JOURNAL OF BACTERIOLOGY, Feb. 1998, p. 759–761 Vol. 180, No. 3
0021-9193/98/$04.00+0

Contact Stimulation of Tgl and Type IV Pili in *Myxococcus xanthus*

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Received 18 August 1997/Accepted 19 November 1997

Myxococcus xanthus tgl **mutants lack social motility and type IV pili but can be transiently stimulated to swarm and to make pili by contacting *tgl*$^+$ cells. The absence of pili in *tgl* mutants is shown not to be due to the absence of pilin. The rate of pilus elongation after Tgl stimulation is shown to be similar to the rate of pilus elongation in wild-type cells, using a new more rapid assay for stimulation.**

Myxococcus xanthus has two genetic systems, called adventurous (A) and social (S) motility, which control its gliding motility (2, 4, 9, 22). Unlike A motility, S motility involves movements of cells which are close to each other, thus implying cell-cell interactions (9, 11). S motility absolutely requires polarly localized type IV pili, since no mutant lacking pili has S motility (10, 13, 18, 25). Homologous type IV pili in *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae* have been shown to be involved in another type of surface translocation called twitching (6). Many of the *pil* gene products in *M. xanthus*, *P. aeruginosa*, and *N. gonorrhoeae* share sequence homologies with the type II, or general secretion, pathway found in enteric bacteria (15).

Mutants for a particular S motility gene, called *tgl* (for transient gliding), lack S motility and type IV pili, but these qualities can be phenotypically rescued by contact with *tgl*$^+$ (donor) cells, a process called stimulation (9, 10). Stimulation does not involve a diffusable factor but instead requires physical contact between live cells. Formerly, to detect *tgl* stimulation, nonswarming (A^- S^-) recipient and donor strains have been used so that the phenotypically stimulated *tgl* recipients were visible as cells that swarmed out of the initial mixture. These stimulated cells have pili (pili$^+$). If the stimulated cells are cultured, their offspring are S^- and pili$^-$; hence, Tgl stimulation is transient and only phenotypic (10). An analogous type of phenotypic rescue occurs for a curli (pili-like) mutant in *Escherichia coli* (3). However, unlike type IV pili (7), curlin subunits are secreted and then are nucleated and polymerized on the cell surface. In the A motility system, which does not involve pili, five groups of mutants lack A motility but can be stimulated for A motility by donor cells which have the corresponding wild-type allele. Stimulation is thus a general phenomenon and may play an important role in the swarming of *M. xanthus.*

The *tgl* gene product contains a signal peptidase II recognition sequence, suggesting that Tgl is a lipoprotein (16). Tgl appears to be localized to the periplasm, probably attached to the outer membrane, and to contain six tandem copies of the tetratrico peptide repeat (TPR) (17). TPR domains are thought to be important in protein-protein interactions (12). Thus, Tgl likely interacts with other proteins involved in pilus assembly. To find the molecular basis of Tgl stimulation, we sought a new assay that would allow stimulation to be monitored on a time scale of hours instead of days and that could use swarming as well as nonswarming strains.

Δ*tgl* mutants express PilA. *tgl* mutants fail to make pili that can be detected by electron microscopy (10). Type IV pili are helical assemblies of pilin monomers, the *pilA* gene product (14). To determine whether the block in pilus biogenesis in *tgl* mutants was at the level of PilA expression or its assembly into pili, whole-cell extracts from a deletion (Δ) *tgl* mutant (16) were probed for expression of PilA with antibody to PilA (24) (see below). As shown in Fig. 1A, a Δ*tgl* mutant expresses near-wild-type levels of PilA protein. In a complementary experiment, the expression of *pilA* was monitored with a *pilA*::*lacZ* transcriptional fusion (24). Transcription of the *pilA*::*lacZ* operon fusion was found to be similar in Δ*tgl* and *tgl*$^+$ cells (Fig. 1B). A Δ*pilB* strain, which also lacks pili and S motility (27), also expresses near-wild-type levels of PilA (Fig. 1). By contrast, expression of *pilA* was blocked in a Δ*pilR* mutant; PilR is known to be a transcriptional activator of *pilA* gene expression (24, 25). We conclude that the absence of pili in *tgl* mutants is due to a failure to assemble pilin monomer subunits into pili.

Stimulation assay for pili. Since S motility absolutely depends on the production of pili, we reasoned that Tgl stimulation might be monitored by measuring the assembly of pili rather than the resultant swarming of S motile cells. Hence, Δ*tgl* recipient cells were mixed with *tgl*$^+$ Δ*pilA* donor cells (which also lack pili) and pili were measured at various times after mixing. To enumerate pili, the cells were grown in CTT medium (8) to a density of 80 to 120 Klett units, concentrated by centrifugation, washed, and resuspended in TPM buffer (10 mM Tris-HCl, 1 mM KPO_4, and 8 mM $MgSO_4$ [pH 7.6]) to a calculated density of 500 Klett units. The cells were then mixed at a ratio of 2 recipient cells to 1 donor cell, and four 20-μl samples were placed on one-half CTT agar plates (8). We observed that stimulation only occurs on a solid surface and not in liquid (10). Cells were harvested from the plates at various times and suspended in 0.4 ml of TPM buffer. Pili were sheared off cells by vortexing the cell suspension in microcentrifuge tubes (1.5 ml) for 2 min (1, 5). The cells were separated from the suspended pili by centrifugation at 14,000 rpm (16,000 × *g*) for 5 min at room temperature. The supernatant was collected, and $MgCl_2$ was added to a final concentration of 100 mM to precipitate pilus fragments (1, 5). After ≥1 h of incubation on ice, which allowed the pilus fragments to form paracrystalline aggregates, the precipitate was collected by centrifugation for 15 min at 4°C, also at 14,000 rpm. The precipitate was resuspended in sample buffer, boiled for 5 min,

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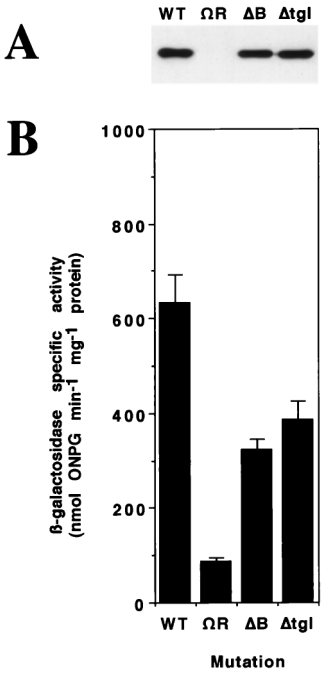

FIG. 1. (A) Immunoblot of *M. xanthus* whole-cell protein probed with anti-PilA serum. Proteins were visualized by anti-rabbit IgG–peroxidase and chemiluminescence (Renaissance; NEN Life Science Products). Samples were prepared from strains containing null mutations of the *pil* genes indicated. Lanes (labeled with the mutation present in each strain): WT, DK1219 (S^+) (9); ΩR, DK10414 (*pilR*-Ω3163, a Tn*5* insertion) (24); ΔB, DK10416 (Δ*pilB*) (27); Δtgl, DK10413 (Δ*tgl*) (16, 24). Protein from 5×10^6 cells was loaded in each lane. (B) β-galactosidase specific activity of the same strains as in panel A, into which a single copy of a *pilA-lacZ* transcriptional fusion has been introduced. The values are averages of four or more independent measurements; error bars indicate standard deviations. ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

and separated overnight by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (19). To detect PilA protein, the gels were blotted and the blots were probed with a 1:2,000 dilution of anti-PilA serum.

As shown in Fig. 2A, the Δ*tgl* recipient cells produce pili that are long enough to become sensitive to shearing when they are mixed with tgl^+ Δ*pilA* (DK10407) donor cells. It is important to note that the PilA protein which makes up these pili can have arisen only from the Δ*tgl* cells, since they are $pilA^+$ while the donor cells are Δ*pilA*. The ability of the PilA protein to assemble into pilus filaments was confirmed by electron microscopy. The PilA protein seen as a doublet in the gels of sheared samples (as in Fig. 2) may reflect incomplete reduction of disulfide bonds or, alternatively, a posttranslational modification of the protein (21). Stimulation is specific to *tgl*, as shown by the following: (i) when the same culture of recipient cells was mixed with an otherwise isogenic donor which lacks Tgl (Δ*tgl* Δ*pilA*) no pilus bands were detected (Fig. 2B) and (ii) when a Δ*pilB* mutant was mixed with the same donor strain DK10407, which is $pilB^+$ tgl^+ Δ*pilA*, no *pilB* stimulation was observed (Fig. 2C). To date, *tgl* mutants are the only type of S motility mutants known which can be stimulated (4, 9, 20).

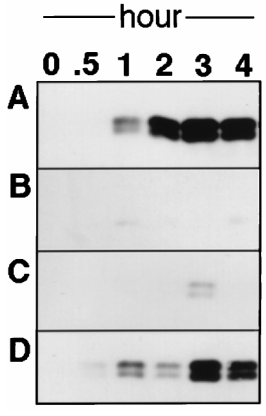

FIG. 2. Immunoblot of sheared pilus samples probed with anti-PilA serum. Cells were incubated on one-half CTT agar plates (9) for the indicated times as described in the text, except that at 0 h cells were directly assayed in CTT medium. (A) DK10405 (Δ*tgl*) (16) mixed with DK10407 (Δ*pilA*) (26); (B) DK10405 mixed with DK8600 (Δ*pilA* Δ*tgl*); (C) DK10407 mixed with DK10416 (Δ*pilB*); (D) sheared DK1622 (wild type) (10) alone.

Time course of Tgl stimulation. The speed of pilus elongation after stimulation of Δ*tgl* cells was compared with the normal extension rate of pili in growing cells. Since wild-type cells constitutively express pili, wild-type (DK1622) cells were first sheared by vortexing for 3 min to remove their pili. Rosenbluh and Eisenbach have shown that sheared cells are able to regrow pili (18). In fact, as shown in figure 2D, the regrowth of pili on sheared wild-type cells was somewhat slower than pilus elongation after the stimulation of Δ*tgl* mutants (Fig. 2A). More rapid growth of pili after stimulation may reflect the accumulation of PilA monomer units in Δ*tgl* cells; wild-type cells may have to synthesize and process their pilin monomers (18, 23). These data argue that *tgl* mutants have all the necessary components for pilus biogenesis except for Tgl protein. The time required for stimulation is comparable to the rate of pilus extension during the growth of wild-type cells. Thus, it is possible that stimulation is involved in the control of swarming. It is hoped that the assay described here will permit the identification of proteins with which Tgl interacts and of the cellular and/or molecular basis of stimulation.

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

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