Brief communication

Ion-swimming speed variation of *Vibrio cholerae* cells

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In the present work we report the variation in swimming speed of *Vibrio cholerae* with respect to the change in concentration of sodium ions in the medium. We have also studied the variation in swimming speed with respect to temperature. We find that the swimming speed initially shows a linear increase with the increase of the sodium ions in the medium and then plateaus. The range within which the swimming speed attains saturation is approximately the same at different temperatures.

[Sen A, Nandi R K and Ghosh A N 2005 Ion-swimming speed variation of *Vibrio cholerae* cells; *J. Biosci*. **30** 465–467]

The bacterial flagellar motor is a rotary molecular engine powered by the flow of ions across the cytoplasmic membrane of a bacterial cell body. The flagellar motor of *Vibrio cholerae*, a human pathogen, derives energy from the sodium ions of the medium (Aizawa 2001), transforms the ionic energy into mechanical form and powers a helical shaped filament to rotate in counterclockwise and clockwise modes that provide the propulsive force for the cell to swim through the medium. However, the relationship of the swimming speed and ion concentration of the medium has not been worked out for *V. cholerae* although the dependence of the swimming speed of *Vibrio alginolyticus*, a marine species, on the concentration of the medium has been explored previously by Liu *et al* (1990). It is therefore relevant to check whether the energizing ion and temperature dependence are the same for both the species, as this implies that these properties are fundamental to the mechanism, rather than environmentally modulatable variables.

Swimming speeds of the *V. cholerae* O1 ElTor MAK 757 cells were measured using a NIKON dark field microscope, model TE300 (Nikon, Tokyo, Japan) at a room temperature of 20ºC. A drop of freshly harvested bacterial cells in late logarithmic phase was re-suspended in freshly prepared double distilled water with a suitable amount of NaCl added to it. A cover slip was gently put on the drop. The sides of the cover slip was sealed with glutaraldehyde to stop the medium flow and to minimize any external disturbance (Magariyama *et al* 1995). After 5–10 min cell movement was observed and linear swimming speeds were determined using a graticule fitted to the microscope. Video imaging was used for short durations. Figure 1a shows the variation of the swimming speed of the cells of *V. cholerae* O1 ElTor MAK 757 with respect to different NaCl concentrations in the medium. The swimming speed shows a typical exponential rise with the increase of Na^+ ion concentration in the medium. To begin with the speed increases linearly with the increase of Na^+ ion concentration in the medium but becomes almost steady after a Na^+ concentration of 50 mM. The swimming speed of *V. alginolyticus* is found to attain saturation within a Na^+ concentration range of 50 mM and 100 mM at a medium viscosity (h) of 0·01 Poise, similar to that in our work (Sowa *et al* 2003).

V. cholerae occur in the environment in nearly 206 different serotypes. Only a few serotypes like O1 ElTor, O139 and sometimes O6 and O34 (Sarkar *et al* 2004) cause cholera in the humans and act as pathogens; the rest of the serotypes occur in the environment as non-pathogenic microbes. On the other hand *V. alginolyticus* is a marine bacterium and needs a high amount of sodium ion concentration to grow. In spite of these differences the two bacteria show similar swimming behaviour comparable sodium ions concentration in the medium. A possible reason for this is due to the presence of Na^+ flagellar motor in the polar flagella of *V. cholerae* and *V. algi-*

Keywords. Flagellar filament; flagellar motor; swimming speed; *Vibrio cholerae*

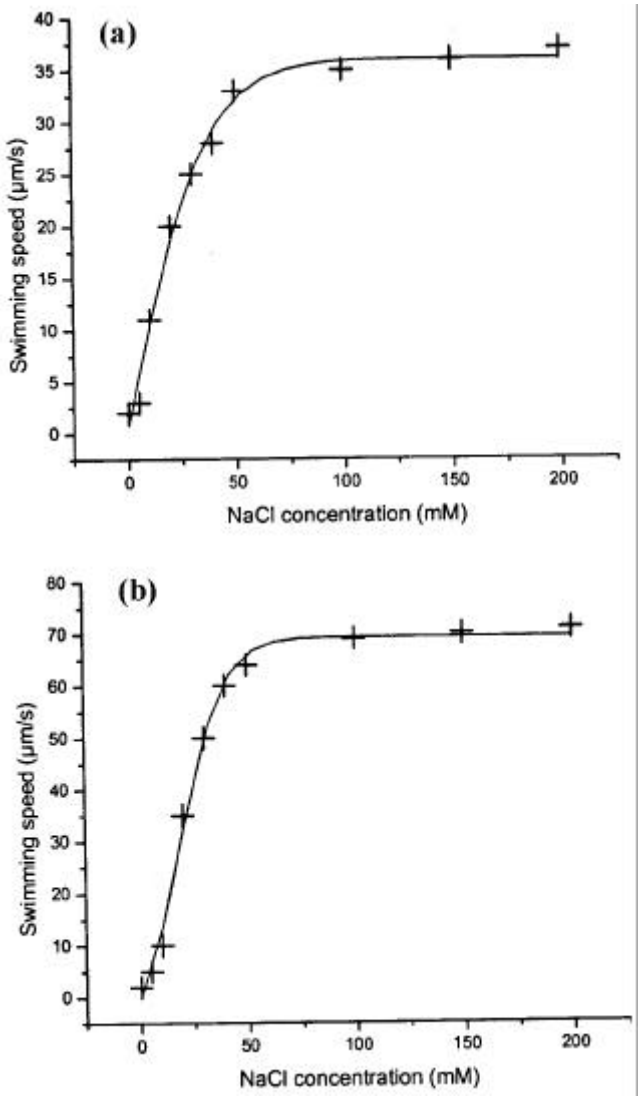

Figure 1. Variation of the swimming speed of the *V. cholerae* cells at different ion concentrations at medium temperature of 20°C (**a**) and 30°C (**b**).

nolyticus (Gosink and Häse 2000), which may possess similar characteristics in spite of living in different habitats. Liu *et al* (1990) show that the swimming speed of *V. alginolyticus* gets saturated within a concentration range of 50 mM to 100 mM of Li^+ similar to *V. cholerae* in the presence of Na^+ ion. This indicates that the motors behave similarly in the presence of different positively charged ions.

In order to investigate whether there is any effect of temperature on the swimming speed of *V. cholerae*, similar experiment was performed at 30ºC (figure 1b). It was found that the nature of ion-swimming speed variation at 30°C is the same as at 20ºC, the only difference being that the swimming speed of *V. cholerae* cells rose to

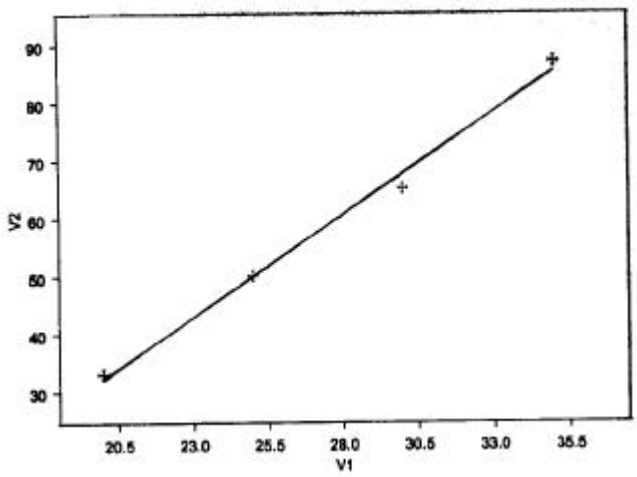

Figure 2. Variation of the swimming speed (V2) (μm/s) of the *V. cholerae* cells at different temperature (V1). The Na^+ ion concentration of the medium is maintained at 50 mM. The curve is fitted by least square method and plotted using "S Plus

65 μm/s (from 33 μm/s at a temperature of 20ºC) at a Na^+ concentration of 50 mM. Studies of the effect of temperature were repeated at 20° and 35°C and 50 mM Na^+.

Figure 2 shows the variation of the swimming speed of *V. cholerae* cells (at a NaCl concentration of 50 mM) at different temperature. It shows that the velocity of the cells varies almost linearly with the temperature (*T*). A similar relationship has been found in the case of *Salmonella enterica* serovar Typhimurium cells (Magariyama *et al* 2001). It has been observed that under favourable conditions (like ion concentration, pH and viscosity), flagellated bacteria with a Na^+ motor swim faster than those that possesses a H^+ flagellar motor (such as *E. coli* or *S. enterica* serovar Typhimurium) (Magariyama *et al* 1995). However it seems unlikely that the linear variation between the temperature and the swimming speed of the *V. cholerae* cells will be the same at very high temperatures (such as 50°C or above) at which question of survival of the bacterial cells come in. Unfortunately we do not have any experimental data showing relationship between the medium temperatures and swimming speed of *V. alginolyticus* that would have been helpful to compare with the experimental data of *V. choleare*. Such experimental data will possibly support the assumption that the flagellar motors of both *V. cholerae* and *V. alginolyticus* are similar.

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MS received 15 May 2004; accepted 31 May 2005

*e*Publication: 22 August 2005

Corresponding editor: SHAHID KHAN