JOURNAL OF BACTERIOLOGY, Jan. 1999, p. 24–33 Vol. 181, No. 1
0021-9193/99/$04.00+0
Copyright © 1999, American Society for Microbiology. All Rights Reserved.

The *Myxococcus xanthus pilQ* (*sglA*) Gene Encodes a Secretin Homolog Required for Type IV Pilus Biogenesis, Social Motility, and Development

DANIEL WALL,[1]* PAUL E. KOLENBRANDER,[1,2] AND DALE KAISER[1]

Departments of Biochemistry and Developmental Biology, Stanford University, Stanford, California 94305,[1] *and Oral Infection and Immunity Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland 20892*[2]

Received 13 August 1998/Accepted 26 October 1998

The *Myxococcus xanthus sglA1* spontaneous mutation was originally isolated because it allowed dispersed cell growth in liquid yet retained the ability to form fruiting bodies. Consequently, most of today's laboratory strains either contain the *sglA1* mutation or were derived from strains that carry it. Subsequent work showed that *sglA* was a gene for social gliding motility, a process which is mediated by type IV pili. Here *sglA* is shown to map to the major *pil* cluster and to encode a 901-amino-acid open reading frame (ORF) that is homologous to the secretin superfamily of proteins. Secretins form a channel in the outer membrane for the transport of macromolecules. The closest homologs found were PilQ proteins from *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*, which are required for type IV pili biogenesis and twitching motility. To signify these molecular and functional similarities, we have changed the name of *sglA* to *pilQ*. The hypomorphic *pilQ1* (*sglA1*) allele was sequenced and found to contain two missense mutations at residues 741 (G→S) and 762 (N→G). In addition, 19 independent social (S)-motility mutations are shown to map to the *pilQ* locus. In-frame deletions of *pilQ* and its downstream gene, *orfL*, were constructed. *pilQ* is shown to be essential for pilus biogenesis, S-motility, rippling, and fruiting body formation, while *orfL* is dispensable for these processes. The *pilQ1* allele, but not the Δ*pilQ* allele, was found to render cells hypersensitive to vancomycin, suggesting that PilQ1 alters the permeability properties of the outer membrane. Many differences between *pilQ1* and *pilQ*$^+$ strains have been noted in the literature. We discuss some of these observations and how they may be rationalized in the context of our molecular and functional findings.

In response to starvation, the gram-negative bacterium *Myxococcus xanthus* initiates a multicellular developmental program that culminates in cells aggregating and forming a fruiting body (19). Within this structure vegetative cells differentiate into spores. This process depends on gliding motility. Gliding is controlled by two distinct genetic systems called adventurous (A)-motility and social (S)-motility (22). S-motility, but not A-motility, depends on polar type IV pili (58, 61). Most of the *M. xanthus pil* genes are homologous to type IV *pil* genes found in *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*, which are also required for a type of motility called twitching and for pathogenesis (33, 53). These pili may retract, as well as polymerize, and they may provide the force for movement by pushing and pulling cells (2, 53). Many type IV *pil* genes are homologous to type II secretion genes (general secretion pathway) (42). In the type IV system, pilin (PilA) is the only known secretion product (33).

Pili and fibrils have been shown to mediate cohesion among cells and adhesion to substrates (4, 49, 61). Cohesive cells clump or aggregate in suspension, and their clumps stick to the walls of culture flasks. Native cultures of *M. xanthus* isolated from soil fail to suspend in liquid culture. To obtain dispersed growth in liquid medium, a spontaneous mutant which retained the ability to form fruiting bodies was isolated after continuous selection; it was named strain FB (15). This mutant of *M. xanthus* was amenable to necessary microbiological manipulations, such as dilutions, and as a result most laboratory strains were derived from strain FB. Later work showed that this mutation was in a social gliding motility gene, named *sglA* (22). Unlike other *sgl* mutations, the *sglA1* mutant retains some S-motility and expresses pili at reduced levels, suggesting that *sglA1* is a hypomorphic allele (23). Strains which carry *sglA1* can form fruiting bodies on agar but not in submerged culture (27). This quality is associated with the decreased cohesiveness of *sglA1* mutants, which consequently are unable to form a mat of cells (biofilm) within which fruiting bodies can develop. Strain DK1622 was constructed from strain FB; DK1622 is *sglA*$^+$, fully S-motile, and capable of developing in submerged culture. Type IV pili are required by *P. aeruginosa* to form a biofilm (40), a process that resembles the formation of fruiting bodies (12).

Here we report the mapping and cloning and the sequence of the *sglA* locus. SglA is found to belong to a large family of proteins called secretins (33), which include PilQ proteins from *P. aeruginosa* and *N. gonorrhoeae*. To indicate the molecular nature of *sglA*, we have changed its name to *pilQ*, as has been done for other *sgl* genes in *M. xanthus* when their functions were recognized. In-frame deletions in *pilQ* and its downstream gene *orfL* were constructed. *pilQ* is shown to be essential for the biogenesis of pili and for S-motility, while *orfL* is not. The origin of the *pilQ*$^+$ DK1622 strain and the role of PilQ in *M. xanthus* are discussed.

MATERIALS AND METHODS

Bacterial strains, phage, plasmids, and DNA manipulations. Bacterial strains and plasmids are listed in Table 1. *M. xanthus* was cultured in CTT medium, CTT agar, or 1/2 CTT agar plates (21). DNA manipulations were done in *Escherichia coli* XL1-Blue cultured in Luria-Bertani medium (46). Antibiotics were added

* Corresponding author. Mailing address: Departments of Biochemistry and Developmental Biology, Stanford University, Stanford, CA 94305. Phone: (650) 723-5685. Fax: (650) 725-7739. E-mail: dwall@cmgm.stanford.edu.

TABLE 1. *M. xanthus* strains and plasmids

Strain or plasmid	Genotype or relevant properties	Phenotype	Construction	Reference
M. xanthus				
DK101	*pilQ1* (*sglA1*)	A^+ S^-	*M. xanthus* FB	15
DK320	*aglB1 pilQ1*	A^- S^-	UV on DK101	21
DK1217	*aglB1*	A^- S^+	Mx8 (YS) × DK320→screen S^+	22
DK1241	*aglR4 pilQ1241*	A^- S^-	UV	20a
DK1242	*cglB pilQ1242*	A^- S^-	UV	20a
DK1243	*agl-12 pilQ1243*	A^- S^-	UV	22
DK1247	*aglK2 pilQ1247*	A^- S^-	UV	20a
DK1287	*agl1 pilQ1287*	A^- S^-	UV	20a
DK1291	*aglK1 pilQ1291*	A^- S^-	UV	20a
DK1600	*pilG* or *pilH*	A^+ S^-	Spontaneous; YS (MD2)	56
DK1609	*agl pilQ1609*	A^- S^-	ICR-191	37a
DK1622	Wild type	A^+ S^+	Mx8 (YS) × DK1217→screen A^+	37a
DK1627	*cglC1 pilQ1627*	A^- S^-	ICR-191	37a
DK1633	*cglC1 pilQ1633*	A^- S^-	ICR-191	37a
DK1636	*cglB2 pilQ1636*	A^- S^-	ICR-191	37a
DK1675	*cglB2 pilQ1675*	A^- S^-	ICR-191	37a
DK1687	*agl pilQ1687*	A^- S^-		37a
DK2136	*aglB1 pilQ2136*	A^- S^-	ICR-191	37a
DK2140	*agl pilQ2140*	A^- S^-	ICR-191	37a
DK2148	*aglB1 pilQ2148*	A^- S^-	ICR-191	37a
DK2149	*aglB1 pilQ2149*	A^- S^-	ICR-191	37a
DK2150	*aglB1 pilQ2150*	A^- S^-	ICR-191	37a
DK2159	*aglB1 pilQ2159*	A^- S^-	ICR-191	37a
DK2167	*cglB2 pilQ10 rif-100*	A^- S^-	Spontaneous; YNS	37a
DK2227	*cglB2 pilQ*Ω2227	A^- S^-		37a
DK4293	Ω4401	A^+ S^+		26
DK4414	Ω4414	A^+ S^+		26
DK8610	Ω3188 *pilQ*$^+$	A^+ S^+	Mx4 (DK10389) × DK101→select Km^r; screen S^+	
DK8611	Ω3188 *pilQ1*	A^+ S^-	Mx4 (DK10389) × DK101→select Km^r; screen S^-	
DK8612	Wild type	A^+ S^+	pDW140 × DK101→select Km^r; select Gal^r; screen Km^s; screen by Southern blotting	
DK8613	Δ*orfL*	A^+ S^+	pDW146 × DK1622→select Km^r; select Gal^r; screen Km^s; screen by Southern blotting	
DK8614	*aglB1* Δ*orfL*	A^- S^+	pDW146 × DK1217→select Km^r; select Gal^r; screen Km^s; screen by Southern blotting	
DK8615	Δ*pilQ*	A^+ S^-	pDW131 × DK1622→select Km^r; select Gal^r; screen Km^s; screen by Southern blotting	
DK8616	*aglB1* Δ*pilQ*	A^- S^-	pDW131 × DK1217→select Km^r; select Gal^r; screen Km^s; screen by Southern blotting	
DK8617	Δ*pilQ* Ω4401	A^+ S^-	Mx4 (DK4293) × DK8615→select Km^r	
DK8618	Δ*pilQ* Ω4414	A^+ S^-	Mx4 (DK4414) × DK8615→select Km^r	
DK10389	Ω3188 *pilQ*$^+$	A^+ S^+	Mx4 (DK3188) × DK1622→select Km^r	
Plasmids				
pBluescript SK	Cloning vector	Ap^r	Stratagene (La Jolla, Calif.)	
pBGS18	Cloning vector	Km^r		51
pKG-2	*nptII galK*	Ap^r Km^r	Km^r Gal^s cassette	52
pDW79	Ω3188; *pil* region	Ap^r Km^r	19-kb (Ω3188) *Cla*I-*Cla*I from DK10389 cloned in pBluescript (*Cla*I)	
pDW81	*pilQ* fragment	Km^r	2.1-kb *Mlu*I-*Mlu*I from pDW79 cloned in pBGS18 (*Sma*I)	
pDW83	*pilQ* fragment	Km^r	Exonuclease III deletion of pDW81, leaving 1.0 kb	
pDW85	*pilQ* fragment	Km^r	Exonuclease III deletion of pDW81, leaving 0.75 kb	
pDW92	*pil* region	Km^r	6.2-kb *Hin*dIII-*Kpn*I from pDW79 cloned in pBGS18 (*Hin*dIII-*Kpn*I)	
pDW94	*pil* region	Km^r	4.5-kb *Hin*dIII-*Kpn*I from pDW79 cloned in pBGS18 (*Hin*dIII-*Kpn*I)	
pDW105	*pil* region	Ap^r	6.2-kb *Hin*dIII-*Kpn*I from pDW79 cloned in pBluescript (*Hin*dIII-*Kpn*I)	
pDW131	Δ*pilQ orfL galK*	Km^r Ap^r	PCR-generated Δ*pilQ*; *nptII galK* cassette cloned in pDW105 (*Bam*HI-*Eco*RI)	
pDW137	*orfL*	Ap^r	2.9-kb *Bam*HI-*Eco*RI from pDW105 in pBluescript	
pDW140	*pilQ* fragment, *galK*	Km^r Ap^r	2.0-kb *Sph*I from pDW81 cloned in pBluescript (pDW96 *Eco*RV); *nptII galK* cassette cloned in *Eco*RI	
pDW146	Δ*orfL galK*	Km^r Ap^r	PCR-generated Δ*orfL* cloned in pDW137 (*Eco*NI-*Eco*RI); *nptII galK* cloned in *Eco*RV	
pDW139	*orfL*	Km^r	5.0-kb *Kpn*I-*Msc*I (blunted and ligated) deletion of pDW92	
pDW167	*pilQ orfL*	Km^r	2.3-kb *Kpn*I-*Not*I (blunted and ligated) deletion of pDW92	
pDW168	*pil* region	Km^r	11-kb *Hin*dIII from pDW79 cloned in pBGS18 (*Hin*dIII)	
pDW169	*pil* region	Km^r	3.3-kb *Psh*AI-*Not*I from pDW79 cloned in pBGS18 (*Sma*I)	
pDW188	*pilQ orfL*	Km^r	2.1-kb *Kpn*I-*Pst*I (blunted and ligated) deletion of pDW92	

when appropriate (kanamycin at 20 μg/ml for *M. xanthus* and at 40 μg/ml for *E. coli* and ampicillin at 100 μg/ml). *M. xanthus* chromosomal DNA preparations, plasmid preparations, DNA manipulations, and Southern hybridizations were all performed as recommend by the manufacturers or as described previously (46, 58).

Mx4 transductions were done as described previously (21). To score for S-motility, Km^r transductants were transferred with toothpicks to fresh CTT-kanamycin agar plates and visually scored for S-motility. Electroporation of plasmid DNA into *M. xanthus* was done as described previously (24, 43). To score for the rescue of S-motility, cells were plated on 0.5% agar CTT-kanamycin plates and visually checked after 7 days.

DNA sequencing and analysis. Double-stranded plasmid DNA was sequenced with the Thermo Sequenase cycle-sequencing kit (Amersham Life Sciences). Restriction fragments were generated and cloned into pBluescript SK or pBGS18 (51) for sequencing. Additional deletion subclones were generated with exonuclease III (46). Primers were designed to cover gaps in the sequence. Both strands were completely sequenced at least once.

Sequence data was compiled and analyzed with DNA Strider and the Genetics Computer Group (Madison, Wis.) Sequence Analysis Software Package version 8.

Development. Cells were grown overnight in CTT and placed at a calculated density of 1,000 Klett units on CF or TPM starvation agar plates (26). Fruiting body formation and rippling were monitored with a Leitz inverted microscope. Spore counts and β-galactosidase assays were performed as described previously (6, 26).

Immunoblotting and autoradiography. Proteins were separated by sodium dodecyl sulfate (SDS)–12% polyacrylamide gel electrophoresis (PAGE) and transferred to Immobilon P membranes (Millipore) (46). For Western blotting the membrane was probed with rabbit anti-PilA serum diluted 1:4,000 (59), followed by peroxidase-conjugated goat anti-rabbit immunoglobulin G (Boehringer Mannheim) diluted 1:2,000. The blots were developed with Renaissance chemiluminescence reagent (NEN Life Science Products).

To label extracellular proteins, 1.8-ml cultures starting at 35 Klett units for CTT and 75 Klett units for A1 medium (3) were grown with 50 μCi of Trans^{35}S-label (ICN Biochemicals)/ml for 6 h in an orbital shaker at 33°C. The cells were then pelleted by centrifugation (12,000 × *g*; 10 min; 4°C). Deoxycholate (0.01%) and 10% trichloroacetic acid were added to the supernatant, mixed, and stored overnight at −20°C. The insoluble proteins were pelleted by centrifugation (12,000 × *g*; 20 min; 4°C) and resuspended in SDS sample buffer (46). To neutralize the pH, a few microliters of sodium hydroxide (1 M) was added to the sample buffer (until it turned blue). The samples were boiled, separated by SDS-PAGE, and blotted as described above. The membranes were treated with En^{3}hance spray (Dupont, NEN) for fluorography and developed overnight on Hyperfilm MP (Amersham Life Science).

Constructing in-frame deletions of *pilQ* and *orfL*. A plasmid, pDW131, which deleted in frame 2,175 bp or 725 codons from the coding region of *pilQ*, was generated via PCR. To construct this *pilQ* in-frame deletion, two primers were designed, one for each end of the gene, oriented in opposite directions. These primers, pQ1 (5′-GCGAAGCTTGCCGGAGCCTGGGCGGCGAC-3′) and pQ2 (5′-GCGAAGCTTCATTGCGCAGACTCTGTAAGG-3′), had unique *Hin*dIII restriction sites (underlined) engineered in their 5′ tails. In separate PCRs, two fragments of *pilQ* DNA were amplified with pQ1 and pQ2, along with corresponding primers that were upstream and downstream of pQ1 and pQ2, respectively. These PCR products were cloned and subsequently ligated together via the *Hin*dIII restriction sites, generating an in-frame deletion with a *Hin*dIII restriction site inserted. The region across this deletion and insertion was verified by sequencing. To avoid PCR complications, a 0.45-kb *Bst*EII-*Mlu*I cassette containing the deletion was swapped with the corresponding cassette of pDW105, generating pDW130. A Km^r-Gal^s cassette (52) was then cloned into the *Eco*RI-*Bam*HI sites of pDW130, generating pDW131. pDW131 was electroporated into DK1622 and DK1217, and homologous recombination into the chromosomal locus was selected for by Km^r. Candidate transformants were screened for the expected tandem duplication of the $pilQ^+$ and Δ*pilQ* alleles by Southern analysis. Recombinants with the expected duplication were then grown in CTT for 1 day to enrich for cells with a second recombination event that lost pDW131 and one of the *pilQ* alleles, thus leaving either a $pilQ^+$ or Δ*pilQ* allele at the chromosomal locus. Such recombinants were selected for by galactose resistance. These Gal^r colonies were screened for Km^s. Southern analysis was used to identify recombinants that had only the Δ*pilQ* allele left at the chromosomal locus.

An *orfL* in-frame deletion was also constructed by the *galK* counterselection method (52). This deletion removed 209 of the 220 codons in *orfL*. To generate this allele, two primers were designed, one at each end of the gene that were oriented in opposite directions. These primers, pL1 (5′-AGGCCTGAGATAGAAGTTCTTCATGAGCG-3′) and pL2 (5′-AGGCCTGAGCCCGAGGAAACGTAGTC-3′), had unique *Stu*I restriction sites (underlined) engineered in their 5′ tails. The primers were used to amplify a 5.2-kb fragment which included pBluescript SK from pDW137. The amplified DNA was digested with *Stu*I, gel purified, and self-ligated, generating pDW144. The region across the *orfL* in-frame deletion and *Stu*I insertion was verified by sequencing. The wild-type *orfL* cassette in pDW137 was then swapped with the Δ*orfL* allele of pDW144 at unique *Eco*NI-*Eco*RI restriction sites (*Eco*RI is in pBluescript SK), generating pDW145. The Km^r-Gal^s cassette of pKG2 was then cloned into the *Eco*RV site of pDW145, generating pDW146. pDW146 was then electroporated into DK1622 and DK1217, and Δ*orfL* strains were subsequently isolated as described above for Δ*pilQ*.

Nucleotide sequence accession number. The nucleotide sequence of *pilQ* and *orfL* has been deposited in GenBank under accession no. AF100157.

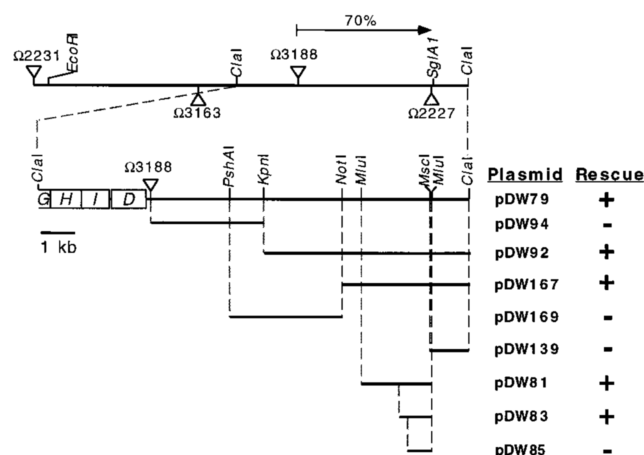

FIG. 1. Genetic map of the *pil* cluster. Triangles above the line represent Tn*5* insertions that do not cause a S-motility defect, while those below the line inactivate S motility. The cotransduction frequency between Ω3188 and *sglA* (*pilQ1*) is given (arrow). The second line shows four *pil* genes that map to the left of Ω3188, and the relevant restriction sites are indicated. The abilities of plasmids to rescue the S-motility defect of *sglA1* are indicated as follows: +, able to rescue; −, unable to rescue.

RESULTS

Mapping and cloning *sglA*. A Tn*5* transposon, Ω3188, linked to the *sglA* locus had been isolated by David Morandi in this laboratory (unpublished). When Ω3188 was transduced into DK101 (*sglA1*), we observed some S^+ transductants. However, Ω3188 is also linked to 10 other *pil* genes (58, 60, 61). To estimate the physical distance separating Ω3188 and the *sglA* locus, transductional crosses were made between DK1217 (A^- S^+) as the recipient and DK8611 (Ω3188 *sglA1*) as the donor. Of 231 Km^r transductants scored, 163 were S^-, a cotransduction frequency of 70% (Fig. 1). Given that bacteriophage Mx4 packages 65 kb of DNA, Wu's formula (62) suggests that the *sglA1* mutation is 7 kb from Ω3188. Three point crosses between Ω2231, Ω3188, and *sglA* implied that the *sglA* locus lies to the right of Ω3188 relative to Ω2231, as depicted in Fig. 1.

A restriction map for the neighborhood of Tn*5* insertion Ω3188 was generated by using Tn*5* DNA as a probe for Southern hybridization. A unique *Cla*I site was found to lie 9.6 kb to the right of Ω3188; according to the cotransduction frequencies, the Ω3188-to-*Cla*I fragment might include the *sglA* locus. There are no *Cla*I sites in Tn*5*, and there is a *Cla*I site 3.4 kb to the left of Ω3188 (Fig. 1) (60). A 19-kb *Cla*I-*Cla*I fragment (including Tn*5*) was cloned into the *Cla*I site of pBluescript SK by selecting for Km^r, generating pDW79. To test if pDW79 contained the $sglA^+$ allele, the plasmid was electroporated into DK320 (*sglA1*), with selection for Km^r. Electroporants of DK320 regained S-motility from pDW79, demonstrating that the $sglA^+$ gene is on this plasmid (Fig. 1). A series of subclones of pDW79 was constructed. As shown in Fig. 1, the *sglA1* mutation could be rescued by a 1-kb subfragment cloned in pDW83.

A 3.6-kb region of DNA surrounding the *sglA* locus was sequenced. Two open reading frames (ORFs) on the same strand and reading frame were identified (Fig. 2). These ORFs

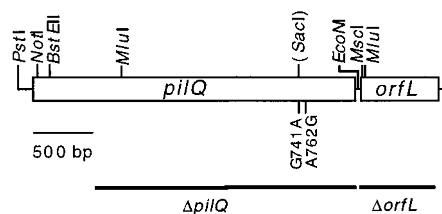

FIG. 2. Genetic organization of *pilQ* and *orfL*. ORFs are read from left to right. The relevant restriction sites are indicated. The two *pilQ1* mutations are shown, along with the new restriction site (*Sac*I) generated by the mutation at codon 741. The black lines below these ORFs represent the regions removed by the in-frame deletions.

had an 81 and a 77% third-codon-position GC bias, respectively, which is diagnostic of *M. xanthus* genes. The intergenic region between these ORFs is 39 bp. Both ORFs are oriented in the same direction as the 10 *pil* genes found immediately to the left of Ω3188 (58, 60, 61).

Since the *sglA1* mutation could be rescued by DNA fragments to the left of the *Msc*I site and not by pDW139 (Fig. 1), the *sglA1* mutation must reside in the ORF labeled *pilQ* in Fig. 2. This ORF encodes a 901-amino-acid protein. A BLAST search of the GENEMBL database showed strong homology to a family of proteins called secretins. Secretins are known to transport macromolecules across the outer membranes of gram-negative bacteria (33). This diverse superfamily includes transporters for type IV pili, for filamentous phage, and for DNA; it has members that belong to type II and III secretion systems (20). Family members share homology in their C-terminal 440 amino acids, which can be subdivided into three parts. The C-terminal 180 amino acids are the most conserved, the middle 120 amino acids are moderately conserved, and the residues 460 to 600 are the least conserved (Fig. 3A). This conserved C-terminal domain includes a signature sequence, (V,I)PXL(S,G)XIPXXGXLF, present in all members of the family (Fig. 3B) (16). The *M. xanthus* sequence includes this signature. The closest matches identified with a BLAST search of the *M. xanthus* sequence were the PilQ proteins of *P. aeruginosa* (35) and *N. gonorrhoeae* (13), 37 and 34% identical over 434 and 430 amino acids, respectively, over the C-terminal region. The gap frequencies were 3.0 and 6.5. The N-terminal region of the secretin family is much less conserved (Fig. 3A) and for that reason is thought to regulate substrate recognition (9). The *P. aeruginosa* and *N. gonorrhoeae* PilQ proteins do contain blocks of similarities to *M. xanthus* PilQ in their N-terminal regions, including 23 and 26% identities over 192 and 128 amino acids (gap frequencies, 3.1 and 5.5%), respectively. In fact, the *P. aeruginosa* PilQ was found to be more similar to *M. xanthus* PilQ than it was to the *N. gonorrhoeae* PilQ.

A Shine-Dalgarno sequence (GAGG) was found 7 bp upstream from the proposed translational start ATG. As has been found for other secretin family members, a putative cleavable signal sequence was identified in the first 29 amino acids by PSORT (discrimination score, 4.33; signal score, 3.05 [Fig. 3]). The resulting mature PilQ protein would be 872 amino acids, making it the largest protein in the secretin family by over 150 amino acids.

***pilQ* mutants.** The rescue data shown in Fig. 1 narrowed the location of the *pilQ1* (*sglA1*) mutation to an approximately 250-bp region. Accordingly, we cloned and sequenced this segment of the DNA from a *pilQ1* mutant. Two closely linked missense mutations were found, one at codon 741 and a second at 762, which would generate Gly→Ser and Asn→Gly amino acid substitutions, respectively (Fig. 2 and 3). The missense

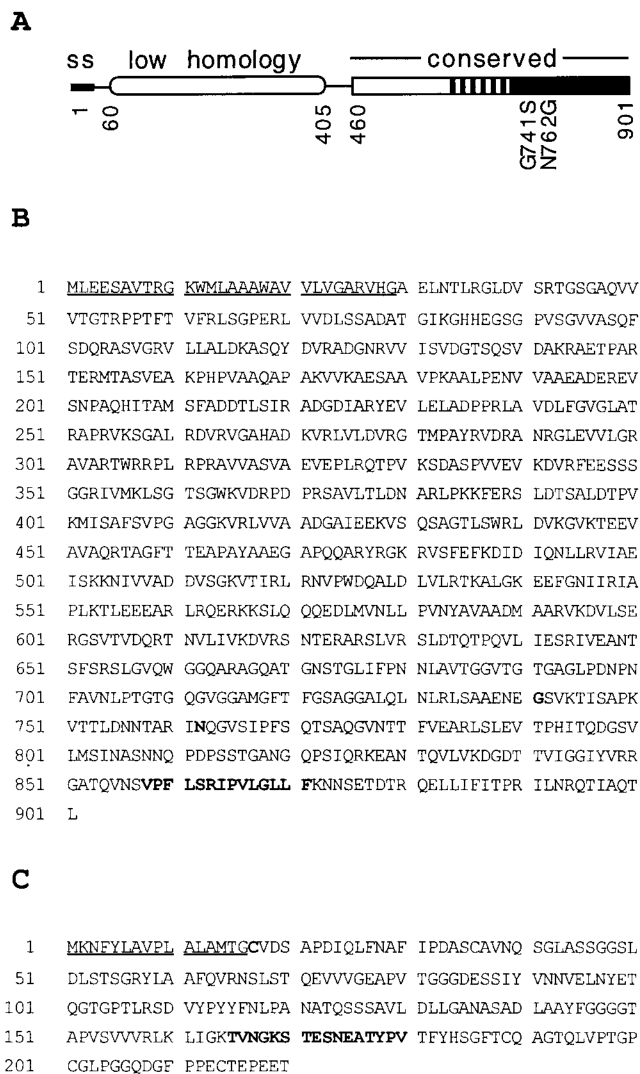

FIG. 3. (A) Modular representation of PilQ. The 29-amino-acid signal sequence (SS), the low-homology region, and the three conserved subdomains are shown. (B) Protein sequence of PilQ. The signal sequence is underlined. The two residues that are changed in PilQ1 are in boldface (residues 741 and 762), as is the signature sequence (V,I)PXL(S,G)XIPXXGXLF. (C) Protein sequence of OrfL. A putative type II signal sequence is underlined, and the signature sequence for a phosphopantetheine attachment site is in boldface.

mutation (G→A) in codon 741 creates a new *Sac*I restriction site (Fig. 2). Both mutations lie in the most highly conserved one-third of the carboxyl end of the protein (Fig. 3). Residues that correspond to 741 and 762 show only limited conservation within the secretin family (9, 16, 35, 44). Some family members contain a Gly-741 and an Asn-762, like the $pilQ^{+}$ allele, in these residues. Other members contain different residues, including the PilQ1 mutant alleles, Ser-741, and/or Gly-762. The natural occurrence of the mutant residues is consistent with the fact that the *pilQ1* allele retains some biological activity. Whether both missense mutations are required to obtain the PilQ1 phenotype is not known.

Hodgkin and Kaiser (22) reported other S-motility mutations which seemed to map to the *sglA* locus by virtue of their transduction linkage to *sglA1*. With the *pilQ* gene in hand, we tested some of these mutants as well as mutants obtained from a more extensive screen for S-motility mutants carried out by

TABLE 2. Rescue of S-motility mutants by *pilQ*

Strain	Rescue by plasmid[a]:						
	pDW168	pDW92	pDW188	pDW81	pDW167	pDW139	pDW169
DK1241		+		+			
DK1242		+	+	−	−	−	−
DK1243		+		+			
DK1247		+	+	−	−	−	−
DK1287	+						−
DK1291		+		+			
DK1609					+		−
DK1627		+	+	−	−	−	−
DK1633	+				+		
DK1636		+		+			
DK1675		+			+		−
DK1687		+		+			
DK2136		+			+		−
DK2140		+	+				−
DK2148		+		+			
DK2149		+		+			
DK2150		+		+			
DK2159	+				+		−
DK2167		+		+			
DK8616 (Δ*pilQ*)		+					

[a] +, able to rescue; −, unable to rescue. pDW168, pDW92, and pDW188 contain the entire *pilQ* gene and flanking DNA. pDW81 and pDW167 contain *pilQ* fragments. pDW169 and pDW139 contain regions upstream and downstream of *pilQ*, respectively. See Table 1 and Fig. 1 for plasmid details.

D. Morandi (unpublished). Both sets of mutants were first tested for cotransduction with Ω2231 (Fig. 1). Mutations found to be linked to Ω2231 and whose cotransduction frequencies suggested that they were in the vicinity of *pilQ* were then tested for the ability of *pilQ*-containing plasmids to rescue their S-motilities. Table 2 summarizes the rescue results for 19 point mutants. Indeed, all of these mutants are rescued by *pilQ* minimal plasmids.

Function of *pilQ*, as deduced from a null mutant. A deletion mutant of *pilQ* was constructed; the deletion was made in frame to avoid potential polar effects. Effects of the Δ*pilQ* mutation on S motility were monitored by constructing the double mutant *aglB1* Δ*pilQ* (DK8616). DK8616 was plated on 1/2 CTT 0.5% agar plates, and as shown in Fig. 4, it failed to swarm. No flares were evident at any time over a 6-day period of observation. DK320 (*aglB1 pilQ1*) also failed to swarm in the absence of $CaCl_2$. The active swarming and flare formation of DK1217 (*aglB1 pilQ*$^+$) are shown at 6 h for comparison (Fig. 4; note the time difference). Even after prolonged incubation (>20 days) flares were never observed, implying a total loss of S motility in DK8616 (Δ*pilQ*). DK320 (*pilQ1*), by contrast, would produce some flares by 20 days (data not shown). Earlier studies have shown that Ca^{2+} is required for gliding motility (57). Although Ca salts are not added to the standard formulations of CTT or 1/2 CTT media, Ca^{2+} is nevertheless present in trace amounts in the agar, casitone, and water that are used for these media. We tested whether Ca^{2+} might be limiting by adding 2 mM $CaCl_2$, and as shown in the bottom row of Fig. 4, the addition of $CaCl_2$ did not dramatically change the swarming of DK1217. However, $CaCl_2$ did enhance the swarming of DK320 (Fig. 4, top row). No swarming of the Δ*pilQ* strain DK8616 was evident with or without $CaCl_2$ addition (Fig. 4, middle row). These results suggest that Ca^{2+} may be limiting in 1/2 CTT agar for a *pilQ1* mutant.

The effect of the Δ*pilQ* mutation on cell movement in an A^- motility background was examined by time lapse microscopy.

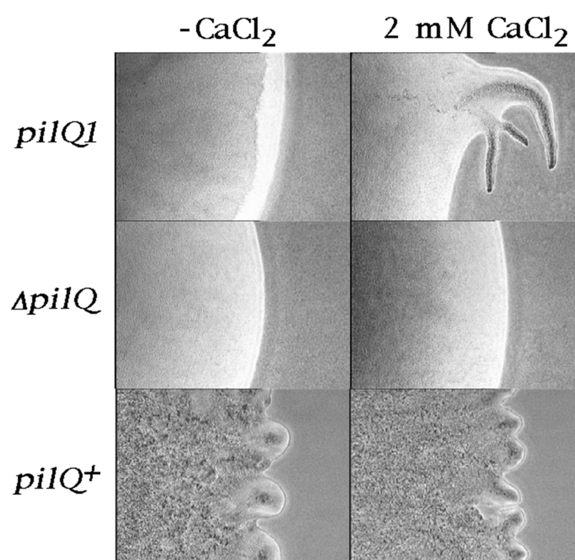

FIG. 4. Swarming in the presence (2 mM $CaCl_2$) and absence ($-CaCl_2$) of $CaCl_2$ on 1/2 CTT 0.5% agar plates. The pictures were taken at 6 days for DK320 (*aglB1 pilQ1*) and DK8616 (*aglB1* Δ*pilQ*) and at 6 h for DK1217 (*aglB1 pilQ*$^+$).

Isolated cells and small groups of 10 to 100 cells were examined over 5-, 10-, and 30-min periods. No longitudinal movement greater than a cell's length was detected in DK8616. Similar results were obtained with other A^- Δ*pil* mutants (61). Hence, in the S-motility system, pili are required not only for macroscopic swarming but also for movement at the cellular level.

The hypomorphic *pilQ1* mutant produces fewer pili than do wild-type cells (23). No pili were evident on DK8616 cells as examined by electron microscopy. The *pilQ* deletion mutant did produce normal levels of pilin, the monomer unit and product of the *pilA* gene, as judged by Western blotting (Fig. 5A). Despite the abundance of pilin, no pili were detected by the sensitive shear assay (55, 59) (Fig. 5B). While Δ*pilQ* mu-

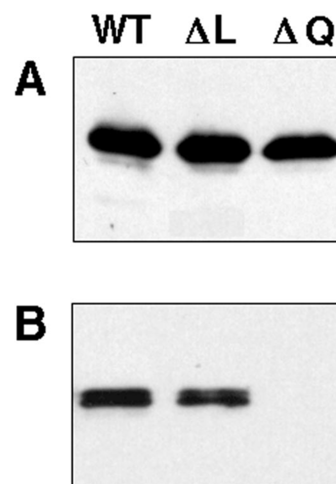

FIG. 5. (A) Western blot for PilA expression from 5×10^6 whole cells. (B) Detection of extracellular pili (PilA) from 2×10^8 cells. The pili were sheared off the cells by passage through a 25-gauge 3.5-inch needle as previously described (59). The strains are DK1622 (WT), DK8613 (ΔL), and DK8615 (ΔQ).

TABLE 3. Sensitivity to vancomycin

Strain	*pil* allele	EOP[a] on vancomycin (20 μg/ml)
DK1217	*pilQ*$^+$	1
DK320	*pilQ1*	6×10^{-4}
DK8611	Δ*pilQ*	1
DK8601	Δ*pilA*	1

[a] EOP, efficiency of plating.

tants make wild-type levels of pilin, they fail to assemble it into filamentous pili.

Studies of the PilQ homolog from *E. coli* bacteriophage f1, called GpIV, have suggested that some secretin point mutants increase the membrane permeability of the cells, so that they are more sensitive to small molecules such as vancomycin (molecular mass, about 1,400 Da) and deoxycholate (DOC), a mild detergent (44, 45). We found that the *pilQ1* mutations did render *M. xanthus* 1,000-fold more sensitive to vancomycin, but the Δ*pilQ* allele did not (Table 3). However, no increase in sensitivity to DOC was found. (It should be noted that wild-type cells are extremely sensitive to DOC; 0.005% is lethal, so it may not be possible to observe a greater sensitivity.) These results show that the *pilQ1* mutation increases the permeability of the outer membrane. Presumably this increased sensitivity results from changes in the multiprotein PilQ channel complex, such that molecules as small as vancomycin can enter the periplasmic space, where they presumably block peptidoglycan synthesis. At higher concentrations *pilQ*$^+$ cells are sensitive to vancomycin, and this antibiotic induces sporulation genes as a consequence of interfering with the recycling of peptidoglycan components (39).

Origin of DK1622. Despite its wide use, a detailed description of the origin of DK1622 has never been published. Although DK1622 is a descendent of DK101 (*pilQ1*), it has a *pilQ*$^+$ allele, resulting in full S-motility, as shown in Fig. 6. DK1622 cells are able to form biofilms and to develop in submerged culture (27). Unlike DK101, DK1622 forms symmetric fruiting bodies, it ripples during development; and it forms fruiting bodies faster than DK101. For these reasons DK1622 is commonly used as a "wild-type" strain. It should be noted, however, that during the construction of DK1622 a 222-kbp deletion occurred that removed several tandem copies of a prophage-like element called Mx alpha without other structural rearrangements, since the physical maps are otherwise identical (5). This deletion may have occurred during the UV irradiation of DK101 used to generate DK320 (22).

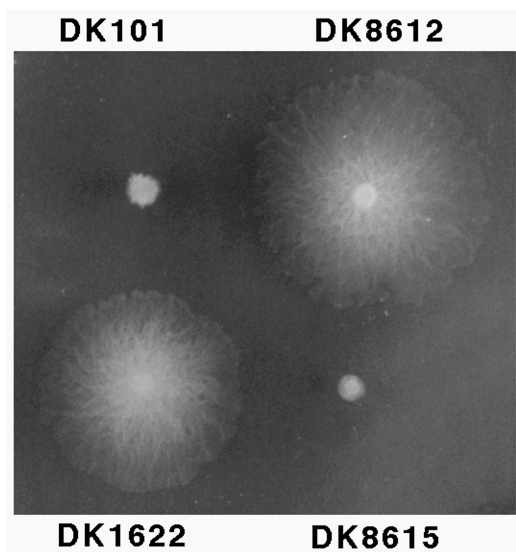

FIG. 6. Role of *pilQ* in swarming. Strains were inoculated on 1/2 CTT 0.5% agar plates and incubated for three days at 33°C. Genotypes: DK101, *pilQ1*; DK8612, *pilQ*$^+$ (isogenic derivative of DK101); DK1622, *pilQ*$^+$; and DK8615, Δ*pilQ* (isogenic derivative of DK1622).

DK320 (*pilQ1*) was rendered *pilQ*$^+$ by Mx8 transduction from a YS (56) (DK1600) donor, thereby generating a strain with full S motility, DK1217 (22; Table 1). DK1217 then served as the recipient for a second Mx8 transduction, again using YS as the donor strain. Transductants were screened for full (A^+ S^+) motility, yielding DK1622 (37a). In the course of our studies we observed that YS (DK1600) was defective in swarming on 0.5% agar plates, where it swarmed slightly faster than DK101 but significantly slower than DK1622. The addition of 2 mM $CaCl_2$ to the agar failed to improve the swarming rate of YS, in contrast to that of DK101. These observations argue that YS contains a mutation in the S-motility system that is different from *pilQ1*.

To identify the mutant locus in YS involved in its S-motility defect, and thereby to clarify the origin of DK1622, we sought to map the S^- mutation in YS. YS was transformed with the overlapping plasmids pDW79 and pSWU257 (58), which together cover the entire known *pil* region. Both plasmids were found to rescue the S-motility defect of YS. These plasmids overlap in a 3.4-kbp region, which contains the *pilG*, *-H*, *-I*, and *-D* genes (Fig. 1). Additional transformants were made to map the YS mutation: plasmid pSWU449 was found to rescue the motility defect of YS, while pSWU402 (60) could not. Thus, the YS mutation can be in either the *pilG* or *-H* gene or both. As shown in Fig. 1, the minimum distance between a *pilG* or *-H* mutation and *pilQ1* is 10 kbp, a distance sufficiently large that a *pilQ*$^+$ transductant from YS would not necessarily receive the *pilG* or *-H* mutation at the same time.

Role of *pilQ* in development. S-motility is necessary for rippling, and it plays an important role in fruiting body development (22, 50, 60). The specific effects of the Δ*pilQ* mutation on development were examined. Figure 7 shows that the aggregation stage of development was greatly delayed in the Δ*pilQ* mutant DK8615: At 72 h, aggregates appeared, with structures similar to those seen 60 h earlier (at 6 to 10 h) in wild-type cells. These aggregates never developed into dark fruiting bodies (Fig. 7). On hard (1.5%) agar, A-motility dominates (Fig. 7, top). On soft (0.5%) agar, S-motility dominates (48). Figure 7 (bottom) shows that aggregation and fruiting body formation were completely blocked in the Δ*pilQ* strain on soft agar, nor did ripples ever form. These results suggest that under certain conditions, i.e., hard agar, A-motility can partially substitute for the lack of S-motility.

Previously the *pilQ1* mutation was found to reduce the developmental expression of the myxobacterial hemagglutinin (MBHA) protein about eightfold (7). Here the effect of the Δ*pilQ* mutation on the expression of two other developmentally regulated reporter fusions, Ω4414 and Ω4401 (26), was examined. Figure 8 shows that the expression of Ω4414, whose expression normally begins at 6 h, was reduced two- to fivefold over the course of development in a Δ*pilQ* background. In contrast, the Δ*pilQ* mutation did not appreciably affect the expression of Ω4401, whose expression starts at the beginning of sporulation (24 h). Interestingly, the Δ*pilQ* mutant sporulated at slightly higher levels than the parental DK1622 strain. Other *pil* null mutants have also been shown to sporulate at ~2-fold-higher levels than wild-type cells (60). This increase in

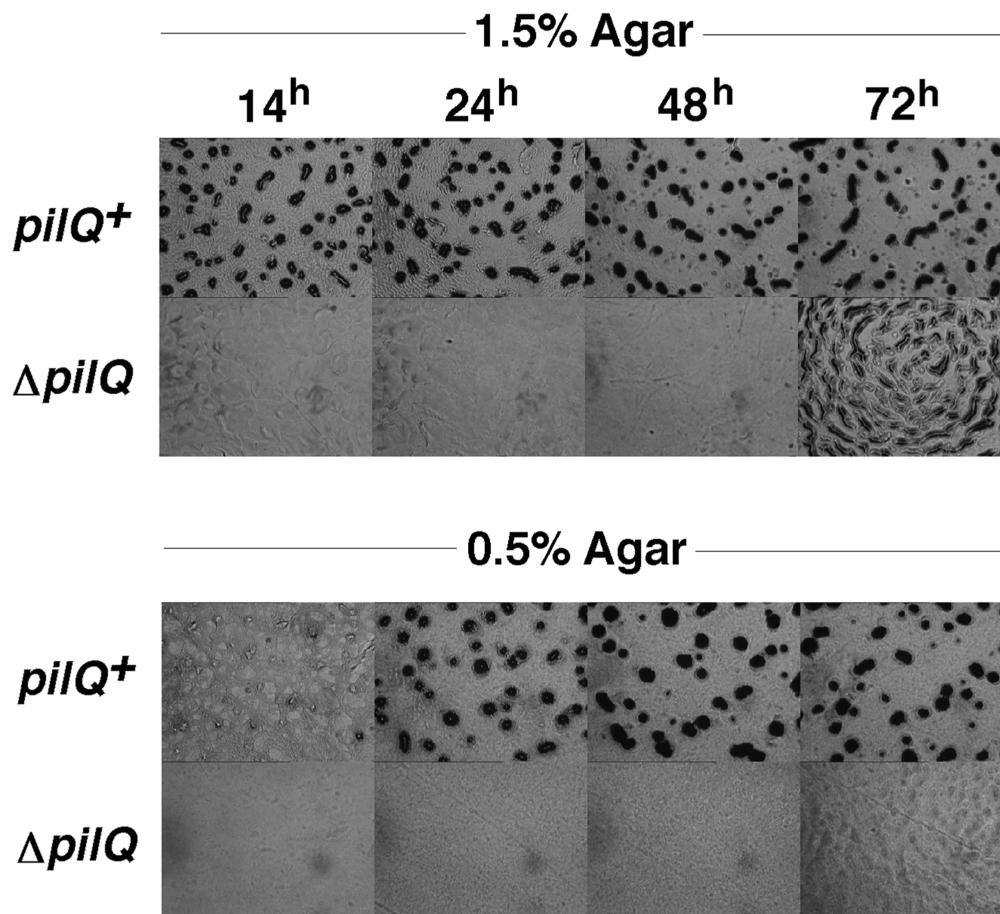

FIG. 7. Development on CF. DK1622 (*pilQ*$^+$) and DK8615 (Δ*pilQ*) were placed on 1.5 or 0.5% CF agar plates, and development was monitored over three days as indicated.

sporulation may be artifactual, since spores are more easily dispersed from *pil* mutants than from *pil*$^+$ cells, which could increase the titer of viable spores.

DK101 (*pilQ1*) forms fruiting bodies, though 1 day later than DK1622, and it ripples infrequently and less extensively. A *pilQ*$^+$ isogenic derivative of DK101 was constructed by the *galK* counterselection method with a *pilQ*$^+$ fragment from pDW140 (DK8612), and it was shown to be fully S-motile (Fig. 6). As illustrated in Fig. 9, DK8612 has a higher propensity to ripple than its parent, DK101. Though restored for S-motility and rippling, DK8612 remained unable to form fruiting bodies with the speed and proficiency of DK1622; it was slow, like DK101 (data not shown).

orfL. Thirty-nine base pairs downstream of *pilQ* is the 220-amino-acid ORF *orfL*. A Shine-Dalgarno site (GGAG) was found 12 bp upstream from its putative translational start ATG. OrfL shows no significant sequence homology to any other protein in the GENEMBL database. However, *orfL* does contain a type II signal sequence (discrimination score, 4.3), suggesting that it may encode a lipoprotein. A lipid moiety would be predicted to be attached to Cys-17 (Fig. 3C). Near the C terminus of OrfL there is a signature sequence for a phosphopantetheine attachment site (Fig. 3C). Phosphopantothenate is the prosthetic group of acyl carrier proteins in some multienzyme complexes, where it functions as a "swing arm" for the attachment of activated fatty acids and amino acid groups. These enzymes produce diverse products, such as

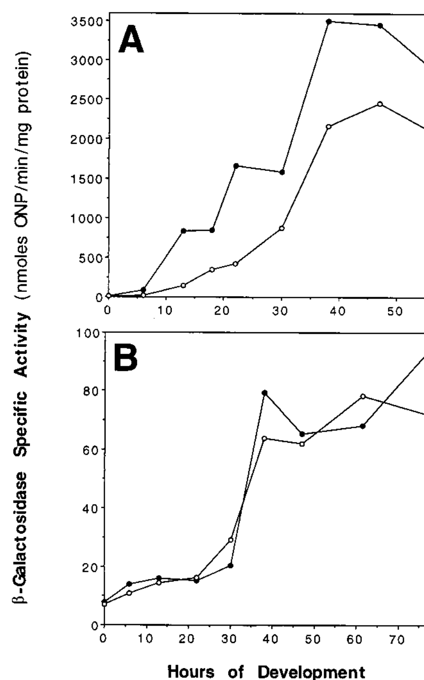

FIG. 8. Developmental expression of Ω4414 (A) and Ω4401 (B) on TPM (1.5%) agar. Reporter β-galactosidase transcriptional fusions are in DK1622 (●) or DK8615 (○). ONP, *o*-nitrophenol.

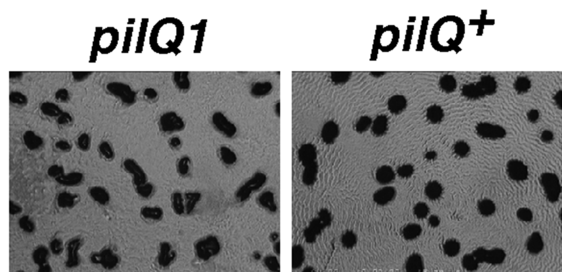

FIG. 9. Rippling on CF agar. DK101 (*pilQ1*) and DK8612 (*pilQ*$^+$) were placed on CF (1.5%) agar at a cell density of 1,000 Klett units and allowed to develop for 5 days at 27°C.

polyketide antibiotics and nodulation factor for *Rhizobium* species. In OrfL the putative pantetheine attachment site is Ser-170 (Fig. 3C).

To ascertain whether OrfL plays a role in S-motility, type IV pilus biogenesis, or fruiting body development, an in-frame deletion of *orfL* was constructed. When the Δ*orfL* allele was introduced into DK1217 (A$^-$ S$^+$), the resulting strain, DK8614, was motile and displayed normal S-motility under a variety of environmental conditions (data not shown). DK8614 was checked for expression of PilA and pilus production; it was similar to the parental strain in both respects (Fig. 5). To test whether the Δ*orfL* allele had any effect on development, it was introduced in DK1622, generating DK8613. This strain exhibited normal fruiting body formation on TPM and CF agars; both rippling and sporulation were at wild-type levels.

Protein secretion. *M. xanthus* secretes many proteins and is one of the most active secreters among gram-negative bacteria (17). Protein secretion appears to be required for (i) transporting catabolic enzymes into the medium for vegetative growth, (ii) intercellular signaling, and (iii) production and assembly of type IV pili. In gram-negative bacteria, type II and III secretion systems are major pathways for protein transport. Both of these pathways employ secretins. In *P. aeruginosa*, there is some overlap between type II secretion and type IV pilus production. The same protein, PilD/XcpA, is used by both systems as a signal peptidase, for example (38). In addition, secretion of type II-dependent proteins is decreased by a *pilA* mutation (31). To test whether a Δ*pilQ* mutation had an effect on protein secretion in *M. xanthus*, the spectrum of proteins secreted into the medium was examined. Figure 10 shows the protein composition of concentrated [^{35}S]methionine-cysteine-labeled culture supernatants separated by PAGE. The Δ*pilQ* mutant had an amount and profile of proteins similar to those of the *pilQ*$^+$ culture when grown in CTT-rich medium (casitone has low levels of methionine and cysteine). The Δ*pilQ* supernatant did contain a >100-kDa protein that was absent in the *pilQ*$^+$ supernatant. When these identical cultures were shifted from CTT to A1 minimal medium (3) for 6 h, there were significant changes in the patterns of proteins found in the culture supernatants. Some proteins were more abundant in A1, e.g., those at 17, 31, 51, 62, and ~120 kDa, while others decreased, e.g., those at 24, 36, 38, and 45 kDa. Compared to growth in CTT, greater differences between *pilQ*$^+$ and Δ*pilQ* strains were seen in A1. Six proteins at 31, 32, 50, 55, 58, and 84 kDa were more abundant, while the protein(s) at ~120 kDa was less abundant in Δ*pilQ* supernatants. In *N. gonorrhoeae*, *pilQ* mutations result in increased levels of PilC (~105 kDa) and S-pilin (soluble truncated pilin, ~16 kDa) in culture supernatants (13, 14). Perhaps some of these more abundant proteins from *M. xanthus* Δ*pilQ* supernatants are Pil proteins.

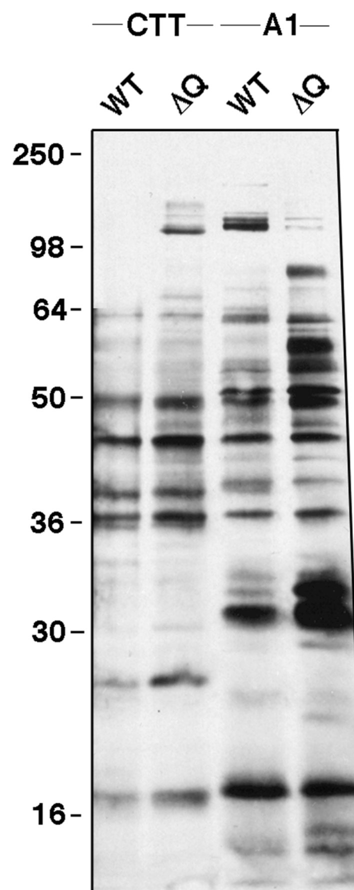

FIG. 10. Profile of proteins found in culture supernatants. DK1622 (WT) and DK8615 (ΔQ) cells were grown in either CTT or A1 minimal medium with Tran^{35}S-label. Culture supernatants were precipitated with trichloroacetic acid, and protein samples were separated by SDS–12% PAGE. The positions of molecular mass standards (in kilodaltons) are given.

DISCUSSION

We have shown that *pilQ* (*sglA*) encodes a secretin homolog. Secretins are an evolutionarily conserved superfamily of proteins involved in macromolecular transport across the outer membrane in three different secretion systems. Secretins are the only proteins common to type II (including type IV pili), type III, and filamentous phage secretion systems, suggesting that they play a fundamental role (33). In all three systems secretins are found in the outer membrane, where they form ring structures of 10 to 18 secretin subunits. These cylindrical structures, as visualized by electron microscopy, have central cavities ranging in size from 50 to 95 Å (1, 25, 30). Such cavities are large enough to accommodate the transport of folded proteins and assembled macromolecular complexes, such as filamentous phage (diameter, 65 Å) or type IV pili (diameter, ~52 Å). Our evidence that Δ*pilQ* mutants lack pili and that *pilQ1* mutants are hypersensitive to vancomycin also suggests that PilQ functions as a channel for type IV pilus export. In *M. xanthus* these polar pili have been observed to extend from "polar holes" (32). It is tempting to speculate that these polar holes are multimeric PilQ channels.

Multimerization of secretin subunits in the outer membrane requires assembly factors or chaperones. For example, the secretins PulD and OutD require their cognate assembly factors, PulS and OutS, for localization and assembly in the outer

membrane (8, 18, 47). The PulS and OutS lipoproteins bind to the C-terminal 65 and 62 amino acids of PulD and OutD. *M. xanthus* PilQ protein does not have a C-terminal binding sequence, nor has a PulS or OutS homolog been identified in *M. xanthus*. Instead, multimerization of PilQ may be mediated by a PilP-like lipoprotein, as has been shown for *N. gonorrhoeae* (14). Upstream of the *M. xanthus pilQ* gene is an ORF that shows homologies to *N. gonorrhoeae* and *P. aeruginosa pilP* (unpublished data). Another candidate for a protein that interacts with PilQ is the Tgl lipoprotein (54), which contains six tandem tetratricopeptide repeats (43), motifs which are known to mediate protein-protein interactions (11). In *M. xanthus* we are interested in how these three proteins might interact.

Secretins can serve as signals for the induction of stress genes. During the course of filamentous bacteriophage (e.g., f1 or M13) infection in *E. coli* or overproduction of the phage secretin GpIV, it was discovered that an operon called *pspABCE* (for phage shock protein) is induced (reviewed in reference 37). This σ^{54}-dependent operon is also specifically induced by the expression of other heterologous secretins, starvation, osmotic shock, heat stress, or ethanol stress (18, 37). Mutations in *psp* result in a loss of viability during stationary phase, and the mutants have defects in protein transport and maintaining membrane potential (37). Model and coworkers explain a diverse set of results by proposing that the secretin signal for *psp* induction is the process of insertion and assembly of a secretin in the outer membrane (37). Conditions which render this insertion process slow or inefficient or which mislocalize it lead to amplification of the signal for *psp* induction. In *M. xanthus* these findings are of interest because many differences have been found between $pilQ^+$ and *pilQ1* strains during development. For example, *frz* (*che* homolog) mutants are defective in sporulation in a $pilQ^+$ background but sporulate at wild-type levels in a *pilQ1* background (24). To explain this suppression, perhaps the PilQ1 mutant protein results in a *psp*-like induction of stress genes, which could compensate for the *frz* sporulation defect.

Fruiting body development depends on cell-cell signaling (6, 19, 28, 29) and on cell movement. Mutational defects in *pilQ* retard aggregation (Fig. 7) by eliminating S-motility. A decrease in the efficiency of C signaling is evident in the decreased expression of the Ω4414 reporter (Fig. 8A). The developmental defects of *asg* and *dsg* mutants worsen in a *pilQ1* background (28). In that background (DK101) *asgB* and *asgC* mutants fruit poorly and produce only 10% as many viable spores as the parental strain (28). In a $pilQ^+$ (DK1622) background, *asgB* and *asgC* mutants sporulate at 43 and 100% efficiencies relative to their parental strain and their morphological defects are less severe. A-factor, which requires *asg* genes, is a set of eight amino acids which are released by the action of extracellular proteases on extracellular proteins (28, 29, 41). Enhancement of the *asg* defect suggests that the PilQ secretin may be involved in releasing peptides, proteins, and proteases from the cell, and hence in A-factor production. If there were such a defect, then when the *pilQ1* allele is combined with *asgB* or *asgC* mutations it could exaggerate their developmental defects.

In addition, *dsg* mutants fail to form fruiting bodies in a *pilQ1* background and their ability to sporulate is reduced >10,000-fold (6). However, in a $pilQ^+$ background *dsg* mutants can sporulate at wild-type levels, though aggregation is delayed. Recently, it has been suggested that the developmental block in *dsg* mutants is not related to a new signaling molecule but instead is a result of lower A-factor levels (6a). Thus, similarly to *asg* mutants, *dsg* would fail to develop due to the secretion defect of *pilQ1*.

Several caveats relating to genetic interactions with *pilQ* should be mentioned. First, *pilQ1* mutants have pleotropic defects, including defects in piliation, S-motility, cell cohesiveness, and permeability properties. Any one or a combination of these defects could have indirect effects on other mutations. Second, the strains used, e.g., DK1622 and DK101 (or DZF1 and DZ2), are less isogenic (see "Origin of DK1622" in Results) than DK101 and DK8612 or DK1622 and DK8615. Third, the molecular natures of the *pilQ* alleles were not previously defined. Here we have constructed an in-frame deletion mutant, sequenced the *pilQ1* mutations, and identified 19 additional *pilQ* alleles. Hopefully, these new alleles and the construction of DK8612 and DK8615 will facilitate understanding the genetic relationships between *pilQ* and other properties of the cell.

Our characterization of PilQ extends the striking similarities between proteins involved in S-motility and those required for twitching motility in *P. aeruginosa* and *N. gonorrhoeae* (53, 58). Additionally, we have found S-motility genes upstream of *pilQ* (downstream of *pilD*) which are homologous to the *pilM*, *-N*, *-O*, and *-P* genes from *Pseudomonas* sp. and *N. gonorrhoeae* (references 14 and 34 and unpublished data). No S-motility mutants or *pil* ORFs have been found downstream of *pilQ*, suggesting that *pilQ* is at the end of the *pil* cluster. Extensive screens for genes required for S-motility have yielded 160 mutants (22, 37a). About 100 of these mutations map to the *pil* cluster described here, and another 7 map to the *tgl* locus (reference 43 and unpublished data), which is also required for pilus assembly. This screen may be approaching saturation, since many of the new mutations are falling into known S-motility genes, e.g., 20 independent mutations map to *pilQ*, 4 map to *pilA* (58), 5 map to *pilT* (61), and the aforementioned 7 map to *tgl*. The genomes of *P. aeruginosa* and *N. gonorrhoeae* are sequenced, and at least in the case of *P. aeruginosa*, a near-saturation screen for twitching motility genes has been completed. Almost all of the twitching genes are either *pil* genes, transcriptional regulators, or, in the case of *P. aeruginosa*, signal transduction genes, i.e., *frz* and *che* homologs (10, 36) (*N. gonorrhoeae* has no obvious *che* homologs). Thus, type IV pilus genes are the major genetic determinant for S-motility and twitching motility. Future work with *M. xanthus* will be aimed at understanding how *pil* gene products interact and how they contribute to S-motility.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Science Foundation (MCB 9423182) to D.K. D.W. was a recipient of an American Cancer Society postdoctoral fellowship (PF-4138).

REFERENCES

1. **Bitter, W., M. Koster, M. Latijnhouwers, H. de Cock, and J. Tommassen.** 1998. Formation of oligomeric rings by XcpQ and PilQ, which are involved in protein transport across the outer membrane of *Pseudomonas aeruginosa*. Mol. Microbiol. **27:**209–219.
2. **Bradley, D. E.** 1980. A function of *Pseudomonas aeruginosa* PAO polar pili: twitching motility. Can. J. Microbiol. **26:**146–154.
3. **Bretscher, A. P., and D. Kaiser.** 1978. Nutrition of *Myxococcus xanthus*, a fruiting myxobacterium. J. Bacteriol. **133:**763–768.
4. **Chang, B. Y., and M. Dworkin.** 1994. Isolated fibrils rescue cohesion and development in the Dsp mutant of *Myxococcus xanthus*. J. Bacteriol. **176:** 7190–7196.
5. **Chen, H. W., A. Kuspa, I. M. Keseler, and L. J. Shimkets.** 1991. Physical map of the *Myxococcus xanthus* chromosome. J. Bacteriol. **173:**2109–2115.
6. **Cheng, Y., and D. Kaiser.** 1989. *dsg*, a gene required for cell-cell interaction early in *Myxococcus* development. J. Bacteriol. **171:**3719–3726.

6a. **Cheng, Y., A. Kuspa, and D. Kaiser.** Unpublished data.

7. **Cumsky, M., and D. R. Zusman.** 1979. Myxobacterial hemagglutinin: a development-specific lectin of *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **76:**5505–5509.

8. **Daefler, S., I. Guilvout, K. R. Hardie, A. P. Pugsley, and M. Russel.** 1997. The C-terminal domain of the secretin PulD contains the binding site for its cognate chaperone, PulS, and confers PulS dependence on pIV^{f1} function. Mol. Microbiol. **24:**465–475.
9. **Daefler, S., M. Russel, and P. Model.** 1997. Module swaps between related translocator proteins pIV(f1), pIV(IKe) and PulD: identification of a specificity domain. J. Mol. Biol. **266:**978–992.
10. **Darzins, A., and M. A. Russell.** 1997. Molecular genetic analysis of type-4 pilus biogenesis and twitching motility using *Pseudomonas aeruginosa* as a model system—a review. Gene **192:**109–115.
11. **Das, A. K., P. T. W. Cohen, and D. Barford.** 1998. The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. EMBO J. **17:**1192–1199.
12. **Davies, D. G., M. R. Parsek, J. P. Pearson, B. H. Iglewski, J. W. Costerton, and E. P. Greenberg.** 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science **280:**295–298.
13. **Drake, S. L., and M. Koomey.** 1995. The product of the pilQ gene is essential for the biogenesis of type IV pili in *Neisseria gonorrhoeae*. Mol. Microbiol. **18:**975–986.
14. **Drake, S. L., S. A. Sandstedt, and M. Koomey.** 1997. PilP, a pilus biogenesis lipoprotein in *Neisseria gonorrhoeae*, affects expression of PilQ as a high-molecular-mass multimer. Mol. Microbiol. **23:**657–668.
15. **Dworkin, M.** 1962. Nutritional requirements for vegetative growth of *Myxococcus xanthus*. J. Bacteriol. **84:**250.
16. **Genin, S., and C. A. Boucher.** 1994. A superfamily of proteins involved in different secretion pathways in gram-negative bacteria: modular structure and specificity of the N-terminal domain. Mol. Gen. Genet. **243:**112–118.
17. **Guespin-Michel, J. F., B. Letouvet-Pawlak, and F. Petit.** 1993. Protein secretion in *Myxobacteria*, p. 235–255. *In* M. Dworkin and D. Kaiser (ed.), Myxobacteria, vol. II. American Society for Microbiology, Washington, D.C.
18. **Hardie, K. R., S. Lory, and A. P. Pugsley.** 1996. Insertion of an outer membrane protein in *Escherichia coli* requires a chaperone-like protein. EMBO J. **15:**978–988.
19. **Hartzell, P. L., and P. Youderian.** 1995. Genetics of gliding motility and development in *Myxococcus xanthus*. Arch. Microbiol. **164:**309–323.
20. **Hobbs, M., and J. S. Mattick.** 1993. Common components in the assembly of type 4 fimbriae, DNA transfer systems, filamentous phage, and protein-secretion apparatus: a general system for the formation of surface-associated protein complexes. Mol. Microbiol. **10:**233–243.
20a. **Hodgkin, J.** Unpublished data.
21. **Hodgkin, J., and D. Kaiser.** 1977. Cell-to-cell stimulation of movement in nonmotile mutants of *Myxococcus*. Proc. Natl. Acad. Sci. USA **74:**2938–2942.
22. **Hodgkin, J., and D. Kaiser.** 1979. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): two gene systems control movement. Mol. Gen. Genet. **171:**177–191.
23. **Kaiser, D.** 1979. Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **76:**5952–5956.
24. **Kashefi, K., and P. L. Hartzell.** 1995. Genetic suppression and phenotypic masking of a *Myxococcus xanthus frzF*$^{-}$ defect. Mol. Microbiol. **15:**483–494.
25. **Koster, M., W. Bitter, H. de Cock, A. Allaoui, G. R. Cornelis, and J. Tommassen.** 1997. The outer membrane component, YscC, of the Yop secretion machinery of *Yersinia enterocolitica* forms a ring-shaped multimeric complex. Mol. Microbiol. **26:**789–797.
26. **Kroos, L., A. Kuspa, and D. Kaiser.** 1986. A global analysis of developmentally regulated genes in *Myxococcus xanthus*. Dev. Biol. **117:**252–266.
27. **Kuner, J. M., and D. Kaiser.** 1982. Fruiting body morphogenesis in submerged cultures of *Myxococcus xanthus*. J. Bacteriol. **151:**458–461.
28. **Kuspa, A., and D. Kaiser.** 1989. Genes required for developmental signalling in *Myxococcus xanthus*: three asg loci. J. Bacteriol. **171:**2762–2772.
29. **LaRossa, R., J. Kuner, D. Hagen, C. Manoil, and D. Kaiser.** 1983. Developmental cell interactions of *Myxococcus xanthus*: analysis of mutants. J. Bacteriol. **153:**1394–1404.
30. **Linderoth, N. A., M. N. Simon, and M. Russel.** 1997. The filamentous phage pIV multimer visualized by scanning transmission electron microscopy. Science **278:**1635–1638.
31. **Lu, H. M., S. T. Motley, and S. Lory.** 1997. Interactions of the components of the general secretion pathway: role of *Pseudomonas aeruginosa* type IV pilin subunits in complex formation and extracellular protein secretion. Mol. Microbiol. **25:**247–259.
32. **MacRae, T. H., W. J. Dobson, and H. D. McCurdy.** 1977. Fimbriation in gliding bacteria. Can. J. Microbiol. **23:**1096–1108.
33. **Manning, P. A., and T. F. Meyer.** 1997. Type-4 pili: biogenesis, adhesins, protein export and DNA import. Proceedings of a workshop. Gene **192:**1–198.
34. **Martin, P. R., A. A. Watson, T. F. McCaul, and J. S. Mattick.** 1995. Characterization of a five-gene cluster required for the biogenesis of type 4 fimbriae in *Pseudomonas aeruginosa*. Mol. Microbiol. **16:**497–508.
35. **Martin, P. R., M. Hobbs, P. D. Free, Y. Jeske, and J. S. Mattick.** 1993. Characterization of *pilQ*, a new gene required for the biogenesis of type IV fimbriae in *Pseudomonas aeruginosa*. Mol. Microbiol. **9:**857–868.
36. **Mattick, J. S., C. B. Whitchurch, and R. A. Alm.** 1996. The molecular genetics of type-4 fimbriae in *Pseudomonas aeruginosa*—a review. Gene **179:**147–155.
37. **Model, P., G. Jovanovic, and J. Dworkin.** 1997. The *Escherichia coli* phage-shock-protein (psp) operon. Mol. Microbiol. **24:**255–261.
37a. **Morandi, D.** Unpublished data.
38. **Nunn, D. N., and S. Lory.** 1992. Components of the protein-excretion apparatus of *Pseudomonas aeruginosa* are processed by the type IV prepilin peptidase. Proc. Natl. Acad. Sci. USA **89:**47–51.
39. **O'Connor, K. A., and D. R. Zusman.** 1997. Starvation-independent sporulation in *Myxococcus xanthus* involves the pathway for beta-lactamase induction and provides a mechanism for competitive cell survival. Mol. Microbiol. **24:**839–850.
40. **O'Toole, G. A., and R. Kolter.** 1998. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. **30:**295–304.
41. **Plamann, L., A. Kuspa, and D. Kaiser.** 1992. Proteins that rescue A-signal-defective mutants of *Myxococcus xanthus*. J. Bacteriol. **174:**3311–3318.
42. **Pugsley, A. P.** 1993. The complete general secretory pathway in gram-negative bacteria. Microbiol. Rev. **57:**50–108.
43. **Rodriguez-Soto, J. P., and D. Kaiser.** 1997. The *tgl* gene: social motility and stimulation in *Myxococcus xanthus*. J. Bacteriol. **179:**4361–4371.
44. **Russel, M.** 1994. Mutants at conserved positions in gene IV, a gene required for assembly and secretion of filamentous phages. Mol. Microbiol. **14:**357–369.
45. **Russel, M., N. A. Linderoth, and A. Sali.** 1997. Filamentous phage assembly: variation on a protein export theme. Gene **192:**23–32.
46. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
47. **Shevchik, V. E., J. Robert-Baudouy, and G. Condemine.** 1997. Specific interaction between OutD, an *Erwinia chrysanthemi* outer membrane protein of the general secretory pathway, and secreted proteins. EMBO J. **16:**3007–3016.
48. **Shi, W., and D. R. Zusman.** 1993. The two motility systems of *Myxococcus xanthus* show different selective advantages on various surfaces. Proc. Natl. Acad. Sci. USA **90:**3378–3382.
49. **Shimkets, L. J.** 1986. Correlation of energy-dependent cell cohesion with social motility in *Myxococcus xanthus*. J. Bacteriol. **166:**837–841.
50. **Shimkets, L. J., and D. Kaiser.** 1982. Induction of coordinated movement of *Myxococcus xanthus* cells. J. Bacteriol. **152:**451–461.
51. **Spratt, B. G., P. J. Hedge, S. te Heesen, A. Edelman, and J. K. Broome-Smith.** 1986. Kanamycin-resistant vectors that are analogues of plasmids pUC8, pUC9, pEMBL8 and pEMBL9. Gene **41:**337–342.
52. **Ueki, T., S. Inouye, and M. Inouye.** 1996. Positive-negative KG cassettes for construction of multi-gene deletions using a single drug marker. Gene **183:**153–157.
53. **Wall, D., and D. Kaiser.** Type IV pili and cell motility. Mol. Microbiol., in press.
54. **Wall, D., and D. Kaiser.** 1998. Alignment enhances the cell-to-cell transfer of pilus phenotype. Proc. Natl. Acad. Sci. USA **95:**3054–3058.
55. **Wall, D., S. S. Wu, and D. Kaiser.** 1998. Contact stimulation of Tgl and type IV pili in *Myxococcus xanthus*. J. Bacteriol. **180:**759–761.
56. **Wireman, J. W., and M. Dworkin.** 1975. Morphogenesis and developmental interactions in myxobacteria. Science **189:**516–523.
57. **Womack, B. J., D. F. Gilmore, and D. White.** 1989. Calcium requirement for gliding motility in myxobacteria. J. Bacteriol. **171:**6093–6096.
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., and D. Kaiser.** 1997. Regulation of expression of the *pilA* gene of *Myxococcus xanthus*. J. Bacteriol. **179:**7748–7758.
60. **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 motility in *Myxococcus xanthus*. Mol. Microbiol. **29:**1249–1261.
61. **Wu, S. S., J. Wu, and D. Kaiser.** 1997. The *Myxococcus xanthus pilT* locus is required for social gliding motility although pili are still produced. Mol. Microbiol. **23:**109–121.
62. **Wu, T. T.** 1966. A model for three-point analysis of random general transduction. Genetics **54:**405–410.