# Effect of Viscosity on Swimming by the Lateral and Polar Flagella of *Vibrio alginolyticus*†

TATSUO ATSUMI,[1] YUKIYO MAEKAWA,[2] TAKU YAMADA,[2] IKURO KAWAGISHI,[2]
YASUO IMAE,[2] AND MICHIO HOMMA[2]*

*Department of Molecular Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464-01,[2]
and Department of Physics, Suzuka College of Technology, Suzuka, Mie 510-02,[1] Japan*

By using mutants of *Vibrio alginolyticus* with only a polar flagellum (Pof$^+$ Laf$^-$) or only lateral flagella (Pof$^-$ Laf$^+$), we examined the relationship between swimming speed and the viscosity of the medium for each flagellar system. Pof$^+$ Laf$^-$ cells could not swim in the high-viscosity environment (ca. 200 cP) in which Pof$^-$ Laf$^+$ cells swam at 20 µm/s. The Pof$^-$ Laf$^+$ cells swam at about 20 µm/s at normal viscosity (1 cP) without the viscous agent, and the speed increased to 40 µm/s at about 5 cP and then decreased gradually as the viscosity was increased further. These results show the functional difference between polar and lateral flagella in viscous environments.

Many bacteria use flagella to move. The helical flagellar filaments extending from the cell body are rotated by a motor located in the proximal portion of the flagellar structure and embedded in the cell membrane (14, 19). The rotation of the helical filaments against the surrounding medium generates the driving force for cell swimming. Swimming speed is dependent on motor torque, cell shape, and the shape, length, number, and location (polar or peritrichous) of the filaments. Those parameters vary among bacterial species.

*Vibrio alginolyticus* has two types of flagella, polar (Pof) and lateral (Laf), on one cell (5). When grown in a liquid environment, it expresses mainly a single polar flagellum at the cell pole and swims rapidly using it. When transferred to the surface of a solid medium, it expresses lateral flagella in addition to polar flagella and the cells elongate and become able to move and spread on the surface (3, 23, 31, 32). By using lateral flagellum-defective mutants (Pof$^+$ Laf$^-$) and polar flagellum-defective mutants (Pof$^-$ Laf$^+$), it has been shown that lateral flagella, but not polar flagella, are essential for movement on a surface such as solid agar (16, 30). It has been proposed that polar flagella act as dynamometers to sense features of the outer environment, such as viscosity, and regulate lateral flagellar expression (15, 22, 23). It has been observed that lateral flagella work better for swimming in a high-viscosity environment than the polar flagellum (27).

Polar flagella are sheathed with a membrane structure which is contiguous to the outer membrane (1). Their diameter is 24 to 30 nm, and the diameter of the central filament is 14 to 16 nm (1, 7). Lateral flagella are not sheathed and have a diameter of 14 to 15 nm (1). The helical wavelengths of polar and lateral flagella are about 1.5 and 0.9 µm, respectively. Their constituent proteins, flagellins, have different molecular weights and antigenicities (22, 29). Moreover, the energy sources of polar and lateral flagellar motors are different, Na$^+$ and H$^+$ motive forces, respectively (2, 16). Thus, polar and lateral flagella are distinct in many regards. The rotational frequency of polar flagella has been observed at over 1,000 Hz in liquid media (21, 24), whereas the rotational frequency of peritrichous flagella of *Salmonella typhimurium* or *Escherichia coli* is much lower, up to 200 or 300 Hz (17, 18). The torque property may be different among the motors.

In this study, we examined the propulsive ability of polar and lateral flagella to clarify the functional difference between the two flagellar systems.

**Effect of viscosity on swimming speed.** The flagellum-defective mutants, YM4 (Pof$^+$ Laf$^-$) and YM19 (Pof$^-$ Laf$^+$), which had been isolated from a parental strain, 138-2 (Pof$^+$ Laf$^+$), were incubated in VC medium or VPG medium (26), and the culture at the late log phase was directly diluted into HEPES (*N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid) buffer (16) or Tris motility buffer (50 mM Tris-HCl [pH 7.5], 300 mM NaCl, 5 mM MgCl$_2$, 5 mM glucose) with 1 mM serine, which is an attractant and prevents the directional change of swimming cells (13, 27). Within 1 min after dilution, the cells were observed under a dark-field microscope at 25°C (maintained by air conditioning) and recorded on videotape. The video images were processed by an image-analyzing system (AVIO Image Σ-II and EXCEL) to generate the motility tracks of cells. The swimming speed of cells was measured from the tracks (Fig. 1). The viscosity of motility buffer containing 300 mM NaCl was varied by addition of polyvinylpyrrolidone K-90 (PVP; Wako Pure Chemical Industries) and was measured by an Ubbelohde viscometer in a water bath at 25°C. YM4 (Pof$^+$ Laf$^-$) swam at about 60 µm/s in the buffer without PVP (viscosity, 0.99 cP). Its swimming speed decreased rapidly as the buffer viscosity was increased. At 200 cP (12% PVP), YM4 hardly moved. YM19 (Pof$^-$ Laf$^+$) swam at 20 µm/s in the buffer without PVP. In contrast with YM4, the swimming speed of YM19 increased with viscosity to about 5 cP, at which YM19 swam maximally at 40 µm/s. Beyond 5 cP, the swimming speed decreased gradually, but even at 200 cP YM19 swam well at about 20 µm/s.

**Swimming speed of wild-type cells.** Viscous agents induce production of lateral flagella, while polar flagella are constitutively expressed (2, 22, 23). So, wild-type cells (138-2) cultured without PVP and with 7% PVP should be Pof$^+$ Laf$^-$ and Pof$^+$ Laf$^+$, respectively. Swimming profiles against viscosity for wild-type cells, which were grown in the absence and the presence of PVP, were similar to those of the Pof$^+$ Laf$^-$ mutant and the Pof$^-$ Laf$^+$ mutant, respectively (Fig. 2). However, Pof$^+$ Laf$^-$

* Corresponding author. Phone: 052-789-2992. Fax: 052-789-3001. Electronic mail address: g44416a@nucc.cc.nagoya-u.ac.jp.
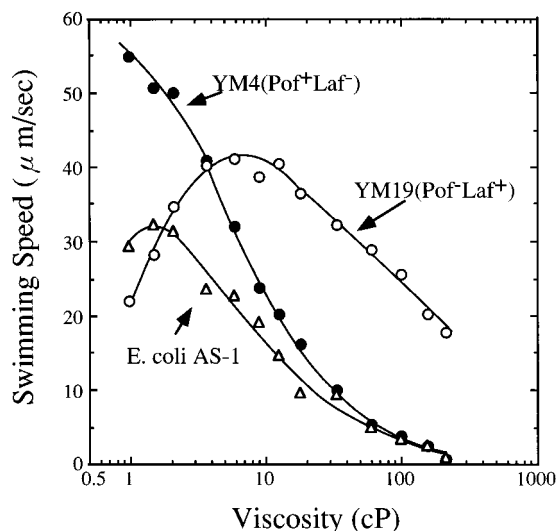
FIG. 1. Functional difference between Laf and Pof with regard to viscosity. YM4 (Pof$^+$ Laf$^-$) (●), YM19 (Pof$^-$ Laf$^+$) (○), and *E. coli* AS-1 (△) cells were grown in VC medium (YM4 and YM19) or TG medium (AS-1). The swimming speed was measured in the HEPES motility buffer (pH 7.0) containing 300 mM NaCl for YM4 and YM19 or in 10 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 0.5% glycerol for *E. coli*. The viscosities of the buffers were changed by the addition of PVP.
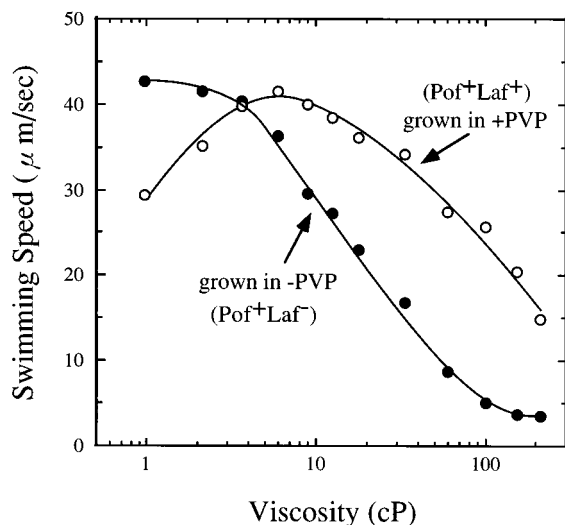


FIG. 2. Effect of viscosity on wild-type cells. Strain 138-2 cells were grown in VC medium without PVP (●) or with 7% PVP (○). The swimming speed was measured in HEPES motility buffer (pH 7.0) containing 300 mM NaCl and supplemented with various concentrations of PVP.
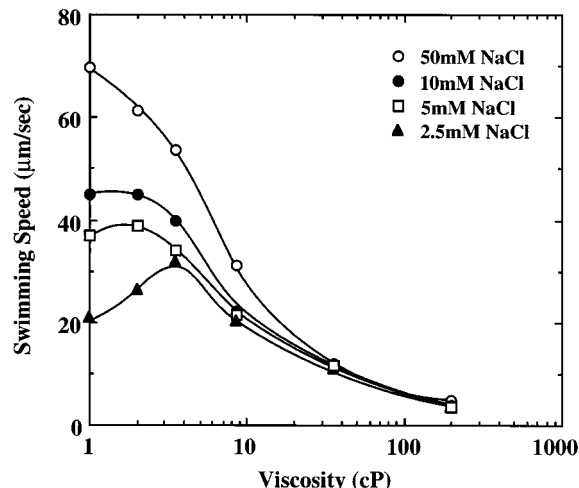


FIG. 3. Effects of viscosity at various sodium concentrations. YM4 (Pof$^+$ Laf$^-$) cells were grown in VPG medium, and their swimming speed was measured in Tris motility buffer (pH 7.5) containing 50 (○), 10 (●), 5 (□), or 2.5 (▲) mM NaCl and supplemented with various concentrations of PVP. The total salt concentration was adjusted to 300 mM by addition of KCl.

cells of the wild-type strain swam more slowly at low viscosity than the Pof$^+$ Laf$^-$ mutant cells, while at high viscosity they swam faster than the mutant cells. These results may be explained by the small amount of lateral flagella expressed even in the absence of PVP. We actually have detected lateral flagellin in extracellular space of low-viscosity medium (25). Pof$^+$ Laf$^+$ cells of the wild-type strain swam at 30 μm/s in the buffer without PVP, which is slower than Pof$^+$ Laf$^-$ mutant cells but faster than Pof$^-$ Laf$^+$ mutant cells. The overall relation of swimming speed and buffer viscosity for Pof$^+$ Laf$^+$ cells was more similar to the Pof$^-$ Laf$^+$ mutant YM19 (Fig. 1). From these results, the polar flagellar function seems to be restricted in the presence of lateral flagella. However, at a low viscosity lateral-flagellum synthesis seems to be effectively down-regulated (22, 30), so the polar flagellar function is not severely disturbed.

**Effect of viscosity on *E. coli* flagella.** In order to further examine lateral flagellar function, we measured the swimming of *E. coli*, a well-studied peritrichous bacterium, as a function of viscosity (Fig. 1). *E. coli* cells grown in TG medium (1% Bacto Tryptone, 0.5% NaCl, 0.5% glycerol) were harvested and resuspended in 10 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 0.5% glycerol. *E. coli* cells showed maximal speed at around 2 cP, their swimming speed rapidly decreased with increasing viscosity, and they could not move at 200 cP. This profile is more similar to the *V. alginolyticus* polar flagella than their lateral flagella except for the lower speed at low viscosity and the slight increase in speed with initial increase in viscosity. In spite of the same peritrichous state of *V. alginolyticus* and *E. coli*, the abilities to propel the cell at high viscosity are very different in the two species.

**Effect of viscosity on various initial swimming speeds produced by Na$^+$-driven polar flagella.** The effects of viscosity on the swimming speed of the Pof$^+$ Laf$^-$ cells were examined at various initial swimming speeds (Fig. 3). The initial swimming speed, which has a roughly linear relation with the flagellar rotation rate (20, 24), was controlled by the external Na$^+$ concentrations. At 50 mM NaCl, swimming speed was ca. 70 μm/s without PVP and decreased with increasing viscosity. On the other hand, at 2.5 mM NaCl, the initial swimming speed was ca. 20 μm/s, increased with increasing viscosity until around 5 cP, and then decreased. Though the initial swimming speeds were very different at 10, 5, and 2.5 mM NaCl, the swimming speeds at around 10 cP were almost the same. These swimming profiles were also observed when the initial swimming speed was reduced by amiloride, which is a specific inhibitor of the Na$^+$-driven flagellar motor, or by the protonophor carbonyl cyanide *m*-chlorophenylhydrazone, which disrupts the H$^+$ motive force-dependent Na$^+$ pumping system (data not shown). The swimming speeds of YM19 and *E. coli* also showed peaks at around 5 and 2 cP, respectively (Fig. 1). Thus, at lower viscosity, the hydrodynamic efficiency of flagellar rotation

seems to increase with viscosity. A peak in swimming speed as a function of various viscosities has been found for many bacteria, both peritrichous and monotrichous (4, 6, 8, 9, 28). It may be speculated that the shape change of rotating flagella occurs at various viscosities and the degree of the shape change dependent on viscosity differs with initial swimming speed.

**Swimming ability of lateral flagella in a high-viscosity environment.** There might be three causes for the swimming ability at high viscosity: peritrichous state, number of filaments, and motor torque itself. First, the comparison of the result for YM19 with *E. coli* suggests that peritrichous state is not a sufficient condition for driving the cell body at high viscosity. Second, in *E. coli* flagella or *Vibrio* lateral flagella, the filaments make bundles to swim. We could assume that the torque of the flagella in a bundle is the summation of the individual torques. It would be possible that many flagella generate a large enhancement of torque, especially in the region of low rotation frequency. Consequently, the swimming speed would be increased with increasing number of flagella especially in the region of high viscosity relative to liquid (no PVP). Other bacteria which can swarm on the surface, e.g., *Proteus mirabilis* and *Serratia marcescens*, have 6 to 10 flagella in liquid. On the surface, they elongate the cell body and increase the number of flagella to hundreds or thousands (10, 12). Recently, it has been reported that *E. coli* and *S. typhimurium* can differentiate into filamentous and hyperflagellated cells that navigate the surface of solid media (10, 11). However, in the high-viscosity medium used, *V. alginolyticus* cells were not hyperflagellated (data not shown) and the number of flagella does not seem sufficient to explain the swimming ability at high viscosity. The third possibility is that different motors have different torque characteristics. Polar motors could apparently generate torque at higher frequencies than the motors of *E. coli* and lateral flagella. Lateral motors apparently generate high torque at low rotational frequency. At this stage, we speculate that both the number of flagella and the torque property of the motor may be responsible for the distinct characters of polar and lateral flagella. To clarify the swimming ability of flagella, their torque properties need to be compared.

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