Motility of *Campylobacter jejuni* in a Viscous Environment: Comparison with Conventional Rod-shaped Bacteria

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The motility of four strains of *Campylobacter jejuni* in solutions of varying viscosity was measured and compared to that of a number of conventional rod-shaped bacteria (CRSB). All the bacteria tested showed an initial increase in velocity in the low viscosity solutions – between 1 and 3 centipoise (1 P = 0.1 Pa s). However, only the campylobacters were actively motile in highly viscous solutions with velocities ranging from 60 to 100 $\mu\text{m s}^{-1}$. All strains of *C. jejuni* tested showed three separate peaks of motility as the viscosity of the solution was increased. A higher proportion of *C. jejuni* cells exhibited longer path lengths when the viscosity of the surrounding medium was increased from 1.4 to 57 cP. The findings of the study suggest that *C. jejuni* has a motility suited to movement in a viscous environment, and that this ability might provide the organism with an ecological advantage when in intestinal mucus. It is proposed that the mechanism of motility changes depending on the viscosity of the supporting environment.

INTRODUCTION

Most studies of bacterial motility have concentrated on flagellated bacteria, particularly *Pseudomonas aeruginosa* and *Escherichia coli* (Shoesmith, 1960; Schneider & Doetsch, 1974; Greenberg & Canale-Parola, 1977*b*). Flagellated bacteria all show an increase in velocity as the viscosity of their environment is increased to values slightly greater than the viscosity of water; however, the mean velocity of conventional rod-shaped bacteria (CRSB) quickly decreases when the viscosity is increased beyond 2–5 centipoise (cP) (Shoesmith, 1960; Schneider & Doetsch, 1974).

In contrast, motile spirochaetes not only demonstrate a noticeable increase in the average velocity of cells negotiating relatively viscous solutions (Kaiser & Doetsch, 1975; Greenberg & Canale-Parola, 1977*a, b*), but also an increase in the percentage of cells exhibiting translational motility (Berg & Turner, 1979). The minimum immobilizing viscosity (MIV) for either *Spirochaeta halophila* P1 or *Spirochaeta aurantia* J4T is approximately 1000 cP (Greenberg & Canale-Parola, 1977*a, b*), and for *Leptospira interrogans* (*biflexa*) B-16, the MIV is greater than 500 cP (Kaiser & Doetsch, 1975). These values compare with an MIV of 60 cP for flagellated bacteria (Greenberg & Canale-Parola, 1977*b*).

The viscosity of the environment can cause conformational changes in the flagellar helix and so reduce the efficiency of propulsion of the flagella (Schneider & Doetsch, 1974). This 'dampening effect' may explain the motility of flagellated bacteria in high viscosity solutions (Kaiser & Doetsch, 1975). Spirochaetes appear to have solved this problem by utilizing a method of locomotion that relies on endocellular organelles. These bacteria have periplasmic fibrils which are enclosed and function within the protoplasmic cylinder and outer sheath, and therefore are not affected by extracellular environmental factors (Canale-Parola, 1978). Kaiser & Doetsch (1975) concluded that the ability to move through viscous environments may confer on spirochaetes important ecological advantages.

Abbreviations: CRSB, conventional rod-shaped bacteria; MIV, minimum immobilizing viscosity.

C. jejuni is a proven cause of human and animal enteritis (Butzler & Skirrow, 1978), yet the pathophysiology of infection is still unknown. Using a mouse caecal model, Lee *et al.* (1986) proposed that the colonization of the intestinal mucosa by *C. jejuni* occurs via the association of the organism with the mucus blanket that lines the gastrointestinal tract, and that this is a major determinant of pathogenicity. *C. jejuni* cells negotiate the intact, viscous strands of gastrointestinal mucus (Lee *et al.*, 1986), often penetrating the length of the crypts in a manner similar to one of the spiral members of the normal rat microbiota (Phillips & Lee, 1983). These findings suggest that, like the spirochaetes, *C. jejuni* has adapted to movement in a viscous milieu.

The aim of this work was to examine the motility of *C. jejuni* in solutions of varying viscosities and to assess the ecological significance of this organism's ability to remain motile in viscous environments. One feature of this project has been the development of a more accurate method for analysing bacterial motility.

METHODS

Bacteria. The four strains of *C. jejuni* used in this study were clinical isolates provided by the following institutions: strain 600 from the ICPMR, Westmead Hospital, NSW, Australia; strains MQ23 and MQ26 from Macquarie Pathology Services, NSW, Australia; and strain VIC from the Department of Microbiology and Biochemistry, the University of Victoria, Victoria, Canada.

The control organisms *Vibrio cholerae* (NW13), *Salmonella enteritidis* (JOR2) and *E. coli* (100B) were supplied by the Culture Collection of the School of Microbiology, the University of New South Wales, NSW, Australia.

Culture media. Slopes of Lysed Blood Agar (LBA) medium were made from Blood Agar Base no. 2 (Oxoid) to which was added 5% (v/v) sterile lysed horse blood (Commonwealth Serum Laboratories).

Tryptone Soya Agar (TSA) slopes contained Tryptone Soya Broth (Oxoid) with 1.2% (w/v) agar.

Brucella Broth (BBL) was supplemented with ferrous sulphate, sodium metabisulphite and sodium pyruvate, each at a final concentration of 0.25% (w/v).

A 0.1% (w/v) peptone solution was made from Proteose Peptone (Difco).

Preparation of viscous solutions. Solutions of varying viscosities were achieved by dissolving an appropriate quantity of methylcellulose (BDH) in either one of the following motility buffers. RF buffer: 10^{-1} M-potassium phosphate buffer (pH 7.0); 10^{-2} M-sodium pyruvate and 2% (v/v) Tween 80 (BDH). Adler's buffer (Adler, 1973): 10^{-2} M-potassium phosphate buffer (pH 7.0); 10^{-4} M-disodium EDTA; 10^{-3} M-magnesium sulphate and 10^{-6} M-L-methionine.

Viscosity measurements were made with a flow viscometer (Kinematic) at 21 °C. These values were converted to centipoise (cP) by the equation: dynamic viscosity (cP) = time required to flow (s) $\times$ constant to flow (s).

Viability of *C. jejuni* in motility buffers. *C. jejuni* was grown microaerophilically overnight on LBA plates at 37 °C, either in an Oxoid anaerobic jar (HP11) with Campylobacter gas-generating kit (BR56) and catalyst, or in a BBL Gaspak jar with anaerobic Gaspak and no catalyst. A loopful of culture from the plates was used to inoculate fresh Brucella Broth in 150 ml conical flasks. *C. jejuni* cells were highly motile after 16 h growth at 42 °C, microaerophilically (see above for conditions). Cells from these cultures were harvested by centrifugation at 2000 g at room temperature, for 15 min. The pellet was washed twice in either RF buffer or Adler's buffer, as appropriate. The washed pellet was resuspended in a quantity of fresh buffer.

Each of the washed suspensions was diluted in triplicate in 0.1% peptone water, 1.5 and 4 h after the initial preparation procedure. These were plated onto LBA (2.5% agar) in duplicate, incubated and viable counts done.

A sample of the centrifuged *C. jejuni* culture was directly resuspended in 0.1% peptone water, and cultured in triplicate as above. This represented the number of viable cells present after the first centrifugation step.

Motility of *C. jejuni* in motility buffers. The motility of *C. jejuni* after washing in the respective motility buffers was semi-quantified using phase-contrast microscopy to examine five large squares of a Neubauer chamber. To eliminate observer bias, the identity of each sample was not known at the time of motility assessment. Motility was recorded at 0.75, 2, 3 and 5 h after the initial preparation procedure.

Bacterial motility in viscous solutions. Bacterial motility was measured by a modification of the method of Schneider & Doetsch (1974). The campylobacters were grown on LBA slopes for 5 h in a microaerophilic environment, whilst the CRSB were grown on TSA slopes for 3 h in air. All cultures were incubated at 37 °C. The methylcellulose solutions were poured over the inoculated slopes, and reincubated under the appropriate conditions for a further 17 h.

Bacteria were washed from the slopes into the methylcellulose solutions, a small drop of which was placed on a microscope slide and covered with a coverslip. Great care was taken to prevent streaming in the specimen. The preparations were examined under the microscope and then videotaped.

Video recordings of the bacteria were obtained by using a $\frac{3}{4}$ inch VCR (Sony U-matic) coupled to a TV camera (National). This was attached to a Zeiss photomicroscope, incorporating a 'phase 3' system ($\times 100$ Neofluar objective lens, $\times 10$ eyepiece and $\times 6.3$ projection lens). The videotape was played back on a monitor, via a JVC Auto Editing Control Unit. The tape speed could be manually slowed so as to allow traces of the paths of individual organisms to be made on a transparent plastic sheet. The use of the editing control unit was a major improvement on the original method, allowing greater accuracy in the measurement of path length and transit times.

(i) *Velocity measurements.* The individual velocities of bacteria were determined by the use of a calibrated planimeter (Koizumi) and stop-watch. Twenty bacteria were recorded for each viscosity reading and the 95% confidence interval calculated; any values outside this range were removed. The standard criterion of the ten greatest velocities was used to calculate the mean velocity of the bacteria at that particular viscosity (Schneider & Doetsch, 1974).

(ii) *Path length measurements.* Individual *C. jejuni* cells were randomly chosen and classified according to the distance that they travelled in a straight and continuous motion, i.e. path length. Distances were converted to micrometers using a calibrated planimeter. The categories were: < 19; 19–37; 37–56; and > 56 μm .

RESULTS

C. jejuni in two types of motility buffer

The viability of *C. jejuni* cells washed in RF buffer was compared to those washed in Adler's buffer. The campylobacters remained viable in the RF buffer for at least 4 h. Conversely, the viable count of cells washed in Adler's buffer decreased to 0.01% over the same time period. Further experiments indicated that whilst the addition of sodium pyruvate did not significantly improve the viability of *C. jejuni*, the presence of Tween 80, and/or absence of EDTA, did have an effect on viability.

The motility of *C. jejuni* in the two buffers was examined semi-quantitatively. After 45 min in the Adler's buffer no motile cells could be seen, whereas the majority of bacteria in the RF buffer were actively motile after 5 h incubation. Furthermore, whilst the addition of sodium pyruvate to Adler's buffer had no discernible effect on motility, the removal of it from the RF buffer caused a rapid decrease in the number of motile cells in that buffer. Adler's buffer has frequently been used in bacterial motility studies (Schneider & Doetsch, 1974), yet was found to be unsuitable for *C. jejuni*. Moreover, the campylobacters moved significantly slower in a solution prepared from Adler's buffer when compared to a solution of similar viscosity that had been prepared in RF buffer ($P < 0.01$). Consequently RF buffer was chosen to study the motility of *C. jejuni*. As preliminary experiments showed there to be no significant difference between the motility of the CRSB in RF buffer compared to that in Adler's buffer, the methylcellulose solutions used in all experiments involving these organisms were prepared in the latter buffer. This allowed a comparison to be made between our results and those of previous studies (Schneider & Doetsch, 1974; Greenberg & Canale-Parola, 1977a).

Effect of viscosity on bacterial motility

Figure 1 shows a plot of velocity versus viscosity for the MQ23 strain of *C. jejuni*, compared to the three types of CRSB tested. The latter were included as representatives of different types of morphology and flagellation (see Table 1).

A difference existed between the mean velocity of the campylobacters compared to that of the CRSB with none of the latter showing a peak velocity greater than the minimum velocity exhibited by the campylobacters. The mean velocity of the three CRSB ranged from 10 to 60 $\mu\text{m s}^{-1}$ as compared to 65 to 100 $\mu\text{m s}^{-1}$ for MQ23. The effect of viscosity on the mean velocity of the peritrichously flagellated bacteria, *E. coli* and *S. enteritidis*, was similar to that reported by others (Schneider & Doetsch, 1974; Greenberg & Canale-Parola, 1977b).

All the bacteria tested exhibited an initial increase in velocity when in the low viscosity solutions (1–3 cP), yet only the campylobacters were actively motile in the highly viscous solutions (> 100 cP). In this respect the type of graph represented in Fig. 1 is inadequate as it does not give an indication of the percentage of cells that remain motile. Thus, although the average velocity of the CRSB in the viscous solutions remained between 10 and 20 $\mu\text{m s}^{-1}$, in

Fig. 1

Fig. 2

Fig. 1. Effect of viscosity on the mean velocity of *C. jejuni* MQ23 (▲), *E. coli* (■), *V. cholerae* (○) and *S. enteritidis* (●).

Fig. 2. Effect of viscosity on the average velocity of three strains of *C. jejuni*: 600 (a); MQ26 (b) and VIC (c). The latter strain is presented with its duplicate (○).

Table 1. *Morphology and flagellar arrangement of the bacteria studied*

The information presented in this table was obtained from *Bergey's Manual of Systematic Bacteriology* (see the references given in this table for further details).

Organism	Average size (μm)	Morphology	Flagellar arrangement	References
<i>C. jejuni</i>	0.2–0.5 × 0.5–5.0	Slender spiral	Single, polar	Smibert (1984)
<i>V. cholerae</i>	0.5–0.8 × 1.4–2.6	Straight or curved rod	Single, polar	Baumann <i>et al.</i> (1984)
<i>E. coli</i>	1.1–1.5 × 2.0–6.0	Straight rod	Peritrichous	Orskov (1984)
<i>S. enteritidis</i>	0.7–1.5 × 2.0–5.0	Straight rod	Peritrichous	Le Minor (1984)

reality, the majority of the cells had been immobilized. Conversely, a very high percentage of the campylobacters were actively motile at similar viscosities.

Effect of viscosity on the motility of C. jejuni

Different strains of *C. jejuni* showed similar patterns of motility in the viscous solutions. This can be seen in Fig. 2, which contains a composite plot of viscosity versus velocity for three *C. jejuni* strains: MQ26, 600 and VIC (the latter in duplicate). The velocities of each of the strains fluctuated from 60 to 100 μm s⁻¹ with an initial peak velocity occurring between 1 and 3 cP. All

Fig. 3. Path lengths of *C. jejuni* MQ23 in two solutions of different viscosity: 1.4 cP (▨) or 57 cP (■). The proportion of cells travelling within four ranges of path lengths are presented for each viscosity, i.e. short path lengths < 19 μm, medium path lengths 19–37 μm and 37–56 μm, and long path lengths > 56 μm.

strains of *C. jejuni* tested demonstrated a multi-peaked velocity versus viscosity curve, with a high proportion of cells remaining motile at 118 cP.

Changes in path length with viscosity

The parameter of path length, i.e. the straight-line distance travelled by *C. jejuni* cells in one continuous motion, was examined at a viscosity of 1.4 cP and 57 cP. Figure 3 illustrates the abrupt increase in the proportion of cells with long path lengths in a high viscosity medium. The same results were obtained when another strain (*C. jejuni* VIC) was used. In both cases, 80 to 90% of the cells had path lengths greater than 56 μm at a viscosity of 57 cP; this compared to between 50 and 60% at 1.4 cP.

DISCUSSION

Using an improved method for analysing bacterial motility, *C. jejuni* was shown to retain its motility in viscous solutions capable of immobilizing the majority of flagellated bacteria. Moreover, the proportion of bacteria showing translational movement in the high viscosities was much greater in the case of the campylobacters.

Many helical bacteria are able to negotiate environments of relatively high viscosity, conferring on them important ecological advantages (Greenberg & Canale-Parola, 1977*a*; Berg & Turner, 1979). More recently, Hazell *et al.* (1986) provided evidence that the spiral morphology and peculiar form of motility of *Campylobacter pyloridis* were important in the organism's ability to colonize the mucus lining the gastric epithelium, and thus to initiate gastritis.

When introduced into a viscous environment, CRSB all behave in a similar manner. After an initial peak in velocity between 1 and 3 cP, their mean velocity decreases as the viscosity of the medium goes beyond 2–5 cP (Shoesmith, 1960; Schneider & Doetsch, 1974). Our results for *E. coli*, *V. cholerae* and *S. enteritidis* correlate well with the findings of previous studies (Shoesmith, 1960; Greenberg & Canale-Parola, 1977*b*; Hazell, 1986). In contrast, *C. jejuni* appears to have overcome the problem of 'flagellar dampening' caused by viscous media – not by developing

endocellular organelles, as the spirochaetes have done, but rather by developing a unique form of motility.

At low viscosities (i.e. 1–10 cP), *C. jejuni* appears to behave much like any other flagellated bacterium, relying primarily on its flagellum for propulsion. When the viscosity becomes so great that the flagellum can no longer overcome the viscous drag on the cell, it is possible that spiral morphology in concert with changes in flagellar conformation and/or rotation become important.

Bacterial flagella are able to assume various helical configurations depending upon the conditions, and this property has implications for the motility of the organism (Shimada *et al.*, 1975). The torque generated by the flagellum of *C. jejuni* may be regulated by alterations in the configuration of the flagellum brought on by increases in the viscosity of the surrounding medium. The multi-peaked motility curves for *C. jejuni* (see Figs 1 and 2) support the notion of a 'torque-dependent' motility for this organism. It is unlikely that the peaks are artefacts, given the number of times the experimental procedure was repeated. Moreover, the pattern was consistent for all four strains of *C. jejuni* tested. Further investigations are required to elucidate how and why the phenomenon occurs.

C. jejuni, with its relatively large surface area to volume ratio and narrow diameter (Table 1), seems suited to movement in a viscous medium (Berg & Turner, 1979). We noted that at high viscosities the spiral morphology of the organism was accentuated and the motility became less random. Studies of the change in path lengths of individual cells with a change in the viscosity of the medium, showed that a greater proportion of the cells travelled further when the viscosity was high (Fig. 3). This pattern of motility is analogous to that of the 'surface mucus-associated' campylobacters, described by Lee *et al.* (1986). Whether the change in path length reflects alterations in the helical configuration of *C. jejuni* cells is purely speculative. Petrino & Doetsch (1978) have argued that mechanical deformations accompanied with increases in viscosity alter the helical configuration of leptospira and so allow the cells to move up a gradient of increasing viscosity ('viscotaxis') because of an increased hydrodynamic efficiency.

The ability of *C. jejuni* to negotiate viscous media is likely to have ecological significance for the organism's colonization of the mucus during pathogenesis. Studies by Lee *et al.* (1986) demonstrated that campylobacters are highly motile in intact mucus strands of mucosal scrapings taken from the caeca of infected mice. This led them to conclude that *C. jejuni* associates with the intestinal tissue by mucus colonization rather than by adhesion. Furthermore, Morooka *et al.* (1985) found that strains of *C. jejuni* showing defective flagellation or motility were less effective than the wild-type in colonizing the intestinal tract of suckling mice. Motility could therefore be an important factor in the colonization of the intestinal tract by *C. jejuni*.

C. jejuni does not exhibit the classic swim/tumble motility characteristic of other micro-organisms (Adler, 1976). According to the theory of chemotaxis, bacterial cells increase the frequency of swims to tumbles when in the presence of an attractant gradient. Allweiss *et al.* (1977) postulated that chemotaxis might play a role in the association and penetration of the mucus gel of the gastrointestinal tract by intestinal pathogens. The increased path length exhibited by *C. jejuni* in viscous environments reported here, together with observations of tracking in mucus preparations (Lee *et al.*, 1986), make it unlikely that the currently accepted theories of chemotaxis are applicable to this organism. If, as is likely, *C. jejuni* penetrates the mucus gel under the attractive influence of oxygen or other chemical attractants, a completely new mechanism for chemotaxis will need to be postulated.

Several authors have discussed the biochemical factors that influence colonization of the many habitats provided by the gastrointestinal tract, i.e. competition for available nutrients (Allweiss *et al.*, 1977; Savage, 1978) and production of toxic metabolites (Lee & Gemmell, 1972). Here, a physical property is shown to be important for a human pathogen with an ability to colonize the intestinal mucus.

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