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DOI: 10.1534/genetics.105.050542

Transposon Insertions of *magellan-4* That Impair Social Gliding Motility in *Myxococcus xanthus*

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Manuscript received September 2, 2005
Accepted for publication October 31, 2005

ABSTRACT

Myxococcus xanthus has two different mechanisms of motility, adventurous (A) motility, which permits individual cells to glide over solid surfaces, and social (S) motility, which permits groups of cells to glide. To identify the genes involved in S-gliding motility, we mutagenized a Δ*aglU* (A^-) strain with the defective transposon, *magellan-4*, and screened for S^- mutants that form nonmotile colonies. Sequence analysis of the sites of the *magellan-4* insertions in these mutants and the alignment of these sites with the *M. xanthus* genome sequence show that two-thirds of these insertions lie within 27 of the 37 nonessential genes known to be required for social motility, including those necessary for the biogenesis of type IV pili, exopolysaccharide, and lipopolysaccharide. The remaining insertions also identify 31 new, nonessential genes predicted to encode both structural and regulatory determinants of S motility. These include three tetratricopeptide repeat proteins, several regulators of transcription that may control the expression of genes involved in pilus extension and retraction, and additional enzymes involved in polysaccharide metabolism. Three insertions that abolish S motility lie within genes predicted to encode glycolytic enzymes, suggesting that the signal for pilus retraction may be a simple product of exopolysaccharide catabolism.

MYXOCOCCUS xanthus is a soil bacterium that can glide over solid surfaces without the use of flagella. The genetic analysis of its ability to glide shows that *M. xanthus* has two different sets of genes involved in two very different mechanisms of motility. Most single mutations affect the ability of *M. xanthus* to glide either as single cells or as groups of cells, but not as both. General searches for mutants of *M. xanthus* with motility defects have shown that single-cell (adventurous, A) motility and group-dependent (social, S) motility are genetically separable and that each type of motility requires the functions of large, nonoverlapping sets of genes (Hodgkin and Kaiser 1979a,b; MacNeil *et al.* 1994a,b; Youderian *et al.* 2003). Mutations in only three genes, *mglA*, which encodes a small GTPase (Stephens and Kaiser 1987; Stephens *et al.* 1989; Hartzell and Kaiser 1991a,b; Hartzell 1997), *agmA*, which is predicted to encode a critical amidase involved in cell wall biogenesis (Youderian *et al.* 2003), and *epsI/nla24*, predicted to encode an activator of transcription (Caberoy *et al.* 2003; Lancero *et al.* 2004; Lu *et al.* 2005), have been shown to abolish both mechanisms of motility simultaneously.

Mutants defective in A motility form colonies with groups of motile cells, but without individual motile cells, at their edges. Adventurous motility in *M. xanthus* requires the functions of at least 30 different genes, many of which have no known homologs in nongliding bacteria (Youderian *et al.* 2003). Loss-of-function mutations in each of these genes do not affect social motility. Although it has been proposed that nozzles that cluster at the cell poles may generate force by directional secretion of polymer (Wolgemuth *et al.* 2002), genetic and biochemical support for this model is forthcoming. In contrast, social motility in *M. xanthus* involves the interaction between two organelles, type IV pili and exopolysaccharide/fibril material, and is much better understood.

Mutants defective in S motility form colonies with individual motile cells, but without groups of motile cells, at their edges. Social motility results from a cycle in which the cell extends a type IV pilus, the pilus tip attaches to a receptor, and the pilus is retracted (Wu and Kaiser 1995; Merz *et al.* 2000; Sun *et al.* 2000; Skerker and Berg 2001; Li *et al.* 2003). This mechanism appears to be conserved among diverse Gram-negative bacteria, including *M. xanthus*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* (see Mattick 2002, for review). For *M. xanthus*, the substrate for pilus attachment is presented on the surface of a partner (receptor) cell within about one cell length of a pilus-producing cell (Hodgkin and Kaiser 1977; Wu and Kaiser 1995). Previous biochemical studies have shown that peritrichous fibrils constitute the receptor organelle required for S motility; these are composed of both protein and exopolysaccharide (EPS) components (Behmlander and Dworkin 1991; Behmlander and Dworkin 1994).

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The EPS component, but not the protein component, of fibrils appears to be essential for social motility and cell–cell adhesion. Mutants defective in EPS production are defective in S motility (Lu *et al.* 2005), and EPS stimulates pilus retraction (Li *et al.* 2003).

Our current understanding of S motility is missing several key elements. Mutants defective in lipopolysaccharide (LPS) production also are defective in S motility (Bowden and Kaplan 1998; Yang *et al.* 2000a); however, as yet, we do not know what role LPS plays in the mechanism of social motility. Also, we do not understand the molecular mechanism of pilus extension and retraction, nor have we identified the full set of genes encoding the cytoplasmic and membrane components of this molecular motor. Other important details missing from our picture of the mechanism of S motility in *M. xanthus* include an understanding of how the signals that trigger pilus retraction are generated and transduced upon the contact of a pilus-bearing cell with a receptor-bearing cell, how the next cycle of pilus extension and retraction is reinitiated after cell–cell contact, and what the signals are precisely for pilus extension and retraction. It is not known whether the cycle of pilus extension and retraction involves the polymerization and depolymerization of the pilus. To begin to define the genes encoding products involved in these processes, we have conducted an extensive screen for mutants with transposon insertions in the nonessential genes required for S motility.

MATERIALS AND METHODS

Bacterial strains and growth conditions: The *M. xanthus* strains we have generated in this study are derivatives of the wild-type strain DK1622, and its A^- mutant derivative MxH1777 (Δ*aglU*) (White and Hartzell, 2000), and are listed in Table 1. *M. xanthus* was grown at 32° in CTPM liquid medium (1% casitone, 10 mm Tris pH 7.6, 1 mm potassium phosphate pH 7.5, 5 mm $MgSO_4$) and CTPM agar (1.5%) plates; CTPM was supplemented with kanamycin (Km; 40 μg/ml). Plasmids were introduced into *M. xanthus* by electroporation (Kashefi and Hartzell 1995; Youderian *et al.* 2003). *Escherichia coli* strain DH5α (λ *pir*) was used for the construction of plasmids and the preparation of plasmid DNA. Plasmids were introduced into this strain by electroporation, and derivatives with plasmids were grown in LB medium supplemented with Km (40 μg/ml). Plasmid pMycoMar, donor of the mini-*mariner* element *magellan-4*, has been described (Rubin *et al.* 1999). Restriction endonucleases and DNA modifying enzymes were from New England Biolabs (Beverly, MA) and were used under recommended conditions for plasmid constructions.

Isolation and phenotypic screening of potential social motility mutants: Electroporation of MxH1777 cells with plasmid pMycoMar was performed as described (Youderian *et al.* 2003). Electroporation mixes were plated on CTPM Km agar and incubated for 5 days at 32°. After incubation, plates were screened visually to identify small colonies with smooth edges, the phenotype of A^-S^- double mutants. Mutants were purified twice, and the phenotypes of single colonies formed by each mutant were compared after each purification step. Colonies displaying reduced motility after the initial plating were chosen conservatively for purification, and, of these, 149/154 colonies displayed a stable A^-S^- phenotype. We found that one of the 149 mutants, MxH1777 *mor-105*, formed colonies with an unusual morphology; these colonies were smaller in diameter and more than twice the height of the colonies formed by the parent or other mutant strains and have a wrinkled appearance. Only 5/154 colonies chosen after the initial plating were found to form colonies with the same size as the parental (A^-S^+) strain after purification. Four of these were not considered in our subsequent analyses, but the fifth mutant was passed through our sequence analysis, because it had a colony morphology strikingly different from that of the parent and formed bright orange colonies. This mutant was designated MxH1777 *mor-115* (for "*mor*phology"). The alleles of *magellan-4* insertions in all other mutants were designated *mis* ("*m*utation *i*n *S*") followed by their isolation number. Five of the 149 mutants, with alleles *mis-121*, *mis-122*, *mis-141*, *mis-146*, and *mis-147*, were found to form colonies with sizes intermediate between those formed by the parental strain and mutants with more severe motility defects (see results).

Cloning of *M. xanthus* genomic DNA flanking *magellan-4* insertions in S genes: To subclone *magellan-4* insertions in S genes, *M. xanthus* genomic DNA was isolated from vegetative cultures of MxH1777 S::*magellan-4* strains, using the method for the rapid isolation of myxophage Mx8 DNA (Magrini *et al.* 1997). Each strain was grown to a density of 5×10^8/ml in CTPM Km medium, and 1 ml of cells were harvested by low speed centrifugation and resuspended in 0.4 ml distilled water. Buffer [50 μl of 2.0 m Tris–HCl (pH 8.0) and 0.1 m ethylenediamine tetraacetate (EDTA)], 2 μl diethylpyrocarbonate, and 10 μl 10% sodium dodecyl sulfate (SDS) were added. Mixtures were incubated at 75° for 5 min, and 50 μl of 5 m potassium acetate was added to precipitate SDS-protein complexes. After incubation at 4° for 2 hr, mixtures were microcentrifuged at $10{,}000 \times g$ for 15 min. Supernatants were precipitated with 2.5 volume ethanol, and pellets were rinsed in 70% ethanol, 10 mm Tris–HCl (pH 8.0), 1 mm EDTA, and 10 mm $MgCl_2$ (DNA wash solution) for 5 min. DNA pellets were air dried, resuspended in 200 μl 10 mm Tris–HCl (pH 8.0) and 0.1 mm EDTA, and stored routinely at −20°. Genomic DNA (2 μl) was cleaved with *Bss*HI or *Not*I for 8–12 hr in a total volume of 12 μl, enzymes were inactivated by incubation at 75° for 15 min, and the mixtures were placed on a 0.025-μm filter (Millipore, Bedford, MA) and dialyzed against 1000 volume distilled water for 30 min. Cleaved DNA (8 μl) was treated with T4 DNA ligase in a total volume of 10 μl at 25° for 4–12 hr, incubated at 65° for 20 min, and drop dialyzed prior to electroporation into *E. coli* DH5α (λ *pir*). Approximately 1–100 Km^R electroporants were obtained after electroporation of 1 μl of each ligated DNA and were recovered on LB Km plates after incubation at 37° for 24 hr. We were successful in cloning and sequencing the *magellan-4* insertions present in 134/154 mutants. The MxH (***M. x****anthus H*artzell) strain names for mutants with *mis* or *mor* insertions are listed in Table 1.

Sequence analysis: Plasmid DNAs with subcloned *Bss*HII fragments were sequenced initially with primers Mar1 and Mar2 (Biosource, Camarillo, TX), complementary to the ends of *magellan-4* (Youderian *et al.* 2003). In several cases, we extended these sequences with additional primers or by sequencing plasmids with *Not*I subclones from the same mutant. Sequences were assembled into contigs and then used in BLASTn searches (Altschul *et al.* 1990) against the DK1622 genome sequence to identify the TA target site for each *magellan-4* insertion, given as the coordinates of the *M. xanthus* sequence (http://cmr.tigr.org/tigr-scripts/CMR/GenomePage.cgi?org=gmx) (Table 1). In all cases, these searches yielded a unique dinucleotide target site of insertion without accompanying deletion or rearrangement. Comparison of our sequences with the *M. xanthus* genome

confirmed the identity of >50 kb of the assembled sequence (our unpublished results).

RESULTS

Genetic screen for *magellan-4* insertions in S motility genes: Transient *cis*-complementation of defective transposons (DNA elements flanked by transposon ends that are lacking an active transposase gene) by transposase has proven to be a powerful method for performing insertion mutagenesis with transposons. This is because stable insertions that do not undergo additional rounds of transposition are generated in the absence of the transposase gene (Hughes and Roth 1988).

Various strategies have been developed for the transient *cis*-complementation of defective transposons carried on either linear or circular DNA donor molecules, using transduction, conjugation, or transformation as mechanisms for their delivery. One such strategy involves the transformation of a recipient cell with a plasmid that cannot replicate in recipient cells and that expresses transposase in *cis* to a defective transposon carrying a selectable antibiotic-resistance determinant. Selection for antibiotic-resistant transformants enriches for recombinants in which the defective transposon has inserted into the target genome; their subsequent outgrowth results in the segregative loss of the donor plasmid molecule, giving rise to mutants with stable insertions.

Plasmid pMycoMar (Rubin *et al.* 1999) is one such plasmid donor for the transposition of the defective *magellan-4* element. This minitransposon has the ends of a *Himar* element, a transposon in the *mariner* superfamily (Hartl *et al.* 1997) that moves from donor to recipient DNA molecules by a "cut and paste" mechanism, dependent only upon transposase (Lampe *et al.* 1996). These ends flank the *npt* gene from bacterial transposon Tn*5*, which confers resistance to kanamycin (Km^R), as well as the R6Kγ origin of replication, which can function only when the Pir initiation protein is supplied in *trans*. Adjacent to the defective *magellan-4* element, pMycoMar can express mariner transposase from the mycobacterial T6 promoter (Barsom and Hatfull 1996). The G + C-rich T6 promoter is active in *Mycobacterium tuberculosis* but inactive in *E. coli* (Rubin *et al.* 1999). We have shown that this promoter also functions well in *M. xanthus* (Youderian *et al.* 2003). In an *E. coli* host that expresses the Pir protein, pMycoMar replicates autonomously, but does not express transposase. In *M. tuberculosis* and *M. xanthus*, pMycoMar cannot replicate autonomously and expresses transposase. After electroporation of *M. xanthus* with pMycoMar, transient *cis*-complementation results in the transposition of *magellan-4* into the target genome and the generation of Km^R insertion mutants; the donor plasmid is lost upon cell division (Youderian *et al.* 2003).

Because *magellan-4* has the R6Kγ origin of replication, it permits the rapid sequence analysis of insertion mutations. When the chromosomal DNA of a mutant with a *magellan-4* insertion is restricted with an endonuclease that does not cleave within the element, ligated, and used to transform an *E. coli pir*+ recipient strain, Km^R recombinants are recovered. Such recombinants carry plasmids with the *magellan-4* element and regions of the target genome flanking the site of *magellan-4* insertion. Sequences of the sites of each insertion in each plasmid can be determined by using primers internal to, and pointing outward from, the ends of transposed *magellan-4* elements.

In another article, we described the use of pMycoMar to generate insertions in adventurous (A) motility genes in *M. xanthus*. We electroporated a strain of *M. xanthus* deficient in S motility with pMycoMar, screened for Km^R recombinants that have a nonmotile colony morphology (resulting from an S–A::*magellan-4* genotype), and found such mutants with a frequency of ~1% (115/12,000). We concluded from this result that *magellan-4* has a much broader spectrum of targets in *M. xanthus* than transposon Tn*5*, because similar approaches with Tn*5* yield only ~0.2% mutants that form nonmotile colonies. This interpretation is supported by the results of sequence analysis, which enabled us to identify 34 different genes disrupted by 115 independent insertions of *magellan-4*. These 34 genes included the four genes previously known to be involved in A motility. Because 15 of the 30 new genes we identified in this search were represented only by single insertions of *magellan-4*, we concluded that we have yet to saturate the genes required for A motility using this transposon (Youderian *et al.* 2003).

In this report, we describe the results of the reciprocal experiment. We started with a mutant defective in A, but not in S, motility. We chose the Δ*aglU-1777* mutant as our starting strain, because we have demonstrated that this allele, when paired with several different S mutant alleles, results in a nonmotile colony phenotype (White and Hartzell 2000). We electroporated this mutant with plasmid pMycoMar and screened for Km^R recombinants that have a nonmotile colony morphology (resulting from a Δ*aglU* S::*magellan-4* genotype). Again, we find such mutants with a frequency of ~1% (149/15,000), a frequency about fivefold higher than that obtained with Tn*5* in similar mutagenesis experiments (MacNeil *et al.* 1994a). These mutants were purified twice, to confirm that their defects in motility breed true, and grown in liquid CTPM medium (or on plates) to prepare genomic DNA for sequence analysis. Chromosomal DNA from nonmotile, Δ*aglU* S::*magellan-4* double mutants was purified, cleaved, and ligated and used to transform an *E. coli* recipient strain that produces the Pir protein; plasmids were prepared from Km^R electroporants of *E. coli* and sequenced. As shown in Table 1, we determined the sequences of the sites of *magellan-4* insertions in each of the plasmids derived from 132 of the 149 independent mutants. All of the insertions were

TABLE 1

Insertions of *magellan-4* that impair social gliding motility

Coordinates	Locus	Insertion	Strain	Gene
0421763–0421764	MXAN0358	*mis-23*	MxH1521	*sgmA; ileS*
0500448–0500449	MXAN0440	*mis-66*	MxH1561	*sgmB*
1287846–1287847	MXAN1106	*mis-15*	MxH1513	*sgmC*
1288161–1288162	MXAN1106	*mis-77*	MxH1572	*sgmC*
1946011–1946012	MXAN1641	*mis-146*	MxH1629	*sgmD*
2122234–2122235	MXAN1795	*mis-17*	MxH1515	*sgmE*
2259176–2259177	MXAN1926	*mis-61*	MxH1557	*mglB*
2462808–2462809	MXAN2128	*mis-114*	MxH1604	*sgmF*
2542123–2542124	MXAN2203	*mis-7*	MxH1505	*sgmG*
2939846–2939847	MXAN2526	*mis-10*	MxH1508	*sgmH*
2981508–2981509	MXAN2561	*mis-96*	MxH1589	*sgmI*
3422255–3422256	MXAN2921	*mis-112*	MxH1603	*sgmJ*
3422987–3422988	MXAN2922	*mis-119*	MxH1609	*sgmK*
3422987–3422988	MXAN2922	*mis-129*	MxH1616	*sgmK*
3589491–3589492	MXAN3060	*mis-141*	MxH1626	*cglB*
3589491–3589492	MXAN3060	*mis-147*	MxH1630	*cglB*
3611423–3611424	MXAN3084	*mis-2*	MxH1501	*tgl*
3611426–3611427	MXAN3084	*mis-85*	MxH1580	*tgl*
3611426–3611427	MXAN3084	*mis-88*	MxH1583	*tgl*
4055186–4055187	MXAN3474	*mor-105*	MxH1598	*stk*
4084951–4084952	MXAN3506	*mis-19*	MxH1517	*sgmL*
4084987–4084988	MXAN3506	*mis-131*	MxH1618	*sgmL*
4085119–4085120	MXAN3506	*mis-26*	MxH1524	*sgmL*
4085119–4085120	MXAN3506	*mis-127*	MxH1614	*sgmL*
4477791–4477792	MXAN3759	*mis-142*	MxH1627	*sgmM*
4563759–4563760	MXAN3797	*mis-51*	MxH1548	*sgmN*
4619415–4619416	MXAN3840	*mis-121*	MxH1611	*scpB*
4672427–4672428	MXAN3886	*mis-116*	MxH1606	*agmA*
5036166–5036167	MXAN4089	*mor-115*	MxH1605	*carR*
5096652–5096653	MXAN4149	*mis-75*	MxH1570	*frzS*
5098300–5098301	MXAN4150	*mis-68*	MxH1563	*sgmO*
5788164–5788165	MXAN4613	*mis-135*	MxH1622	*sgmP*
5791219–5791220	MXAN4616	*mis-130*	MxH1617	*sgmQ*
5792644–5792645	MXAN4616	*mis-101*	MxH1594	*sgmQ*
5792644–5792645	MXAN4616	*mis-120*	MxH1610	*sgmQ*
5800139–5800140	MXAN4620	*mis-70*	MxH1565	*sgmR*
5801403–5801404	MXAN4621	*mis-43*	MxH1540	*rfbC*
5804537–5804538	MXAN4621	*mis-102*	MxH1595	*rfbC*
5805964–5805965	MXAN4622	*mis-41*	MxH1538	*rfbB*
5807214–5807215	MXAN4623	*mis-21*	MxH1519	*rfbA*
5819677–5819678	MXAN4639	*mis-28*	MxH1526	*sgmS*
5822620–5822621	MXAN4640	*mis-9*	MxH1507	*sgmT*
5901872–5901873	MXAN4707	*mis-55*	MxH1551	*sgmU*
6413442–6413443	MXAN5134	*mis-122*	MxH1612	*mutS*
6640018–6640019	MXAN5333	*mis-24*	MxH1522	*sgmV*
6947398–6947399	MXAN5592	*mis-42*	MxH1539	*sgmW*
7140653–7140654	MXAN5766	*mis-100*	MxH1593	*sgmX*
7142162–7142163	MXAN5766	*mis-13*	MxH1511	*sgmX*
7145304–7145305	MXAN5770	*mis-73*	MxH1568	*sgmY*
7146533–7146534	MXAN5772	*mis-97*	MxH1590	*pilQ*
7147173–7147174	MXAN5772	*mis-63*	MxH1559	*pilQ*
7148427–7148428	MXAN5772	*mis-50*	MxH1547	*pilQ*
7148895–7148896	MXAN5772	*mis-29*	MxH1527	*pilQ*
7148895–7148896	MXAN5772	*mis-30*	MxH1528	*pilQ*
7149164–7149165	MXAN5773	*mis-103*	MxH1596	*pilP*
7149521–7149522	MXAN5772	*mis-31*	MxH1529	*pilP*
7149933–7149934	MXAN5774	*mis-78*	MxH1573	*pilO*
7149933–7149934	MXAN5774	*mis-79*	MxH1574	*pilO*

(*continued*)

TABLE 1

(Continued)

Coordinates	Locus	Insertion	Strain	Gene
7151318–7151319	MXAN5776	*mis-39*	MxH1536	*pilM*
7151453–7151454	MXAN5776	*mis-44*	MxH1541	*pilM*
7152059–7152060	MXAN5776	*mis-118*	MxH1608	*pilM*
7152059–7152060	MXAN5776	*mis-128*	MxH1615	*pilM*
7152136–7152137	MXAN5776	*mis-94*	MxH1588	*pilM*
7152564–7152565	MXAN5777	*mis-108*	MxH1601	*nla23*
7152889–7152890	MXAN5777	*mis-58*	MxH1554	*nla23*
7152889–7152890	MXAN5777	*mis-86*	MxH1581	*nla23*
7152889–7152890	MXAN5777	*mis-89*	MxH1584	*nla23*
7156382–7156383	MXAN5780	*mis-64*	MxH1560	*pilI*
7156382–7156383	MXAN5780	*mis-133*	MxH1620	*pilI*
7156717–7156718	MXAN5780	*mis-69*	MxH1564	*pilI*
7156717–7156718	MXAN5780	*mis-104*	MxH1597	*pilI*
7157002–7157003	MXAN5781	*mis-74*	MxH1569	*pilH*
7157880–7157881	MXAN5782	*mis-144*	MxH1628	*pilG*
7157964–7157965	MXAN5782	*mis-99*	MxH1592	*pilG*
7158021–7158022	MXAN5782	*mis-76*	MxH1571	*pilG*
7158154–7158155	MXAN5782	*mis-80*	MxH1575	*pilG*
7158204–7158205	MXAN5782	*mis-33*	MxH1531	*pilG*
7158273–7158274	MXAN5782	*mis-83*	MxH1578	*pilG*
7158288–7158289	MXAN5782	*mis-4*	MxH1502	*pilG*
7158344–7158345	MXAN5782	*mis-1*	MxH1500	*pilG*
7158429–7158430	MXAN5782	*mis-106*	MxH1599	*pilG*
7158429–7158430	MXAN5782	*mis-107*	MxH1600	*pilG*
7159294–7159295	MXAN5783	*mis-91*	MxH1585	*pilA*
7159294–7159295	MXAN5783	*mis-92*	MxH1586	*pilA*
7159632–7159633	MXAN5783	*mis-84*	MxH1579	*pilA*[a]
7160010–7160011	MXAN5784	*mis-132*	MxH1619	*pilR*
7162816–7162817	MXAN5786	*mis-14*	MxH1512	*pilC*
7162902–7162903	MXAN5786	*mis-32*	MxH1530	*pilC*
7163164–7163165	MXAN5786	*mis-134*	MxH1621	*pilC*
7163494–7163495	MXAN5786	*mis-11*	MxH1509	*pilC*
7163494–7163495	MXAN5786	*mis-60*	MxH1556	*pilC*
7163494–7163495	MXAN5786	*mis-123*	MxH1613	*pilC*
7164417–7164418	MXAN5787	*mis-34*	MxH1532	*pilT*
7164529–7164530	MXAN5787	*mis-82*	MxH1577	*pilT*
7164529–7164530	MXAN5787	*mis-87*	MxH1582	*pilT*
7166434–7166435	MXAN5788	*mis-36*	MxH1534	*pilB*
7231003–7231004	MXAN5831	*mis-148*	MxH1631	*sgmZ; glgP*
7572031–7572032	MXAN6125	*mis-109*	MxH1602	*sgnA*
8039216–8039217	MXAN6518	*mis-40*	MxH1537	*sgnB*
8154610–8154611	MXAN6627	*mis-52*	MxH1549	*sgnC*
8200210–8200211	MXAN6671	*mis-38*	MxH1535	*sglK*
8200360–8200361	MXAN6671	*mis-45*	MxH1542	*sglK*
8200465–8200466	MXAN6671	*mis-48*	MxH1545	*sglK*
8200465–8200466	MXAN6671	*mis-49*	MxH1546	*sglK*
8461333–8461334	MXAN6908	*mis-35*	MxH1533	*sgnD; pgi*
8461447–8461448	MXAN6908	*mis-71*	MxH1566	*sgnD; pgi*
8461447–8461448	MXAN6908	*mis-93*	MxH1587	*sgnD; pgi*
8982735–8982736	MXAN7360	*mis-59*	MxH1555	*sgnE*[a]
9039673–9039674	MXAN7415	*mis-18*	MxH1516	*epsZ*
9039673–9039674	MXAN7415	*mis-20*	MxH1518	*epsZ*
9039673–9039674	MXAN7415	*mis-47*	MxH1544	*epsZ*
9040255–9040256	MXAN7415	*mis-57*	MxH1553	*epsZ*
9040369–9040370	MXAN7415	*mis-72*	MxH1567	*epsZ*
9042228–9042229	MXAN7417	*mis-117*	MxH1607	*epsY*
9042228–9042229	MXAN7417	*mis-136*	MxH1623	*epsY*
9042228–9042229	MXAN7417	*mis-138*	MxH1624	*epsY*

(*continued*)

TABLE 1

(Continued)

Coordinates	Locus	Insertion	Strain	Gene
9045962–9045963	MXAN7421	*mis-62*	MxH1558	*epsV*
9046689–9046690	MXAN7422	*mis-22*	MxH1520	*epsU*
9047313–9047314	MXAN7422	*mis-46*	MxH1543	*epsU*
9063422–9063423	MXAN7441	*mis-81*	MxH1576	*epsH*
9065683–9065684	MXAN7442	*mis-6*	MxH1504	*sgnF*
9065986–9065987	MXAN7442	*mis-27*	MxH1525	*sgnF*
9066004–9066005	MXAN7442	*mis-67*	MxH1562	*sgnF*
9070550–9070551	MXAN7445	*mis-25*	MxH1523	*epsE*
9071704–9071705	MXAN7448	*mis-56*	MxH1552	*epsD*
9072040–9072041	MXAN7448	*mis-53*	MxH1550	*epsD*
9072433–9072434	MXAN7448	*mis-8*	MxH1506	*epsD*
9072508–9072509	MXAN7448	*mis-16*	MxH1514	*epsD*
9072734–9072735	MXAN7449	*mis-5*	MxH1503	*epsC*
9072805–9072806	MXAN7449	*mis-12*	MxH1510	*epsC*
9072805–9072806	MXAN7449	*mis-139*	MxH1625	*epsC*
9076128–9076129	MXAN7451	*mis-98*	MxH1591	*epsA*

The 132 insertions of *magellan-4* in the *M. xanthus* genome described in this report are listed in order of their sites within the genome sequence, which can be found at http://cmr.tigr.org/tigr-scripts/CMR/GenomePage.cgi?org=gmx. Given are the gene numbers in which the insertions are situated, the allele numbers of the insertions, the strain numbers of the derivatives of MxH1777 with each insertion, and the gene names.

[a] An insertion lies upstream of the coding sequence indicated.

found within the preferred TA dinucleotide target sequence duplicated by *magellan-4* upon insertion. For the majority of these insertions, we determined that the TA target site had been duplicated by insertion, by sequencing both insertion junctions.

Insertions of *magellan-4* in genes required for type IV pilin biogenesis: Among the sequenced insertions of *magellan-4* that affect S motility, 50 were found in one cluster of genes required for type IV pilin biosynthesis, located in a 27-kb region of the *M. xanthus* genome (Figure 1). These insertions are distributed among 40 different target sites, and insertions that occur at the same site are often found in opposite orientations. The most frequent target for insertion in this region was the *pilG* gene, in which 10 insertions were recovered at 9 different sites (with the pair of transposon insertions at the same site found in opposite orientations). These results confirm that the spectrum of insertions made by *magellan-4* in *M. xanthus* is extremely broad and shows little preference in choosing AT target sites within the same gene.

As shown in Figure 1, The 23 predicted coding sequences in this region of the genome are transcribed in the same direction, most likely as at least five separate operons. These include the *pilA* gene (*MXAN5783*), which encodes pilin (Wu and Kaiser 1996); the *pilG*

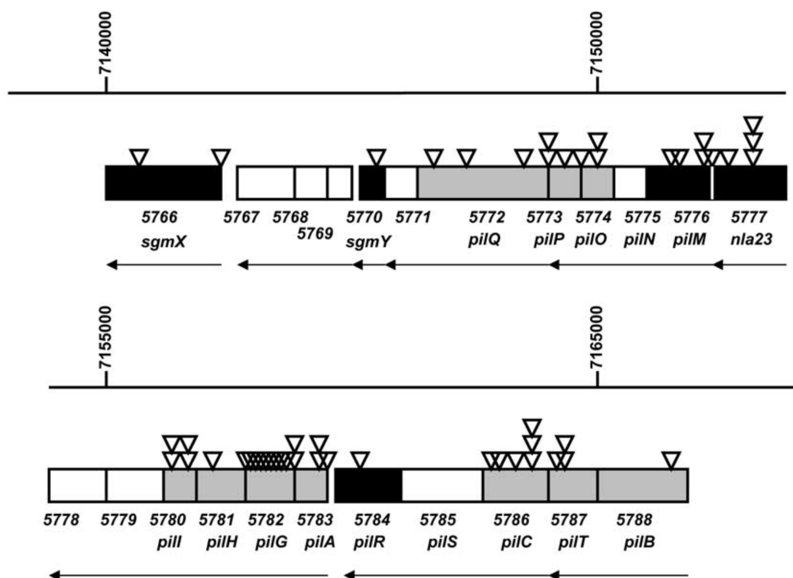

Figure 1.—*magellan-4* insertions identify 15 different genes required for S motility in the *M. xanthus pil* gene cluster. The 27-kb region of the *M. xanthus* genome with the *pilA* (pilin) and adjacent genes (boxes) is shown; the directions of transcription are indicated by arrows. Coordinates and gene numbers are those of the *M. xanthus* genome sequence (http://cmr.tigr.org/tigr-scripts/CMR/GenomePage.cgi?org=gmx). Positions of *magellan-4* insertions are indicated by triangles. Shaded boxes indicate genes shown previously to be required for S motility; solid boxes indicate the new genes identified in this study. The top and bottom represent contiguous sequence.

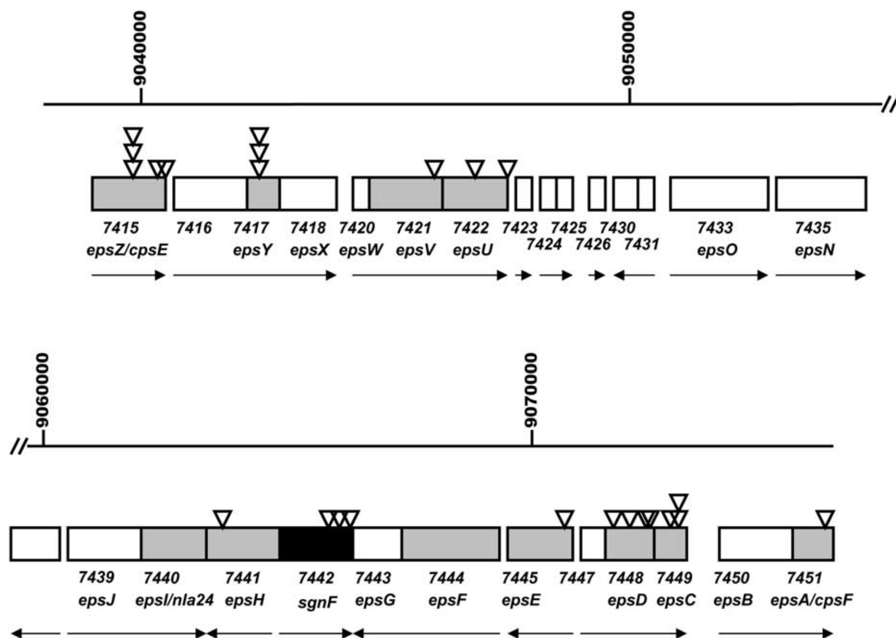

FIGURE 2.—*magellan-4* insertions identify 10 different genes required for S motility in the *M. xanthus eps* gene cluster. The details are as in Figure 1; note that the central portion of the *eps* gene cluster, which does not contain genes required for S motility, is not shown.

(*MXAN5782*), *pilH* (*MXAN5781*), and *pilI* (*MXAN5780*) genes (WU *et al.* 1998); and the *pilB* (*MXAN5788*), *pilT* (*MXAN5787*), *pilC* (*MXAN5786*), and *pilQ* (*MXAN5772*) genes, each of which has been shown to be essential for S motility (WU *et al.* 1997; WALL *et al.* 1999). Insertions of *magellan-4* were obtained in each of these genes, as well as in *pilP* (*MXAN5773*), *pilO* (*MXAN5774*), *pilM* (*MXAN5776*), *pilR* (*MXAN5784*), and *nla23*(*MXAN5777*), predicted to encode a response regulator homologous to PilR (WALL *et al.* 1999; CABEROY *et al.* 2003). No insertions were found in the large *pilS* (*MXAN5785*) gene, consistent with the finding that it is not essential for S motility (WALL *et al.* 1999).

In addition to genes in this cluster known or suspected to be required for S motility, insertions were recovered in gene *MXAN5770*, immediately downstream from *MXAN5771*, which is not required for S motility (WU and KAISER 1996). *MXAN5770* is predicted to encode a conserved hypothetical protein. Two different insertions were recovered in nearby gene *MXAN5766*, predicted to encode a product with tetratricopeptide (TPR) repeats (see below).

Insertions of *magellan-4* in genes required for exopolysaccharide biosynthesis: Among the independent *magellan-4* insertions that disrupt S motility, 24 disrupt genes known to be required for exopolysaccharide (EPS) biosynthesis. Recently, LU *et al.* (2005) identified a large group of genes involved in EPS biosynthesis, which also is required for S motility. Their strategy to identify these genes was to mutagenize *M. xanthus* by electroporation with a plasmid library made with a Km^R vector that carries random, ~500-bp inserts of *M. xanthus* DNA prepared by physical shearing. Because their plasmid vector cannot replicate in *M. xanthus*, Km^R recombinants must arise due to the integration of the plasmid, which occurs most frequently by homologous recombination in this host, as in the yeast *Saccharomyces cerevisiae*. Thus, this strategy is equivalent to that of inactivating a yeast gene by the integration of a plasmid that carries an insert missing the 5′ and 3′ ends of a gene; integration of a plasmid results in a merodiploid in which the integrated vector is sandwiched between two incomplete copies of the gene, one missing its 5′ end and one missing its 3′ end, required for function (NEFF *et al.* 1983).

Using this strategy, they screened among 5000 "integrative disruption" mutants and found 68 with reduced motility. Four of these 68 mutants are unable to form fruiting bodies, a phenotype of many S motility mutants. Also, they do not bind calcofluor white, which is an indicator dye for EPS production (RAMASWAMY *et al.* 1997). The integrated plasmids in three of these four integrative disruption mutants were found to flank a 37-kb region of the *M. xanthus* genome, which they designated the "*eps*" region.

Sequence analysis shows that this region includes genes predicted to encode products homologous to those of the genes known to be involved in EPS biosynthesis in other bacteria. In-frame deletion and insertion mutations in 11 genes within this region impair social motility (Figure 2). These are: *epsA* (*MXAN7451*), *epsC* (*MXAN7449*), *epsD* (*MXAN7448*), *epsE* (*MXAN7445*), *epsF* (*MXAN7444*), *epsH* (*MXAN7441*), *epsI/nla24* (*MXAN7440*), *epsU* (*MXAN7422*), *epsV* (*MXAN7421*), *epsY* (*MXAN7417*), and *epsZ* (*MXAN7415*) (LU *et al.* 2005). As shown in Table 1, 24/131 of the independent *magellan-4* insertions that disrupt S motility fall within 9 of these 11 genes. Three insertions interrupt the coding sequence *MXAN7442*, which lies between *epsG* and *epsH* and is predicted to encode a membrane protein of unknown function. The predicted products of these 12 genes fall into four functional categories. The majority

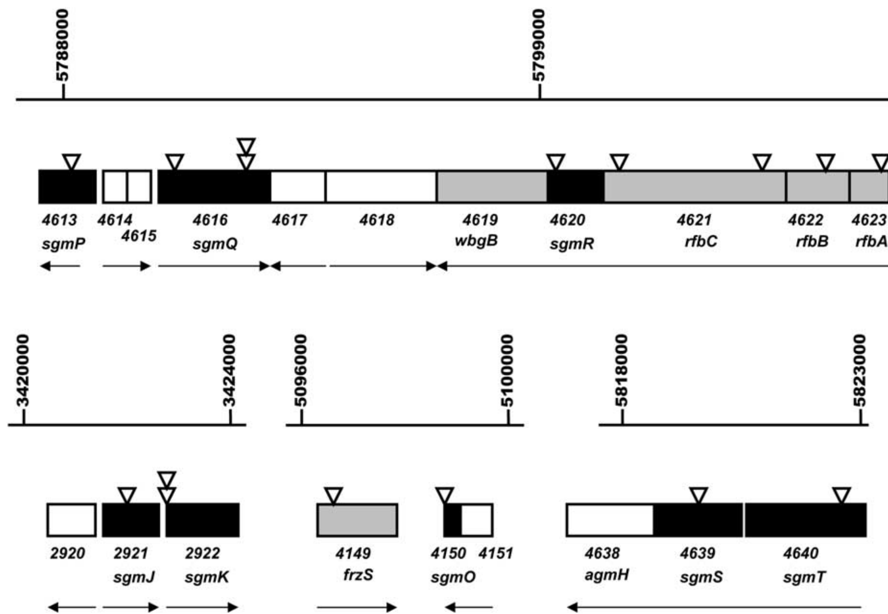

Figure 3.—*magellan-4* insertions identify six different genes required for S motility in the *M. xanthus rfb* gene cluster. (Top) Shown are the positions of *magellan-4* insertions in the *rfb* gene cluster, and adjacent genes, as in Figures 1 and 2. (Bottom) Three additional regions of the *M. xanthus* genome have adjacent genes with multiple insertions of *magellan-4* that confer the loss of S motility. The *sgmJ* and *sgmK* genes define a new cluster of genes presumably required for LPS biogenesis, *sgmO* is adjacent to *frzS*, and the *sgmT* (histidine kinase/response regulator) and *sgmS* genes lie immediately upstream of *agmH*, required for A motility (Youderian *et al.* 2003). One or both of these genes may be required for both S and A motility.

(7/12) of these are sugar transferases presumably required for EPS assembly, two have response regulator domains (EpsF and EpsI), one is presumably involved in EPS export (EpsY), and two (EpsV and the product of *MXAN7442*) have as yet unknown functions. We did not recover *magellan-4* insertions in the *easA* (*MXAN2293*) gene, which encodes an accessory protein essential for EPS biogenesis (Lu *et al.* 2005).

Insertions of *magellan-4* in genes required for LPS biosynthesis: Among the independent *magellan-4* insertions that disrupt S motility, four fall into a cluster of five adjacent genes. Four of these five genes, *rfaA* (*MXAN4623*), *rfaB* (*MXAN4622*), *rfaC* (*MXAN4621*), and *wbgB* (*MXAN4619*), are known to be required for LPS biosynthesis (Bowden and Kaplan 1998; Yang *et al.* 2000a) (Figure 3, top). Also, one *magellan-4* insertion interrupts *MXAN4620*, predicted to encode a hypothetical protein with unknown function. Four *magellan-4* insertions lie within the nearby genes *MXAN4616*, predicted to encode a glycosyl transferase, and *MXAN4613*, predicted to encode a second dTDP-glucose 4,6-dehydrogenase similar to that of the *rfbB* (*MXAN4622*) gene. These adjacent genes also are likely involved in the biosynthesis of LPS.

Insertions of *magellan-4* in additional genes known to be required for S motility: Many additional genes have been characterized that encode products essential for S motility. These include the three genes known to be required for both S and A motility, *mglA* (*MXAN1925*), which is the second gene in an operon with its interacting partner, *mglB* (*MXAN1926*) (Hartzell and Kaiser 1991a,b; Hartzell 1997), *agmA* (*MXAN3886*), which encodes one of five different *N*-acetylmuramoyl l-alanine transamidases predicted to be made by *M. xanthus* (Youderian *et al.* 2003), and *epsI/nla24* (*MXAN7440*), which encodes a response regulator essential for EPS biosynthesis (Caberoy *et al.* 2003; Li *et al.* 2003). Among our nonmotile derivatives of parent strain MxH1777, we recovered one mutant with an insertion of *magellan-4* in the *mglB* gene; such mutations also were recovered in our reciprocal hunt for *magellan-4* insertions that reduce the motility of a parental S^- strain and are defective in A motility; these insertions have polar effects on the expression of *mglA* (Youderian *et al.* 2003). In the current hunt, we also recovered one *magellan-4* insertion in the *agmA* gene, confirming that its function is required for both A and S motility (Youderian *et al.* 2003), but no insertions in the *epsI/nla24* gene (Figure 2).

Genes identified previously that are essential for S motility also include *tgl* (*MXAN3084*), which encodes an outer membrane lipoprotein (Rodriguez-Soto and Kaiser 1997a,b) that can be transferred among cells upon contact (Nudleman *et al.* 2005); *sglK* (*MXAN6671*), which encodes a DnaK homolog essential for S motility (Weimer *et al.* 1998); *frzS* (*MXAN4149*), which encodes a cytoplasmic protein with a coiled-coil domain that can assemble into myosin-like lattices (Ward *et al.* 2000); *nla1* (*MXAN5853*) and *nla19* (*MXAN1078*), which encode two different response regulators (Caberoy *et al.* 2003); *masK* (*MXAN1922*), which encodes an essential tyrosine kinase (Thomasson *et al.* 2002); and three genes in the *dif* operon (*MXAN6696–MXAN6691*) encoding a methyl-accepting chemotaxis protein (*difA*, *MXAN6696*), a histidine kinase (*difE*, *MXAN6692*), and a CheW homolog (*difC*, *MXAN6694*) (Yang *et al.* 2000b; Bellenger *et al.* 2002). We recovered *magellan-4* insertions in *tgl*, *frzS*, *sglK*, and *nla23*, but not in *masK*, *nla1*, *nla19*, *difA*, *difC*, or *difE* (Table 1).

Recently, Pham *et al.* (2005) showed that integrative disruption of the *MXAN4150* (*sgmO*) gene, which they named *rasA*, results in defects in fibril production, S motility, and multicellular development. Our result that

a *magellan-4* insertion in this gene impairs S motility confirms their results independently. In addition, Kimura *et al.* (2004) found that the simultaneous disruption of the cotranscribed *rppA* (*MXAN5907*) and *mmrA* (*MXAN5906*) genes also results in an S motility defect. Therefore, we might predict that (polar) insertions of *magellan-4* in the *rppA* gene would also disrupt S motility; however, we did not recover insertions in this possible target.

Insertions of *magellan-4* in genes previously not known to be required for S motility: Among the 132 *magellan-4* insertions that we characterized, seven are from mutants that form colonies with distinct morphologies or display only partial S motility defects. One of these mutants, MxH1777 *mor-115*, forms bright orange colonies with a motility phenotype indistinguishable from that of the MxH1777 parent. Sequence analysis of this mutant shows that its *magellan-4* insertion lies in the very 3′ end of the *carR* gene, which encodes a regulator of carotenoid biosynthetic gene expression, consistent with the observation that it overproduces the bright orange carotenoids made by *M. xanthus* (Hodgson 1993; Martinez-Laborda *et al.* 1990). A second morphology mutant forms colonies with a wrinkled appearance that are smaller than, and more than twice the height of, those formed by its parent. The insertion in this mutant, *mor-105*, lies within the *stk* (*MXAN3474*) gene, which encodes a DnaK homolog involved in cell-cell adhesion. Loss-of-function mutations in the *stk* gene suppress a subset of S motility defects (Dana and Shimkets 1993).

Five mutants were found to form colonies with sizes intermediate between those formed by their A^- parent and its A^-S^- mutant derivatives. One of these carries an insertion in *MXAN1641*, predicted to encode a protein of unknown function. A second has an insertion in *MXAN3840*, predicted to encode a homolog of the ScpB protein, which regulates chromosome segregation in Gram-positive bacilli (Soppa *et al.* 2002). A third has an insertion in the *M. xanthus* homolog of the *mutS* gene (*MXAN5134*), required for mismatch repair. It is possible that defects in these three genes have pleiotropic effects on colony size and affect S motility indirectly by impairing central cell processes. In addition, we recovered two insertions in the *cglB* gene (*MXAN3060*), known to be involved in A motility, but not in S motility (Rodriguez and Spormann 1999), that confer partial motility defects. These results confirm that the Δ*aglU cglB* double mutants have a more severe defect than their Δ*aglU* parent (White and Hartzell 2000), consistent with our recent results that several other alleles in A motility genes, when paired with the Δ*aglU* mutation, result in more severe A motility defects (our unpublished results).

The remaining 41 insertions of *magellan-4* confer profound defects in S motility; these likely affect the expression of genes whose products are directly involved in S motility. These insertions inactivate genes encoding proteins involved in central metabolic process, in both cytoplasmic and membrane proteins that may be components of the pilus motor, in regulators of transcription, and in polysaccharide metabolism. Three *magellan-4* insertions inactivate genes *MXAN0358*, *MXAN3759*, and *MXAN3797*, predicted to encode central metabolic functions. The first of these genes encodes one of two isoleucine tRNA synthetases found on the *M. xanthus* genome, demonstrating that this *ileS* gene is not essential for growth. The effect of the *magellan-4* insertion in this gene on S motility could be direct, but it is quite possible that this insertion affects the expression of the adjacent *MXAN0359*–*MXAN0362* genes. These four genes are predicted to encode homologs of the *Pseudomonas aeruginosa* genes *fimT*, *pilW*, *pilV*, and *pilY1*, which contribute to the stability of type IV pili (Alm and Mattick 1995, 1997; Alm *et al.* 1996; Carbonnelle *et al.* 2005). The second of these is in a gene, *MXAN3759*, predicted to encode one of two propionyl-CoA carboxylases made by *M. xanthus* (Kimura *et al.* 1997). The third of these is in a gene, *MXAN3797*, predicted to encode a short-chain acyl-CoA dehydrogenase.

Fourteen *magellan-4* insertions define 13 new genes predicted to encode proteins of unknown function (see Table 2). These include *MXAN0440*, predicted to encode an integral membrane exporter, and *MXAN1795* and *MXAN7360*, predicted to encode lipoproteins; the insertion that affects *MXAN7360* lies upstream of its potential start codons and likely inactivates its promoter. Three genes, *MXAN1106*, *MXAN4639*, and *MXAN5766*, are predicted to encode proteins with TPR motifs; the first among these is a protein that also has a DnaJ motif adjacent to its C-terminal TPR motif; the latter two have pairs of adjacent TPR motifs. These TPR repeat domain pairs compose a larger domain similar to that of the *Pseudomonas aeruginosa* PilF protein, which is required for the assembly of type IV pili (fimbrae) (Watson *et al.* 1996), a domain shared by the Tgl lipoprotein (Rodriguez-Soto and Kaiser 1997a,b). Four *magellan-4* insertions define four new genes predicted to encode transcription regulators; three of these have response-regulator domains.

Finally, the *magellan-4* insertions define 12 new genes predicted to encode proteins involved in polysaccharide metabolism, 9 of which are likely critical for EPS biosynthesis, LPS biosynthesis, or both. Three *magellan-4* insertions affect genes likely to encode products involved in polysaccharide catabolism; *MXAN2203* is predicted to encode a glycosyl hydrolase with an Asp box motif (β hairpin), suggesting that its activity may be regulated by a complex phosphorelay system (Zhang and Shi 2005). The product of *MXAN2561* has a fibronectin type III domain, found among several bacterial polysaccharide hydrolases (Folders *et al.* 2001; Kataeva *et al.* 2002). The *glgP* gene (*MXAN5831*) encodes glycogen phosphorylase.

TABLE 2

***magellan-4* insertions identify 31 new genes required for S motility**

Gene	Name	Putative function
		Central metabolic functions
MXAN0358 (1)	*sgmA; ileS*	Isoleucyl tRNA synthetase; downstream of *fimT, pilW*
MXAN3759 (1)	*sgmM; pccB2*	Propionyl-CoA carboxylase
MXAN3797 (1)	*sgmN*	Short-chain acyl-CoA dehydrogenase
		Cytoplasmic and membrane proteins of unknown function
MXAN0440 (1)	*sgmB*	Inner membrane protein; RND exporter
MXAN1106 (2)	*sgmC*	DnaJ/TPR repeat domain protein
MXAN1641 (1)[a]	*sgmD*	Hypothetical protein
MXAN1795 (1)	*sgmE*	Lipoprotein
MXAN2526 (1)	*sgmH*	Hypothetical protein
MXAN4150 (1)	*sgmO*	Conserved hypothetical protein; downstream of *frzS*
MXAN4620 (1)	*sgmR*	Hypothetical protein; downstream of *rfbC*; methyltransferase
MXAN4639 (1)	*sgmS*	TPR repeat protein; PilF domain
MXAN5766 (1)	*sgmX*	TPR repeat protein; PilF domain
MXAN5770 (1)	*sgmY*	Conserved hypothetical protein
MXAN6125 (1)	*sgnA*	Hypothetical protein; helicase?
MXAN6518 (1)	*sgnB*	Conserved hypothetical protein; ABC transporter
MXAN7360 (1)	*sgnE*	Lipoprotein
MXAN7442 (2)	*sgnF*	Membrane protein
		Regulators of transcription
MXAN2128 (1)	*sgmF*	LysR family activator
MXAN4640 (1)	*sgmT*	Histidine kinase/response regulator
MXAN5592 (1)	*sgmW*	Response regulator
MXAN6627 (1)	*sgnC*	Response regulator
		Polysaccharide metabolism
MXAN2203 (1)	*sgmG*	Asp box motif protein; glycosyl hydrolase?
MXAN2561 (1)	*sgmI*	Fibronectin type III domain protein; hydrolase?
MXAN2921 (1)	*sgmJ; wbaZ*	Glycosyl transferase group I domain
MXAN2922 (2)	*sgmK*	LPS biosynthesis; WcaJ C-terminal domain
MXAN3506 (4)	*sgmL*	Polysaccharide biosynthesis
MXAN4613 (1)	*sgmP; rfbB2*	dTDP-glucose 4,6-dehydrogenase
MXAN4616 (3)	*sgmQ*	Glycosyl transferase group I and II domains
MXAN4707 (1)	*sgmU; rfaF*	Heptosyl transferase 9 domain
MXAN5333 (1)	*sgmV; rfaG*	Glycosyl transferase group I domains (2)
MXAN5831 (1)	*sgmZ; glgP*	Glycogen phosphorylase
MXAN6908 (3)	*sgnD; pgi*	Glucose-6-phosphate isomerase

Genes are divided into four functional categories as shown and are numbered in accord with the *M. xanthus* genome sequence available at http://cmr.tigr.org/tigr-scripts/CMR/GenomePage.cgi?org=gmx. After each gene number, in parentheses is shown the number of *magellan-4* insertions characterized in each gene; new genes involved in S motility were designated *sgmA* through *sgnF*; alternative suggestions for gene names are also listed. The predicted function of each gene is listed.

[a] We have included *MXAN1641* as an S gene although its direct role in motility has yet to be determined (see RESULTS).

DISCUSSION

Among 128 independent insertions of the defective transposon *magellan-4* in genes required for S motility in *M. xanthus*, two-thirds lie within 27 of the 37 nonessential genes whose functions have been shown to be required for S motility. These include genes involved in the biogenesis of type IV pili, EPS and LPS. Our results show that the mechanism of S motility is more complex genetically than that of flagellar motility, which requires the products of <60 genes in Salmonella and *E. coli* (Chilcott and Hughes 2000). If we estimate that we have identified ∼75% of the nonessential genes involved in S motility by our mutant hunt, then we predict that there should be at least 80 nonessential genes whose products are required for S motility.

This number likely is an underestimate. Although the spectrum of *magellan-4* insertions in S genes is very broad, it is not random and likely shows both site and regional preferences in *M. xanthus*, as does any other transposon and as is suggested by the results shown in

Figures 1–3. Another indication that we have yet to reach saturation of the S motility genes with insertions of *magellan-4* is that previously we found 12/115 nonmotile derivatives of an S mutant with insertions of *magellan-4* in the *mglBA* genes (among 36 target genes) (Youderian *et al.* 2003), but only 1 of the 128 insertions in S motility genes described in this report falls within the *mglBA* operon (among 58 target genes). We note that some of the genes we have identified with insertions of *magellan-4* may not be involved directly in S motility, due to polar and other effects that insertions may have on the expression of neighboring genes. This is certainly the case for insertions of *magellan-4* in the *mglB* gene, which are polar in the expression of *mglA* (Youderian *et al.* 2003), and may also be the case for insertions in *MXAN4620*, upstream of *wbgB* (Figure 3, top), and in *MXAN0358* (*ileS*), adjacent to homologs of the *P. aeruginosa fimT*, *pilW*, *pilV*, and *pilY1* genes. These homologs may represent a new *pil gene* cluster.

Two of the new genes we have identified as social motility genes are likely involved in fatty acid metabolism. One of the *magellan-4* insertions identifies a second *M. xanthus* gene, *MXAN3759*, predicted to encode a propionyl-CoA acyl carboxylase. A defect in its paralogue, *pccB* (*MXAN1113*), results in a reduction in the relative amount of long-chain fatty acids present in the membranes of vegetative and developing cells, but not in their abolition. Thus, although a *pccB* mutant can aggregate, it cannot form heat-resistant spores in response to carbon starvation (Kimura *et al.* 1997), a phenotype that can be masked partially by the addition of methylmalonyl-CoA to developing cells. The fact that a second propionyl-CoA acyl carboxylase isozyme also is required for social motility underscores the importance of long-chain fatty acids in the integrity of the *M. xanthus* membrane. The gene *MXAN3797* is predicted to encode one of the >20 short-chain acyl-CoA dehydrogenases (SCADs) made by *M. xanthus*. Whether fatty acid metabolism plays a direct role in S motility or whether the *magellan-4* insertions in *MXAN3759* and *MXAN3797* result in pleiotropic effects on membrane biogenesis remains to be determined.

magellan-4 insertions that impair S motility identify four new genes predicted to encode regulators of transcription. Three of these genes are predicted to encode products with response regulator domains. Among these, the product of one (*MXAN4640*) also is predicted to have a histidine kinase domain. This gene may be cotranscribed with *MXAN4639*, which also is required for S motility (Figure 3, bottom). The *M. xanthus* genome includes genes for 150 different proteins with response regulator domains. An earlier mutant hunt using integrative disruption to probe the functions of 27 of these genes showed that four are required for S motility (Caberoy *et al.* 2003). That we obtained *magellan-4* insertions in only 2 of these 4 genes among the 27 again demonstrates that our *magellan-4* insertions have not saturated the genes required for S motility. The fact that we obtained *magellan-4* insertions in 3 additional genes among the 123 predicted to encode additional response regulators and that have yet to be tested for function shows that at least seven different response regulators are required for S motility. The systematic functional analysis of the 120 remaining genes predicted to encode response regulators may reveal additional genes required for S motility. We also obtained an insertion in gene *MXAN2128*, which encodes 1 of the 24 proteins in the LysR family of transcription regulators predicted to be encoded by genes present on the *M. xanthus* genome.

Among the genes of unknown function identified as social motility genes by *magellan-4* insertions are three new genes predicted to encode proteins with TPR repeat motifs. We suspect that these proteins will play central roles in the mechanism of *S motility* for several reasons. The *M. xanthus* genome has >60 genes predicted to encode proteins with TPR repeat domains, at least three of which, *agmK* (*MXAN4863*), *aglT* (*MXAN4869*), and *agmU* (*MXAN4870*), are required for A motility (Youderian *et al.* 2003). The Tgl lipoprotein, which is transferred from cell to cell upon contact (Nudleman *et al.* 2005), also has a TPR repeat domain. Such domains are critical for the function of kinesin-dependent eukaryotic motors (Gindhart and Goldstein 1996). One or more of these proteins may play a structural role in the mechanism of pilus movement in *M. xanthus*.

During the mechanics of pilus extension and retraction, ATP is predicted to be hydrolyzed to energize the pilus motor, as is the case for the variety of motors that drive the mechanisms of eukaryotic motility involving the cycle of macromolecular sliding and/or polymerization/depolymerization. The analysis of S mutants suggests that the proteins involved in ATP hydrolysis may include not only PilT and PilB, the ATPases thought to be involved in pilus extrusion, but also the homologs of DnaJ and DnaK involved in S motility. Two of the *M. xanthus* genes predicted to encode >12 homologs of HSP70 (DnaK), *sglK* (Weimer *et al.* 1998; Yang *et al.* 1998), and *stk* (Dana and Shimkets 1993), play central roles in S motility. Also, one of the genes we have identified by *magellan-4* insertions (*MXAN1106*) encodes a TPR repeat protein with a DnaJ domain.

Many of the new S motility genes that we have identified are involved in LPS and/or EPS polysaccharide biosynthesis. Three of these, *MXAN4613*, *MXAN4616*, and *MXAN4620*, are clustered near three genes previously shown to be involved in S motility (Figure 3, top); additional genes within this cluster are predicted to be involved in LPS production and may be required for S motility. The two adjacent genes *MXAN2921* and *MXAN2922* lie within an additional cluster of genes likely required for LPS biosynthesis and S motility (Figure 3, bottom). The role that LPS plays in S motility could be direct or indirect, because many bacteria

regulate the balance between LPS and EPS biogenesis, and defects in LPS biosynthesis may cause dramatic changes in the structure or amount of EPS produced by cells. LPS mutants of *M. xanthus* produce more EPS antigen than do wild-type cells (Bowden and Kaplan 1998).

It is clear that EPS plays a direct role in the mechanism of S motility. Mutants defective in EPS production are hyperpiliated, a phenotype that can be rescued by the addition of EPS or chitin, a polymer of *N*-acetylglucosamine, but not by cellulose, a polymer of glucose (Li *et al* 2003). *N*-acetylglucosamine is a major component of *M. xanthus* EPS. The addition of chitin to wild-type *M. xanthus* cells results in pilus retraction. In contrast, the addition of *N*-acetylglucosamine to wild-type *M. xanthus* cells has the opposite effect and results in hyperpiliation. Glucose and galactose have no effect on pilus extension (Li *et al.* 2003). Our results suggest that the signals that govern pilus extension and retraction are the products of polysaccharide catabolism. The *magellan-4* insertions identified three genes potentially involved in polysaccharide catabolism that are essential for S motility. These three genes include *MXAN2561*, predicted to encode a protein with a fibronectin type III domain, implicated in the hydrolysis of polysaccharides; *MXAN2203*, predicted to encode a glycosyl hydrolase; and, most important, the *glgP* gene (*MXAN5831*), predicted to encode glycogen phosphorylase.

Although social motility appears to be a mechanism conserved among diverse Gram-negative bacteria, very few genetic determinants of this mechanism are conserved in sequence among the bacteria that display social (or "twitching") motility, *M. xanthus* and *P. aeruginosa* (Peabody *et al.* 2003). The gene encoding glycogen phosphorylase (EC 2.4.1.1), which cleaves polysaccharides with $\alpha(1 \rightarrow 4)$ glucan linkages, is conserved among *M. xanthus*, *P. aeruginosa*, and *Haemophilus influenzae*, which has been shown recently to display S motility (Bakaletz *et al.* 2005). Glycogen phosphorylase is an economical enzyme that uses the cofactor PLP to carry out the phosphorylation of monosaccharides concomitant with their hydrolysis from polysaccharides, without consuming ATP to generate its monosaccharide phosphate products. We propose that the signal for the retraction of the *M. xanthus* pilus is likely *N*-acetylglucosamine phosphate, the predicted product of glycogen phosphorylase acting on chitin as a substrate. *N*-acetylglucosamine phosphate is an ideal signal for pilus retraction, because it can be cleaved to form *N*-acetylglucosamine by the phosphatases made by *M. xanthus* (Weinberg and Zusman 1990). Indeed, a mutation in the *pph1* gene, encoding a Ser/Thr protein phosphatase, affects vegetative swarming on 0.3% agar but not on 1.5% agar (Treuner-Lange *et al.* 2001), a phenotype indicative of a defect in S motility. Currently, we are testing the hypothesis that glycogen phosphorylase generates *N*-acetylglucosamine phosphate from EPS as a signal for pilus retraction.

The authors thank Larry Shimkets for careful reading of the manuscript and helpful comments and Neal Burke for excellent technical assistance. This work was supported by grants MCB0242191 from the National Science Foundation to P.L.H. and GM59336 from the National Institutes of Health to P.Y.

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Communicating editor: S. Gottesman