Arch Microbiol (2000) 174 : 399–405
DOI 10.1007/ s002030000226

ORIGINAL PAPER

**Zhaomin Yang · Dongchuan Guo · M. Gabriela Bowden
Hong Sun · Leming Tong · Zhuo Li · Alfred E. Brown
Heidi B. Kaplan · Wenyuan Shi**

The *Myxococcus xanthus wbgB* gene encodes a glycosyltransferase homologue required for lipopolysaccharide O-antigen biosynthesis

Received: 29 March 2000 / Revised: 30 August 2000 / Accepted: 20 September 2000 / Published online: 9 November 2000

Abstract *Myxococcus xanthus* is a gram-negative soil bacterium that initiates a complex developmental program in response to starvation. A transposon insertion (Tn*5*-*lac* Ω109) mutant with developmental deficiencies was isolated and characterized in this study. A strain containing this insertion mutation in an otherwise wild-type background showed delayed developmental aggregation for about 12 h and sporulated at 1–2% of the wild-type level. Tn*5*-*lac* Ω109 was found to have disrupted the *M. xanthus wbgB* gene, which is located 2.1 kb downstream of the *M. xanthus* lipopolysacharide (LPS) O-antigen biosynthesis genes *wzm wzt wbgA*. The deduced polypeptide sequence of WbgB shares significant similarity with bacterial glycosyltransferases including *M. xanthus* WbgA. The *wbgB*::Tn*5*-*lac* Ω109 mutant was found to be defective in LPS O-antigen synthesis by immunochemical analysis. Further mutational analysis indicated that the defects of the *wbgB*::Tn*5*-*lac* Ω109 mutant were not the result of polar effects on downstream genes. Various motility assays demonstrated that the Tn*5*-*lac* Ω109 mutation affected both social (S) and adventurous (A) gliding motility of *M. xanthus* cells. The pleiotrophic effects of *wbgB* mutations indicate the importance of LPS O-antigen biosynthesis for various cellular functions in *M. xanthus*.

Keywords Myxobacteria · *Myxococcus* · Lipopolysaccharide · O-antigen · Gliding motility · Development · Fruiting body

Z. Yang (✉) · Z. Li · A. E. Brown
Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA
e-mail: yangzha@auburn.edu,
Tel.: +1-334-8441656, Fax: +1-334-8441645

D. Guo · G. B. Bowden · H. B. Kaplan
Department of Microbiology and Molecular Genetics, University of Texas Medical School, Houston, TX 77030, USA

H. Sun · L. Tong · W. Shi
School of Dentistry, Molecular Biology Institute and Dental Research Institute, University of California, Los Angeles, CA 90095-1668, USA

Abbreviations. *LPS* Lipopolysaccharide · *A-motility* Adventurous motility · *S-motility* Social motility

Introduction

Myxococcus xanthus is a gram-negative soil bacterium that can move on a solid surface without the aid of flagella (Hartzell and Youderian 1995; Spormann 1999). Two genetically and behaviorally distinct systems have been defined for the control of *M. xanthus* gliding motility (Hodgkin and Kaiser 1979a, b), adventurous (A)-motility and social (S)-motility. Mutations in A- or S-motility genes inactivate the corresponding system, but cells are still motile by virtue of the remaining system. A^+S^- (A-motile) cells retain A-motility behavior and the ability to move as single cells. A^-S^+ (S-motile) cells retain S-motility behaviors and can only move as apparent cell groups. The two motility systems show different selective advantages on various surfaces (Shi and Zusman 1993). S-motility is particularly effective on relatively soft and wet surfaces. In addition, S, but not A, motility is central to *M. xanthus* development because almost all of the known S-motility null mutants are defective in fruiting body development (Hodgkin and Kaiser 1979b; MacNeil et al. 1994b). Many of the A-motility mutants, on the other hand, can still undergo developmental aggregation without obvious defects in aggregation (Hodgkin and Kaiser 1979a, b; MacNeil et al. 1994a, b). In both motility systems, some classes of mutants can be stimulated to transiently gain motility when they are in contact with wild-type cells or cells of another class. The stimulatable A-motility mutants are designated *cgl*, and the non-stimulatable are designated *agl* (Hodgkin and Kaiser 1979a). The stimulatable S-motility mutants are designated *tgl* and the non-stimulatable are designated *sgl* (Hodgkin and Kaiser 1979b)

Previous studies indicate that *M. xanthus* S-motility requires the cell-surface components type IV pili (Kaiser 1979; Wu and Kaiser 1995) and extracellular matrix fibrils (Arnold and Shimkets 1988b; Shimkets 1986a, b). Most of the known S-motility mutations affect one of

these two components. The *pil* and the *tgl* mutants, for example, are defective in the biogenesis and/or function of type IV pili (Rodriguez-Soto and Kaiser 1997; Wu and Kaiser 1995; Wu et al. 1997, 1998). The *dsp* (Arnold and Shimkets 1988b; Shimkets 1986a, b), *sglK* (Weimer et al. 1998), and *dif* (Yang et al. 1998b, 2000) mutants are defective in *M. xanthus* fibril production. All of these S-motility mutants are defective in development and in cellular cohesion to various degrees, validating the ideas that S-motility is the primary system for developmental aggregation and that cellular cohesion is a critical component of *M. xanthus* S motility.

The lipopolysaccharide (LPS) O-antigen is another cell-surface component found to be required for normal *M. xanthus* gliding motility (Bowden and Kaplan 1998). The *M. xanthus* LPS has a structure typical of a gram-negative bacterium. It consists of three regions: lipid A, which is present in the outer leaflet of the outer membrane; the core oligosaccharide attached to lipid A, and a repeated oligosaccharide of variable length, termed the O-antigen, attached to the core (Fink and Zissler 1989). The precise structure of the *M. xanthus* LPS has not been determined; however, the LPS O-antigen appears to be methylated during early development (Panasenko 1985) and to contain a novel sugar derivative (Panasenko et al. 1989). Among the LPS O-antigen mutants isolated, the best characterized are those that map to the *wzm wzt wbgA* operon (Bowden and Kaplan 1998; Guo et al. 1996). The *wzm wzt* genes code for homologues of the ATP-binding cassette (ABC) transporters, which presumably transfer the O-antigen from the cytoplasm to the periplasm, where it is attached to the lipid A and core components of the LPS. Although the function of the *wbgA* gene in O-antigen biosynthesis is unknown, part of the deduced amino acid sequence of WbgA displays high sequence similarity to bacterial glycosyltransferases. Mutations in each of the *wzm wzt wbgA* genes generate mutants that have similar defects in LPS O-antigen biosynthesis, gliding motility, fruiting body formation, and developmental gene expression (Kaplan et al. 1991; Guo et al. 1996; Bowden and Kaplan 1998; M. Bowden, Ph.D. Thesis, University of Texas Houston Health Science Center, 1999).

In this report, we describe the identification and characterization of the *M. xanthus wbgB* gene, which encodes a glycosyltransferase homologue required for LPS O-antigen biosynthesis. The *wbgB* gene maps 2.1 kb downstream of the *M. xanthus* LPS O-antigen biosynthesis genes *wzm wzt wbgA*. Similar to the other LPS O-antigen mutants, *wbgB* mutants were found to be defective in fruiting body development, sporulation and gliding motility.

Materials and methods

Bacterial strains, plasmids, phages, media, and growth conditions

Myxococcus xanthus strains and plasmids used in this study are listed in Table 1. *Escherichia coli* strain XL1-blue from Stratagene was used for general cloning purposes. *M. xanthus* phage Mx4 (*ts*18 *ts*27) was used for generalized transduction (Campos et al. 1978). Phage P1::Tn*5*-*lac* was used for transposon mutagenesis (Kroos and Kaiser 1984). *M. xanthus* was grown and maintained at 32 °C in CYE or CTT medium (Campos et al. 1978; Hodgkin and Kaiser 1977). Development-inducing media used in this study were MOPS medium and CF medium (Hagen et al. 1978). *E. coli* strains were grown and maintained at 37 °C in LB medium (Miller 1972).

Table 1 Bacterial strains and plasmids

Strains/plasmids	Genotype/description	Reference/source
Strains		
DK1217	*aglB1*	Hodgkin and Kaiser (1979a)
DK1218	*cglB2*	Hodgkin and Kaiser (1979a)
DK1253	*tgl-1*	Hodgkin and Kaiser (1979b)
DK1300	*sglG1*	Hodgkin and Kaiser (1979b)
DK1622	Wild-type	Kaiser (1979)
DK4167	*mgl9*	Hodgkin and Kaiser (1979a)
DZF1	*sglA1*	Morrison and Zusman (1979)
HK1199	*wbgB*::pDG282	This study
HK1304	*wzm wzt wbgA sglA1*	Bowden and Kaplan (1998)
SW109	*wbgB*::Tn*5*-*lac* Ω109 *sglA1*	This study
SW511	*wbgB*::Tn*5*-*lac* Ω109	This study
SW512	wbgB::Tn*5*-*lac* Ω109 *aglB1*	This study
SW513	*wbgB*::Tn*5*-*lac* Ω109 *cglB2*	This study
SW514	*wbgB*::Tn*5*-*lac* Ω109 *tgl-1*	This study
SW515	*wbgB*::Tn*5*-*lac* Ω109 *sglG1*	This study
Plasmids		
pBGS18	Cloning vector	Spratt et al. (1986)
PUC18	Cloning vector	Yanisch-Perron et al. (1985)
pYG114	See text	This study
pDG282	See text	This study
6B2E	Cosmid	Unpublished (see text)

Mutant isolation and strain construction

Myxococcus xanthus SW109 was isolated in a genetic screen for developmental mutants as previously described (Yang et al. 1998a). Briefly, *M. xanthus* DZF1 was first mutagenized by a Tn*5* transposon derivative Tn*5*-*lac* by P1 transduction (Kroos and Kaiser 1984). The transductants, selected on CYE plates with 100 μg kanamycin/ml, were screened on CF plates for developmental deficiencies. Mx4-mediated generalized transduction was used to transfer mutations between *M. xanthus* strains (Campos et al. 1978). Strain SW511 was constructed by the transfer of Tn*5*-*lac* Ω109 to the wild-type strain DK1622. The double mutants SW512, SW513, SW514 and SW515 were constructed by the introduction of the Tn*5*-*lac* Ω109 insertion into the relevant parental strains DK1217, DK1218, DK1253, and DK1300. A strain containing *aglB* and Δ*wzm wzt wbgA* was constructed by infecting DK1217 with an Mx4-transducing phage stock from HK1304. Strain HK1199 was constructed by the homologous integration of pDG282 into *M. xanthus* DK1622 by electroporation (Kashefi and Hartzell 1995).

Gene cloning, plasmid construction, DNA sequencing and computer analysis

An *Eco*RI DNA fragment from mutant SW109 chromosomal DNA containing the Kan^r determinant from Tn*5*-*lac* Ω109 and the adjacent *M. xanthus* DNA was cloned into the *Eco*RI site of pUC18 (Yanisch-Perron et al. 1985) to generate pYG114. A 0.54-kb *Eco*RI-*Pst*I fragment from pYG114 was then used as a probe and identified the cosmid clone 6B2E from the *M. xanthus* genomic DNA library constructed by R. Gill (University of Colorado, unpublished data). Plasmid pDG282 was constructed by cloning into pBGS18 (Spratt et al. 1986) a 2.0-kb *Eco*RI-*Apa*I fragment that contains sequences starting from codon 284 of *wbgB* to 410 base pairs (bp) beyond the predicted stop codon. DNA was sequenced by the automated DNA sequencing facilities at the University of California (Davis, Calif., USA) and at the Department of Microbiology and Molecular Genetics, University of Texas Medical School (Houston, Tex., USA). Sequence assembly and analysis were performed with BCM Search Launcher, BLAST, GCG and DNAMAN programs (Altschul et al. 1990; Devereux et al. 1984; Smith et al. 1996). The nucleotide sequences of *M. xanthus wbgB* gene reported in this paper are available in the GenBank database under the accession number AF194331.

Phenotypic characterization

For the characterization of developmental phenotypes, cells from overnight cultures (1–5×10^8 cells ml^{-1}) were resuspended in MOPS medium at about 2.5×10^9 cells ml^{-1}, spotted at time zero on MOPS plates and incubated at 32 °C. Developmental aggregation over 48 h was examined and photographed with a 4× objective lens under bright field. The formation of developmental spores was quantitated after 4 days of incubation on MOPS plates. Samples were scraped off MOPS plates, treated with 1% SDS, and SDS-resistant and refractile myxospores were counted under a microscope using a Petroff-Hausser chamber. For colony expansion or the swarming assays, cells from overnight culture were resuspended in CYE medium at a cell density of 5×10^9 cells ml^{-1}. The cell suspension (10 μl) was spotted onto CYE plates with 0.3% or 1.5% agar and incubated at 32 °C. The diameters of the swarming colonies were measured daily.

M. xanthus O-antigens were immunodetected using cells grown exponentially as previously described (Guo et al. 1996). The appropriate monoclonal antibodies (MAbs) against *M. xanthus* LPS O-antigens (generously provided by M. Dworkin) were used as the primary antibodies, and a goat anti-mouse IgG-alkaline phosphatase conjugate (Promega) was used as the secondary antibody.

Results

Tn*5*-lac Ω109 insertion results in *M. xanthus* developmental defects

To identify genes necessary for *M. xanthus* development, Tn*5*-*lac* transposon mutants were screened for deficiencies in fruiting body formation. *M. xanthus* DZF1 was used as the parental strain in the mutagenesis because, compared with the wild-type, it is more easily infected by transducing phages, grows dispersed in liquid culture and forms more compact colonies on agar plates (Campos et al. 1978). Mutant SW109, harboring the Tn*5*-*lac* Ω109 insertion, was defective in development. It showed no sign of aggregation even after prolonged starvation (Fig. 1A). In contrast, the parental strain DZF1 formed well-organized fruiting bodies after 24 h of starvation as expected. The number of myxospores formed by mutant SW109 is less than 0.1% of that by strain DZF1. Mx4-mediated transduction (O'Connor and Zusman 1986) using mutant SW109 as the donor and strain DZF1 as the recipient indicated that the developmental defect of *M. xanthus* SW109 was 100% linked to the Tn*5*-*lac* Ω109 insertion.

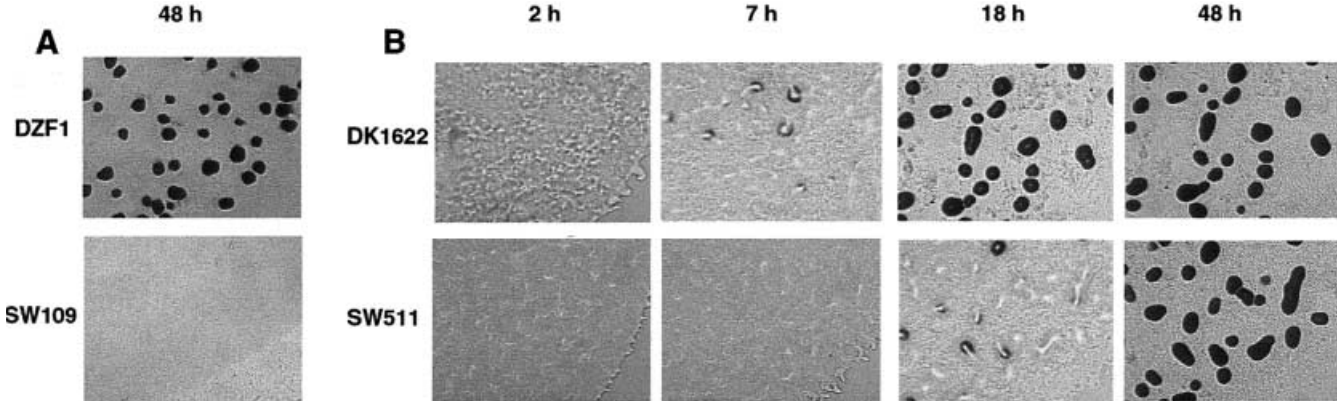

Fig. 1 Developmental defects of Tn*5*-*lac* Ω109 insertion mutants in DZF1 (**A**) and DK1622 (**B**) backgrounds. Fruiting body development was examined on MOPS plates and photographed with a 4× objective lens under bright field. For each strain, a 10-μl cell suspension at 2.5×10^9 cells ml^{-1} was spotted onto developmental plates and incubated at 32 °C. The strains and the time at which the photographs were taken are indicated

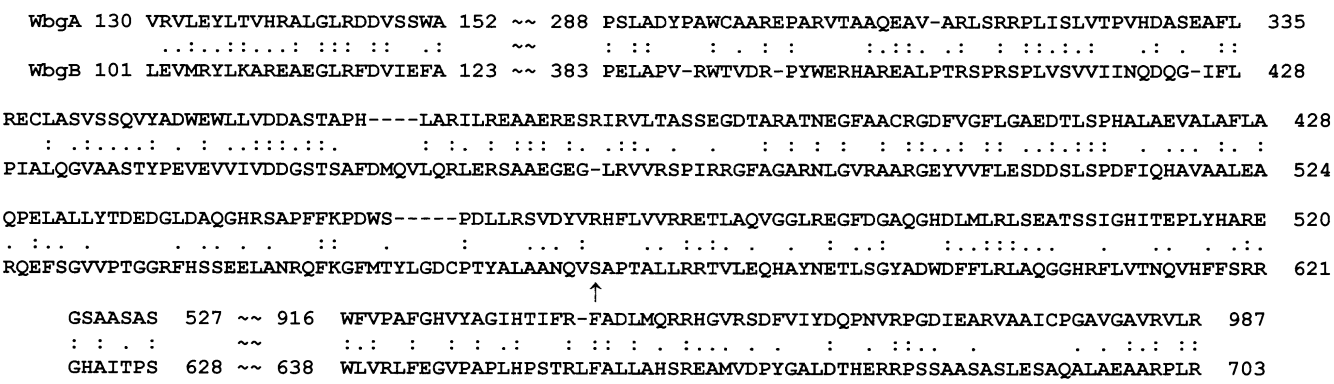

Fig. 2 Comparison of the *Myxococcus xanthus* WbgA and WbgB amino acid sequences. Identical residues are indicated by *colons* (:) and conserved residues are indicated by *periods* (.). Breaks in the sequences are indicated by *tildes* (~). WbgA and WbgB share over 28% identity and 55% similarity over the aligned regions. Tn*5-lac* Ω109 inserted into codon 570 of *wbgB*, which codes for a serine (*S*) residue indicated by the *arrow*

To further characterize the phenotypes of the Tn*5*-*lac* Ω109 insertion mutation, strain SW511, containing the Tn*5*-*lac* Ω109 insertion in an otherwise wild-type background, was generated and examined. The ability of mutant SW511 to form fruiting bodies and to sporulate was different from both its parental strains DK1622 and SW109. Compared to wild-type DK1622, developmental aggregation of mutant SW511 was delayed about 10–12 h (Fig. 1B). Aggregation of *M. xanthus* DK1622 cells became obvious after 6–7 h on MOPS plates, and after 18 h *M. xanthus* DK1622 formed well-aggregated fruiting bodies containing spherical myxospores. On the other hand, mutant SW511 showed no sign of aggregation after 12 h of starvation and visible aggregation appeared only after about 18 h. In contrast to the mutant SW109 that remained non-aggregated, mutant SW511 formed wild-type-like fruiting bodies by 48 h. The sporulation efficiency of SW511, at 1–2% of the wild-type level, was higher than SW109. Compared with strain SW511, mutant SW109 displayed more severe developmental defects, which is likely due to the combined effects of Tn*5*-*lac* Ω109 and the leaky *sglA* mutation in DFZ1 background (Table 1).

Tn*5*-lac Ω109 insertion disrupts *M. xanthus wbgB* gene

To further characterize the locus identified by Tn5-*lac* Ω109, the gene disrupted by the transposon was cloned to generate plasmid pYG114, and a cosmid (6B2E) containing this region, which also contained the *M. xanthus* LPS O-antigen biosynthesis genes *wzm wzt wbgA* (Guo et al. 1996; Bowden and Kaplan 1998), was identified. Southern analyses indicated that the *M. xanthus* DNA in pYG114 hybridized to a region about 3–4 kb downstream of *wbgA* (data not shown). DNA sequencing and analyses indicated that the Tn*5*-*lac* Ω109 disrupted a predicted ORF of 780 amino acids (accession no. AF194331). Codon preference analysis (Devereux et al. 1984) revealed that this ORF has a strong codon bias toward GC at the third position, as expected for genes in *M. xanthus*, which has a 67.5% G+C content (Shimkets 1993). This ORF reads in the same direction as *wzm wzt wbgA*, and its predicted initiation codon (ATG) is 2.1 kb downstream of the stop codon of *wbgA*.

The deduced polypeptide sequence of the ORF is significantly similar to putative bacterial glycosyltransferases including *M. xanthus* WbgB. Its C-terminus has the highest percent identity to a *Leptospira borgpetersenii* ORF (accession no. AF078135.1) within the LPS O-antigen biosynthetic locus (28% over 231 amino acids), and to a putative glycosyltransferase (accession no. AP000002.1) from a thermophilic archaeon *Pyrococcus horikoshii* (25% over 205 amino acids). The N-terminal portion of *wbgB* is 25% identical over 259 amino acids to the N-terminus of a putative glycosyltransferase from the cyanobacterium *Synechocystis* sp. (accession no. D90914.1). As shown in Fig. 2, this *M. xanthus* ORF is highly similar to *M. xanthus* WbgA, with its C-terminus 27.6% identical and 54.4% similar to residues 288–527 of WbgA, which is predicted to contain 1276 amino acid residues. The *M. xanthus wbgA* gene is known to be required for LPS biosynthesis because *wbgA* mutations block LPS O-antigen production (Guo et al. 1996). The gene that Tn*5*-*lac* Ω109 disrupted was designated *wbgB* because of its similarity and proximity to *wbgA* and their shared functions in LPS O-antigen biosynthesis (see below).

The *wbgB* gene is essential for *M. xanthus* LPS O-antigen biosynthesis

On the basis of the similarity and proximity of *wbgB* to *wbgA*, we decided to investigate whether *wbgB* was involved in *M. xanthus* LPS O-antigen biosynthesis. The biogenesis of LPS was examined by immunoblot analysis with MAbs against *M. xanthus* LPS O-antigens as shown in Fig. 3 (Gill and Dworkin 1986; Guo et al. 1996). The immunoblot analysis revealed that SW511 reacted very weakly with all the O-antigen-specific MAbs, as did the negative control mutant HK1304, whereas the wild-type strain reacted strongly with all the MAbs examined. These data suggested that a wild-type *wbgB* gene is required for the biosynthesis of LPS O-antigens in *M. xanthus*.

Fig. 3 Immunoblot analysis of whole *M. xanthus* cells with monoclonal antibodies (MAbs) against *M. xanthus* LPS O-antigen. Approximately 1×10^6 exponentially growing cells were transferred to nitrocellulose membranes and reacted with the MAbs listed at the *top*. The strains used are indicated on the *left*. DK1622 was used as the positive control, HK1304 as the negative control

The defects in O-antigen biosynthesis of mutant SW511 could be attributed to the absence of the *wbgB* gene product or to a polar effect of the Tn*5-lac* Ω109 insertion on downstream genes. To resolve this uncertainty, pDG282, which contains sequences from 852 bp downstream of the predicted translation start site of *wbgB* to 410 bp past its stop codon, was integrated into the chromosomal *wbgB* locus of wild-type strain DK1622 by single cross-over homologous recombination. The resulting merodiploid strain HK1199 contains a wild-type *wbgB* gene, but the expression of any downstream genes cotranscribed with *wbgB* in the wild-type would have been disrupted (Gill and Shimkets 1993). Strain HK1199 appeared wild-type for all of the characteristics examined, including O-antigen biosynthesis (Fig. 3), fruiting body formation and motility (data not shown). This indicates that the mutant phenotype of the *wbgB* insertion mutant SW511 is due to the alteration of the *wbgB* gene, and not to any polar effect on downstream genes. In addition, recent sequencing efforts revealed that the gene immediately downstream of *wbgB* is transcribed in the opposite direction from *wbgB* (M. Esmaeiliyan and H.B. Kaplan, unpublished observations). A wild-type *wbgB* is therefore necessary for *M. xanthus* O-antigen biosynthesis.

wbgB mutants are defective in *M. xanthus* gliding motility

In the initial characterization of *M. xanthus* mutant SW109, extracellular complementation experiments (Hagen et al. 1978) demonstrated that fruiting body formation could not be restored to the *wbgB* mutant SW109 by mixing with cells of either the wild-type or any known signaling mutant (data not shown). In addition, neither *wbgB* mutant SW109 nor SW511 showed the characteristic phenotypes of the "frizzy" mutants, which are altered in their reversal frequency(Blackhart and Zusman 1985) (Fig. 1). One remaining possibility to explain the observed developmental abnormalities is that *wbgB* mutations cause motility defects. Suggestive of and consistent with such a possibility are the physical proximity of *wbgB* to *wzm wzt wbgA*, the similar defects of these mutants in LPS O-antigen biogenesis and their delayed developmental phenotypes (Figs. 1, 3) (Bowden and Kaplan 1998).

The effect of *wbgB* mutations on motility was compared with the known LPS O-antigen mutation Δ*wzm wzt wbgA* (Bowden and Kaplan 1998). When the *wbgB*::Tn*5-lac* Ω109 mutation was introduced into A^+S^- mutants DK1253 and DK1300, the resulting double mutants SW514 and SW515 showed a colony-edge morphology similar to their A^+S^- parents and the Δ*wzm wzt wbgA* S^- transductants (data not shown), indicating that these LPS O-antigen mutations do not appear to effect A-motility by this assay. This is consistent with previous observations of the Δ*wzm wzt wbgA* mutations (Bowden and Kaplan 1998). When the *wbgB*::Tn*5-lac* Ω109 mutation was introduced into the A^-S^+ mutant DK1217 (*aglB1*), the colonies of the fresh transductants (SW512) showed smooth edges similar to those of the fresh Δ*wzm wzt wbgA aglB1* transductants, indicating that the *wbgB* mutations caused S-motility defects (Fig. 4A, C). Surprisingly, when toothpick-transferred to new plates, the colonies of the *aglB wbgB* double mutant expanded faster than those of the Δ*wzm wzt wbgA aglB1*, mutant and the colony edges of the *aglB wbgB* double mutant (SW501) also showed extensive flaring (Fig. 4B, D).

Since it is known that *M. xanthus* S-motility is more effective on soft surfaces whereas A-motility is preferentially utilized on hard surfaces, the motility of the *wbgB* mutant (SW511) was further analyzed by comparing its swarming ability with that of various strains or transduc-

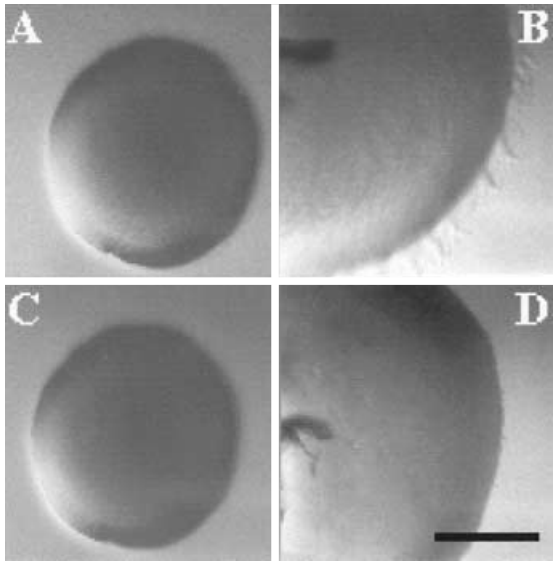

Fig. 4 Colony morphology of *M. xanthus* LPS O-antigen mutants. Shown are colony-edge morphologies of double mutants resulting from the transduction Ωinto strain DK1217 (*aglB1*) of the Tn*5-lac* Ω109 (**A**, **B**) and Δ*wzm wzt wbgA* (**C**, **D**) mutations. Initial transductants were photographed 1 week after transduction (**A**, **C**). The transductants were transferred by toothpick onto 1.5% agar CTT plates with 50 μg kanamycin/ml, grown at 32 °C and photographed 5 days after the transfer (**B**, **D**). *Bar* 1 mm

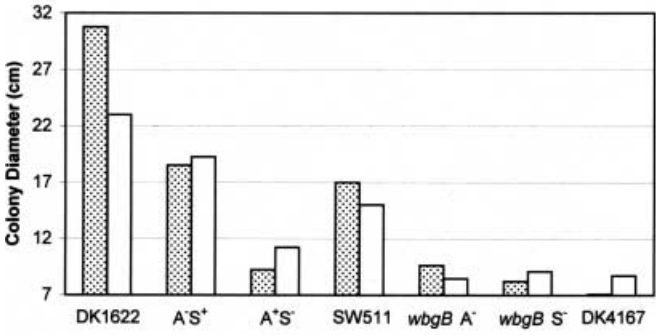

Fig. 5 Expansion of swarming colonies of *M. xanthus* strains. The experiment was conducted as described in Materials and methods. *Filled bars* show the expansion on 0.3% agar plates, and *open bars* on 1.5% agar plates. The results presented here are the diameters of the colonies after 120 h of incubation at 32 °C. The *bars* labeled A^+S^- represent the average from DK1253 and DK1300, A^-S^+ from DK1217 and DK1218, *wbgB* A^- from SW512, and SW513, and *wbgB* S^- from SW514 and SW515. SW511 is the *wbgB* mutant in an otherwise wild-type background. DK1622 was used as the wild-type control and DK4167 as the A^-S^- negative control. Duplicate samples were run for each strain

tants on both soft (0.3% agar) and hard (1.5% agar) surfaces as described in Materials and methods. The results are shown in Fig. 5. A few conclusions may be drawn from these results. First, these assays confirmed the earlier observation that *wbgB* mutants have defects in S-motility. Cells of propagated *wbgB* A^- double mutants displayed diminished motility in comparison with its S-motile (A^-S^+) parents on both soft and hard agar surfaces. Since only S-motility is present in the S-motile (A^-S^+) parents, the reduced swarming of the double mutants (*wbgB* A^-) should be due to a reduction of S-motility and to the addition of the *wbgB* mutation. Second, the motility displayed by propagated *wbgB* mutant cells is at least partially S-motility because SW511 displayed better swarming than A^+S^- mutants on both types of surfaces, especially on soft agar plates (Fig. 5). Furthermore, when S-motility mutations were combined with a *wbgB* mutation, the double mutants (*wbgB* S^-) showed further reduction in swarming when compared with the *wbgB* parental mutant SW511. This further confirms that the propagated *wbgB* mutant SW511 possesses S-motility that can be eliminated by the introduction of additional S-motility mutations. Third, the swarming expansion of the propagated *wbgB* mutant SW511 displayed an intermediate motility phenotype (Fig. 5) in that it is unlike either a simple A^+S^- or A^-S^+ mutant. When the mutation *wbgB*::Tn*5-lac* Ω109 was introduced into A^+S^- strains, *wbgB* S^- double mutants displayed a reproducible reduction in swarming on 1.5% agar surfaces compared to the A^+S^- parents. The reduction in swarming of *wbgB* S^- double mutants can be attributed to a reduction in A-motility resulting from the *wbgB* mutation. These observations suggest that the *wbgB* mutation affects *M. xanthus* A-motility as well. The defects of LPS O-antigen mutants in single-cell motility have been documented and will be published elsewhere. The results here indicate that colony edge morphology alone may not be adequate to define the complex motility behaviors of *M. xanthus* cells.

Discussion

We report here the identification and characterization of *M. xanthus wbgB*, a gene that is required for wild-type fruiting body development, wild-type gliding motility, and LPS O-antigen biosynthesis. The phenotypes of the *wbgB* mutants are largely consistent with the phenotypes of other LPS mutants reported by Bowden and Kaplan (1998). The *wbgB* gene was identified by a Tn*5* insertion mutation resulted in defects in fruiting body formation and sporulation. Under our experimental conditions, the transposon insertion caused a delay of 10–12 h in developmental aggregation in an otherwise wild-type background. The formation of developmental spores by *wbgB* mutants was reduced to 1–2% that of the wild-type level despite the formation of fruiting-body-like aggregates after prolonged incubation. Moreover, *wbgB* mutants were shown to have motility defects in both S and A systems. Molecular cloning and sequence analysis indicate that the deduced WbgB protein is similar to putative bacterial glycosyltransferases including *M. xanthus* WbgA, which is known to be necessary for *M. xanthus* LPS O-antigen biogenesis and wild-type motility (Bowden and Kaplan 1998). In addition, *wbgB* was located to 2.1 kb downstream of *wbgA*, and we showed that LPS O-antigen biosynthesis in *M. xanthus* requires a wild-type *wbgB* gene.

Although the exact structure and composition of *M. xanthus* LPS O-antigen is not known, multiply glycosyltransferases are expected to be involved in O-antigen biosynthesis. Generally speaking, there is one specific glycosyltransferase for every sugar molecule present in the repeating unit of LPS O-antigens (Whitfield 1995). The wide variety of LPS O-antigen mutant phenotypes reflects the biological importance of LPS for *M. xanthus*. The requirement of LPS O-antigen for wild-type motility is unique in that the lack of O-antigens affects both S- and A-motility. Prior to this report, *mglA* and *mglB* were the only genes known to affect both S- and A-motility in *M. xanthus* (Hartzell and Kaiser 1991a, b).

The mechanism by which *mgl* genes regulate *M. xanthus* motility is not yet understood. In the cases of both *mgl* and LPS O-antigen biosynthesis genes, the underlying mechanisms must explain their unusual effects on both A- and S-motility. It is noted that all LPS O-antigen mutants produce limited slime trails (M. Bowden, Ph.D. Thesis, University of Texas Houston Health Science Center, 1999; W. Shi, unpublished observations), which suggests that LPS O-antigen mutations may alter the interactions between bacterial cells and their gliding substrata. An alteration of this type may explain the effects of those mutations on *M. xanthus* gliding motility. The defects in motility may in turn adversely affect fruiting body formation and sporulation. Many other possibilities exist however. Further studies are necessary to understand the function of this complex surface polysaccharide in the biology of *M. xanthus*.

Acknowledgments We would like to thank Dr. D. R. Zusman for helpful discussions. We thank Drs. B. Julien, R. Gill and M. Dworkin for kindly providing experimental materials. This work was supported by NIH grants GM54666 to W. Shi, GM47444 to H. Kaplan and NIH training grant 5-T32-DE-07296 and grants from Auburn University to Z. Yang.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Arnold JW, Shimkets LJ (1988b) Cell surface properties correlated with cohesion in *Myxococcus xanthus*. J Bacteriol 170:5771–5777

Blackhart BD, Zusman DR (1985) "Frizzy" genes of *Myxococcus xanthus* are involved in control of frequency of reversal of gliding motility. Proc Natl Acad Sci USA 82:8767–8770

Bowden MG, Kaplan HB (1998) The *Myxococcus xanthus* lipopolysaccharide O antigen is required for social motility and multicellular development. Mol Microbiol 30:275–284

Campos JM, Geisselsoder J, Zusman DR (1978) Isolation of bacteriophage MX4, a generalized transducing phage for *Myxococcus xanthus*. J Mol Biol 119:167–178

Devereux JP, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for VAX. Nucleic Acids Res 12:378–395

Fink JM, Zissler JF (1989) Characterization of lipopolysaccharide from *Myxococcus xanthus* by use of monoclonal antibodies. J Bacteriol 171:2028–2032

Gill JS, Dworkin M (1986) Cell surface antigens during submerged development of *Myxococcus xanthus* examined with monoclonal antibodies. J Bacteriol 168:505–511

Gill RR, Shimkets LJ (1993) Genetic approaches for analysis of myxobacterial behavior. In: Dworkin M, Kaiser D (eds) Myxobacteria II. ASM, Washington DC, pp 129–155

Guo D, Bowden MG, Pershad R, Kaplan HB (1996) The *Myxococcus xanthus rfbABC* operon encodes an ATP-binding cassette transporter homolog required for O-antigen biosynthesis and multicellular development. J Bacteriol 178:1631–1639

Hagen DC, Bretscher AP, Kaiser D (1978) Synergism between morphogenetic mutants of *Myxococcus xanthus*. Dev Biol 64: 284–296

Hartzell P, Kaiser D (1991a) Function of MglA, a 22-kilodalton protein essential for gliding in *Myxococcus xanthus*. J Bacteriol 173:7615–24

Hartzell P and Kaiser D (1991b) Upstream gene of the mgl operon controls the level of MglA protein in *Myxococcus xanthus*. J Bacteriol 173:7625–35

Hartzell PL, Youderian P (1995) Genetics of gliding motility and development in *Myxococcus xanthus*. Arch Microbiol 164: 309–323

Hodgkin J, Kaiser D (1977) Cell-to-cell stimulation of movement in nonmotile mutants of *Myxococcus*. Proc Natl Acad Sci USA 74:2938–2942

Hodgkin J, Kaiser D (1979a) Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): genes controlling movement of single cells. Mol Gen Genet 171:167–176

Hodgkin J, Kaiser D (1979b) Genetics of gliding motility in *Myxococcus xanthus*: two gene systems control movement. Mol Gen Genet 171:177–191

Kaiser D (1979) Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. Proc Natl Acad Sci USA 76: 5952–5956

Kaplan HB, Kuspa A, Kaiser D (1991) Suppressors that permit A-signal-independent developmental gene expression in *Myxococcus xanthus*. J Bacteriol 173:1460–1470

Kashefi K, Hartzell PL (1995) Genetic suppression and phenotypic masking of a *Myxococcus xanthus frzF*$^-$ defect. Mol Microbiol 15:483–494

Kroos L, Kaiser D (1984) Construction of Tn5 lac, a transposon that fuses *lacZ* expression to exogenous promoters, and its introduction into *Myxococcus xanthus*. Proc Natl Acad Sci USA 81:5816–5820

MacNeil SD, Calara F, Hartzell PL (1994a) New clusters of genes required for gliding motility in *Myxococcus xanthus*. Mol Microbiol 14:61–71

MacNeil SD, Mouzeyan A, Hartzell PL (1994b) Genes required for both gliding motility and development in *Myxococcus xanthus*. Mol Microbiol 14:785–795

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Morrison CE, Zusman DR (1979) *Myxococcus xanthus* mutants with temperature-sensitive stage-specific defects: evidence for independent pathways in development. J Bacteriol 140:1036–1042

O'Connor KA, Zusman DR (1986) Genetic analysis of *Myxococcus xanthus* and isolation of gene replacements after transduction under conditions of limited homology. J Bacteriol 167: 744–748

Panasenko SM (1985) Methylation of macromolecules during development in *Myxococcus xanthus*. J Bacteriol 164:495–500

Panasenko SM, Jann B, Jann K (1989) Novel change in the carbohydrate portion of *Myxococcus xanthus* lipopolysaccharide during development. J Bacteriol 171:1835–1840

Rodriguez-Soto JP, Kaiser D (1997) The *tgl* gene: social motility and stimulation in *Myxococcus xanthus*. J Bacteriol 179:4361–4371

Shi W, Zusman DR (1993) The two motility systems of *Myxococcus xanthus* show different selective advantages on various surfaces. Proc Natl Acad Sci USA 90:3378–3382

Shimkets LJ (1986a) Correlation of energy-dependent cell cohesion with social motility in *Myxococcus xanthus*. J Bacteriol 166:837–841

Shimkets LJ (1986b) Role of cell cohesion in *Myxococcus xanthus* fruiting body formation. J Bacteriol 166:842–848

Shimkets LJ (1993) The myxobacterial genome. In: Dworkin M, Kaiser D (eds) Myxobacteria II. ASM, Washington DC, pp 85–108

Smith RF, Wiese BA, Wojzynski MK, Davison DB, Worley KC (1996) BCM Search Launcher-an integrated interface to molecular biology data base search and analysis services available on the World Wide Web. Genome Res 6:454–462

Spormann AM (1999) Gliding motility in bacteria: insights from studies of *Myxococcus xanthus*. Microbiol Mol Biol Rev 63: 621–641

Spratt, BG, Hedge PJ, Heesen S, Edelman A, Broome-Smith JK (1986) Kanamycin-resistant vectors that are analogues of plasmids pUC8, pUC9, pEMBL8 and pEMBL9. Gene 41:337–342

Weimer RM, Creighton C, Stassinopoulos A, Youderian P, Hartzell PL (1998) A chaperone in the HSP70 family controls production of extracellular fibrils in *Myxococcus xanthus*. J Bacteriol 180:5357–5368

Whitfield C (1995) Biosynthesis of lipopolysaccharide O antigens. Trends Microbiol. 3:178–85

Wu SS, Kaiser D (1995) Genetic and functional evidence that Type IV pili are required for social gliding motility in *Myxococcus xanthus*. Mol Microbiol 18:547–558

Wu SS, Wu J, Kaiser D (1997) The *Myxococcus xanthus pilT* locus is required for social gliding motility although pili are still produced. Mol Microbiol 23:109–121

Wu SS, Wu J, Cheng YL, Kaiser D (1998) The *pilH* gene encodes an ABC transporter homologue required for type IV pilus biogenesis and social gliding motility in *Myxococcus xanthus*. Mol Microbiol 29:1249–1263

Yang Z, Geng Y, Shi W (1998a) A DnaK homologue in *Myxococcus xanthus* is involved in social motility and fruiting body formation. J Bacteriol 180:218–224

Yang Z, Geng Y, Xu D, Kaplan HB, Shi W (1998b) A new set of chemotaxis homologs is essential for *Myxococcus xanthus* social motility. Mol Micriobiol 30:1123–1130

Yang Z, Ma X, Tong L, Kaplan HB, Shimkets LJ, Shi, W (2000) The *Myxococcus xanthus dif* genes are required for the biogenesis of cell surface fibrils essential for social motility. J Bacteriol 182:5793–5798

Yanisch-Perron C, Vieira J, Messing J (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33:103–119