New Locus Important for *Myxococcus* Social Motility and Development

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The *mts* locus in salt-tolerant *Myxococcus fulvus* HW-1 was found to be critical for gliding motility, fruiting-body formation, and sporulation. The homologous genes in *Myxococcus xanthus* are also important for social motility and fruiting-body development. The *mts* genes were determined to be involved in cell-cell cohesion in both myxobacterial species.

Myxobacteria are gram-negative gliding bacteria with a multicellular developmental life cycle (20, 23). Gliding motility in these cells plays a crucial role in myxobacterial predatory and developmental aggregation (23). There are two distinct motility systems controlling *Myxococcus* gliding: the adventurous (A) system, which controls the movement of individual and isolated cells, and the social (S) system, which is essential for the swarming and aggregation of cells (7, 8). With the dual motility system, myxobacteria are able to adapt to different environments, such as dry and highly hydrated agar surfaces.

Although most myxobacteria are found in soil, there are salt-tolerant species from marine environments (5, 9–11, 17, 28). In contrast to the salt sensitivity of terrestrial strains (19), salt-tolerant myxobacteria are able to withstand a wide range of salinity. In response to changes in salinity, some salt-tolerant myxobacteria show altered vegetative and developmental characteristics (29). Differences in morphogenetic characteristics appear to indicate that salt-tolerant myxobacteria are the result of the adaptation of soil myxobacteria to marine environments (29). Analysis of salt-tolerant *Myxococcus* strains has revealed that they may have retained dual gliding motility systems. Some of the high-salt-tolerant strains exhibited enhanced S motility in the presence of seawater, as indicated by increased swarming on soft agar (24).

To identify the S motility genes in the salt-tolerant *Myxococcus fulvus* strain HW-1, a genetic screen was performed using transposon MiniHimar1lacZ electroporation (14), which yielded a transformation efficiency of 10 to 10^2 CFU/μg DNA. Of more than 2,000 insertion mutants, 21 were deficient in motility and formed small colonies compared to those of the wild-type parent strain HW-1. One of the mutants showed a significant reduction in colony expansion on a 0.3% agar surface, which is indicative of an S motility defect. The mutated gene led to the discovery of the *mts* locus, which is the focus of this report.

**Phenotypic characteristics of the mutant HL-1.** The mutant HL-1 (Table 1) was assessed for motility phenotypes by standard methods (21). As shown in Fig. 1A to D, the swarming colony sizes of HL-1 were 90.4% and 84.2% smaller than those of HW-1 on hard and soft agar, respectively. On soft agar, the mutant produced small colonies with a rough, dentate swarm edge (Fig. 1C), in contrast to the large colonies with the translucent smooth lacy swarm edge of the wild-type strain (Fig. 1D). At their swarming edges on hard agar, HL-1 cells moved mainly as individuals, with a few in small groups (Fig. 1E), whereas HW-1 cells translocated over the agar surfaces either as individuals or in groups (Fig. 1F). The phenotypes of the mutants mimic those of some *Myxococcus xanthus* A^+ S^- mutants deficient in extracellular polysaccharides (EPS), such as DK3468 (dsp) (22) and YZ603 (adiE) (2), suggesting that the mutant HL-1 is defective in social motility.

The mutant HL-1 was assessed for developmental ability on TPM starvation medium by methods described previously (15). The mutant cells formed a weak and rudimentary fruiting-body structure (Fig. 1G), compared to the mature fruiting bodies of wild-type cells (Fig. 1H). The sporulation frequency of HL-1 was only 0.67% that of HW-1, which was able to develop about 3.0 × 10^6 spores from an initial input of 5 × 10^7 cells after a 5-day incubation. These results indicate that the mutant HL-1 is also significantly defective in developmental aggregation and sporulation.

Cells of the mutant HL-1 dispersed easily in liquid culture, indicating possible defects in cell cohesion. The amount of EPS was assessed by scanning electron microscopy, which revealed less extracellular matrix on the surfaces of HL-1 cells than on those of HW-1 cells. The dyes Congo red and trypan blue, which bind to EPS (3, 26), were employed for quantitative analysis of the extracellular matrices of the wild-type and mutant strains by the method described previously (2). The wild type strain HW-1 bound 69.8% and 48.4% of Congo red and trypan blue, respectively, compared to 49.8% and 22.9% for HL-1, indicating less cohesion ability of the mutant. A clumping assay, using the method described previously by Shimkets (22), also confirmed that the mutant cells exhibited less cohesion than the wild-type cells, and the relative absorbance readings at the 100-min end point for the mutant and wild-type cells in morpholinepropanesulfonic acid (MOPS) buffer were 0.563 ±
We reported previously that the salt-tolerant Myxococcus strains exhibited enhanced S motility in the presence of seawater on either soft or hard CYE agar (24). Interestingly, the effect of seawater on swarming ability was significantly decreased by the mutation (Fig. 2). These results suggest that the mutated gene(s) is involved in or responsible for the enhancement of surface translocation in response to the presence of seawater.

The mutagenized gene in HL-1 and the related genes in this locus. The MiniHimar1-\(lacZ\) transposon contains the Escherichia coli replication origin R6K. To identify the gene mutated in HL-1, its genomic DNA was digested with SphI and BamHI, self-ligated for \(E. coli\) transformation, and then sequenced. Two thermal asymmetric interlaced PCR amplifications (18)
were then performed. The nested specific primers and arbitrary degenerate primers (AD primers) used in this study are listed in Table 2. An upstream 6.3-kb segment and a downstream 6.7-kb segment flanking the insertion were obtained. After sequencing, the junction sequence between the two segments was further PCR amplified from the wild-type strain HW-1 and sequenced. By using the FramePlot program, version 2.3.2 (http://www.nih.go.jp/%7Ejun/cgi-bin/frameplot.pl), the 13-kb segment (deposited in GenBank with the accession number EF371498) was predicted to contain six open reading frames (ORFs), which likely form a gene cluster (Fig. 3). Blastx search against the GenBank database revealed that the sequences are significantly homologous to the corresponding ORFs from M. xanthus DK1622 and Stigmatella aurantiaca (accession numbers EF371498 and H11032, respectively). Blastx analysis of the potential cleavage sites is at residues 24, 25, 21, 20, and 22, respectively. Bioinformatics analysis of the predicted mts gene, together with the phenotypes of the mutant, suggested that the Mts proteins are probably involved in the construction of the cell surface matrix for S motility and development.

**Characteristics of M. xanthus mts mutants.** Genetic manipulation of HW-1 is difficult. Attempts to make targeted mts mutations in HW-1 using established protocols for M. xanthus (12) were unsuccessful (data not shown). Instead, an in-frame deletion of mtsC (429 amino acid residues were deleted from 496 amino acids of MXAN1334) was performed for M. xanthus strains DK1622 (A+ S+), DK1217 (A+ S−), and DK10410 (A− S−) to determine the function of mtsC. The mutants in the A+ S+ and A− S− backgrounds formed smaller colonies on soft agar than their parent strains, whereas the mutation in the A+ S− background did not lead to changes in the colonies on either hard or soft agar (Fig. 4). The results indicated that mtsC is likely also involved in S motility in M. xanthus.

Interestingly, the effect of the in-frame deletion on motility in M. xanthus seems to be less marked than that of the insertion in mtsC in M. fulvus HW-1. To determine whether the Mts proteins are important for S motility and development in M. xanthus, we completely deleted the sequence of MXAN1332 to MXAN1337 from DK1622. Plasmid pZCY9, which contains a deletion of all the mts genes, was transformed into DK1622 to give rise to the mutant ZC16-23. ZC16-23 produced smaller colonies (about 75% the size of colonies of the wild-type strain DK1622) on hard CTT (1% (wt/vol) Casitone, 8 mM MgSO4, 0.5% (wt/vol) D-glucose, 1 mM K2HPO4, KH2PO4 [pH 7.6], and 10 mM Tris-HCl [pH 7.6]) agar (Fig. 4). Compared to the change of the colony size

**FIG. 4.** Swarming colony sizes of the mutant HL-1 and the wild-type strain HW-1 on the nutrient medium CTT without (dashed lines) or with (solid lines) 20% seawater and with different concentrations of agar.

![FIG. 4](http://example.com/figure4.png)

**TABLE 2. Primers used for TAIL-PCR amplification**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Length (bp)</th>
<th>Sequence (5’ to 3’)</th>
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<td>Specific primers</td>
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<td>AD4</td>
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<td>TG(A/T)GNAG(A/T)ANCA(G/C)AGA</td>
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<tr>
<td>AD7</td>
<td>16</td>
<td>CA(A/T)CGICNGA(G/C)GAA</td>
<td>43.0</td>
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a TAIL-PCR, thermal asymmetric interlaced PCR; T_m, melting temperature.
in *M. fulvus* HL-1 by the insertion in *mtsC*, the effect of the complete deletion of *mts* in *M. xanthus* is not as prominent. The MXAN1334 insertion mutant exhibited motility phenotypes similar to those of the deletion mutant ZC16-4 (data not shown). These results indicate that the motility differences between the *M. fulvus* HW-1 insertion mutant and the *M. xanthus* DK1622 deletion mutant were not likely due to polar effects of transposon insertion. The *mts* products obviously play a more important role in *S* motility in *M. fulvus* HW-1 than they do in *M. xanthus* DK1622.

In contrast to their role in *S* motility, Mts proteins are essential for fruiting-body formation and sporulation in *M. xanthus*. When inoculated onto TPM (10 mM Tris HCl [pH 7.6], 8 mM MgSO₄, 1 mM K₂HPO₄·K_H₂PO₄ [pH 7.6], and 1.5% agar) plates, DK1622 cells formed visible fruiting-body structures from the second day of incubation; whereas the mutants ZC16-4 and ZC16-23 did not form fruiting bodies even after 5 days of incubation (Fig. 4). Under our assay conditions, DK1622 cells produced 1.3 × 10⁶ spores from an initial input of 5 × 10⁷ cells after 5 days of incubation. The sporulation frequency of ZC16-4 and ZC16-23 was only 0.01% that of DK1622 cells, and no spores were detected for ZC16-23. The effects of *mts* genes on development are rather similar in *M. fulvus* HW-1 and *M. xanthus* DK1622.

**Concluding remarks.** *S* motility is a cell-cell contact-dependent mode of movement that is essential for myxobacterial predation and development. Youderian and Hartzell recently suggested that at least 25% of the nonessential genes involved in *S* motility had not yet been identified, probably due to preferential mutation hot spots (27). Different *Myxococcus* species or strains may possess genotypes with subtle differences in motility. The diversification of the motility genotypes thus provides a useful source and also an efficient approach to discovering the hypomorph motility genes in the model strain *M. xanthus* DK1622. This paper describes a new genetic locus (*mts*) that is required for *S* motility and development in *Myxococcus*. The *mts* locus is predicted to contain six ORFs. Four components—MtsB, MtsC, MtsD, and MtsE—were predicted to be homologous to the type 3 thrombospondins (Goldman et al. [6] predicted two, MtsC and MtsE), which are multifunctional proteins with affinity for cell surfaces, calcium ions, and many matrix macromolecules (16). The thrombospondins have been reported previously only in eukaryotes. In this paper we determined that the *mts* locus is also involved in *Myxococcus* cellular cohesion. Although the *mts* products are required in development, they affect *S* motility to different extents in different *Myxococcus* strains. The Mts proteins probably function cooperatively, serving as components for intercellular cohesion, like the thrombospondins in eukaryotic cells (16). However, the predicted myxobacterial thrombospondin-like proteins are similar to thrombospondin 3 only in the variable region, not in the highly conserved region or the calcium binding motifs. The structure and function of Mts are under further investigation.

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**REFERENCES**


