JOURNAL OF BACTERIOLOGY, July 1997, p. 4372–4381 Vol. 179, No. 13
0021-9193/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Identification and Localization of the Tgl Protein, Which Is Required for *Myxococcus xanthus* Social Motility

JORGE P. RODRIGUEZ-SOTO[1]† AND DALE KAISER[1,2]*

Department of Biochemistry[1] and Department of Developmental Biology,[2] Stanford University School of Medicine, Stanford, California 94305-5427

Received 3 October 1996/Accepted 23 April 1997

Tgl protein is required for the production of the type IV pili found at a pole of the *Myxococcus xanthus* cell. These pili are essential for social motility. Evidence is presented that Tgl is a membrane protein, based on experiments with polyclonal antibody specific for Tgl that was raised against the fusion proteins β-galactosidase–Tgl and TrpE-Tgl. Immunoaffinity-purified antibody reacted with a protein in *M. xanthus* having an apparent molecular mass of 27.5 kDa as measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, while the sequence of the *tgl* gene translates into a polypeptide of 27 kDa. Although these numbers are close, it is likely that the primary *tgl* translation product is processed and modified in *M. xanthus*. The N terminus has a signal peptidase II recognition sequence, cleavage of which is expected to remove 19 amino acid residues. When the *tgl* gene is expressed in *Escherichia coli*, the protein product consistently migrates faster in the gel than mature Tgl expressed in *M. xanthus*, suggesting a second modification by addition which slows migration of the protein from *M. xanthus*. Tgl, as detected by its specific antibody, sediments with the membrane fraction of cells. It can be extracted with detergents but not with salt or by the addition of chelators for divalent cations. In an equilibrium gradient, Tgl bands at the buoyant density of membranes and with the NADH-oxidase activity. Intact cells failed to bind anti-Tgl antibody, and less than 2% of the total Tgl is released in soluble form from the periplasm. Yet, cells that had been osmotically shocked and treated with paraformaldehyde were able to react with the specific antibody—a reaction absent from cells with a deletion of the *tgl* transcription unit. Assuming that osmotic shock disrupts the outer membrane, the fractionation and localization data imply that Tgl is attached to the inner or outer membranes, from which it is exposed to the intermembranous space. Tgl is necessary for synthesis of pili in *M. xanthus* and is the only pilus protein that can be donated by other cells (stimulation). Tgl contains six tandem copies of the tetratrico peptide repeat structural motif. Its membrane localization, capacity for stimulation, and content of tetratrico structural repeats together suggest that Tgl may be necessary for the assembly of pilin subunits into filaments.

Myxococcus xanthus cells, which have no flagella and are unable to swim, nevertheless spread efficiently by swarming on surfaces such as agar, plastic, and glass (7). Swarming on agar is evident at the edge of the colony, which spreads outward by means of the gliding movements of individual cells (47) and of coordinate groups of cells organized as peninsulas and islands (39). Genes have been identified in *M. xanthus* that control particular aspects of these cell movement and swarming behaviors. One set of genes work together to provide a function known as social motility, which can be distinguished morphologically from adventurous motility (18). A recent review summarizes *Myxococcus* motility (16). The products of the social motility genes apparently provide cell-cell interactions that give rise to the strongly social quality of socially motile swarming. Socially motile cells are usually found in contact with each other; apparently, single socially motile cells fail to move when they are deposited on agar after having been completely isolated from each other by extensive dilution (22). Social motility is also more efficient on soft, wet surfaces than on dry ones (45).

Currently about 10 social motility genes are known, but this gene system is not yet saturated genetically since several new S^- (lack of social motility) alleles were recently described (29). One social motility gene, *dsp* (dispersed), is involved in the production of an extracellular meshwork of fibrils (2). Fibrils are polysaccharide filaments of various diameters to which a number of proteins bind specifically and which loosely link cells to each other (2–4). Other social motility genes encode pili, which are polar structures. No mutant lacking pili retains social motility (21, 56). Social motility is also lost when pili are detached by violent shearing and reappears when pili are allowed to grow back (41). Several social motility genes, including *tgl* (transient glider), the subject of this paper, are needed for production of pili. At least six other pili genes are clustered in the *sglI* region of the *M. xanthus* chromosome (56, 57). The *tgl* gene is not linked to the *sglI* region; it is linked to *cglB* and *rpoB* (46).

Among the pili genes, loss of *tgl* gene function produces a unique quality in addition to the loss of social motility. When *tgl* mutant cells are mixed with tgl^+ cells, the social motility and piliation of the *tgl* mutant cells is transiently restored—a process called stimulation. After several hours of incubation, mixed donor and recipient cells spotted on an agar surface form the distribution of peninsulas and islands characteristic of socially motile swarming (21). Reisolation of individual cells from the swarm has shown that the cells whose gliding has been rescued remain genotypically *tgl* mutants. No stimulation has been observed when donor and recipient cells are placed as separate patches on an agar surface, even when their separation is of the order of 10 cell lengths. However, if two patches are allowed to intersect, stimulation is observed in the mixing region, indicating that stimulation requires either contact or close proximity between the donor and recipient cells (17).

* Corresponding author.
† Present address: Department of Pathology, Palo Alto Medical Foundation, Palo Alto, CA 94301.

TABLE 1. Strains and plasmids

Strain or plasmid	Description	Reference or source
M. xanthus strains		
DK1250	*aglB1 tgl-1*	18
DK1251	*aglJ1 tgl-2*	18
DK1252	*cglF1 tgl-3*	18
DK2700	*cglB2 sglA1 tgl-4*	46
DK1622	Reconstructed wild type	21
DK3930	*aglB1* Tc^r::ΔORFA *tgl*	pJPR510xDK1250[a]
DK3931	*aglB1* Tc^r::Δ*tgl*	pJPR541xDK1250
DK3952	*cglB2 tgl*::Ω3950	40
DK3956	Tc^r::ΔORFA *tgl*	Mx4(DK3930)xDK1622[b]
DK3958	Tc^r::Δ*tgl*	Mx4(DK3931)xDK1622[b]
DK4162	Ω1935	40
DK4170	*mgl-7*	49
DK7501	Ω7501	42
DK8020[c]	*tgl*::Ω3950	Mx4(DK3952)xDK1622[b]
E. coli strains		
HB101	Protease$^+$	5
D1210	Protease$^+$, *lacI*^{q}	1
CAG597	Protease$^-$	C. Gross
CAG626	Protease$^-$	C. Gross
Plasmids		
pKK240-11	Expression vector	1
pUC19tet	pUC19 with Tc^r gene from pBR322	S. Inouye
pUR291	β-Galactosidase hybrid protein vector	43
pJPR126	1.7-kb *tgl* transcription unit in pUC18	40
pJPR312	1.1-kb *Pst*I fragment from pJPR126 into *Pst*I site of pUR291	This work
pJPR329	1.2-kb *Nco*I-*Hin*dIII fragment from pJPR126 into pKK240-11 at same sites	This work
pJPR345	TrpE-Tgl hybrid protein expression vector	40

[a] Recipient strains are indicated after the x.
[b] Mx4 phage was grown in the strain shown in parentheses, and phage lysates were used to transduce tetracycline or kanamycin resistance into the recipient.
[c] Strain DK8020 was constructed by A. Rosenbluh.

Stimulation is transient, and the stimulated *tgl* mutant cells that move away from the donor cells stop moving after a time. Stimulation does not involve stable acquisition of genetic material because progeny of the stimulated cells retain the S$^-$ phenotype and *tgl* mutant genotype of the parents (21).

The genetic properties and structure of the *tgl* gene were considered in the accompanying paper (40). Here we will be concerned with the identification of the Tgl protein, its location within the cell, and its likely role in pilus assembly and stimulation.

MATERIALS AND METHODS

Strains and phages. The bacterial strains used in this study are listed in Table 1. The conditions for growth, transduction by phage, and DNA electroporation have been described previously (40).

Construction of plasmids for deletion of the *tgl* region. The chromosomal DNA region in *M. xanthus* corresponding to either the entire ORFA *tgl* transcription unit or only the *tgl* gene was deleted and replaced with a tetracycline resistance (Tc^r) marker. The DNA regions flanking the sequences targeted for deletion were subcloned, in the proper orientation, adjacent to the Tc^r determinant in plasmid pUC19tet (S. Inouye, Department of Biochemistry, R. W. Johnson Medical School) (see Fig. 1). The chromosomal regions adjacent to Ω1935 were isolated from pJPR102, which contains 16 kb of chromosomal DNA and the *tgl* locus, and from pJPR103, which contains 25 kb of DNA from the other side of Ω1935 (40). A 2.5-kb *Pst*I fragment from pJPR103, which contains 1.8 kb of chromosomal DNA and 685 bp of the adjacent IS*50* from the transposon at Ω1935, was ligated into the unique *Pst*I site of pUC19tet to produce pJPR505. To obtain chromosomal fragments from the other side of Ω1935, the intermediate plasmid pJPR502 was constructed by the ligation of an 8.4-kb *Xho*I fragment from pJPR102, which contains 7.9 kb of chromosomal DNA including the *tgl* locus and 490 bp of IS*50* DNA, into the unique *Sal*I site of the plasmid pBGS18 (48). pJPR502 was used to obtain two different fragments for the deletion of the *tgl* region as follows. A 4.2-kb *Nar*I fragment was isolated, treated with the Klenow preparation of DNA polymerase I, and ligated into the unique *Bam*HI site adjacent to the Tc^r gene of pJPR505, after treatment with the Klenow protein, to produce the deletion plasmid pJPR510. This results in the deletion of the 1.7-kb region that contains the entire ORFA *tgl* transcription unit. The second deletion plasmid was constructed by the ligation of a 3.2-kb *Sac*I fragment into the *Sac*I site of pJPR505 adjacent to the Tc^r gene, after partial digestion of pJPR505 and isolation of linearized plasmid, to produce pJPR541. The deletion in this plasmid consists of a 633-bp region of the *tgl* locus which is required for the rescue of the *tgl-1*, *tgl-2*, and *tgl-3* mutations and encodes ~20 kDa of the C terminus of the Tgl protein (see Fig. 1).

Introduction of plasmids into *M. xanthus*. The deletion plasmids pJPR510 and pJPR541 were introduced into DK1250 by electroporation (40). Transformants in which the Tc^r gene integrated by double recombination events that resulted in the replacement of chromosomal regions should not contain any pUC19-derived sequences. These transformants were identified by colony hybridization (34) with nick-translated pUC18 as a probe. To generate strains with deletions isogenic to the wild type, Mx4 phage lysates of the two deletion strains DK3930 and DK3931 were prepared and Tc^r transductants of DK1622, DK3956, and DK3958 were isolated. Southern blots confirmed that substitutions had been made in the *tgl* transcription unit; a new 4-kb *Eco*RI fragment harboring the Tc^r gene in strain DK3956 and a new 2.9-kb *Eco*RI fragment with the Tc^r gene and loss of a *Pst*I site in strain DK3958 were revealed. A new 2.5-kb *Pst*I fragment harboring IS*50* sequences was found in both strains of this isogenic pair.

Expression of proteins from cloned genes. For this purpose, *Escherichia coli* cells harboring the expression plasmids were grown in Luria-Bertani liquid cultures supplemented with carbenicillin (100 μg/ml); in general, the procedures described elsewhere (40) were followed except as noted. To prepare whole-cell extracts, cells were harvested by centrifugation (10,000 × *g*, 4°C, 10 min) and pellets were resuspended in 1/10 volume of TEBP (10 mM Tris [pH 7.6], 5 mM EDTA, 0.5 mM benzamidine [Sigma], 0.5 mM phenylmethylsulfonyl fluoride [PMSF; Boehringer Mannheim]) and sonicated in ice by using a Microson sonifier with a microtip set at a power output of 12 with three to five 15-s bursts. The destruction of rod-shaped cells was confirmed microscopically.

D1210, CAG597, and CAG626 cells harboring the plasmid pKK240-11, pUR291, pJPR312, or pJPR329 were grown to an optical density at 550 nm of 0.6 to 0.8, expression was induced (where indicated) by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM, and cells were harvested after 2 to 4 h of additional growth. Expression of TrpE hybrid proteins

in HB101 and their purification from inclusion bodies has been previously described (24).

Immunization of rabbits and immunochemical purification of antisera. Approximately 100 mg of protein from β-galactosidase–Tgl or TrpE-Tgl hybrid protein inclusion body preparations was fractionated in sodium dodecyl sulfate (SDS)-polyacrylamide gels (8 and 12.5% acrylamide, respectively), and blocks of the gel corresponding to the hybrid proteins were cut out and macerated to prepare them for injection (24). Two female New Zealand White rabbits were immunized subcutaneously with 2 mg of protein in complete Freund's adjuvant. Reimmunizations with 1 to 2 mg of protein, but no adjuvant, were separated by at least 1-month intervals. Blood was sampled by auricular-vein phlebotomies at 1, 2, and 3 weeks after each reimmunization. Whole blood was stored at 4°C overnight and the clot was removed by centrifugation, leaving crude serum to test for anti-Tgl activity.

Purification of the antiserum made against the TrpE-Tgl protein followed the method of Redding et al. (38). TrpE and β-galactosidase–Tgl inclusion body preparations were adjusted to a protein concentration of 5 mg/ml, and affinity columns were prepared as described previously (38). The ammonium sulfate immunoglobulin G fraction was passed over an affinity column made with TrpE protein. Proteins that did not bind were collected in the flowthrough by elution with 150 mM NaCl–50 mM HEPES (pH 7.5)–1 mM EDTA–0.5 mM benzamidine–0.5 mM PMSF –10 mg of bovine serum albumin (BSA; Miles Laboratory) per ml. This material, which contained antibodies reactive against Tgl protein and lacked >95% of the antibodies reactive against the TrpE protein, was applied to a second affinity column made with β-galactosidase–Tgl inclusion body proteins. Antibodies specific for Tgl proteins were eluted and dialyzed (38).

Western immunoblotting. To obtain *M. xanthus* protein extracts, cells were grown in liquid culture to 80 to 120 Klett density units, harvested by centrifugation (16,000 × *g*, 4°C, 10 min), and resuspended at 1,000 Klett units (~4×10^9 cells per ml) in TEBP buffer. Cells were disrupted by sonication as described above. By using 10 μl of the extracts, containing 100 μg of total protein, the proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) (26). The transfer to nitrocellulose, reaction with antibodies, and visualization of enzymatic activity have been described previously (24). Antisera were diluted 1:2,000 to react with proteins from inclusion bodies and were diluted 1:100 to react with proteins in whole-cell extracts. The intensity of the bands obtained from the development of alkaline phosphatase activity in Western immunoblots was measured by reflectance densitometry and quantified by cutting out and weighing the peaks of the density scans.

Harvesting of growth media and osmotic shock treatment. Supernatants were obtained from cells grown in liquid suspension or as films on the bottom of stationary tissue culture flasks (23). Cells in tissue culture flasks were dislodged from the plastic into the overlaying medium by gently scraping with a bent Pasteur pipette. Cells were removed by centrifugation (15,000 × *g*, 10 min, 4°C) followed by filtering through 0.45-μm-pore-size filters (Syrfil-MF; Nucleopore). Supernatant fractions were adjusted to the following concentrations of protease inhibitors: 5 mM EDTA, 0.5 mM benzamidine-HCl, and 0.5 mM PMSF. Aliquots were frozen in liquid nitrogen, lyophilized, resuspended in water (1/11 original volume), and dialyzed in 6- to 8-kDa molecular mass cutoff dialysis tubing (Spectropore) with 1 liter of TEBP.

For the isolation of membrane fractions, cells were grown in liquid CTT (17) and concentrated to 1,000 Klett units per ml, and then 0.5 ml of the cell suspension was spread on 15-cm-diameter plates containing 1.5% agar. After two days of incubation, cells were harvested by gently scraping the agar surface with a razor blade. Cells grown in liquid media or tissue culture flasks were enumerated by measuring cell density by using a Klett-Sommersen colorimeter with a red filter. Wet weight was determined for cells grown on agar, and the number of cells was calculated from the mass.

Osmotic shock was performed following the method of Nossal and Heppel (33) with a 10-ml cell culture or similar amounts of cells grown in tissue culture flasks. All solutions were cooled in ice, EDTA was omitted, and the cells were gently resuspended by pipetting. After removal of cells by centrifugation, all supernatants were filtered and supplemented with protease inhibitors as described above. The cell pellet was resuspended in 0.5 ml of TEBP to a concentration of ~4×10^9 cells/ml, and cells were disrupted by sonication (four 15-s bursts with a Microson sonifier with a microtip set at 10 to 14). Alkaline phosphatase was assayed by hydrolysis of *o*-nitrophenyl phosphate (8), and β-galactosidase (25) was measured as described previously, except that cells were resuspended in TEBP.

Fractionation of cell membranes. Cells grown on agar were scraped with a razor blade, weighted (wet weight), washed with 1 ml of CTT per 0.1 g of wet cells, and resuspended to ~4×10^9 cells/ml in 10 ml of TEBP. Cells were disrupted by sonication in ice (Branson sonifier 450 with a microtip at a power output of 4 to 6 and with a 50% duty cycle). Complete disruption was verified by phase-contrast microscopy. Cell wall components and debris were removed by centrifugation (15,000 × *g*, 10 min, 4°C) to obtain S15 and P15 fractions. P15 was resuspended in TEBP to the original volume. A membrane fraction was isolated from S15 by centrifugation (200,000 × *g*, 1 h, 4°C). The pellet (P200) was resuspended in TEBP to the original volume by sonication (described above) until no visible particulate material remained. Fractionation was also carried out in 0.5 ml of TEBP with different concentrations of NaCl (0.1 M, 0.5 M, 1 M, and 4 M).

Membrane fractions were extracted with detergents as follows. Aliquots (10 to 20 μl) of membrane fractions (100 to 200 μg of protein) were adjusted to 50 μl with TEBP and different final concentrations of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS; Sigma) (0.05, 0.1, 0.5, and 1%) or Sarkosyl (Sigma) (0.5, 1.0, and 1.5%). Samples were incubated in ice for 1 h, insoluble material was removed by centrifugation (200,000 × *g*, 1 h, 4°C) with a 100.1 rotor and Beckman TLA centrifuge, and pellets were resuspended in TEBP with 1% SDS by sonication or incubation in boiling water for 5 min. Treatments with EDTA and EGTA were performed as described above by diluting 0.5 M EDTA (pH 8) or 0.5 M EGTA (pH 8) to final concentrations of 25 and 20 mM, respectively.

Isopycnic sedimentation. The membrane fraction from 4×10^9 cells was resuspended by sonication in 1 ml of 57% sucrose (wt/vol)–TEBP, and the layers of a step gradient consisting of 1 ml of 55% sucrose and 2 ml (each) of 50, 45, 40, 35, and 30% sucrose were added successively on top. The tube was centrifuged in a SW41 rotor at 38,000 rpm and 4°C for 70 h. Fractions of 1 ml were collected from the bottom of the tube, and the density of each fraction was determined from the measured refractive index. NADH oxidase (35) and β-galactosidase (25) activities were measured as described previously.

Fluorescence-activated cell sorter (FACS) experiments. Purified specific anti-Tgl was immunoabsorbed with *tgl*-deleted *M. xanthus* cells. DK3956 was grown in liquid, osmotically shocked to remove periplasmic proteins, and fixed with 4% paraformaldehyde. Fixed cells were resuspended to a concentration of ~4×10^9 cells/ml in the antiserum and agitated by rotation for 4 h at room temperature or 12 to 16 h at 4°C, after which the cells were removed by centrifugation. Eight to ten sequential absorptions were required to remove the reactivity against DK3956 cells.

For fixation, cells were resuspended in 4% paraformaldehyde–25 mM K_2HPO_4–KH_2PO_4 (pH 7.5) and incubated for 1 h at room temperature with rotation. Cells were harvested by centrifugation and washed with TPM (10 mM Tris-HCl [pH 7.5], KH_2PO_4, 8 mM $MgSO_4$) three times, 5 min per wash. After fixation, 95% of the cells appeared as morphologically normal rods by phase-contrast microscopy.

Fixed cells were incubated with anti-Tgl antibody as follows. Cells (4×10^7) were pelleted by centrifugation and resuspended in 45 μl of phosphate-buffered saline (PBS)–3% BSA, and 5 μl of the immunoabsorbed antiserum was added. After incubation for 4 to 6 h at room temperature, or 18 h at 4°C, cells were washed with 100 μl of PBS–3% BSA three times and resuspended in 50 μl of goat anti-rabbit antibody conjugated to fluorescein diluted 1:100 in PBS–3% BSA. This mixture was incubated for 4 h at room temperature, and then the cells were washed with 100 μl of TPM three times. Finally, 2×10^6 cells were diluted in 2 ml of TPM for analysis in a FACS (FACSTAR Plus; Becton Dickenson). The amplifier settings, data compilation, and analysis have been described previously (42).

RESULTS

Protein fusions and generation of antibodies against Tgl protein. A fragment containing three quarters of the *tgl* transcription unit was subcloned in the correct reading frame to produce fusion proteins with N termini (N fragments) from the TrpE or β-galactosidase protein of *E. coli* and C termini (C fragments) from Tgl. The left end of the Tgl C fragment is the *Pst*I site shown in the top two panels of Fig. 1. Plasmid pJPR345, containing a TrpE-Tgl fusion gene (40), and plasmid pJPR312, containing a β-galactosidase–Tgl fusion gene constructed in the plasmid pUR291, were introduced into *E. coli*. The TrpE-Tgl and β-galactosidase–Tgl hybrid proteins were isolated from inclusion bodies that were produced in the *E. coli* strains. The fusion proteins were partially purified by SDS-PAGE and injected into rabbits for the production of polyclonal antibodies. It was expected that some of the antibodies would be directed against the Tgl portion of the hybrid proteins.

Figure 2, lane 3, shows a Western blot of the proteins obtained from purified inclusion body preparations after induction of hybrid protein expression in *E. coli* cultures harboring plasmid pJPR345 (TrpE-Tgl) developed with an antiserum against TrpE. This anti-TrpE serum reacts with a protein of 58 kDa (Fig. 2, lane 2), close to the 56 kDa predicted from the DNA sequence of the TrpE-Tgl fusion gene. This protein also reacts with an antiserum generated against the β-galactosidase-Tgl protein (Fig. 2, lane 4).

Figure 2, lanes 5 to 8, shows the proteins produced from pJPR312 after induction of β-galactosidase–Tgl fusion gene

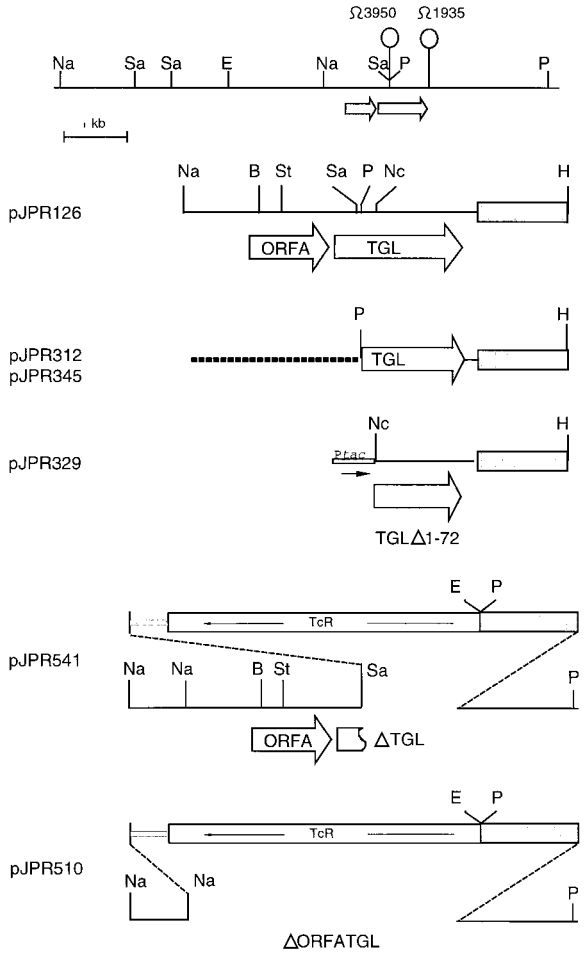

FIG. 1. The top panel shows the restriction map for and the transposon insertion sites in the *tgl* region. The nucleotide sequence between the *Nar*I site and the transposon insertion Ω1935 predicts the two open reading frames ORFA and *tgl*, shown as open arrows below the line (40). ORFA and *tgl* are transcribed from left to right. The transposon Tn*5lac* (open lollipop) insertion at Ω3950 inactivates *tgl* function; the Tn*5* insertion at Ω1935 (shaded lollipop) leaves *tgl* function unaltered. pJPR126 (40), shown in the next panel, contains a 1.7-kb DNA fragment that includes the complete *tgl* transcription unit and ORFA. The next panel shows the region encoding the Tgl carboxy, or C, fragment (see text); this region was used to generate fusion proteins from pJPR312 and pJPR345. The dashed line represents the region encoding the N fragment of the fusion proteins. The region in pJPR329 used for the expression of the TglΔ1–72 protein is shown. P*tac* is the expression plasmid promoter region. The last two panels give the structure of plasmids pJPR541 and pJPR510, showing the replacement of the *tgl* sequences by a tetracycline resistance gene obtained from pUC19tet. These regions were inserted into the *M. xanthus* chromosome to produce deletions of the *tgl* locus. The thin solid line represents *Myxococcus* chromosomal DNA. Sequences from the IS*50* elements of Tn5Ω1935 are shown as large shaded boxes. The regions from the *Sac*I to *Nar*I sites in pJPR510 and from *Nar*I to *Nar*I in pJPR510 and from IS*50* to *Pst*I in both plasmids are not drawn to scale. The thin, shaded box indicates pUC19tet sequences. TcR, tetracycline resistance gene (with its direction of transcription indicated by an arrow). Restriction sites: B, *Bal*I; E, *Eco*RI; Na, *Nar*I; Nc, *Nco*I; P, *Pst*I; Sa, *Sac*I; St, *Stu*I.

expression with IPTG. Fusion plasmid pJPR312 specifies three novel proteins (Fig. 2, lane 6), the largest of which has an interpolated mass of 142 kDa, close to that predicted from the sequence (136 kDa) for the hybrid protein. Figure 2, lane 8, shows a Western immunoblot reaction of the anti-TrpE-Tgl serum with the 136-kDa protein. Two faster-migrating proteins, at 132 and 120 kDa, specific to the plasmid pJPR312 are not recognized by anti-Tgl antibodies in the anti-TrpE-Tgl serum.

These results demonstrate the presence of antibodies specific for the Tgl portion of the hybrid proteins in both the polyclonal anti-TrpE-Tgl serum and the polyclonal anti-β-galactosidase–Tgl serum. The antiserum generated against the TrpE-Tgl hybrid protein was selected for further purification because Western blots of extracts of *E. coli* cells which carried no *tgl* gene showed that the anti-TrpE-Tgl serum reacted with fewer bands and with lower intensity overall than the antiserum generated against the β-galactosidase–Tgl fusion. Specific anti-Tgl antibodies were then purified in three steps. After precipitation of the immunoglobulin G protein from the serum, the anti-TrpE activity was removed by passage over a TrpE affinity column. The column flowthrough, enriched by removal of >95% of the antibodies reactive against TrpE, was then passed over a second affinity column made with a β-galactosidase–Tgl fusion protein as detailed in Materials and Methods. Specific anti-Tgl antibodies were eluted from this second column.

Specificity of the immunoaffinity-purified anti-Tgl antibodies. The antiserum generated against the TrpE-Tgl hybrid protein and purified as described above reacts with a single protein with a nominal molecular mass of 27.5 kDa in the *tgl*$^+$ *M. xanthus* strain DK4162 (Fig. 3, lane 1). If this is the *tgl* gene product, then expression of the *tgl* transcription unit in *E. coli* should produce a similar protein that reacts with this antiserum. The plasmid pJPR126, derived from pUC18, contains the *tgl* gene in the correct orientation for transcription from an *E. coli* (*lac*) promoter (Fig. 1). pJPR126 was introduced into two protease-deficient *E. coli* strains, CAG626 (*lonB* mutation) and CAG597 (*htpR* mutation). Cells harboring pJPR126 or the parental plasmid pUC18 were harvested from cultures in their exponential phase of growth. Figure 3 (lane 2) shows the reaction of the immunoaffinity-purified antibody with a protein produced in CAG626 cells harboring pJPR126. The protein is absent from cells harboring pUC18 (Fig. 3, lane 3). The same protein was observed in CAG597 cells (data not shown). The protein produced in *E. coli* migrates at 27 kDa, slightly faster than the protein produced in *tgl*$^+$ *M. xanthus* (Fig. 3, lane 1). This same difference in the apparent mass of the two proteins

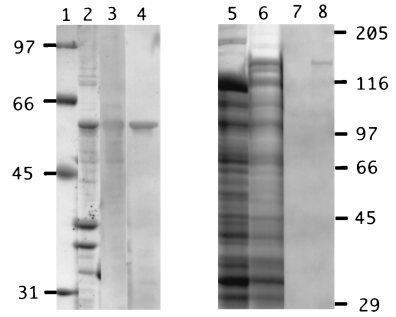

FIG. 2. SDS-polyacrylamide gels of Tgl fusion proteins. Roughly 50 μg of total protein was loaded in each lane. Lane 1, molecular weight standards for lanes 1 to 4; lane 2, Coomassie blue stain of protein from the TrpE-Tgl fusion specified by pJPR345; lane 3, Western immunoblot of the TrpE-Tgl fusion specified by pJPR345 probed with anti-TrpE antibody; lane 4, Western immunoblot of the TrpE-Tgl fusion specified by pJPR345 probed with anti-β-galactosidase–Tgl antibody; lane 5, Coomassie blue stain of protein from *E. coli* cells carrying the β-galactosidase expression vector, pUR291, after induction with IPTG; lane 6, Coomassie blue stain of protein from *E. coli* cells carrying pJPR312 that encoded β-galactosidase–Tgl; lane 7, Western immunoblot of the proteins from the β-galactosidase strain (pUR291) probed with anti-TrpE-tgl antibody; lane 8, Western immunoblot of the proteins from the β-galactosidase–Tgl hybrid strain (pJPR312) probed with anti-TrpE-Tgl antibody. Positions of molecular weight standards for lanes 5 to 8 are marked at the right. Molecular weight is indicated in thousands.

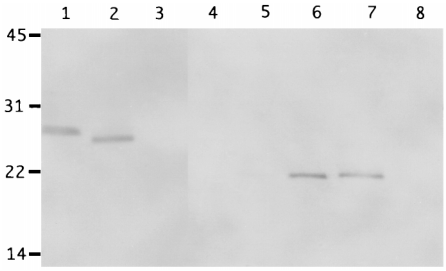

FIG. 3. Western immunoblot of Tgl polypeptides in *E. coli* and *M. xanthus*. After separation by SDS-PAGE and transfer to nitrocellulose, the blot was incubated with immunoaffinity-purified anti-Tgl antibodies. Lane 1, *M. xanthus* DK 4162 (*tgl*$^+$) cells; lane 2, *E. coli* CAG626 cells with pJPR126 (with *tgl*); lane 3, *E. coli* CAG626 cells with pUC18 (no *tgl*); lane 4, *E. coli* D1210 cells with pKK240-11 (no *tgl*) after induction; lanes 5 and 6, *E. coli* D1210 cells with pJPR329 (that has *tgl*) before (lane 5) and after (lane 6) induction with IPTG; lane 7, *E. coli* CAG626 cells with pJPR329 (with *tgl*); lane 8, *E. coli* CAG626 cells with pKK240-11 (no *tgl*). Molecular weights (in thousands) are indicated on the left.

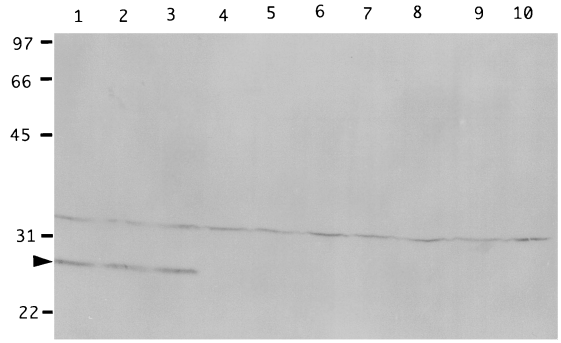

FIG. 4. SDS-PAGE and Western immunoblot of *M. xanthus* whole-cell extracts, incubated with immunoaffinity-purified anti-Tgl antibody. Lane 1, DK1622 (wild type); lane 2, DK4162 (wild type with the transposon insertion Ω1935); lane 3, DK4170 (*mgl-7*); lane 4, DK1250 (*aglB1 tgl-1*); lane 5, DK1251 (*aglJ1 tgl-2*); lane 6, DK1252 (*cglF1 tgl-3*); lane 7, DK2700 (*cglB2 sglA1 tgl-4 rifR*); lane 8, DK3956 (Tc^r::ΔORFA *tgl*); lane 9, DK3958 (Tc^r::TglΔ60–241); lane 10, DK8020 (*tgl*Ω3950). Molecular weights (in thousands) are indicated on the left. ▶, position of Tgl.

was observed after electrophoresis by the systems of Laemmli (26) and Schagger and von Jagow (44) (data not shown).

An additional argument that the antiserum recognizes the protein encoded by the *tgl* gene is that alterations in *tgl* change the protein expressed. A fragment of the *tgl* coding region was cloned into the plasmid pKK240-11 for overexpression from the highly inducible P*tac* promoter (Fig. 1). The plasmid constructed, pJPR329, placed an AUG codon in frame at the position corresponding to amino acid 72 from the N terminus of the Tgl protein, seven nucleotides downstream from a ribosomal binding site (AGGA) characteristic of abundantly produced proteins. Induction of transcription from the P*tac* promoter was expected to produce a *tgl* polypeptide truncated at its N terminus (the TglΔ1–72 protein) with a predicted mass of 20 kDa. Figure 3 shows Western blots of extracts of *E. coli* cells harboring pJPR329. The addition of IPTG to D1210 cells (which have the *lacI*^{q} allele) results in the accumulation of a protein with a mass of 21.5 kDa (Fig. 3, lane 6), and the same protein is detected constitutively in *lonB* mutant cells (lane 7). This protein is encoded by the *tgl* transcription unit because it is not produced when *E. coli* cells harboring pKK240-11, which lacks *tgl*, are similarly induced (Fig. 3, lane 4) or in pJPR329-containing cells that have not been exposed to IPTG (lane 5) but does accumulate in pJPR329 cells after exposure to IPTG (lane 6).

Detection of the *tgl* gene product in *M. xanthus*. Proteins in cell extracts of *M. xanthus* strains that contain a wild-type or a mutated allele of *tgl* were screened with the affinity-purified anti-Tgl serum. Apart from an immunoreactive band at about 34 kDa that is present in all strains whether or not they have a *tgl*$^+$ gene, Fig. 4 illustrates the presence of a 27-kDa protein in *tgl*$^+$ strains DK1622, DK4162, and DK4170 that reacts with the affinity-purified polyclonal anti-Tgl serum. On the other hand, strains DK1250, DK1251, DK1252, DK2700, DK3956, DK3958, and DK8020, each of which carries a different mutant allele of *tgl*, show no reaction. The protein recognized by this anti-Tgl serum is absent in all three classes of *tgl* mutants tested: (i) those derived by UV mutagenesis, which are likely point mutants (DK1250, DK1251, DK1252, and DK2700 [18, 46]); (ii) a null mutant generated by a Tn*5lac* transposon insertion in *tgl* (DK8020); and (iii) the isogenic pair of mutants derived by the replacement of the chromosomal *tgl* region with a Tc^r determinant (DK3956 and DK3958).

Is Tgl protein secreted? Tgl$^+$ cells can stimulate (transiently induce motility in) the *tgl* mutant cells of a mixture of *tgl*$^+$ and *tgl* mutant cells (18, 40). To test whether Tgl protein might be secreted by *tgl*$^+$ cells into the surrounding fluid, the culture supernatants of cells grown in liquid suspension were examined for Tgl protein by Western immunoblotting. Lane 5 of the immunoblot shown in Fig. 5 was loaded with supernatant protein from 10^8 cells. No Tgl protein was detected, even though it was evident in 2×10^6 whole cells, as shown in Fig. 5, lane 4. If Tgl protein is secreted, its steady-state level is less than 2% of the protein present in extracts of whole cells. The fluid washes of growing cells harvested from a submerged culture concentrated 10-fold as well as a fluid wash of a culture growing on agar were examined without finding any Tgl protein (data not shown).

To test whether Tgl protein might be secreted into the periplasm, cells were subjected to an osmotic shock regimen that releases periplasmic proteins from *E. coli* (33). *M. xanthus* DK7501 was used because it is *tgl*$^+$, makes β-galactosidase that localizes to the cytoplasm (15), and also makes a phosphatase that localizes to the periplasm (53). The latter two proteins would mark cytoplasm and periplasm. More than 90% of the DK7501 cells survived the shock procedure as judged by a count of viable cells, and phase-contrast microscopy showed that >98% of the cells retained their morphologically normal rod shape. Apparently, few cells lysed as a consequence of the osmotic shock. The distribution of enzyme activity and Tgl protein among the shock fractions is shown in Table 2. The shock procedure released 77% (average from two independent experiments) of the phosphatase activity in the periplasmic fraction. The periplasmic fluid contained 7% of the total cellular protein but less than 1% of the β-galactosidase activity. No Tgl protein was detected in the periplasmic fraction obtained from 10^8 cells (Fig. 5, lane 6). Since the Tgl protein from

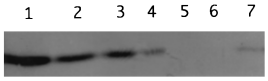

FIG. 5. Western immunoblot of *M. xanthus* cell extracts, showing the location of Tgl. Lanes 1 to 4 contain sonicates of cells after their periplasmic contents had been washed out. Lanes and the quantities that were loaded in them: lane 1, 2×10^7 cells; lane 2, 1×10^7 cells; lane 3, 5×10^6 cells; lane 4, 2×10^6 cells. Lane 5 contains culture supernatant from 10^8 cells concentrated by lyophilization. Lane 6 contains the periplasmic fraction from 2×10^8 cells. Lane 7 contains the sucrose wash from 10^8 cells.

TABLE 2. Fractionation by osmotic shock[a]

Suspension	% Total activity			
	Cell protein	β-Galacto-sidase	Alkaline phosphatase	Tgl
Sucrose wash	<1	0	<1	4
Periplasm	7	<1	77	<1
Cell pellet	93	100	23	96

[a] Fractionation of DK7501 cells grown in liquid suspension by the osmotic shock procedure. The values given are averages of the values obtained in two experiments.

2×10^6 whole cells can be detected, we calculate that less than 2% of the Tgl protein is present in soluble form in the periplasm.

Is Tgl protein associated with the membrane? Table 2 indicates that a small amount of protein having the size and antigen reactivity of Tgl protein was found in the 20% sucrose wash fraction. The intensity of this sucrose wash gel band (Fig. 5, lane 7) indicates an amount of Tgl protein that would be obtained from 2×10^6 whole cells, even though the wash of 10^8 cells was applied to the gel. This suggests that a low percentage of Tgl protein is released by washing cells with a 20% sucrose solution. The Tgl protein in the sucrose wash is unlikely to have been produced by cell lysis because this fraction contains no β-galactosidase activity and <1% of the phosphatase activity present in cells. When a sucrose wash fraction was sedimented at 200,000 × *g*, all the Tgl protein was recovered in the sediment (data not shown), suggesting that it may reside in membrane vesicles or another particulate form.

Cells grown in liquid suspension or on agar were disrupted by sonication. Figure 6 shows a Western immunoblot of fractions resulting from differential centrifugation. Roughly half of the Tgl protein was present in the supernatant (S15) left by sedimentation at 15,000 × *g*, and the remainder was present in the pellet, consisting of cell debris and large membrane fragments (Fig. 6, lanes 1 and 4). When the 15,000 × *g* pellet fraction (P15) was resuspended in buffer and disrupted by passing it through a French pressure cell, additional Tgl protein was released into the supernatant (data not shown). Treatment of P15 with lysozyme failed to release Tgl protein (data not shown). All the Tgl protein present in the S15 fraction was sedimented at 200,000 × *g* for 1 h (P200, shown in lane 3 of Fig. 6, and S200, shown in lane 2).

The sedimentation of Tgl protein with the particulate membrane fraction, P200, may indicate an association with the membrane or the formation of Tgl protein aggregates. These two possibilities can be distinguished by sedimentation in an equilibrium sucrose density gradient. Membrane-associated proteins are expected to comigrate in the gradient with the lipid vesicles, while protein aggregates would band elsewhere in the gradient according to their particular buoyant densities. The P200 fraction resuspended in 57% sucrose was placed at the bottom of a preformed sucrose gradient. This P200 fraction also contained 30% of the β-galactosidase activity expressed in this strain, because β-galactosidase is a tetramer with a molecular mass of 544 kDa that is partially pelleted under the conditions used. Figure 7 shows the distribution of Tgl protein, β-galactosidase, and NADH oxidase activities throughout the sucrose gradient. The immunoblot in the bottom panel of Fig. 7 shows the peak of Tgl protein in fraction 5. This coincides with the peak of NADH oxidase activity that marks the inner membrane (35). The measured density of this fraction was 1.18 g/cm^3, close to the value of 1.19 g/cm^3 reported for the mixture of inner and outer *M. xanthus* membranes (35). In contrast, the fractions from the bottom of the gradient that would contain materials with buoyant densities of 1.22 g/cm^3 and above have 70% of the β-galactosidase activity recovered in the gradient. Therefore, Tgl protein cofractionates with membrane fragments, not with protein aggregates.

Membrane proteins can often be solubilized with detergents, which replace the lipid molecules through interactions with the hydrophobic regions of these proteins. A P200 fraction of *tgl*$^+$ cells was exposed to increasing concentrations of the zwitterionic nondenaturing detergent CHAPS and then sedimented at 200,000 × *g*. As shown in Fig. 8, increasing the concentration

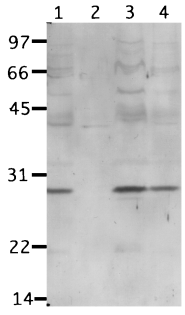

FIG. 6. Western immunoblot of *M. xanthus* cells fractionated by centrifugation. Supernatant (S15) (lane 1) and pellet (P15) (lane 4) from centrifugation at 15,000 × *g*. Supernatant (S200) (lane 2) and pellet (P200) (lane 3) from centrifugation at 200,000 × *g*. About 50 μg of total protein was loaded in each lane. Molecular weights (in thousands) are indicated on the left.

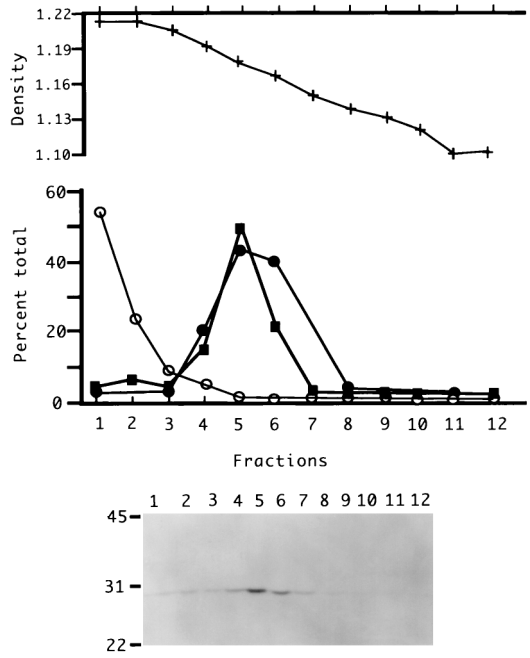

FIG. 7. Isopycnic density gradient centrifugation of *M. xanthus* membranes. Membrane fractions from DK7501 were isolated and floated in a sucrose gradient, as described in Materials and Methods. Fraction 1 contains the bottom of the gradient, and fraction 12 contains the top of the gradient. The top panel shows the density of each fraction. The middle panel shows the percent of total enzymatic activity recovered in each fraction for NADH oxidase (solid circles), β-galactosidase (open circles), and Tgl (solid squares). The amount of Tgl in each fraction was determined by densitometry of a Western immunoblot probed with affinity-purified anti-Tgl antibodies (bottom panel). Molecular weights (in thousands) for the immunoblots are indicated.

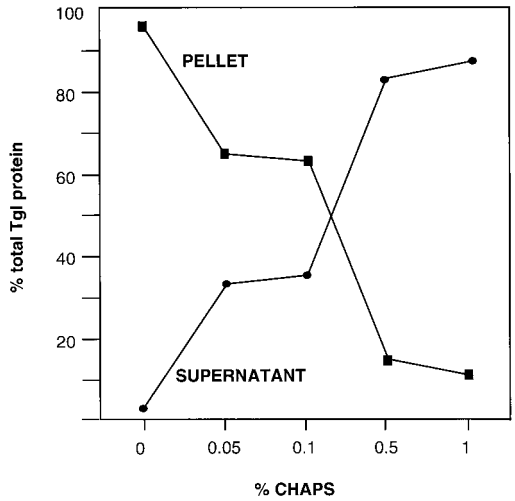

FIG. 8. Release of Tgl protein by detergent from *M. xanthus* membranes. Aliquots of the membrane fraction were treated with increasing concentrations of CHAPS detergent. The abscissa indicates the percentage of the total Tgl recovered in the supernatant (circles) or pellet (squares) as determined by densitometry of Western immunoblots.

of CHAPS to 1% increased the solubility of Tgl protein. Similar results were obtained with Sarkosyl, an anionic detergent. If Tgl is associated with the membrane through ionic bonds, those bonds might be weakened by exposure to high salt concentrations. However, when disrupted cells were exposed to 0.1 M, 0.5 M, 1 M, or 4 M NaCl, Tgl protein remained associated with the membrane fraction. Similar results were obtained when either EDTA (20 mM) or EGTA (25 mM) was included with the NaCl (data not shown). Apparently, Tgl protein associates with the membrane through hydrophobic, not ionic, interactions.

Cellular localization of Tgl protein by immunofluorescence. To look for Tgl protein on whole cells, immunoaffinity-purified antibody was extensively absorbed with the strain DK3956, chosen because it has a deletion of *tgl* but is otherwise isogenic to the wild type. The absorbed antibody preparation retained full activity against Tgl protein, as determined by immunoblots. Intact cells fixed with paraformaldehyde or cells that had been treated with an osmotic shock before fixation were incubated with the purified and absorbed anti-Tgl specific antibodies. Binding of these antibodies was detected by reaction with a secondary antibody that had been conjugated to fluorescein. Finally the fluorescent cells were detected in a FACS. Several experiments were performed and a typical result is shown in Fig. 9. The anti-Tgl antibodies failed to react with cells that had been treated only by washing and fixation (Fig. 9A). However, after cells had been exposed to the osmotic shock procedure (Fig. 9B), binding of the anti-Tgl antibodies to wild-type cells could be detected. Binding to the *tgl* deletion strain (DK3956) was absent (Fig. 9C). The detected binding to wild-type cells is mediated by the anti-Tgl antibodies, because their omission before the incubation with secondary antibody left no binding

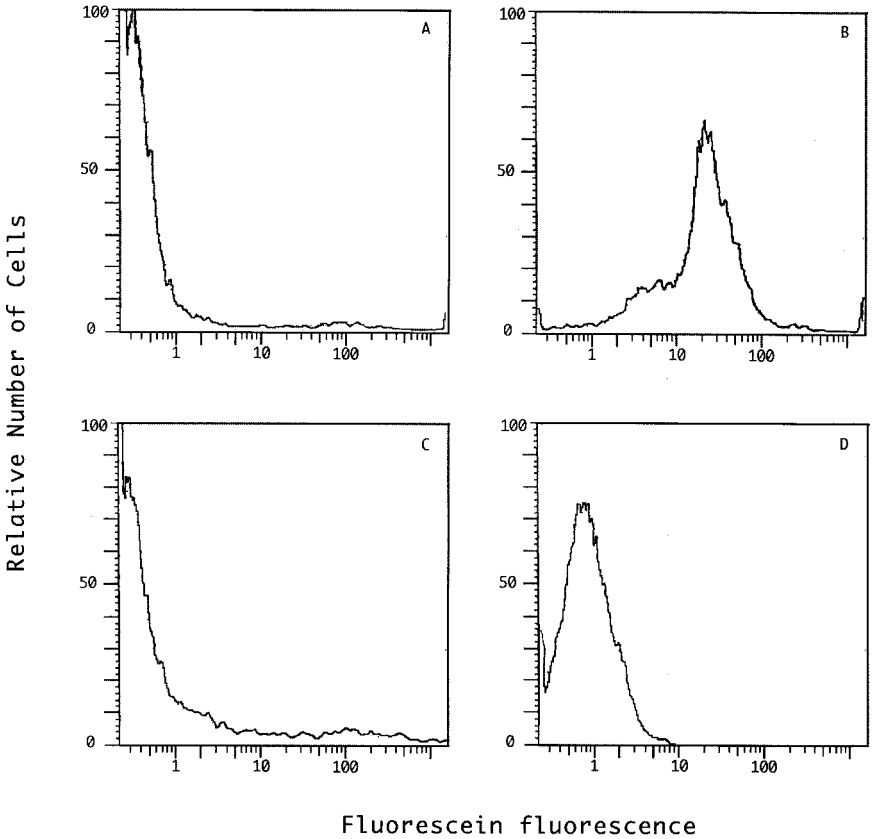

FIG. 9. Detection of Tgl protein in whole cells. *tgl*$^+$ (DK7501) or Δ*tgl* (DK3956) cells were washed, fixed, and exposed to affinity-purified rabbit anti-Tgl antibody that had also been absorbed eight times with Δ*tgl* cells. Details of the absorption are given in Materials and Methods. Following reaction with anti-Tgl, the cells were exposed to fluorescein-conjugated goat anti-rabbit antibody and then analyzed in the FACS. (A) *tgl*$^+$ cells (DK7501) were washed and fixed (without shock treatment). (B) *tgl*$^+$ cells were osmotically shocked and then fixed. (C) Δ*tgl* cells (DK3956) were washed and fixed (without shock treatment). (D) Δ*tgl* cells were osmotically shocked and then fixed.

(data not shown), and when the *tgl* deletion strain was shocked a significant but low intensity of fluorescence (Fig. 9D) compared with that for the tgl^+ strain (Fig. 9B) was observed. A low-level background fluorescence has been reported previously (42). Therefore, an osmotic shock along with fixation rendered the Tgl protein epitope in whole cells accessible from the outside for specific binding of the rabbit antibody. After treatment, 95% of these cells appeared as morphologically normal rods by phase-contrast microscopy.

DISCUSSION

The *tgl* gene is essential for biogenesis of type IV pili, which are polar structures on *Myxococcus* cells (40). Since *tgl* is required for pili it is also required for social motility (56, 57). This paper describes the identification of *tgl* protein (Tgl) in cell extracts and takes the first steps toward its cellular localization. Both identification and localization rely critically on the specificity of anti-Tgl antibody, which was raised against a TrpE-Tgl fusion protein, checked against a β-galactosidase–Tgl fusion protein, and purified immunochemically. In extracts of tgl^+ *M. xanthus* cells, purified antibody identified a protein whose motility in SDS-PAGE gave it a nominal molecular mass of 27.5 kDa. This protein was absent from null mutants (either a *tgl*::Tn*5* insertion mutation or an engineered internal deletion of *tgl*). The sequence of the *tgl* gene translates into a polypeptide of 27 kDa (40).

Tgl is unique among the social motility proteins which have been identified in that the swarm pattern characteristic of social motility can be transiently but efficiently restored to *tgl* mutant cells by contact with tgl^+ cells (18). The same is true of the pili, which are present at a pole of the tgl^+ *M. xanthus* cell but are absent in *tgl* mutants (21). Since the pili are essential constituents for social motility (56), restoration of piliation is sufficient to explain the restoration of social motility. But how can the transient, nonheritable, yet efficient transfer of the capacity to assemble pili be interpreted, and what is the role of Tgl?

The positive reactions with the immunochemically purified anti-Tgl antibody and the amino acid sequence of Tgl imply that it is a membrane protein. In the first place, fractionation of sonically disrupted *M. xanthus* cells showed that Tgl sedimented with the cell membranes. The protein could be solubilized by detergents but not by the addition of salt or chelators of divalent cations. In *M. xanthus* cells, association of Tgl with membrane lipids was confirmed by showing that Tgl floats in a sucrose gradient with membrane vesicles that carry NADH-oxidase activity from electron transport proteins in the cytoplasmic membrane (Fig. 7).

The amino acid sequence of Tgl predicted from its base sequence (40) has no region expected to form a membrane-spanning α-helix. However, expression of the complete *tgl* gene in *E. coli* yielded a protein whose apparent molecular weight was less than that of the corresponding *M. xanthus* protein (Fig. 3). Although the difference in electrophoretic mobility was not large (about 0.5 kDa), it was found consistently. The lower mobility in *M. xanthus* suggests either that Tgl may be modified by addition of a substituent in *M. xanthus* and the modification either does not occur or occurs differently or that the protein is degraded in *E. coli.*

Membrane proteins are often synthesized with N-terminal signal peptides that are removed by signal peptidases as they pass through the cytoplasmic membrane on their way to insertion in the proper target membrane. Indeed, the deduced N terminus of Tgl resembles a signal sequence with a positively charged residue, Arg, at position 3 and then a run of 12 hydrophobic amino acids (STASCSLALLLV, in single-letter code) followed by a signal peptidase recognition site (Fig. 10). If removal of a signal peptide were the end of processing, then the electrophoretic mobility in *M. xanthus* extracts would have been expected to be higher than in *E. coli* since there was normal targeting in *M. xanthus* and thus presumably signal peptide cleavage. However, the signal peptidase recognition site in the deduced sequence of Tgl more closely resembles that for signal peptidase II (SPII) than that for the more common SPI. SPII cleaves nascent lipoproteins between highly conserved Gly-Cys residues (6). The N-terminal cysteine is also modified by the addition of glycerol and fatty acids (6). Such a hydrophobic modification could anchor one end of Tgl to a lipid bilayer. At positions 17 to 20 of its nascent sequence, Tgl has the tetrapeptide SSGC, which might be the lipoprotein processing site. Apart from the serine residue at position 17, the Tgl sequence corresponds to the lipoprotein consensus (6). Moreover, *Serratia marcescens* murein lipoprotein and *E. coli* RplB protein, which have serine and threonine residues at this position, respectively, are cleaved by signal peptidase II and have been shown to be modified by the addition of fatty acids (31, 50). Evidently lipoproteins can include serine or threonine at this position.

```
(1)          M F R+L S T A S C S L A L L L V S S G C S
(2)        M K+A T K+L V L G A V I L G S T L L A G C S
(3)          M N R+T K+L V L G A V I L G S H S A G C S
(4)            M R+Y L A T L L L S L A V L I T A G C G
```

FIG. 10. N-terminal amino acid sequence alignment of Tgl with several lipoproteins. Features characteristic of N termini processed by signal peptidase II are discussed in the text. The proteins shown are Tgl protein (40) (sequence 1), *E. coli* lpp (30) (sequence 2), *S. marcescens* lpp (31) (sequence 3), and *E. coli* RplB (50) (sequence 4). $^+$, positively charged amino acid.

The suggestion is that Tgl is cleaved between residues 19 and 20 and then further modified by the addition of a lipid; this double modification could explain a mature Tgl whose electrophoretic mobility is close to that predicted for the unmodified peptide. Other type IV pilus systems include lipoproteins that are necessary for pilus biogenesis. Examples are BfpB, a 58-kDa lipoprotein located primarily in the outer membrane of enteropathogenic *E. coli* cells (37), and PilP, a lipoprotein and pilus assembly factor in *Neisseria gonorrhoeae* (11). No amino acid sequence homology has been detected between these proteins and Tgl, nor has stimulation been reported with these pilus systems.

Tgl is a membrane protein but not a cell surface antigen. In cell extracts, Tgl sediments with a fraction that contains mixed inner and outer membrane fragments (Fig. 7). Wash fluids from *M. xanthus* cells, grown either in suspension or on plastic culture flasks, fail to react with the anti-Tgl serum. Whole cells also do not react, even when a very sensitive indirect immunofluorescence assay is employed (Fig. 9). After cells are osmotically shocked and fixed, however, the antibody can react with whole cells. This reaction is Tgl-specific since there is no reaction with cells with a deletion of the *tgl* transcription unit. Fixation with paraformaldehyde, following a gentle osmotic shock that conserved viability, prevented cell lysis, and the cells retained their normal gross morphology. Fixation alone did not expose the epitopes. It is not excluded that the shock or fixation had altered the localization of Tgl or changed its conformation so that reactive epitopes became exposed. However, considering the cell fractionation results, it is more likely that the shock had rendered the outer membrane permeable to

anti-Tgl antibodies. Orndorff and Dworkin (35) showed that the peptidoglycan of *M. xanthus* becomes lysozyme sensitive in cells exposed to 20% sucrose in the presence of EDTA. A similar shock treatment (without fixation) renders the peptidoglycan of many gram-negative bacteria, including *E. coli* and *Pseudomonas aeruginosa*, accessible to lysozyme (8, 32). Assuming that fixation and osmotic shock can alter the outer membrane to give antibodies access to the periplasmic space, then the cell fractionation and FACS experiments imply that Tgl is exposed within that space and associated with one or both membranes.

Recently a ladle-shaped pilin structure has been proposed for the monomer units within a type IV pilus fiber of *N. gonorrhoeae*, based on 2.6-Å resolution X-ray diffraction data (36). All type IV pili have a fiber diameter of 60 Å and a high degree of conservation of the N-terminal 32 amino acids (36). This conserved sequence forms an α-helix, the handle of the ladle, and these handles are proposed to assemble a coiled-coil core common to all type IV pilus fibers. The *M. xanthus* PilA protein, the pilin monomer, is identical in 23 of its N-terminal amino acids to those of the *N. gonorrhoeae* type IV pilus fiber (56). Critical residues conserved within the otherwise variable globular domain which forms the head of the ladle, such as a disulfide bond, are also shared between *M. xanthus* PilA and the *N. gonorrhoeae* pilus fiber (56). Inactivation of social motility by mutations in the *pilA* gene is accompanied by loss of *M. xanthus* pili. *pilA* and two genes that regulate its expression, *pilR* and *pilS*, as well as other *pil* genes are clustered; *tgl* is genetically distant from this cluster. Deletion of the *pilA* gene and its genetic rescue when an intact *pilA* gene is introduced into the deletion mutants has established that pili are absolutely necessary for social motility; the possibility that pili and social motility are associated indirectly has been eliminated by these experiments (56). Tgl lacks the conserved type IV pilin sequence, consequently it must be otherwise involved in filament assembly, perhaps as a component of the base of the pilus or as an essential assembly cofactor that does not become a part of the pilus shaft or base. A membrane localization of Tgl would accommodate either role.

The predicted amino acid sequence of Tgl is composed of six similarly oriented copies of the tetratrico peptide repeat (TPR) structural motif (40). This motif has been identified in a heterogeneous group of proteins with such diverse cellular functions as protein transport, cell cycle control, transcription repression, and formation of chaperonin macromolecular complexes (12). Each TPR unit could form two α-helices with hydrophobic surfaces. One element common to these diverse proteins is their participation in multiprotein complexes (10, 27, 51, 52). The identification of six TPR repeats in Tgl suggests that it may complex with other proteins that participate in pilus assembly.

The data presented here combined with earlier results offer one way to think about the unanticipated phenomenon of motility stimulation (17, 18). By means of stimulation, any strain that is tgl^+, irrespective of the strain's capacity to assemble pili or to spread by swarming, can lead a *tgl* mutant strain with which it is mixed to assemble pili or to create a swarm with the specific socially motile pattern (18). The stimulated social motility and pilus assembly are both transient, however, and the *tgl* mutant recipients emerge after stimulation genetically unchanged (21). These qualities of *tgl* stimulation might be explained by postulating transfer of Tgl or of a patch of membrane that contains it from a tgl^+ donor cell to a *tgl* mutant recipient cell with which it makes contact. Though such a process to our knowledge has not been observed in gram-negative bacteria, both vesicle reception (20) and vesicle donation do separately have precedents. As to donation, virulence factors have been documented in vesicles from *Actinobacillus* (14), *Bacteroides* (now *Porphyromonas*) (13), *Borrelia* (54), *Capnocytophaga* (28), *Hemophilus* (55), *Neisseria* (9), and *Pseudomonas* (19).

Extrapolating from the low intensity of Tgl bands in Western blots relative to the intensities of the bands of TrpE and β-galactosidase expressed in *E. coli*, the protein appears to be of low abundance. A role in an early step of initiating or facilitating pilus assembly would not require much Tgl protein. The TPR sequences in Tgl would be involved in the protein-protein interactions necessary for these purposes. Once initiated, a pilus filament could polymerize, drawing on a pool of pilin, the *pil*A product (56), which should have accumulated in the absence of Tgl. The availability of a variety of characterized *tgl* mutants and of Tgl-specific antibodies opens the way to experimental tests of this proposal as well as a more precise cellular localization of Tgl protein.

REFERENCES

1. **Amann, E., and J. Brosius.** 1985. 'ATG vectors' for regulated high-level expression of cloned genes in *Escherichia coli*. Gene **40:**183–190.
2. **Arnold, J. W., and L. J. Shimkets.** 1988. Cell surface properties correlate with cell cohesion in *Myxococcus xanthus*. J. Bacteriol. **170:**5771–5777.
3. **Behmlander, R. M., and M. Dworkin.** 1991. Extracellular fibrils and contact-mediated cell interactions in *Myxococcus xanthus*. J. Bacteriol. **173:**7810–7821.
4. **Behmlander, R. M., and M. Dworkin.** 1994. Biochemical and structural analysis of the extracellular matrix fibrils of *Myxococcus xanthus*. J. Bacteriol. **176:**6295–6303.
5. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41:**459–469.
6. **Braun, V., and H. C. Wu.** 1994. Lipoproteins, structure, function, biosynthesis and model for cell export, p. 319–340. *In* J.-M. Ghuysen and R. Hakenbeck (ed.), Bacterial cell wall. Elsevier Science Publishing, Inc., New York, N.Y.
7. **Burchard, R. P.** 1980. Gliding motility of bacteria. BioScience **30:**157–162.
8. **Cheng, K.-J., J. M. Ingram, and J. W. Coster.** 1971. Interactions of alkaline phosphatase and the cell wall of *Pseudomonas aeruginosa*. J. Bacteriol. **107:** 325–336.
9. **DeVoe, I. W., and J. E. Gilchrist.** 1973. Release of endotoxin in the form of cell wall blebs during in vitro growth of *Neisseria meningitidis*. J. Exp. Med. **138:**1156–1167.
10. **Dodt, G., N. Braverman, C. Wong, A. Moser, H. W. Moser, P. Watkins, and S. J. Gould.** 1995. Mutations in the PTS1 receptor gene, PXR1, define complementation group 2 of the peroxisome biogenesis disorder. Nat. Genet. **9:**115–125.
11. **Drake, S. L., S. A. Sandstedt, and M. Koomey.** 1997. PilP, a pilus biogenesis lipoprotein in *Neisseria gonorrhoeae*, affects expression of PilQ as a high-molecular-mass multimer. Mol. Microbiol. **23:**657–668.
12. **Goelb, M., and Y. Mitsuhiro.** 1991. The TPR snap helix: a novel protein repeat motif from mitosis to transcription. Trends Biochem. Sci. **16:**173–177.
13. **Grenier, D., and D. Mayrand.** 1987. Functional characterization of extracellular vesicles produced by *Bacteroides gingivalis*. Infect. Immunol. **55:**111–117.
14. **Hammond, B. F., and R. H. Stevens.** 1982. *Capnocytophaga* and *Actinobacillus actinomycetemcomitans*: occurrence and pathogenic potential in juvenile periodontitis, p. 46–61. *In* R. J. Genco and S. E. Mergenhagen (ed.), Host-parasite interactions in periodontal diseases. American Society for Microbiology, Washington, D.C.
15. **Hartzell, P., and D. Kaiser.** 1991. Function of MglA, a 22-kilodalton protein essential for gliding in *Myxococcus xanthus*. J. Bacteriol. **173:**7615–7624.
16. **Hartzell, P. L., and P. Youderian.** 1995. Genetics of gliding motility and development in *Myxococcus xanthus*. Arch. Microbiol. **164:**309–323.
17. **Hodgkin, J., and D. Kaiser.** 1977. Cell-to-cell stimulation of movement in nonmotile mutants of *Myxococcus*. Proc. Natl. Acad. Sci. USA **74:**2938–2942.
18. **Hodgkin, J., and D. Kaiser.** 1979. Genetics of gliding motility in *M. xanthus* (Myxobacterales): two gene systems control movement. Mol. Gen. Genet. **171:**177–191.
19. **Kadurugamuwa, J. L., and T. J. Beveridge.** 1995. Virulence factors are released from *Pseudomonas aeruginosa* in association with membrane vesicles during normal growth and exposure to gentamicin: a novel mechanism of enzyme secretion. J. Bacteriol. **177:**3998–4008.
20. **Kahn, M. E., F. Barany, and H. O. Smith.** 1983. Transformasome: specialized membranous structures that protect DNA during *Haemophilus* transformation. Proc. Natl. Acad. Sci. USA **80:**6927–6931.
21. **Kaiser, D.** 1979. Social gliding is correlated with the presence of pili in

Myxococcus xanthus. Proc. Natl. Acad. Sci. USA **76:**5952–5956.
22. **Kaiser, D., and C. Crosby.** 1983. Cell movement and its coordination in swarms of *Myxococcus xanthus*. Cell Motil. **3:**227–245.
23. **Kalos, M., and J. Zissler.** 1990. Transposon tagging of genes for cell-cell interactions in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **87:**8316–8320.
24. **Koelle, M. R., W. S. Talbot, W. A. Segraves, M. T. Bender, P. Cherbas, and D. S. Hogness.** 1991. The Drosophila EcR gene encodes an ecdysone receptor, a new member of the steroid receptor superfamily. Cell **67:**59–77.
25. **Kroos, L., A. Kuspa, and D. Kaiser.** 1986. A global analysis of developmentally regulated genes in *Myxococcus xanthus*. Dev. Biol. **117:**252–266.
26. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
27. **Lamb, J. R., S. Tugendreich, and P. Heiter.** 1995. Tetratrico peptide repeat interactions: to TPR or not to TPR? Trends Biochem. Sci. **20:**257–259.
28. **London, J., R. Celesk, and P. Kolenbrander.** 1982. Physiological and ecological properties of the oral gram-negative gliding bacteria capable of attaching to hydroxyapatite, p. 76–85. *In* R. J. Genco and S. E. Mergenhagen (ed.), Host-parasite interactions in periodontal diseases. American Society for Microbiology, Washington, D.C.
29. **MacNeil, S. D., F. Calara, and P. L. Hartzell.** 1994. New clusters of genes required for social motility in *Myxococcus xanthus*. Mol. Microbiol. **14:**61–71.
30. **Nakamura, K., and M. Inouye.** 1979. DNA sequence of the gene for the outer membrane lipoprotein of *E. coli*: an extremely AT-rich promoter. Cell **18:**1109–1117.
31. **Nakamura, K., and M. Inouye.** 1980. DNA sequence of the *Serratia marcescens* lipoprotein gene. Proc. Natl. Acad. Sci. USA **77:**1369–1373.
32. **Neu, H. C., and L. A. Heppel.** 1964. The release of ribonuclease into the medium when *Escherichia coli* cells are converted to spheroplasts. J. Biol. Chem. **239:**3893–3900.
33. **Nossal, N. G., and L. A. Heppel.** 1966. The release of enzymes by osmotic shock from *Escherichia coli* in exponential phase. J. Biol. Chem. **241:**3055–3062.
34. **O'Connor, K. A., and D. R. Zusman.** 1986. Genetic analysis of *Myxococcus xanthus* and isolation of gene replacements after transduction under conditions of limited homology. J. Bacteriol. **167:**744–748.
35. **Orndorff, P. E., and M. Dworkin.** 1980. Separation and properties of the cytoplasmic and outer membranes of *Myxococcus xanthus*. J. Bacteriol. **141:**917–947.
36. **Parge, H. E., K. T. Forest, M. J. Hickey, D. A. Christensen, E. D. Gertzoff, and J. A. Tainer.** 1995. Structure of the fibre-forming protein pilin at 2.6 A resolution. Nature **378:**32–38.
37. **Ramer, S. W., D. Bieber, and G. K. Schoolnik.** 1996. BfpB, an outer membrane lipoprotein required for the biogenesis of bundle-forming pili in enteropathogenic *Escherichia coli*. J. Bacteriol. **178:**6555–6563.
38. **Redding, K., C. Holcomb, and R. S. Fuller.** 1991. Immunolocalization of Kex2 protease identifies a putative late Golgi compartment in the yeast *Saccharomyces cerevisiae*. J. Cell Biol. **113:**527–538.
39. **Reichenbach, H., H. H. Heunert, and H. Kuczka.** 1965. Myxococcus spp. (Myxobacteriales) Schwarmentwicklung und Bildung von Protocysten. Film E778. Institut Wissenschaftlichen Film, Gottingen, Germany.
40. **Rodriguez-Soto, J., and D. Kaiser.** 1997. The *tgl* gene: social motility and stimulation in *Myxococcus xanthus*. J. Bacteriol. **179:**4361–4371.
41. **Rosenbluh, A., and M. Eisenbach.** 1992. The effect of mechanical removal of pili on gliding motility in *Myxococcus xanthus*. J. Bacteriol. **174:**5406–5413.
42. **Russo-Marie, F., M. Roederer, B. Sager, and L. A. Herzenberg.** 1993. Beta-galactosidase activity in single differentiating bacterial cells. Proc. Natl. Acad. Sci. USA **90:**8194–8198.
43. **Ruther, U., and B. Muller-Hull.** 1983. Easy identification of cDNA clones. EMBO J. **12:**1791–1794.
44. **Schagger, H., and G. von Jagow.** 1988. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range 1 to 100 kDa. Anal. Biochem. **166:**368–379.
45. **Shi, W., and D. R. Zusman.** 1993. The two motility systems of *Myxococcus xanthus* show different selective advantages on various surfaces. Proc. Natl. Acad. Sci. USA **90:**3378–3382.
46. **Sodergren, E., and D. Kaiser.** 1983. Insertions of Tn5 near genes that govern stimulatable cell motility in *Myxococcus*. J. Mol. Biol. **167:**295–310.
47. **Spormann, A. M., and D. Kaiser.** 1995. Gliding movements in *Myxococcus xanthus*. J. Bacteriol. **177:**5846–5852.
48. **Spratt, B. G., P. J. Hedge, S. T. Heesen, A. Edelman, and J. K. Broome-Smith.** 1986. Kanamycin-resistant vectors that are analogues of plasmids pUC8, pUC9, pEMBL8 and pEMBL9. Genes **41:**337–342.
49. **Stephens, K., and D. Kaiser.** 1987. Genetics of gliding motility in *Myxococcus xanthus*: molecular cloning of the mgl locus. Mol. Gen. Genet. **207:**256–266.
50. **Takase, I., F. Ishino, M. Wachi, H. Kamata, M. Doi, S. Asoh, H. Matsuzawa, T. Ohta, and M. Matsuhashi.** 1987. Genes encoding two lipoproteins in the *leuS-dacA* region of the *Escherichia coli* chromosome. J. Bacteriol. **169:**5692–5699.
51. **Terlecky, S. R., W. M. Nuttley, D. McCollum, E. Stock, and S. Subramani.** 1995. The *Pichia pastoris* peroxisomal PAS8p is the receptor for the C-terminal tripeptide peroxisomal targeting signal. EMBO J. **14:**3627–3634.
52. **Tzamarias, D., and K. Struhl.** 1995. Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. Genes Dev. **9:**821–831.
53. **Voelz, H., and R. O. Ortigoza.** 1968. Cytochemistry of phosphatases in *Myxococcus xanthus*. J. Bacteriol. **96:**1357–1365.
54. **Whitmire, W. M., and C. F. Garon.** 1993. Specific and nonspecific responses of murine B cells to membrane blebs of *Borrelia burgdorferi*. Infect. Immunol. **61:**1460–1467.
55. **Wispelwey, B., E. J. Hansen, and W. M. Scheld.** 1989. *Haemophilus influenzae* outer membrane vesicle-induced blood-brain barrier permeability during experimental meningitis. Infect. Immunol. **57:**2559–2562.
56. **Wu, S. S., and D. Kaiser.** 1995. Genetic and functional evidence that Type IV pili are required for social gliding motility in *Myxococcus xanthus*. Mol. Microbiol. **18:**547–558.
57. **Wu, S. S., J. Wu, and D. Kaiser.** 1997. The *Myxococcus xanthus* pilT locus is required for social gliding although pili are still produced. Mol. Microbiol. **23:**109–121.