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Morphology of isolated MvspI, a putative antigenic variation protein of *Mycoplasma mobile*, the fastest gliding mycoplasma

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Mycoplasma mobile featured with robust gliding motility (2.0-4.5 micrometer/s, 3-7 cell length/s) is the pathogen of freshwater fishes. It has a cluster of genes, named *mvsp*s (mobile variable surface protein). These genes are likely to be responsible for antigenic variation essential for pathogenicity. Here, we isolated from *M. mobile* cells the largest, MvspI protein localized at other parts than the neck specialized for gliding motility on the cell surface. Gel filtration analysis showed that the isolated MvspI protein was dimeric. Rotary-shadowing electron microscopy (EM) revealed its molecular shape, featured with a globular core 30 nm long in diameter and 0-2 arms. The distribution in arm length of one-arm and two-arm type images showed three peaks. The epitope of a monoclonal antibody against MvspI was determined by partial digestion of MvspI, immunoblotting and peptide mass fingerprinting. This monoclonal antibody recognized the C-terminal end in the amino acid sequence and the tip of arm in the EM images, suggesting that the core corresponds to the N-terminal region. The amino acid sequence analysis revealed that MvspI had thirteen repeats, consisted with of 90 amino acids in its N-terminal half region, suggesting that the core is composed of the repeats. The features of structure of MvspI are similar to those of gliding proteins, Gli349 and Gli521, clarified recently. Antigenic and gliding proteins may be derived from the common ancestors.

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Isolation and characterization of P1 adhesin involved in gliding motility of *Mycoplasma pneumoniae*.

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Mycoplasma pneumoniae, a cause of human pneumonia, adheres to the respiratory epithelium and glides at the maximum speed of 1 μ /s. It forms a membrane protrusion, so-called "the attachment organelle", featured with clustering of proteins involved in the cytoadherence and gliding motility. P1 adhesin, a 168 kDa protein localizing at the attachment organelle functions also as the "leg" of gliding motility, although its amino acid sequence does not show any similarities with that of the leg protein, Gli349 of fastest gliding species, *Mycoplasma mobile*. P1 adhesin is also the key protein as the immunodominant surface structure of this species. To characterize this protein directly, we isolated it by consecutive application of 4 purification steps, i.e. (1) extractions by CHAPS, (2) extraction by octylglucoside, (3) salting out, and (4) ion exchanger column chromatography. Rotary-shadowing electron microscopy showed two types of molecular images. One is spherical shape of 21 ± 3.3 nm diameter, likely monomer, and the other is ring structure of with 47.2 ± 9.6 nm diameter, likely oligomer. Partial digestion by several proteases showed that the molecule can be divided into 3 major domains. We will discuss the whole images of P1 adhesin molecule based on the experimental and sequence information.

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Experimental analysis of the motion of mono-flagellated bacteria in aqueous solutions containing polymers

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Bacteria such as *Vibrio alginolyticus* have helical flagellar filaments, and swim in aqueous environments by rotating them. The filament is thin and long. The role of the filament is to convert the motor torque to thrust force like a screw propeller. Some peculiar phenomena have been observed when they swim in aqueous solutions containing polymer molecules. That is, swimming speed of *Pseudomonas aeruginosa* does not decrease monotonically rather shows a peak at some concentration of a linear polymer, polyvinylpyrrolidone (PVP). In addition, swimming speed of *V. alginolyticus* shows no peak in the PVP solution supplemented with 50 mM NaCl, while it shows a peak in the solution with lower concentration of NaCl. In the present study, we simultaneously measured the flagellar rotation rate (f) and swimming speed (v) of cells of *V. alginolyticus* that have single polar flagella per cell by using the laser dark-field microscopy. The ratio of swimming speed to flagellar rotation rate (v - f ratio), which means how far a cell progresses per flagellar rotation, was almost constant with viscosity in the solution containing spherical polymer, Ficoll. On the other hand, it increased with viscosity in the solution containing linear polymer, methylcellulose. In the methylcellulose solution supplemented with 300 mM NaCl, the v - f ratio gradually increased to nearly double the value. In the solution with 20 mM NaCl, it increased faster at lower viscosity and also to double the value.

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How does high pressure affect on the bacterial motility?

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Flagellated bacteria sense a variety of environmental factors and swim toward their favorable environment by controlling their flagellar rotation. The chemotaxis is caused not only by the chemical material, but also the mechanical stimuli. Hydrostatic pressure is one of the mechanical stimuli that change the bacterial behaviors, but the detailed observation has not been done yet. Here, we have studied the pressure-induced changes on the motility of *Escherichia coli* by using a high-pressure microscope. *E. coli* cells were enclosed in the high-pressure chamber equipped with a small optical window. This high-pressure chamber could be available up to 200 MPa (~2000 bar) and it was mounted on a commercial inverted microscope. We monitored the pressure-induced effect on the behavior of cells that swim freely in solution. Below 20 MPa, the *E. coli* cells kept the normal motility same as 0.1 MPa (normal ambient pressure). At 20~40 MPa, the *E. coli* cells could swim with the similar speed to 0.1 MPa, but were prevented from changing the swimming direction, as non-chemotactic mutant. At 40~80 MPa, the fraction and velocity of swimming cells decreased with the pressure. Above 80 MPa, all cells stopped to swim, and diffused in solution. After the decompression, most cells, which were applied the pressure of less than 100 MPa, recovered the normal motility. The high hydrostatic pressure is the reversible stimuli to modify the multiple processes on the bacterial motility.