# Effect of cellular filamentation on adventurous and social gliding motility of *Myxococcus xanthus*

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**Filamentous bacterial cells often provide biological information that is not readily evident in normal-size cells. In this study, the effect of cellular filamentation on gliding motility of *Myxococcus xanthus*, a Gram-negative social bacterium, was investigated. Elongation of the cell body had different effects on adventurous and social motility of *M. xanthus*. The rate of A-motility was insensitive to cell-body elongation whereas the rate of S-motility was reduced dramatically as the cell body got longer, indicating that these two motility systems work in different ways. The study also showed that filamentous wild-type cells glide smoothly with relatively straight, long cell bodies. However, filamentous cells of certain social motility mutants showed zigzag, tangled cell bodies on a solid surface, apparently a result of a lack of coordination between different fragments within the filaments. Further genetic and biochemical analyses indicated that the uncoordinated movements of these mutant filaments were correlated with the absence of cell surface fibril materials, indicating a possible new function for fibrils.**

*Myxococcus xanthus* moves on solid surfaces by gliding, a motility mechanism for movement without flagella on a solid surface (1, 2). Genetic and behavioral analyses reveal that *M. xanthus* has two different types of motility systems: adventurous (A) motility (cells move as single cells or as small cell groups) and social (S) motility (cells move as large cell groups) (3, 4). Mutations in A- or S-motility genes inactivate the corresponding system; however, the cells are still motile by means of the remaining system. Previous studies have shown that cells exhibiting A-motility move better on a hard, dry surface, whereas those with S-motility move better on a soft, moist surface (5). The selective advantage of A- and S-motility systems over different surfaces enables the bacterium to adapt to a variety of physiological and ecological environments.

S-motility is very important for the complex, social lifestyle of this bacterium. It is required for fruiting body formation, a developmental process in which hundreds of thousands of starved *M. xanthus* cells aggregate to form a well organized multicellular structure as a means of protection against adverse conditions (3, 6). It is also thought to be beneficial to the predatory nature of this bacterium, because a cell group presumably can secrete more extracellular enzymes than a single cell, thus facilitating the digestion of their prey (e.g., an *Escherichia coli* colony) (7). Little is known about the physiological role of A-motility other than that it confers an adventurous nature onto single cells.

Extensive genetic studies have been performed to analyze the genes required for both A- and S-motility. Initial studies by Kaiser and colleagues (3, 4, 8) indicated that there were more than 10 genetic loci (such as *sgl, tgl,* etc.) involved in S-motility and 21 genetic loci (such as *agl, cgl*, etc.) involved in A-motility. In addition, an *mgl* locus was required for both A- and S-motility (3). A recent study by Hartzell and colleagues (9) found additional loci for *M. xanthus* gliding motility. Many of the genetic loci related to gliding motility have been characterized further at the molecular level. For example, the *sgl* locus encodes many genes homologous to the *pil* genes of *Pseudomonas* (10). The *sglK* locus encodes a protein homologous to DnaK (11, 12). The *tgl* and *cglB* loci also have been cloned and sequenced recently (13, 14). A group of S-motility mutants were mapped at the *dsp* locus even though the molecular nature and functions of the *dsp* genes remain to be elucidated (15). Our group recently characterized a genetic locus (*dif*) that is also required for S-motility (16). The *dif* locus encodes a set of chemotaxis homologues, including DifA (MCP homologue), DifC (CheW homologue), DifD (CheY homologue), and DifE (CheA homologue). The *dif* locus maps near the known *dsp* region (Z.Y., H. Kaplan, and W.S., unpublished data).

Two *M. xanthus* cellular surface appendages, pili and fibrils, are related to S-motility based on biochemical and genetic analyses (8, 17). *M. xanthus* pili are located at the cell poles and belong to the type IV family of bacterial pili (8, 10). The extracellular matrix fibrils of *M. xanthus* are located over the entire bacterial cell body (18). Viewed with the electron microscopy, they appear to form a mesh-like structure or network linking cells together or linking cells to the solid substratum over which the *M. xanthus* cells glide. The fibrils are composed of approximately equal amounts of protein and carbohydrate (19). Many characterized S-motility-related genes (such as *sgl*) are involved in the biogenesis or function of the pili (10, 13). The *dsp* and *dif* mutants lack fibrils (ref. 17; Z.Y. and W.S., unpublished data). Some S-motility mutants (such as *tgl*) are defective in both pili and fibrils (20). It is evident from previous studies that fibrils are required for cellular adhesion (17). Recent studies demonstrate that pili also are involved in cellular adhesion, even though it is still unclear whether the pilus mutants also lack fibrils (21).

Despite intensive research efforts to elucidate the mechanism of gliding motility in *M. xanthus*, many questions remain. What is the difference between the A- and S-motility systems? Does S-motility require more than cellular adhesion? Are there additional cellular functions for pili and fibrils besides their involvement in cellular adhesion? In this study, we have used an antibiotic-induced cellular filamentation method to address some of these questions. Our results provide some useful information about the difference between two motility systems and reveal some possible new physiological functions for fibrils and pili.

## Materials and Methods

**Bacterial Growth Conditions and Strains.** *M. xanthus* cells were grown in CYE medium (10 g/liter casitone/5 g/liter yeast extract/8 mM $MgSO_4$ in 10 mM Mops buffer, pH 7.6) (22) at 32°C on a rotary shaker at 225 rpm. To produce filamentous cells of *M. xanthus* (called myxo-filaments in this paper), 100 $\mu$M cephalexin was added to the growth medium to block cell wall septation. Most myxo-filaments used in this study were cultured for at least 8 hr, and the cell body was at least four times longer than normal cells unless specified.

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Abbreviations: CYE medium, 10 g/liter casitone/5 g/liter yeast extract/8 mM $MgSO_4$ in 10 mM Mops buffer, pH 7.6; wt, wild type.

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**Table 1. Bacterial strains**

| Strains | Relevant genotype | Motility phenotype | Ref. or source |
|---|---|---|---|
| DK1622 | wt | $A^+S^+$ | (8) |
| DZ4148 | *frzE*::Tn5Ω226 | $A^+S^+$ | (5) |
| DK1253 | *tgl-1* | $A^+S^-$ | (3) |
| DK1300 | *sglG1* | $A^+S^-$ | (3) |
| DK10405 | Δ*tgl*::*Tc*$^r$ | $A^+S^-$ | Dale Kaiser |
| DK10407 | Δ*pilA*::*Tc*$^r$ | $A^+S^-$ | Dale Kaiser |
| DK10409 | Δ*pilT* | $A^+S^-$ | Dale Kaiser |
| DK10416 | Δ*pilB* | $A^+S^-$ | Dale Kaiser |
| LS300 | *dsp* | $A^+S^-$ | Larry Shimkets |
| DK3470 | *dsp*-1693 | $A^+S^-$ | (15) |
| SW501 | *difE*::*Km*$^r$ | $A^+S^-$ | (16) |
| SW504 | Δ*difA* | $A^+S^-$ | (16) |
| DK1217 | *aglB1* | $A^-S^+$ | (3) |
| DK1218 | *cglB2* | $A^-S^+$ | (3) |
| MXH1216 | A::Tn5-*lac*Ω1215 | $A^-S^+$ | (6) |
| CDS | *lfp1*:20 | $A^+S^+$ | (24) |
| SW506 | *aglB1, difE*::*Km*$^r$ | $A^-S^-$ | (16) |
| SW538 | Δ*pilA*::*Tc*$^r$, A::Tn5-*lac*Ω1215 | $A^-S^-$ | This study |
| SW590 | *difE*::*Km*$^r$, Δ*pilA*::*Tc*$^r$ | $A^+S^-$ | This study |
| SW596 | *aglB1, difE*::*Km*$^r$, Δ*pilA*::*Tc*$^r$ | $A^-S^-$ | This study |

The *M. xanthus* strains used in this study are listed in Table 1. Strains DK1622 [wild type (wt)], SW504 (Δ*difA*), SW501 [*difE*::*Km*$^r$ (kanamycin resistance)], SW506 (*aglB1*, *difE*::*Km*$^r$), MXH1216 (A::Tn5-*lac*Ω1215), DK1217 (*aglB1*), DK1218 (*cglB2*), DK1300 (*sglG1*), DK10405 [Δ*tgl*::*Tc*$^r$ (tetracycline resistance)], and DK10407 (Δ*pilA*::*Tc*$^r$) have been described previously (3, 6, 8, 16, 23). CDS (*ifp*1:20) was obtained from Dworkin's lab (24). DK10409 (Δ*pilT*) and DK10416 (Δ*pilB*) were obtained from Dale Kaiser's lab (Stanford Univ., Stanford, CA). Strain LS300 (*dsp*) was obtained from Larry Shimkets' lab (Univ. of Georgia, Athens). Mx4-mediated generalized transduction (22) was used to construct strains SW538 (A::Tn5-*lac*Ω1215, Δ*pilA*::*Tc*$^r$), SW590 (*difE*::*Km*$^r$, Δ*pilA*::*Tc*$^r$), and SW596 (*aglB1*, *difE*::*Km*$^r$ Δ*pilA*::*Tc*$^r$). SW538 was constructed by transducing Mx4 lysate of MXH1216 into DK10407 and selecting for Km$^r$. SW590 was constructed by transducing Mx4 lysate of SW501 into DK10407 and selecting for Km$^r$. SW596 was constructed by transducing Mx4 lysate of DK10407 into SW506 and selecting for Tc$^r$.

**Microscopic Analysis of A- and S-Motility.** To analyze A-motility of normal or filamentous cells, $A^+S^-$ cells at $2.5 \times 10^7$/ml were spotted onto Mops medium hard agar (8 mM $MgSO_4$/10 mM MOPS, pH 7.6/1.5% agar) for analysis as described (5). Gliding motility of individual cells on agar surfaces was observed with a Leica microscope with a ×32 objective lens. For the study of bacterial gliding motility and cellular reversal frequency, microscopic images were captured by using time-lapse video photography [Hyper HAD video camera (Sony) and time-lapse video cassette recorder, AG6040 (Panasonic)]. Because of the slow movement of the *M. xanthus* cells, bacterial movements were recorded at a slower rate (60×) and played back at the normal rate. The cellular speed and the reversal frequency were estimated through frame-by-frame analysis.

To analyze S-motility of normal-size or filamentous cells, 10 μl of $A^-S^+$ cells ($2.5 \times 10^7$ cells/ml) was spotted onto a Mops medium-soft agar (0.4% agar) for analysis. As reported by Shi and Zusman (5), the S-gliding motility is much increased (up to 15 μm/min) on 0.4% agar surface. Also under that condition, $A^-S^+$ cells still can move even when they are one cell distance apart. Thus, when cells were spotted at $2.5 \times 10^7$ cells/ml, the cell density is low enough that we were able to clearly follow movement of single $A^-S^+$ cells and calculate their moving speed by using video microscopy as described above. To further confirm these social motility data, we also used the method described by Shi *et al.* (25). A small portion of $A^-S^+$ cells was labeled with tetrazolium chloride (red cells) and then mixed with a high density of unlabeled $A^-S^+$ cells at the ratio of 1:100. The mixture then was spotted onto 0.4% agar at $5 \times 10^8$ cells/ml. The movement of single cells within social groups then was analyzed by tracking the few red cells within large cell groups.

**Other Assays.** For cell growth rate and cellular elongation rate, *M. xanthus* cells starting at $2.5 \times 10^7$ cells/ml were grown in CYE with or without 100 μM cephalexin with vigorous shaking. Samples of each culture were measured every 60 min for increased OD at 600 nm. After 8 hr, the cell body length of normal cells and myxo-filaments was measured manually through magnified images on a monitor screen. The presence of fibrils and pili was examined by using Western blot analyses. For fibrils, whole cells of myxo-filaments were pelleted, lysed using SDS/PAGE loading buffer, and adjusted to an equivalent of $5 \times 10^9$ cells/ml. Ten microliters of cell lysate was loaded for each strain, and the proteins were separated electrophoretically. Fibril proteins were detected by using mAb 2105 (18). To purify pili, myxo-filaments were collected and resuspended at $5 \times 10^7$ cells/ml in TPM buffer (10 mM Tris, pH 7.6/1 mM $KH_2PO_4$/8 mM $MgSO_4$), vortexed for 2 min, then centrifuged at 13,000 rpm for 5 min in a bench-top microcentrifuge. The supernatant was collected in a clean tube and $MgCl_2$ was added to a final concentration of 100 mM. After incubation on ice for 1 hr, the solution was centrifuged at 14,000 rpm for 20 min at 4°C. The pilus pellet (invisible) was dissolved in SDS/PAGE loading buffer that was one-fourth of the supernatant volume; 10 μl of the dissolved pilus pellet was loaded for SDS/PAGE and Western blot analyses. Extracellular pili were detected by using PilA antibody (26). To study the effect of purified fibrils on *dif* and *dsp* mutants, fibrils were purified and quantified according to Chang and Dworkin (27). Myxo-filaments were mixed with fibril at 3.2 μg of carbohydrate per cell, incubated at 32°C for 30 min, then spotted onto Mops agar plate containing 100 μM cephalexin for motility analysis. For transmission electron microscopic analysis, the myxo-filaments were fixed in 2% glutaraldehyde in phosphate buffer, washed with physiological saline, and postfixed in 1% $OsO_4$ buffered with phosphate. The specimens then were placed for 1 hr in 0.5% uranyl acetate, dehydrated in graded acetone, and embedded in Spurr Embedding medium. Sections 1- to 2-μm thick were obtained with a Reicher $OmU_2$ ultramicrotome by using a diamond knife and stained with toluidine blue for orientation. The thin sections were stained with uranyl acetate and Reynold's lead citrate and examined with a Siemens Elmiskop 1A electron microscope (Siemens, Iselin, NJ); photographs were taken at an original magnification of ×14,000 and subsequently were enlarged.

## Results

**Cephalexin-Induced Filamentous Cells of *M. xanthus*.** The aim of the study is to examine the effect of cellular filamentation on gliding motility of *M. xanthus*. For this purpose, a method that can effectively produce filamentous cells of *M. xanthus* is required. Cephalexin, an antibiotic, blocks bacterial cell wall septation and has been used to generate filamentous cells of other bacterial species (28, 29). In this study, we examined the effect of cephalexin on the cellular physiology of *M. xanthus*. We found that cephalexin effectively inhibited cell wall septation of *M. xanthus* and produced filamentous cells referred to as "myxo-filaments" in this paper (Fig. 1). Testing of various concentrations of cephalexin revealed that 20 μM cephalexin was the minimal concentration that had some inhibitory effect on cell

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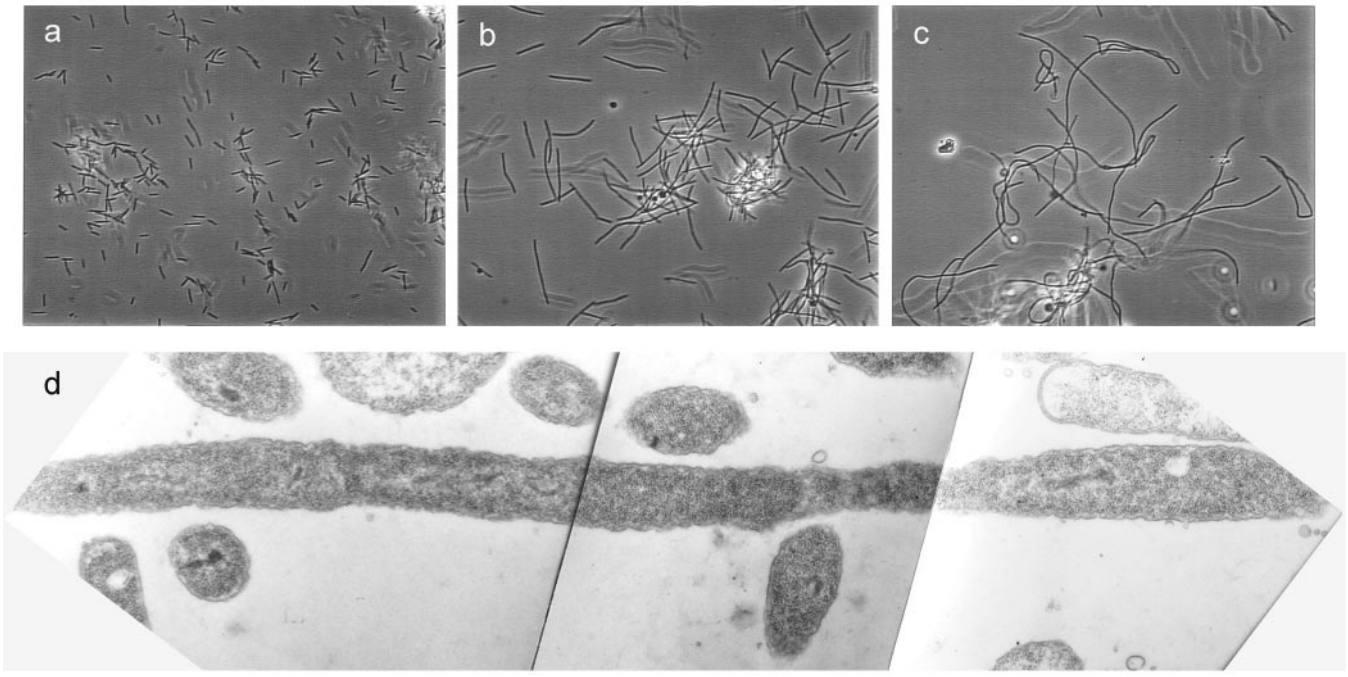


**Fig. 1.** Effect of cephalexin on *M. xanthus*. Phase-contrast images of wt *M. xanthus* cells (DK1622) grown in CYE without cephalexin (*a*), with 100 $\mu$M cephalexin after 6 hr (*b*), and with 100 $\mu$M cephalexin after 12 hr (*c*). Pictures were taken through a $\times$32 objective lens. (*d*) Electron microscopy analysis of DK1622 myxo-filaments grown in CYE with 100 $\mu$M cephalexin for 12 hr ($\times$14,000).

septation. At 100 $\mu$M cephalexin, all septum formation was blocked. Cephalexin at 200 $\mu$M was toxic to *M. xanthus*, i.e., the cells became spheroplasts or lysed. The normal doubling time for *M. xanthus* wild-type DK1622 in CYE medium was about 4 hr. In the presence of 100 $\mu$M cephalexin, the doubling time was also around 4 hr. Thus, although 100 $\mu$M cephalexin completely inhibited cell septation, it had a minimal effect on cell growth. Because of blocked cell wall septation, the cell length was doubled every 4 hr. Therefore, most myxo-filaments used in this study were about four times longer than the regular single cells, after 8 hr of growth in 100 $\mu$M cephalexin.

Transmission electron microscopic analyses indicated that myxo-filaments were elongated cells without obvious septa (Fig. 1*d*). However, these long myxo-filaments separated into individual, normal-size cells, even in nongrowth medium, when cephalexin was removed (data not shown). This likely is due to cephalexin blocking a very late step of cell wall septation after most cell division events have taken place (29). To maintain the cells as filaments, cephalexin was added not only to the growth medium, but also to the Mops agar used in the motility analyses.

**Effect of Filamentation on A- and S-Motility.** The rationale of these experiments is that the movement of filamentous *M. xanthus* cells could provide some additional information about gliding motility that would be hard to obtain with normal-size cells. With increased cell length, cell mass is also increased. If the "gliding motors" are located along the cell body, they would be doubled as the cell length and mass doubles. In this case, the gliding speed of filamentous cells may not change much. However, if the "gliding motors" are located at the cell poles, they would remain the same as the cell length and mass increased, in which case the gliding speed of filamentous cells is expected to decrease.

Cells with different body lengths were generated by growing *M. xanthus* in CYE plus cephalexin for various times. Although treatment with cephalexin for different periods of time resulted in a relatively uniform, graded population, we measured the actual cell length of every individual cell studied to ensure accuracy. A-motility was measured by analyzing the gliding movement of isolated $A^+S^-$ cells on hard agar. S-motility was measured on 0.4% agar as described in *Materials and Methods*.

Filamentous $A^+S^-$ strains (DK10407 and DK1300) and $A^-S^+$ strains (DK1217 and DK1218) were tested for the effect of filamentation on gliding motility. As shown in Fig. 2, the myxo-filaments of $A^+S^-$ cells (DK10407 and DK1300) are as motile as the normal-size cells (Fig. 2*a*), indicating that elongation of the cell body had little effect on A-motility. In contrast, the gliding speed of myxo-filaments of $A^-S^+$ cells (DK1217 and DK1218) was reduced dramatically, suggesting that elongation of the cell body had a negative effect on S-motility (Fig. 2*b*).

Both DK10407 and DK1300 are defective in pili production. Because S-motility also involves fibril materials, we constructed additional motility mutants (Tables 1 and 2) to examine further the interesting phenomenon described above. The results are shown in Table 2. For A-motility, gliding speed was insensitive to elongation of the cell body regardless of the fibril$^-$pilus$^+$, fibril$^+$pilus$^-$, or fibril$^-$pilus$^-$ background. Thus, both fibrils and pili have little to do with A-gliding motility. It is likely, then, that the number of "gliding motors" for A-motility increases as the cell body elongates because myxo-filaments moved at a similar speed as the normal-size cells (Fig. 2*a* and Table 2). In contrast, the myxo-filaments of A$^-$fibril$^-$pilus$^+$, A$^-$fibril$^+$pilus$^-$, or A$^-$fibril$^+$pilus$^+$ were nonmotile, indicating that elongation of the cell body disrupts normal function of S-motility (Fig. 2*b* and Table 2).

**Fibrils May Mediate Cellular Coordination.** When we were performing the studies listed in Table 2, we noticed the following interesting behavior of myxo-filaments. Wild-type myxo-filaments (A$^+$fibril$^+$pilus$^+$) glided as single units even though they were much longer than normal size. In other words, the filaments were arranged in long, relatively straight cell bodies as they moved forward or backward (Fig. 3*a*). To achieve this, one would imagine that the different fragments within the filaments had the same gliding speed and reversed the gliding direction at

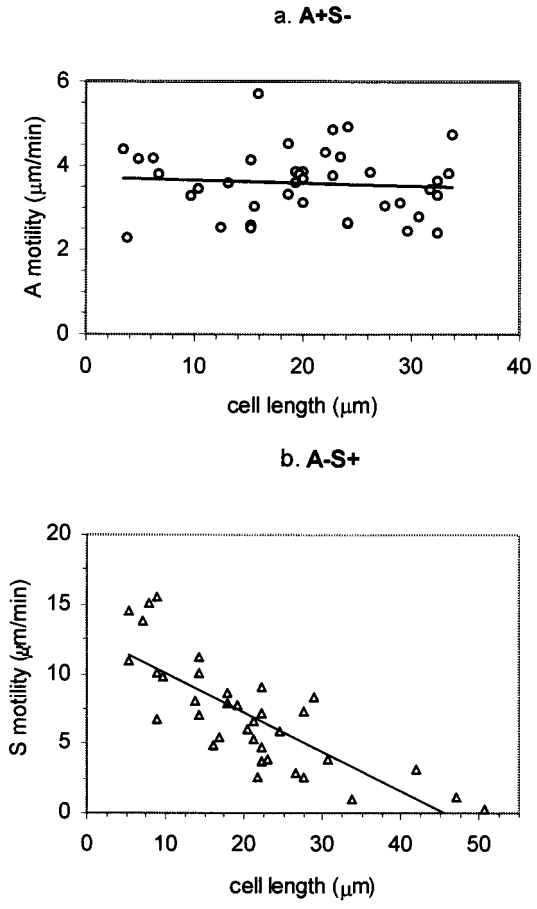


**Fig. 2.** Effect of elongation of cell body on gliding speed of A- and S-motility. DK10407 ($A^+S^-$) (*a*) and DK1217 ($A^-S^+$) (*b*) were used in this study. Cells with different cell body lengths were obtained by growing cells in CYE with 100 $\mu$M cephalexin for various times. The gliding speed was analyzed with time-lapse video microscopy as described in *Materials and Methods*. Similar results were obtained with other $A^+S^-$ and $A^-S^+$ strains, including DK1300 and DK1218 (data not shown).

the same time. We observed similar behavior for filamentous DK10407 cells ($A^+$fibril$^+$pilus$^-$) (Fig. 3*c*). However, the behavior of filamentous SW504 cells ($A^+$fibril$^-$pilus$^+$) was totally different. They had zigzag-shaped, elongated cell bodies rather than relatively straight cell bodies (Fig. 3*b*). Detailed behavior analyses revealed that these SW504 filaments ($A^+$fibril$^-$pilus$^+$) behaved as several uncoordinated fragments physically linked together. Each fragment was fully motile, but moved forward or backward with no apparent coordination among the neighboring fragments. Consequently, the net movement of the whole filament was almost zero because of a lack of coordination among the fragments. Considering that these fragments were connected, sometime tangled with each other, it was very hard to exactly measure the speed and reversal frequency of every fragment within the filaments. However, on many occasions, we were still able to follow the movement of certain fragments, especially the fragments at either end of the filaments. Based on analysis of these end fragments whose movement and reversal frequency could be measured, movement appeared to be at normal speed (about 5 $\mu$m/min) with regular reversal frequency (about once every 4–6 min). The best analogy to describe the above phenomenon is to imagine several cars chained together. In $A^+$fibril$^+$pilus$^+$ or $A^+$fibril$^+$pilus$^-$ background, the drivers within these cars somehow get a synchronous signal, which enables them to move forward and backward together as one unit. In $A^+$fibril$^-$pilus$^+$ background, each driver acts on its own will, so that, although every car is moving, the whole chain is zigzagged and going nowhere. Similar uncoordinated movement also was observed in filamentous cells of SW590 ($A^+$fibril$^-$pilus$^-$) (Table 2). It is also worthwhile to note that uncoordinated movement of fibril$^-$ filaments appeared even when they were twice longer than normal size.

**Table 2. The behavior of myxo-filaments of A- and S-motility mutants**

| Strain | Phenotype | Motility of myxo-filaments |
|---|---|---|
| DK10407 | $A^+$Fibril$^+$Pilus$^-$ | Yes |
| SW504 | $A^+$Fibril$^-$Pilus$^+$ | Yes* |
| SW590 | $A^+$Fibril$^-$Pilus$^-$ | Yes* |
| DK1217 | $A^-$Fibril$^+$Pilus$^+$ | No |
| SW538 | $A^-$Fibril$^+$Pilus$^-$ | No |
| SW506 | $A^-$Fibril$^-$Pilus$^+$ | No |
| SW596 | $A^-$Fibril$^-$Pilus$^-$ | No |

Movement of myxo-filaments was studied with video microscopy as described in *Materials and Methods* and shown in Fig. 2. "Yes" indicates motility of filaments. "No" indicates no motility of filaments.

*Uncoordinated movement of myxo-filaments as described in text and shown in Fig. 3.

The above study implies that the absence of fibril materials may have something to do with the lack of coordination within myxo-filaments. Therefore, we performed more detailed analyses to study the relationship between fibril materials and uncoordinated movement of myxo-filaments. We examined a group of known social motility mutants for coordination of filament movement by using video microscopy and for the level of fibril and pilus production in myxo-filaments by Western blot analyses using a mAb against fibrils and a polyclonal antibody against PilA. The results are presented in Table 3 and Fig. 4. In general, the correlation was very strong: wild-type or some pilus$^-$ filaments expressed normal levels of fibrils and exhibited coordinated movement, whereas the *dsp* and *dif* filaments had no detectable fibrils and exhibited uncoordinated movement. The *tgl* (defective in both fibrils and pili) (20), CDS (missing fibril protein 20 kDa) (24), and *pilB* filaments had reduced yet detectable fibril materials, and they were still largely coordinated, but to a lesser degree than the wild type. Fig. 4 indicates that the levels of fibril and pilus production in myxo-filaments are basically the same as what have found in normal-size cells (refs. 26 and 30; Z.Y. and W.S., unpublished data). The data presented in Table 3 and Fig. 4 also showed that the presence or absence of pili was not related to the observed uncoordinated movement and that many pilus$^-$ myxo-filaments (such as *pilA* and *sgl* mutants) still possessed fibrils.

Previous studies have shown that purified fibril material can partially rescue some of the defects of the *dsp* and *dif* mutants (ref. 27; Z.Y. and W.S., unpublished data). Therefore, we examined the effect of purified fibrils on the behavior of *dsp* and *dif* filaments. The extracellular fibrils from the wild-type organism were purified and added to the *dsp* and *dif* mutants. Under our experimental conditions, we did not observe any rescue effect. The movement of *dsp* and *dif* mutant filaments treated with purified fibrils was still uncoordinated. Thus, it is not the absence of fibrils that is responsible for the uncoordinated movement.

## Discussion

Cephalexin is an antibiotic that blocks cell wall septation during cell division and produces filamentous cells. It has been used by various investigators to address some very interesting biological questions. Cephalexin-treated *E. coli* cells have been used to examine the chemotactic signal relay (28) and to generate giant

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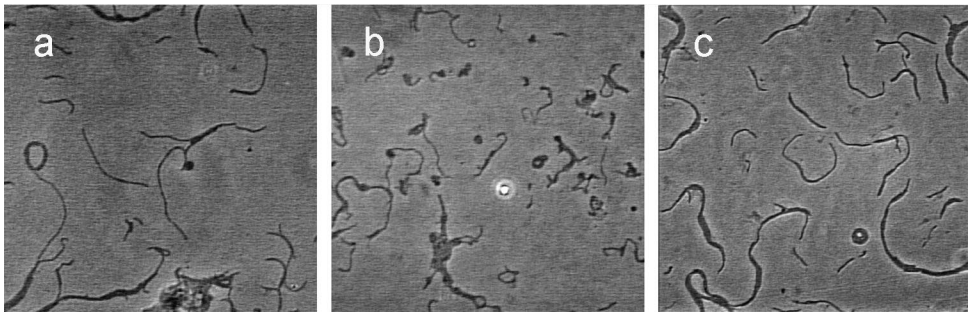


**Fig. 3.** Cellular behavior of *M. xanthus* myxo-filaments. Cells were grown overnight in CYE with 100 $\mu$M cephalexin and then spotted onto Mops hard agar containing 100 $\mu$M cephalexin for 5 hr before the pictures were taken. (*a*) Wt DK1622 myxo-filaments. (*b*) Fibril-deficient mutant SW504 myxo-filaments. (*c*) Pilus-deficient mutant DK10407 myxo-filaments. Pictures were taken through a ×32 objective lens.

bacterial cells for the study of ion channels (31, 32). In this study, we present a new application of cephalexin for studying the gliding motility of *M. xanthus*.

In analyzing the effects of cellular filamentation on *M. xanthus* A- and S-motility mutants, we found that elongation of the cell body had different effects on A- and S-motility. A-motility was insensitive to cell body elongation, whereas S-motility was dramatically reduced as the cell body got longer. This clearly indicates a difference between A- and S- motility systems in *M. xanthus*. That $A^+S^-$ myxo-filaments moved at the same speed as $A^+S^-$ single cells suggests that the number of "gliding motors" for A-motility increases as the cell body elongates. It is likely, then, that gliding motors for A-motility are distributed along the cell body. In contrast, social gliding speed decreased as the cell body became longer, suggesting that elongation of the cell body disrupts normal function of S-motility. One possible explanation is that "gliding motors" for S-motility are located at the cell poles and the number of "gliding motors" for S-motility is unchanged as the cell body elongates. Because it is known that type IV pili are located at cell poles and required for social motility (8, 10, 21), it is possible that the pili could be the actual S-motility "motors" or at least closely associated with the S-motility "motors."

One interesting outcome of this study is the observation that the fragments within wild-type myxo-filaments exhibited coordinated movement, whereas fragments within fibril-deficient myxo-filaments were uncoordinated (Fig. 3). Purified fibrils failed to rescue this uncoordinated movement of *dsp* and *dif* filaments, indicating that the presence of fibrils alone is not sufficient to reconstitute the function. Thus, it could be some other functions of fibrils (such as temporal or spatial expression of fibril materials) that are required for coordinated movement. Alternatively, *dsp* and *dif* genes could have additional physiological functions for cellular coordination unrelated to fibrils. If it is indeed the fibril that is responsible for coordinated movements, at this point we do not know exactly how fibrils materials are involved in this process. It could be a purely physical effect because fibril-coated surfaces may be much smoother for gliding motility. It is also likely that fibrils mediate some type of cellular coordination signal(s). Before this study, the only known function for fibrils of *M. xanthus* was cellular adhesion. This study clearly demonstrates that fibrils have other functions beyond simple cell–cell adhesion. Somehow, they may play a key role in coordinating the movement of fragments within myxo-filaments. This function could be expanded to fibrils acting as a coordination mediator for adjacent cells during social movement of large groups. At this point, we do not know whether fibrils are the actual signal molecules or whether they merely mediate a signal within the fibril materials. Furthermore, it remains to be seen

**Table 3. Phenotypes of S-motility mutants**

| Strain | Relevant genotype | Pili | Fibrils | Coordinated movement of myxo-filaments |
|---|---|---|---|---|
| DK1622 | wt | + | + | Yes |
| DZ4148 | *frzE* | + | + | Yes |
| SW504 | *difA* | + | − | No |
| SW501 | *difE* | + | − | No |
| LS300 | *dsp* | + | − | No |
| DK3470 | *dsp* | + | − | No |
| DK10407 | *pilA* | − | + | Yes |
| DK10409 | *pilT* | # | + | Yes |
| DK10416 | *pilB* | − | +/− | * |
| DK10405 | *tgl* | − | +/− | * |
| DK1253 | *tgl* | − | +/− | * |
| DK1300 | *sgl* | − | + | Yes |
| CDS | *ifp* | − | +/− | * |

With the exception of wt DK1622, all other strains are known social motility mutants. The presence of pili and fibril materials was examined by Western blot analyses using anti-PilA antibody and antifibril antibody, respectively. Part of these data is presented in Fig. 4. +, Presence of pili or fibrils; −, absence of pili or fibrils; +/−, reduced fibrils. #, Presence of nonfunctional pili on the cell surface. Coordinated movement of myxo-filaments was examined by time-lapse video microscopy as described in the text. "Yes" indicates coordinated movement. "No" indicates uncoordinated movement. *, Largely coordinated movement with some uncoordinated movement. The myxo-filaments of the *frzE* mutant exhibited coordinated movement, but moved without cellular reversal. $A^-S^+$ filamentous cells were not included because of their nonmotility.

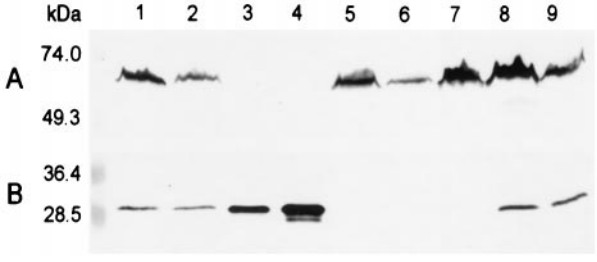


**Fig. 4.** Western blot analyses of fibrils and pili. (*A*) Western blot analysis of fibrils. The same amount of whole-cell lysate of myxo-filaments was loaded for each sample. Fibrils were detected by using mAb 2105. Shown is one major band of 66 kDa. Similar results were obtained with other minor bands that were also recognized by mAb2105, except for CDS, which did not contain one of the minor bands (24). (*B*) Western blot analysis of pili. Pili were sheared off myxo-filaments and detected with polyclonal antibody against PilA. Lanes 1–9: DK1622, DZ4148, SW504, LS300, DK10407, DK10405, DK1300, DK10409, and CDS. SW501 (same result as SW504), DK3470 (same as LS300), DK10416, and DK1253 (same as DK10405) are not shown.

how fibrils are involved in cellular coordination. Our recent studies indicated that the *dif* mutants are defective in both sensing and producing fibrils (unpublished data). Thus, we propose a model in which *M. xanthus* cells sense the fibrils produced by other cells and then produce more fibrils to relay a coordination signal in a manner similar to cAMP signaling in *Dictyostelium discoideum* (33). We hypothesize that such a fibril-mediated signal-relay system may play a coordinating role for social motility.

In summary, the data presented in this paper provide some new insight into the gliding motility of *M. xanthus.* It is clear that social motility is much more than a group of cells physically linked to each other; rather, it involves sophisticated intercellular signaling and coordination. In addition to their adhesive function, fibrils and pili seem to play important roles in social motility. The fibrils likely are required for intercellular coordination, whereas the pili could be directly involved in S-motility "motors." Further investigation into the molecular mechanisms of these two cell surface appendages and the interaction between them will provide greater understanding of both A- and S-motility of *M. xanthus*.

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