BACTERIAL PATHOGENICITY

Spirochaete-like swimming mode of Campylobacter jejuni in a viscous environment

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The swimming patterns of Campylobacter jejuni in environments of low and high viscosity were examined by a video tracking method. In media of low viscosity, C. jejuni swam with an average velocity of 39.3 μm/s with frequent changes in direction. The velocity of C. jejuni increased in a medium at a little higher viscosity than that of a low viscosity buffer. In addition to this, C. jejuni showed a second increase of velocity in media of a high viscosity of about 40 centipoise. The swimming patterns at these two velocity peaks were compared. In the second peak the wild-type C. jejuni exhibited repeated back and forth swimming patterns which were more like the swimming pattern of spirochaetes than that of monotrichous bacteria. Thus C. jejuni may presumably use a different swimming mode in media of high viscosity than the original swimming mode mediated by the propelling force of the flagella. The spiral shape of this bacterium like that of spirochaetes may strongly influence its swimming ability in media of high viscosity such as the mucous layer of the intestinal tract.

Introduction

Campylobacter jejuni, a gram-negative spiral-shaped bacterium, is now recognised as the leading cause of diarrhoeal disease in man and is often found in the intestinal tracts of mammals or birds that are believed to be the epidemiological reservoirs [1, 2]. Yet the pathophysiological mechanisms of this infection are still not clearly understood [3].

It has been well documented that, for the colonisation of the mammalian intestinal tract by this bacterium, the motility mediated by rotation of their flagella is of primary importance [4–7]. Morooka et al. reported that the non-motile mutant strains they isolated did not colonise the intestinal tract of suckling mice when inoculated orally [4, 7]. Ueki et al. showed that anti-flagellar antibodies, especially of the IgM and IgA types, inhibited colonisation [6]. Furthermore, Takata et al. isolated non-chemotactic mutants of C. jejuni, demonstrated that they did not colonise the mouse intestinal tract and excluded the possibility that the flagellar filament is an adhesive organ [5, 8]. These and other reports together indicate that the chemotactic motility by flagella is the major colonisation factor of this species.

The flagellum of C. jejuni consists of three morphological parts, the helical filament, the hook, which is an intermediate structure connecting the filament and the basal structure, and the basal body which is attached to one end of the spiral-shaped bacterial cell [9]. The specialised structure for driving the flagellar filament is located in the basal body. Intracellularly, a complicated structure that may play a role in the transfer of information or energy for the movement of the flagellar organelle is found at one end of the cell [9].

Various analyses of the bacterial motility of Escherichia coli or Salmonella enterica serotype Typhimurium have revealed that the bacterial cell often changes its swimming direction randomly after a brief interval of vigorous erratic movement which is called ‘tumbling’ motion [10–12]. The combination of these two patterns of movement, swimming and tumbling may lead the bacteria toward suitable substances called chemo-attractants. The non-chemotactic mutant strain of C. jejuni is reported to have lost its tumbling motion during swimming [8].

For colonisation of the intestinal epithelium, the bacteria have to move and adhere in the viscous environment of the thick mucus layer lining the epithelial cell surface of the intestinal tract of humans or mammals [13–17]. Thus, examining the behaviour of the bacteria in such a viscous environment is important for understanding the mechanisms of
colonisation and infection by motile bacteria. To simulate the bacterial motility in the mucous layer, artificial viscous solutions have been prepared by dissolving viscous agents in buffer, and motility of the bacteria in these viscous environments has then been examined. Analysis of the bacterial motility in viscous environments has indicated that the bacteria can move well in conditions of high viscosity. Furthermore, it has been recognised as a general behavioural phenomenon of flagellate bacteria that an increase in the swimming velocity is observed as the viscosity increases [18--21].

The aim of this study was to examine the motility of C. jejuni in solutions of various viscosities to assess the ecological significance of this organism's ability to remain motile in viscous environments.

Materials and methods

Bacterial strains and culture conditions

The strain of C. jejuni used in these experiments was strain FUM158432; its origin and characteristics have been described previously [4, 8]. C. jejuni was cultured at 42°C on Brucella Agar (Difco) supplemented with defibrinated sheep blood (Nippon Bio-Supply Center, Tokyo, Japan) 10% or in Brucella Broth (Difco) under micro-aerophilic conditions in a GasPak jar without catalysts (BBL Microbiology Systems, Cockeysville, MD, USA). Vibrio cholerae O139 strain VO18 [22], Pseudomonas aeruginosa strain K [23] and S. Typhimurium strain LT-2 (laboratory collection) were also used in this experiment. These strains were maintained and cultured on Luria nutrient agar or in broth at 37°C. All these strains originated from our own stock cultures.

Examination and imaging of the swimming patterns

The bacteria were cultured in liquid media with constant shaking to their log phase (for 17 h under micro-aerobic conditions at 42°C for C. jejuni and for 2 h with aeration at 37°C for the other bacteria). The culture was diluted with 0.1 M phosphate-buffered saline (PBS: NaCl 8 g, Na2HPO4 29 g, KCl 2 g, KH2PO4 2 g/L; pH 7.0) up to a concentration of 10⁶ cfu/ml. The method for specimen preparation for motility examination has been described previously [24]. Briefly, one drop of the bacterial suspension was placed on a clean glass slide and then immediately covered with a thin cover glass. Then the edges were sealed with nail polish to prevent the evaporation of the medium. The movement of the bacteria was examined with a light microscope (Diaplan Leitz, Ernst Leiz, Wetzlar, Germany) under dark field illumination with a 10× ocular and a 20× objective lens. The observations were usually completed within 5 min and it was possible to maintain the temperature of the sample on the microscope stage at 30°C.

The image of the bacterial movement was tracked by an image analysis system DVS-1000 (Hamamatsu Photonics, Sizuoka, Japan) on video tape through a high resolution video camera (monochrome type, Hamamatsu Photonics) as described previously [24].

Preparation of viscous solutions

Viscous solutions were prepared by dissolving an appropriate quantity of the following ingredients either in PBS or in modified RF buffer [18] comprising 0.1 M potassium phosphate buffer, pH 7.0, and Tween 80 (Wako Pure Chemical Industries, Osaka, Japan) 2% v/v: methylcellulose (MC; Sigma, mol. wt 4000), polyvinylpyrrolidone (PVP; Sigma, mol. wt 360 000) and carb-oxymethylcellulose (CMC; Katayama Kagaku Kogyo, Osaka, Japan). As these three ingredients all showed similar effects in respect of both the swimming pattern and the speed of C. jejuni, MC was mainly employed in this study.

The kinematic viscosity of the solutions was measured with a Cannon-Fenske routine type viscometer (Sibata Chemical App. Mfg Co. Ltd, Japan) and an Ubbelohde type viscometer (Kusano Scientific Instrument Mfg Co. Ltd, Tokyo, Japan). The density of the solutions was measured with a Gay-Lussac type flask. The kinematic viscosity of the sample (in centistokes, cSt) was obtained by multiplying the efflux time (in seconds) by the viscometer constant at 30°C. To determine the viscosity (in centipoise, cP), the kinetic viscosity was multiplied by the density of the solutions (in g/ml).

Scanning electron microscopy

The shape of the bacteria was observed by scanning electron microscopy (SEM) as described previously [25]. Briefly, the bacteria cultured on a thin agar layer on a glass fibre filter (Whatman GF/F) were fixed and dehydrated in a small petri dish, dried by the critical point drying method, cemented to an aluminium stub with silver paste or paper bond and then coated with a thin layer of gold-palladium (Au 60%, Pd 40%). The observations were done with an Hitachi HFS-2 field-emission-source SEM operated at 25 kV.

Results

Swimming pattern and velocity of C. jejuni in a medium of low viscosity

In this experiment 0.1 M PBS (pH 7.0) was employed as a medium of low viscosity. The swimming pattern of C. jejuni in this medium was recorded and analysed by the video tracking method. The average velocity of the wild-type strain was 39.3 µm/s and was slower than that of V. cholerae, but faster than that of P. aeruginosa [24] and S. Typhimurium (22 µm/s).

The typical swimming pattern of strain FUM158432 is
shown in Fig. 1. Zigzag movements which represent frequent changes of swimming direction were evident (Fig. 1). The change in direction in the swimming pattern was supposed to be a phenomenon resembling the tumbling motion reported among peritrichously flagellate bacteria [10–12].

Movement in media of high viscosity

As an in-vitro model system of the intestinal mucous layer, the high viscosity media were prepared by dissolving methylcellulose in RF buffer [18, 19, 26]. The experiments were repeated in triplicate and the swimming patterns of >30 cells were examined in each experiment.

The average swimming velocities of several flagellate bacteria in the viscous media were plotted against the change in viscosity (Fig. 2). The graphs revealed that as the concentration of methylcellulose increased, the swimming velocity of the bacteria gradually decreased. However, the highest swimming velocity was recorded at a slightly higher viscosity than 1.0 cP (1.5–3 cP) in all the bacterial species examined in this experiment. The graphs of V. cholerae, P. aeruginosa and S. Typhimurium had only one peak of highest velocity in the viscous media and this type of graph was designated as the 'single peak pattern'. On the other hand, in contrast to the other bacteria, C. jejuni showed a second increase in velocity at a higher viscosity of c. 40 cP and thus formed a second peak. Thus it was confirmed that C. jejuni had a 'double peak pattern' of swimming velocity as has been reported in campylobacters by other researchers with a different measuring system [18, 21].

Fig. 1. Video tracking image of C. jejuni wild type in PBS. The swimming path was recorded for 15 s. Bar = 100 μm.

Fig. 2. The relationship between swimming velocity and the viscosity of the medium. (a) C. jejuni FUM158432; (b) ▲, V. cholerae, ●, P. aeruginosa and ○, S. Typhimurium.
In these two peaks, as demonstrated in Fig. 3A and B, the C. jejuni wild-type strain showed different swimming patterns. In the solution of low viscosity, at the first peak, the bacteria lost the typical swimming pattern of the wild type, and swam in a rather straight manner in one direction as was seen in the tracks of non-chemotactic mutants [8]. This swimming pattern was characteristic of the strain up to a viscosity of \( \leq 40 \) cP. In the second peak at 40 cP, the bacteria showed a different pattern from that in the medium of lower viscosity. In this high viscosity medium, C. jejuni frequently reversed its swimming direction in a back and forth pattern with a relatively short straight moving path (Fig. 3). In a viscous solution >40 cP, the bacteria almost ceased their movement. These results revealed that this bacterium uses a different moving mode in solutions of high viscosity from that generally used in media of low viscosity.

**Discussion**

The swimming patterns and the velocity of C. jejuni in media of low and high viscosity were examined by means of a video tracking method. This new method can demonstrate the swimming patterns of a single cell of C. jejuni in clear photocopies.

The analysis of the pattern recorded in a low viscosity salt solution revealed that C. jejuni exhibited frequent directional changes in its swimming pattern. In contrast to peritrichous bacteria, polar flagellate monotrichous bacteria are supposed only to change the rotational direction of their flagella and then move in another direction. They do not need time to rewind the bundles of the flagella in a new direction. In the track of the wild-type strain the pattern of the backup movement and the pause in swimming was rarely seen and this might also make it difficult to observe the

![Fig. 3. Video tracking images of C. jejuni wild type in highly viscous media. (A) Image of the wild type in the first peak; (B) wild type in the second peak; all were recorded for 3 s. Bars = 100 \( \mu \)m.](image-url)
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Fig. 4. Scanning electron micrograph showing the characteristic spiral shaped C. jejuni with the flagella at both ends of its body. Bar = 500 nm.

tumbling movement with a method other than the video tracking method.

The initial increase in the velocity of the bacteria with the increase in viscosity was confirmed in this study [18–20]. This is supposed to be a general behavioural phenomenon of motile bacteria in viscous solutions. However, Lowe et al. have reported that in a viscous solution prepared with an agent such as ficoll, a high molecular material without long unbranched chains, Escherichia coli or Streptococcus spp. showed movement without any increase in the swimming speed at high viscosity range [27]. This observation suggested that the increase of swimming velocity in highly viscous solutions is greatly affected by the shape of the molecule in the solution. This study confirmed that C. jejuni showed a small increase in velocity at c. 3.5 cP in ficoll solution (unpublished observations). In addition to this, the velocity of C. jejuni increased again in a high viscosity zone whereas that of the other bacteria decreased. Ferrero and Lee also demonstrated an increase in the swimming velocity of C. jejuni in a medium of high viscosity with a video recording method [18]. The increase in the velocity again at 40 cP is a characteristic phenomenon found in C. jejuni. It has also been reported that in a medium of high viscosity, the bacteria with spiral shapes, like spirochaetes, are able to overcome the problem of the dampening action of viscous media on their flagella because of their characteristic shapes [18, 19, 21, 28, 29]. C. jejuni has a spiral shaped body and two flagella, one at each end (Fig. 4). At a low viscosity, C. jejuni behaved just like any other flagellate bacterium, while at a high viscosity it swam by a different mechanism. Therefore, the assumption was made that the shift in the swimming pattern from a flagella-dominated-type to the spiral shape-dominated-type might occur at a viscosity of c. 40 cP. The video tracking images recorded in these two zones, in the first peak and the second peak, showed different patterns. The pattern at the first peak was straight swimming with low tumbling, but at the second peak the organism showed frequent back and forward movements. This different pattern may represent the use of different swimming modes by C. jejuni.

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References

5. Newell DG, McBride H, Dolby JM. Investigations on the role of flagella in the colonization of infant mice with Campylo-


