL*, *gldM*, and *gldN* were deficient in chitin utilization, and complementation restored the ability to digest chitin (Fig. 6). Although the *gldN* mutant was deficient in chitin utilization, it did digest a small amount of chitin (Fig. 6D). This may be explained by the fact that cells of *gldN* mutants retain some motility.

Mutations in *gldK*, *gldL*, *gldM*, and *gldN* do not affect levels of GldJ protein. A previous study demonstrated that cells with mutations in *gldA*, *gldB*, *gldD*, *gldF*, *gldG*, *gldH*, or *gldI* had normal levels of *gldJ* transcript but dramatically reduced levels of GldJ protein (5). It was proposed that GldJ interacts directly or indirectly with the other Gld proteins to form a complex and that removal of any of the Gld proteins destabilized GldJ. Western blot analyses of wild-type and mutant strains revealed that, unlike the previously identified *gld* mutants, the levels of GldJ protein were not dramatically affected by mutations in *gldK*, *gldL*, *gldM*, and *gldN* (Fig. 7).

DISCUSSION

Novel *mariner* transposons, *HimarEm1* and *HimarEm2*, were constructed to allow mutagenesis of *F. johnsoniae*. Erythromycin-resistant colonies arose as a result of transposition,

FIG. 7. Effect of *gld* mutations on levels of GldJ protein. Western blot analysis of whole-cell extracts using antiserum to GldJ. Lane 1, wild-type *F. johnsoniae* FJ1. Lane 2, wild-type *F. johnsoniae* MM101. Lane 3, *gldJ* mutant FJ123. Lane 4, *gldA* mutant CJ288. Lane 5, *gldK* mutant CJ1372. Lane 6, *gldL* mutant CJ1300. Lane 7, *gldM* mutant FJ113. Lane 8, *gldN* mutant CJ1304. Equal amounts of protein were loaded in each lane.

and motility mutants with insertions in novel genes (*gldK*, *gldL*, *gldM*, and *gldN*) were identified. *Himar* insertions occur at positions containing the target nucleotide sequence "TA" and are usually described as being otherwise random (39, 56). *Himar* insertions in *F. johnsoniae* were far from random. Eleven independent insertions in the region spanning *gldK*, *gldL*, *gldM*, and *gldN* were isolated and characterized in this study. The 4,125-bp region between HMM1361, which lies 17 bp upstream of the *gldK* start codon, and HMM1304, which lies within *gldN*, contains 690 copies (345 on each strand) of the target sequence "TA," but the 11 insertions were found at only seven sites. Targets with more than one insertion were found in *gldK* (HMM1372 and HFJ131 at position 159 numbered from the "A" of the *gldK* start codon and HMM1373 and HFJ150 at position 544), *gldL* (HMM1300 and HMM1253 at position 486 numbered from the "A" of the *gldL* start codon), and *gldN* (HMM1304 and HMM1382 at position 345 numbered from the "A" of the *gldN* start codon). At each site the orientation of the transposon insertions was conserved, and in each case the mutants with identical transposon insertions were isolated from independent experiments, so that they could not have been siblings. This high frequency of multiple insertions at individual sites would not be expected if the transposons inserted randomly. Although the *HimarEm* transposons were not completely random, they exhibited different biases than Tn4351 and thus constitute an important addition to the genetic toolbox for *F. johnsoniae*.

GldK, GldL, GldM, and GldN are predicted to localize to the cell envelope, where the bulk of the motility apparatus is expected to reside. GldL, GldM, and GldN are not similar to proteins of known function, but GldK is similar in sequence to the *F. johnsoniae* lipoprotein GldJ. GldJ is required for motility and is present in helical arrays in the cell envelope (5). Interestingly, expression of *gldJ* or *gldK* and *gldL* in wild-type cells from plasmids with a copy number of approximately 10 resulted in decreased gliding motility and colony spreading (reference 5 and this study). Overexpression of *gldK* and *gldL* together also adversely affected cell growth. The defects in colony spreading and cell growth resulting from the presence of *gldK* and *gldL* on a plasmid may explain why identification of *gld* genes by cosmid complementation of spontaneous and chemically induced *gldK*, *gldL*, *gldM*, and *gldN* mutants was unsuccessful.

Transposon insertions in *gldN* resulted in severe motility

defects, but occasionally cells were observed to move. The observation that overexpression of *gldO*, which is similar in sequence to *gldN*, suppressed mutations in *gldN* suggests that partial redundancy between GldN and GldO may be responsible for the occasional cell movements observed for *gldN* mutants.

Strains with mutations in *gldK*, *gldL*, *gldM*, and *gldN* exhibited increased resistance to bacteriophages that infect wild-type cells and were deficient in chitin utilization. Previous studies have demonstrated that *gldA*, *gldB*, *gldD*, *gldF*, *gldG*, *gldH*, *gldI*, and *gldJ* mutants also exhibit bacteriophage resistance and deficiencies in chitin utilization (1, 5, 15–17, 27, 28). Although the connection between bacteriophage resistance, chitin utilization, and gliding motility is not understood, it has been suggested that they may each rely on one or more transporters that are defective in *gld* mutants (28).

Twelve *gld* genes (*gldA*, *gldB*, *gldD*, *gldF*, *gldG*, *gldH*, *gldI*, *gldJ*, *gldK*, *gldL*, *gldM*, and *gldN*) that play important roles in gliding motility have been identified (references 1, 5, 15–17, 27, and 28 and this study). Introduction of these genes into 50 spontaneous and chemically induced nonmotile mutants restored motility to each of them. This suggests that few if any additional *F. johnsoniae* *gld* genes required for gliding motility remain to be discovered. *gld* genes which are essential for viability would have been difficult to identify by transposon mutagenesis but presumably could have been found by complementation of spontaneous or chemically induced mutants. *gld* genes with redundant or overlapping functions, however, may have been missed by our analyses.

GldA, GldF, and GldG are thought to constitute an ABC transporter (1, 15). GldB, GldD, GldH, GldI, and GldJ and perhaps GldK are lipoproteins whose exact roles in gliding are not known (references 5, 27, and 28 and this study). Many of the Gld proteins (GldB, GldD, GldH, GldL, GldM, and GldN) exhibit no significant similarity to proteins of known function, suggesting that the bacteroidete gliding "motor" may be a novel structure. The gliding motor converts chemical energy into mechanical work. Previous studies indicate that proton motive force (PMF) is required for gliding (12, 34). ATP may also be needed, since it has been reported that cells depleted for ATP fail to propel latex spheres (12). The motor presumably has one or more components that span the cytoplasmic membrane to harvest cellular energy. The only Gld proteins required for movement that are likely to span the cytoplasmic membrane are GldF, GldG, and GldL. GldF and GldG interact with GldA and are thought to form an ABC transporter. This transporter presumably uses ATP to perform its essential function in gliding, but it is possible that it also utilizes the PMF. The *E. coli* HlyA hemolysin exporter is an example of an ABC transporter that requires PMF and ATP hydrolysis for transport (20). GldL may span the cytoplasmic membrane and could be in a position to perform a role in energy transduction. One possible explanation for the inhibition of growth and motility by plasmids carrying *gldK* and *gldL* is that these proteins are involved in harvesting PMF, and moderate overexpression results in the formation of an unregulated channel that dissipates PMF. The fact that neither GldK alone (encoded by pTB99) nor GldL alone (encoded by pTB81a) inhibited growth or motility indicates that overexpression of both proteins is needed for inhibition and suggests that these pro-

teins interact. The suppression of the *gldK* mutant UW102-141 by pTB81a also suggests that GldK and GldL interact.

Many of the genes required for *F. johnsoniae* gliding motility have been identified. Some of the genes involved in gliding of the δ -proteobacterium *Myxococcus xanthus* and of the mollusc *Mycoplasma mobile* have also been identified (37, 38, 41, 44, 46, 47, 53, 55, 56). Complete genome sequence information is available for *M. xanthus* (The Institute for Genomic Research, personal communication) and *M. mobile* (The Broad Institute, Cambridge, MA), facilitating comparative studies. Analysis of the known motility genes from *F. johnsoniae*, *M. mobile*, and *M. xanthus* reveals few similarities. *M. mobile* gliding motility requires large surface proteins and may involve the cytoskeleton (19, 31, 41). *M. mobile* has no obvious homologs to any of the proteins known to be required for *F. johnsoniae* motility with the exception of GldA. Proteins similar to GldA are found in virtually all organisms, so the presence of a distantly related protein in *M. mobile* is probably not meaningful with respect to gliding motility. *M. xanthus* has two motility systems. Social motility relies on type IV pili, whereas the mechanism of adventurous motility may involve polysaccharide secretion (45, 50, 53). Analysis of the *M. xanthus* genome reveals apparent homologs to *gldA*, *gldF*, *gldG*, *gldI*, *gldJ*, and *gldK*. GldA is similar to many putative ATP-binding components of ABC transporters of *M. xanthus*. One of these, PilH, is involved in social motility. There is no evidence linking the *M. xanthus* proteins related to GldF, GldG, GldI, GldJ, and GldK to gliding. Obvious homologs to the other *gld* genes that are required for *F. johnsoniae* cell movement (*gldB*, *gldD*, *gldH*, *gldL*, *gldM*, and *gldN*) are not found in the *M. xanthus* genome. While there may be some underlying similarities between the mechanisms of gliding employed by *F. johnsoniae* and *M. xanthus*, many of the components of the gliding machineries do not appear to be genetically related.

Complete genome sequences are available for several non-gliding (6, 21, 33, 54) and gliding (*C. hutchinsonii*, DOE-Joint Genome Institute and Los Alamos National Labs) members of the phylum Bacteroidetes. *C. hutchinsonii*, which is only distantly related to *F. johnsoniae*, has homologs to each of the *F. johnsoniae* *gld* genes and presumably uses the products of these genes to propel itself. In contrast the nonmotile bacteroidetes *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, and *Porphyromonas gingivalis* each have homologs to some *gld* genes but lack others. For example, obvious homologs of *gldD* and *gldG* are lacking in each of these bacteria, which may account for their inability to glide. Close homologs of *gldJ* are also lacking, although *B. fragilis* and *P. gingivalis* each have a *gldK* homolog which exhibits some similarity to *gldJ*. *B. fragilis* has apparent homologs to *gldA*, *gldB*, *gldF*, *gldH*, *gldK*, *gldL*, and *gldM*; *B. thetaiotaomicron* has homologs to *gldA*, *gldB*, and *gldH*; and *P. gingivalis* has *gldA*, *gldB*, *gldH*, *gldI*, *gldK*, *gldL*, *gldM*, and *gldN* homologs. *F. johnsoniae* *gld* mutants are deficient in chitin utilization, indicating that Gld proteins may function in both gliding and chitin utilization. The Gld homologs in nonmotile bacteroidetes may perform similar roles in nutrient utilization. Further analysis of the Gld proteins will help elucidate the mechanism of gliding motility employed by *F. johnsoniae* and other gliding bacteria of the phylum Bacteroidetes and may also identify the functions of similar proteins in nonmotile members of this large and diverse phylum.

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REFERENCES

- 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.
- Bayley, D. P., E. R. Rocha, and C. J. Smith. 2000. Analysis of *cepA* and other *Bacteroides fragilis* genes reveals a unique promoter structure. *FEMS Microbiol. Lett.* **193**:149–154.
- Bouhenni, R., A. Gehrke, and D. Saffarini. 2005. Identification of genes involved in cytochrome *c* biogenesis in *Shewanella oneidensis*, using a modified *mariner* transposon. *Appl. Environ. Microbiol.* **71**:4935–4937.
- Braun, T. F., and M. J. McBride. 2005. *Flavobacterium johnsoniae* GldJ is a lipoprotein that is required for gliding motility. *J. Bacteriol.* **187**:2628–2637.
- Cerdeno-Tarraga, A., S. Patrick, L. Crossman, G. Blakely, V. Abratt, N. Lennard, I. Poxton, B. Duerden, B. Harris, M. Quail, A. Barron, L. Clark, C. Corton, J. Doggett, M. Holden, N. Larke, A. Line, A. Lord, H. Norbertczak, D. Ormond, C. Price, E. Rabinowitsch, J. Woodward, B. Barrell, and J. Parkhill. 2005. Extensive DNA inversions in the *B. fragilis* genome control variable gene expression. *Science* **307**:1463–1465.
- 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.
- Cho, S. G., and Y. J. Choi. 1998. Characterization of the *xaiF* gene encoding a novel xylanase-activity-increasing factor, XaiF. *J. Microbiol. Biotechnol.* **8**:378–387.
- Cosma, M., S. Pepe, I. Annunziata, R. Newbold, M. Grompe, G. Parenti, and A. Ballabio. 2003. The multiple sulfatase deficiency gene encodes an essential and limiting factor for the activity of sulfatases. *Cell* **113**:445–456.
- Dierks, T., B. Schmidt, L. Borissenko, J. Peng, A. Preusser, M. Mariappan, and K. von Figura. 2003. Multiple sulfatase deficiency is caused by mutations in the gene encoding the human C(alpha)-formylglycine generating enzyme. *Cell* **113**:435–444.
- Doering, D. 1992. Ph.D. thesis. Massachusetts Institute of Technology, Cambridge.
- Dzink-Fox, J. L., E. R. Leadbetter, and W. Godchaux III. 1997. Acetate acts as a protonophore and differentially affects bead movement and cell migration of the gliding bacterium *Cytophaga johnsonae* (*Flavobacterium johnsoniae*). *Microbiology* **143**:3693–3701.
- Fellay, R., J. Frey, and H. Krisch. 1987. Interposon mutagenesis of soil and water bacteria: a family of DNA fragments designed for in vitro insertional mutagenesis of Gram-negative bacteria. *Gene* **52**:147–154.
- Hofmann, K., and W. Stoffel. 1993. TMbase—a database of membrane spanning protein segments. *Biol. Chem. Hoppe-Seyler* **374**:166.
- Hunnicutt, D. W., M. J. Kempf, and M. J. McBride. 2002. Mutations in *Flavobacterium johnsoniae* *gldF* and *gldG* disrupt gliding motility and interfere with membrane localization of GldA. *J. Bacteriol.* **184**:2370–2378.
- 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.
- 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.
- Korolev, E. V., A. V. Nikonov, M. S. Brudnaya, E. S. Snigirevskaya, G. V. Sabinin, Y. Y. Komissarchik, P. I. Ivanov, and S. N. Borchsenius. 1994. Tubular structures of *Mycoplasma gallisepticum* and their possible participation in cell motility. *Microbiology* **140**:671–681.
- 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.
- Kuwahara, T., A. Yamashita, H. Hirakawa, H. Nakayama, H. Toh, N. Okada, S. Kuhara, M. Hattori, T. Hayashi, and Y. Ohnishi. 2004. Genomic analysis of *Bacteroides fragilis* reveals extensive DNA inversions regulating cell surface adaptation. *Proc. Natl. Acad. Sci. USA* **101**:14919–14924.
- Lapidus, I. R., and H. C. Berg. 1982. Gliding motility of *Cytophaga* sp. strain U67. *J. Bacteriol.* **151**:384–398.
- Li, L.-Y., N. B. Shoemaker, and A. A. Salyers. 1995. Location and charac-

- terization of the transfer region of a *Bacteroides* conjugative transposon and regulation of the transfer genes. *J. Bacteriol.* **177**:4992–4999.
24. Lupas, A. 1996. Prediction and analysis of coiled-coil structures. *Methods Enzymol.* **266**:513–525.
 25. McBride, M. J. 2001. Bacterial gliding motility: multiple mechanisms for cell movement over surfaces. *Annu. Rev. Microbiol.* **55**:49–75.
 26. McBride, M. J. 2004. Cytophaga-flavobacterium gliding motility. *J. Mol. Microbiol. Biotechnol.* **7**:63–71.
 27. McBride, M. J., and T. F. Braun. 2004. GldI is a lipoprotein that is required for *Flavobacterium johnsoniae* gliding motility and chitin utilization. *J. Bacteriol.* **186**:2295–2302.
 28. McBride, M. J., T. F. Braun, and J. L. Brust. 2003. *Flavobacterium johnsoniae* GldH is a lipoprotein that is required for gliding motility and chitin utilization. *J. Bacteriol.* **185**:6648–6657.
 29. 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.
 30. McGowan, S. J., M. Sebahia, S. O'Leary, K. R. Hardie, P. Williams, G. S. Stewart, B. W. Bycroft, and G. P. Salmond. 1997. Analysis of the carbapenem gene cluster of *Erwinia carotovora*: definition of the antibiotic biosynthetic genes and evidence for a novel beta-lactam resistance mechanism. *Mol. Microbiol.* **26**:545–556.
 31. Miyata, M., H. Yamamoto, T. Shimizu, A. Uenoyama, C. Citti, and R. Rosengarten. 2000. Gliding mutants of *Mycoplasma mobile*: relationships between motility and cell morphology, cell adhesion and microcolony formation. *Microbiology* **146**:1311–1320.
 32. Nakai, K., and M. Kanehisa. 1991. Expert system for predicting protein localization sites in Gram-negative bacteria. *Proteins Struct. Funct. Genet.* **11**:95–110.
 33. Nelson, K., R. Fleischmann, R. DeBoy, I. Paulsen, D. Fouts, J. Eisen, S. Daugherty, R. Dodson, A. Durkin, M. Gwinn, D. Haft, J. Kolonay, W. Nelson, T. Mason, L. Tallon, J. Gray, D. Granger, H. Tettelin, H. Dong, J. Galvin, M. Duncan, F. Dewhirst, and C. Fraser. 2003. Complete genome sequence of the oral pathogenic bacterium *Porphyromonas gingivalis* strain W83. *J. Bacteriol.* **185**:5591–5601.
 34. 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.
 35. 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.
 36. Pearson, W. R. 1990. Rapid and sensitive sequence comparison with FASTP and FASTA. *Methods Enzymol.* **183**:63–98.
 37. Rodriguez, A. M., and A. M. Spormann. 1999. Genetic and molecular analysis of *cglB*, a gene essential for single-cell gliding in *Myxococcus xanthus*. *J. Bacteriol.* **181**:4381–4390.
 38. Rodriguez-Soto, J. P., and D. Kaiser. 1997. The *tgl* gene: social motility and stimulation in *Myxococcus xanthus*. *J. Bacteriol.* **179**:4361–4371.
 39. Rubin, E., B. Akerley, V. Novik, D. Lampe, R. Husson, and J. Mekalanos. 1999. In vivo transposition of *mariner*-based elements in enteric bacteria and mycobacteria. *Proc. Natl. Acad. Sci. USA* **96**:1645–1650.
 40. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 41. Seto, S., A. Uenoyama, and M. Miyata. 2005. Identification of a 521-kilodalton protein (Gli521) involved in force generation or force transmission for *Mycoplasma mobile* gliding. *J. Bacteriol.* **187**:3502–3510.
 42. 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.
 43. Stanier, R. Y. 1947. Studies on non-fruiting myxobacteria. I. *Cytophaga johnsonae*, n. sp., a chitin-decomposing myxobacterium. *J. Bacteriol.* **53**:297–315.
 44. Stephens, K., P. Hartzell, and D. Kaiser. 1989. Gliding motility in *Myxococcus xanthus*: *mgl* locus, RNA, and predicted protein products. *J. Bacteriol.* **171**:819–830.
 45. 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.
 46. Uenoyama, A., A. Kusumoto, and M. Miyata. 2004. Identification of a 349-kilodalton protein (Gli349) responsible for cytoadherence and glass binding during gliding of *Mycoplasma mobile*. *J. Bacteriol.* **186**:1537–1545.
 47. White, D. J., and P. L. Hartzell. 2000. AglU, a protein required for gliding motility and spore maturation of *Myxococcus xanthus*, is related to WD-repeat proteins. *Mol. Microbiol.* **36**:662–678.
 48. Wilson, K., A. Sessitsch, J. Corbo, K. Giller, A. Akkermans, and R. Jefferson. 1995. β -Glucuronidase (GUS) transposons for ecological and genetic studies of rhizobia and other gram-negative bacteria. *Microbiology* **141**:1691–1705.
 49. Wolf, E., P. Kim, and B. Berger. 1997. MultiCoil: a program for predicting two- and three-stranded coiled coils. *Protein Sci.* **6**:1179–1189.
 50. Wolgemuth, C., E. Hoiczky, D. Kaiser, and G. Oster. 2002. How myxobacteria glide. *Curr. Biol.* **12**:369–377.
 51. 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.
 52. 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.
 53. 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.
 54. Xu, J., M. K. Bjursell, J. Himrod, S. Deng, L. K. Carmichael, H. C. Chiang, L. V. Hooper, and J. I. Gordon. 2003. A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis. *Science* **299**:2074–2076.
 55. Yang, Z., Y. Geng, D. Xu, H. B. Kaplan, and W. Shi. 1998. A new set of chemotaxis homologues is essential for *Myxococcus xanthus* social motility. *Mol. Microbiol.* **30**:1123–1130.
 56. Youderian, P., N. Burke, D. J. White, and P. L. Hartzell. 2003. Identification of genes required for adventurous gliding motility in *Myxococcus xanthus* with the transposable element *mariner*. *Mol. Microbiol.* **49**:555–570.