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Relationship Between Cell Coiling and Motility of Spirochetes in Viscous Environments

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The lowest viscosity that stops translational motility of cells (minimum immobilizing viscosity [MIV]) was determined for various spirochetes. The viscous agent used was polyvinylpyrrolidone. The MIV for either *Spirochaeta halophila* P1 or *Spirochaeta aurantia* J4T was approximately 1,000 centipoise (cp), and for *Leptospira interrogans* (*biflexa*) B16 the MIV was greater than 500 cp. In comparison, the MIV for the flagellated bacteria *Escherichia coli* and *Spirillum serpens* was 60 cp. MIV values for two *S. halophila* mutant strains lacking the characteristic cell coiling (Hel$^-$ mutants) were 70 and 120 cp, approximately one-tenth the MIV for the wild-type strain. MIV values for cells of *S. aurantia* strains with fewer coils than comparably long cells of *S. aurantia* J4T were 300 to 600 cp. The average velocity of strains of *S. aurantia* and *S. halophila* decreased at viscosities higher than 2 to 3 cp. At 2 cp the average velocity of *S. halophila* P1 was 16 μm/s, whereas the average velocities of Hel$^-$ mutant strains were 7 to 9 μm/s. This study indicates that the coiling of spirochetes plays a role in their ability to move through environments of relatively high viscosity. Among the spirochetes we investigated, this ability is greater in the more extensively coiled strains.

Spirochetes are chemoheterotrophic bacteria characterized by a distinctive morphology. Typically, the spirochetal cell is helical in shape, its main structural component being the "protoplasmic cylinder," which comprises the nuclear and cytoplasmic regions, as well as the cell membrane-wall complex. Filamentous structures, called axial fibrils, are wound around the protoplasmic cylinder and are inserted near the cell poles. The axial fibrils are similar to bacterial flagella in fine structure and chemical composition, but are entirely endocellular inasmuch as they are enclosed, together with the protoplasmic cylinder, by a triple-layered membrane called "outer sheath" or "outer cell envelope" (3). Probably as a result of their cellular architecture, spirochetes possess a type of motility unique among bacteria. The spirochetal cell, which has no exoflagella, has translational motility in liquids, without being in contact with a solid surface. Furthermore, at least certain spirochetes are able to "creep" or "crawl" on solid surfaces (2, 5).

It is possible that their unique fine-structural organization and type of motility have conferred to spirochetes evolutionary and ecological advantages which have enabled these organisms to compete successfully with other bacteria in nature. Studies by Kaiser and Doetsch (12) and Schneider and Doetsch (14) support this possibility. These authors reported that the velocity of a strain of *Leptospira interrogans*, an obligately aerobic spirochete, was markedly enhanced in relatively viscous environments (e.g., 300 centipoise [cp] or higher), whereas the velocity of flagellated bacteria rapidly decreased at viscosities greater than 5 cp. Subsequently, we observed that a viscosity of 60 cp immobilized flagellated bacteria such as *Escherichia coli* and *Spirillum serpens* (E. P. Greenberg and E. Canale-Parola, Abstr. Annu. Meet. Am. Soc. Microbiol., 1977, I42, p. 161).

One aim of the study reported here was to determine whether the ability to move through environments of relatively high viscosity is present in spirochetes other than leptospires. Furthermore, we isolated mutant spirochetes that were motile but had little or no cell coiling as compared with their parental strain. These mutants were used to investigate the role of cell coiling in the translational movement of spirochetes through viscous environments.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Strains of *Spirochaeta aurantia* (Table 1) were grown as described elsewhere (7). Strains of *Spirochaeta halophila* (Table 1) were grown for 48 h at 37°C in 5-ml volumes of ISM broth (6) contained in 16- by 150-mm test tubes. The inoculum was 0.1 ml of a similar 48-h

TABLE 1. *Designation, phenotype, and source of strains used in this investigation*

Strain	Relevant phenotype	Source and reference
S. halophila RS1	Hel+ Mot+	This lab (6)
S. halophila P1	Hel+ Mot+	Nonpigmented mutant from RS1[a] (6)
S. halophila M2	Hel+ Mot-	From RS1; this study[a]
S. halophila DC1	Hel- Mot-	From P1; this study[a]
S. halophila R51	Hel- Mot+	From DC1; this study[a]
S. halophila R532	Hel- Mot+	From DC1; this study[a]
S. halophila R752	Hel- Mot+	From DC1; this study[a]
S. aurantia J4T	Tightly coiled	This lab (4)
S. aurantia J4L	Loosely coiled	This lab (4)
S. aurantia M1	Loosely coiled	This lab (4)
L. interrogans (*biflexa*) B16		C. D. Cox and R. Corin[b]

[a] Occurred spontaneously.
[b] University of Massachusetts, Amherst.

culture inoculated with cells from an ISM agar plate culture (6). *L. interrogans* (*biflexa*) B16 was grown without shaking as described previously (8) in SM-74 medium (C. D. Cox, personal communication). This medium consisted of SM-4 salts (8) and SM-6 supplements (17), which contained 10 times the reported amount of vitamin B12.

The migration medium for *S. halophila* was ISM agar (6) modified as follows: (i) the concentration of yeast extract was 0.2% (wt/vol); (ii) 0.02% (wt/vol) D-glucose replaced maltose; and (iii) the agar concentration was 0.5% unless otherwise specified.

ISM Sloppy Agar medium consisted of ISM broth to which 0.1% (wt/vol) agar was added.

Isolation of nonmotile mutants of *S. halophila*. To enrich for nonmotile mutants of *S. halophila*, cultures were repeatedly transferred in test tubes (16 by 150 mm) containing 10 ml of ISM Sloppy Agar. The inoculum for each tube was 0.1 ml, drawn from near the air-medium interface of an ISM Sloppy Agar culture. Inocula were gently layered on the surface of the ISM Sloppy Agar. The cultures were incubated at 37°C. The spirochetes grew not only at the air-medium interface but also below the surface of the medium, where they formed growth disks or rings similar to those reported for *S. aurantia* (4). Thus, cells that did not migrate from the area of inoculation (including nonmotile mutant cells) were enriched for by repeated transfer. To isolate the nonmotile mutants, cells from the surface of these enrichment cultures were streaked on ISM agar plates. Spontaneous nonmotile mutants, which formed small glistening surface colonies (Fig. 1B) rather than the diffuse colonies of the wild type (Fig. 1A), were occasionally observed and were cloned by streaking them on ISM agar plates. None of the mutants isolated by this procedure exhibited any form of motility.

Preparation of cell suspensions. Cells were harvested by centrifugation (3,000 × *g* for 5 min) at room temperature (22 to 24°C) and were washed once by centrifugation. Unless otherwise specified, strains of *S. aurantia* were washed in chemotaxis buffer (7), strains of *S. halophila* in inorganic salts solution (6), and *L. interrogans* B16 in SM-74 medium. Each final cell suspension was prepared by using the appropriate wash solution to which either polyvinylpyrrolidone (PVP) (Nutritional Biochemicals Co., K-90; molecular weight, 360,000) or methyl cellulose (Fisher Scientific Co. M-280, 400 cp) was added in amounts yielding the desired viscosity. Final cell densities were in the range of 1×10^8 to 2×10^8 cells per ml. Viscosities were measured using modified Ostwald viscometers (14).

Motility measurements. Unless otherwise specified, cell suspensions were equilibrated at room temperature for at least 5 min, placed in microscope observation chambers (7), and observed immediately. Velocities were measured by determining the time required by individual cells to travel a predetermined distance (generally 45 μm). This was accomplished using a Zeiss GFL phase-contrast microscope equipped with an eyepiece micrometer. Time was measured with a stopwatch calibrated in 0.1 s. Average velocities were calculated by the methods of Kaiser and Doetsch (12). As a control experiment, the average velocities at various viscosities for *Pseudomonas aeruginosa* (from the University of Maryland culture collection, kindly sent to us by R. N. Doetsch) were determined by the procedures described above. The velocities we determined were essentially identical to those previously reported for this organism (14).

The minimum immobilizing viscosity (MIV) is defined as the lowest viscosity that stops translational movement of cells. The MIV for any given strain was extrapolated by plotting the viscosity in cp on a logarithmic scale versus the average velocity (Fig. 2). Maximum velocity is defined as the highest average velocity.

Migration of *S. halophila* in agar gels. Four spot inoculations were made on each migration medium plate. Each inoculum consisted of 0.005 ml of a 48-h ISM broth culture. The spots were measured (zero-time diameter), and then the plates were kept at room temperature for 30 min to allow the inocula to become absorbed into the agar gel. The plates were placed in a humid incubator at 37°C for 96 h. Finally, the diameters of the growth rings were measured. The total increase in diameter was the difference between the diameter at 96 h and the diameter at zero time.

Microscopy. Equipment and methods for phase-contrast microscopy, electron microscopy, and photography were described previously (6).

RESULTS

Mutant strains of *S. halophila*. Two morphologically distinct types of nonmotile (Mot-) mutants of *S. halophila* were isolated. One type is represented by *S. halophila* strain M2 (Table 1). This strain had the characteristic cell coiling of *S. halophila* (Fig. 4A) and was morphologi-

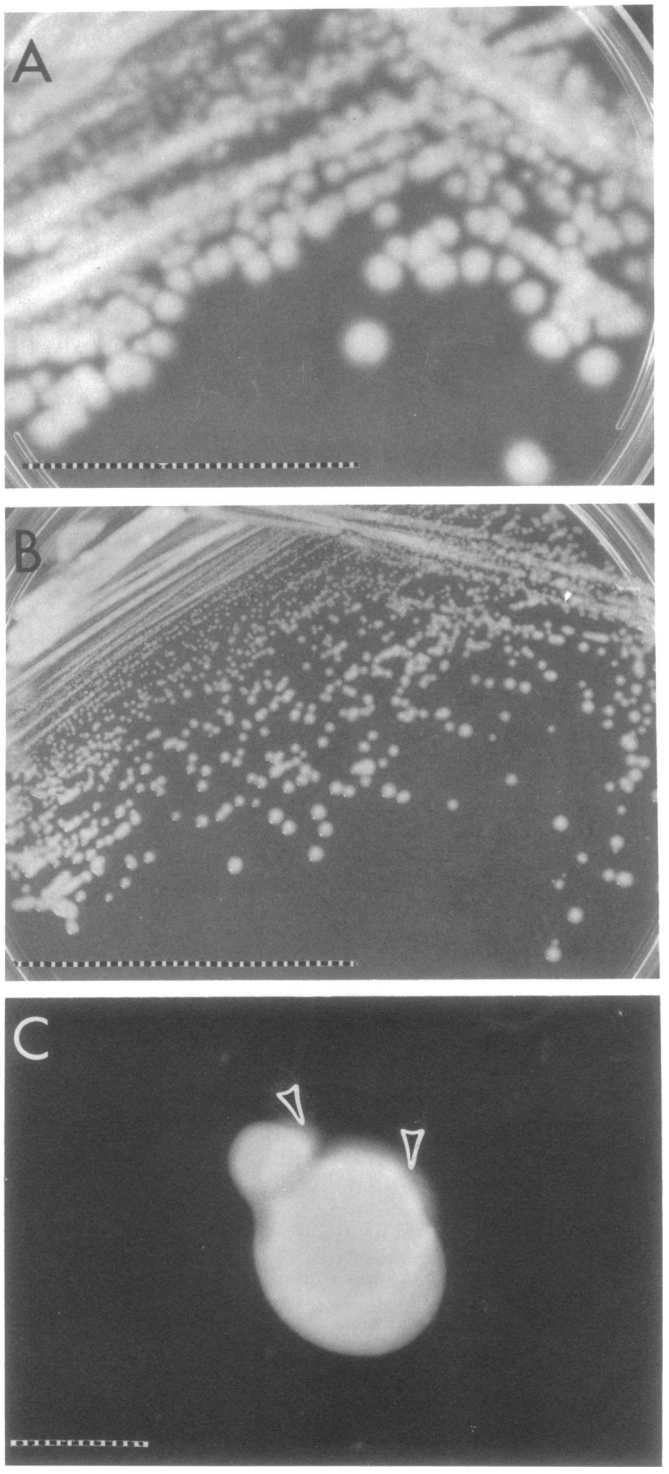

FIG. 1. *Colony morphology of S. halophila P1 (A) and S. halophila DC1 (B) on ISM agar plates incubated at 37°C for 5 days. Bars = 5 cm. (C) Colonies of S. halophila DC1 on migration medium plate incubated at 37°C for 14 days. Note motile sectors diffusing through agar gel (arrows). Bar = 5 mm.*

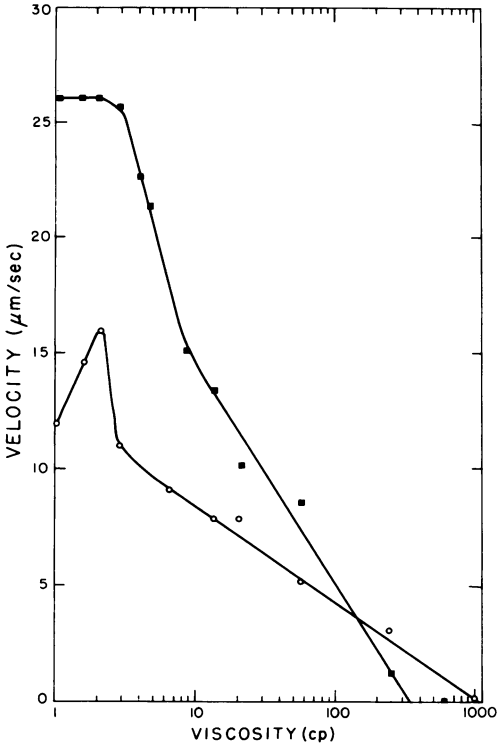

FIG. 2. *Effect of viscosity on the average velocity of S. aurantia M1 (■) and S. halophila P1 (○). The viscous agent was PVP.*

cally indistinguishable from its motile parent. Electron microscopy revealed the presence of axial fibrils and an outer sheath in cells of strain M2 (Fig. 3). Only one strain of the second type of nonmotile mutant was isolated. This strain, called DC1 (Table 1), was morphologically distinct from the parental strain in that it lacked the characteristic cell coiling of *S. halophila* (Fig. 4). In general, cells of strain DC1 were relatively straight, often with one or both ends bent or hooked (Fig. 4B). A very small percentage of cells with a few loose, irregular, shallow coils or waves was observed in cultures of strain DC1 (Fig. 4B). Cells of DC1 were similar in size to those of the parental strain (0.4 by 15 to 30 μm) and had axial fibrils and an outer sheath (Fig. 5).

Motile revertants of strain DC1 were obtained by spot-inoculating cells of this mutant on migration medium plates. During the initial period of incubation, glistening surface colonies with well-defined edges were observed. After 1 to 2 weeks of incubation, sectors of some of these colonies began to diffuse through the agar medium (Fig. 1C). Cells from these sectors were cloned on ISM agar plates. Colonies of the motile strains obtained from the diffusing sectors were similar in appearance to those of the wild-type strain P1 (Fig. 1A) but were smaller. The motile revertant strains isolated by this procedure resembled strain DC1 in morphology

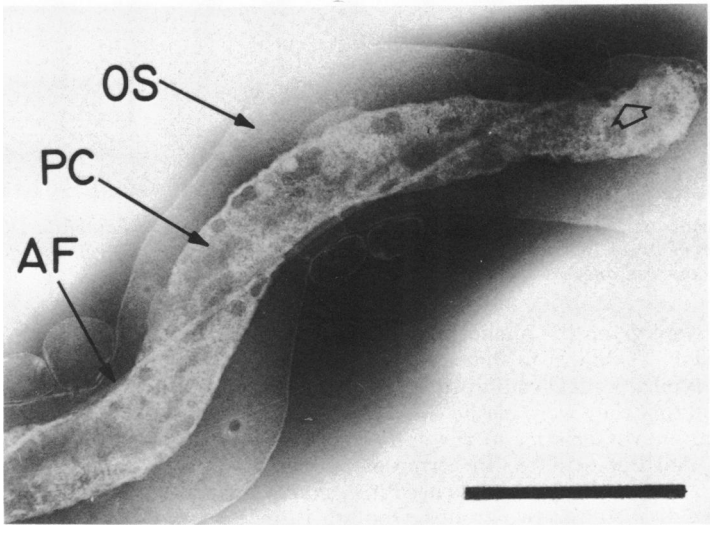

FIG. 3. *Electron micrograph of a portion of a cell of S. halophila M2. Negative-stain preparation. Note the disk-shaped structure (arrow) anchoring the axial fibril (AF) subterminally in the protoplasmic cylinder (PC). The outer sheath (OS) appears to surround both AF and PC. Bar = 0.5 μm.*

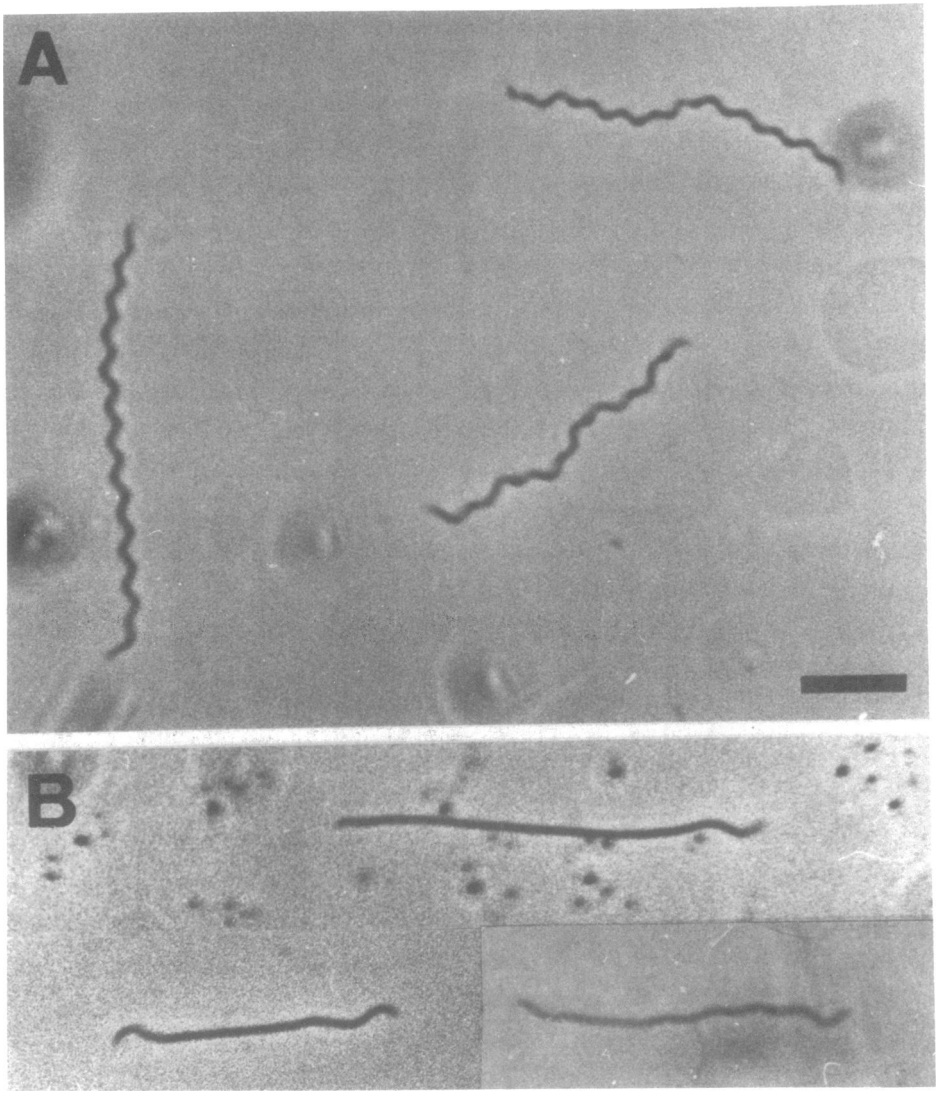

FIG. 4. *Phase-contrast photomicrographs of S. halophila P1 (A) and S. halophila DC1 (B). Wet-mount preparations. Cells of strain DC1 with a few shallow waves or coils, such as the cell shown in the lower right-hand corner, are observed only rarely. Bar = 5 μm.*

(Fig. 4B). They were given the phenotypic designation Hel$^-$ Mot$^+$ (Table 1) to indicate that they lacked the typical helical configuration or coiling (Hel$^-$) and that they were motile (Mot$^+$).

Morphological characteristics of the spirochetes used in motility studies. In terms of their cell coiling, the motile spirochetes used in this study may be divided into two groups. One group includes the typically coiled spirochetes *S. halophila* strain P1, *S. aurantia* strains M1, J4L, and J4T, and *L. interrogans* strain B16. Cells of *S. halophila* P1 and *S. aurantia* J4T are "tightly coiled"; that is, the wavelength (9) of their cell coils ranges from 1.2 to 1.7 μm. *S. aurantia* strains M1 and J4L are "loosely coiled," with coil wavelengths of 2.5 to 2.6 μm. Thus, cells of these loosely coiled strains have fewer coils than equally long cells of the tightly coiled strains. Whereas the strains of *Spirochaeta* have a cell diameter ranging from 0.3 to 0.4 μm, the cells of *L. interrogans* B16 measure approximately 0.2 μm in diameter. On the basis of their coil wavelength, the leptospiral cells may be regarded as tightly coiled.

The second group consists of the motile mutants (Hel$^-$ Mot$^+$) of *S. halophila* (Table 1) ob-

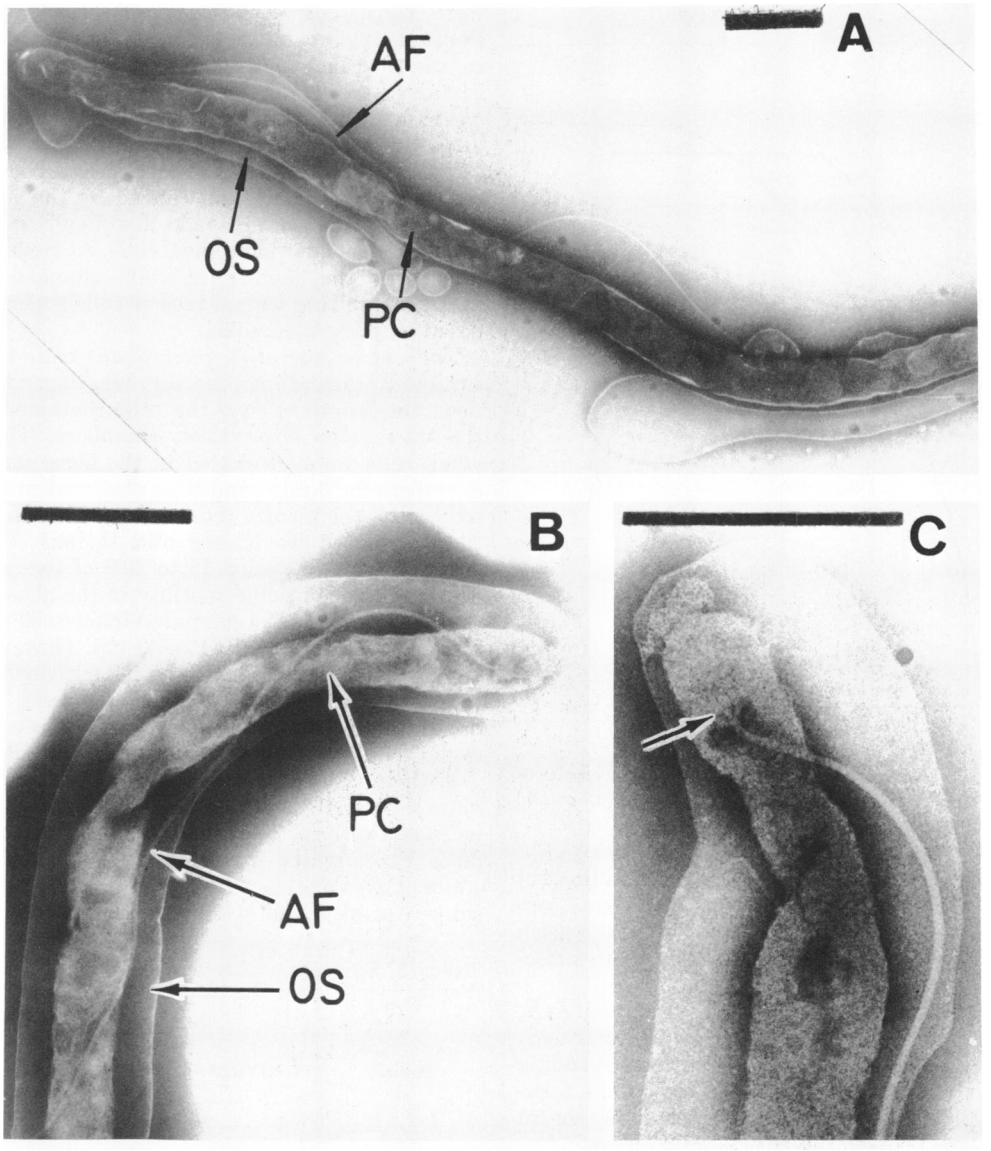

FIG. 5. *Electron micrographs of portions of cells of S. halophila DC1. Negative-stain preparations. (A) Portion of cell showing general lack of coiling. An axial fibril (AF), the protoplasmic cylinder (PC), and the outer sheath (OS) are visible. (B) Hooked or bent end of a cell. The OS appears to surround both AF and PC. (C) Higher magnification of an end of a cell. Note the disklike structure (arrow) anchoring the AF subterminally. Bars = 0.5 μm.*

tained from strain DC1 as described above. As previously mentioned, cells of these mutants lacked the characteristic coiling of *S. halophila*.

Motility of spirochetes in viscous solutions. Velocities of *S. aurantia* M1 in solutions of different viscosities were measured by using either methyl cellulose or PVP as the viscous agent. At the lower viscosities tested, the average velocity was not appreciably affected, but at higher viscosities the average velocity declined (Fig. 6). When methyl cellulose rather than PVP was used as the viscous agent, higher viscosities were required to effect the same decreases in average velocity (Fig. 6). Schneider and Doetsch (14) obtained similar results in comparing the effect of these two viscous agents on the velocity of *Serratia marcescens*. Our

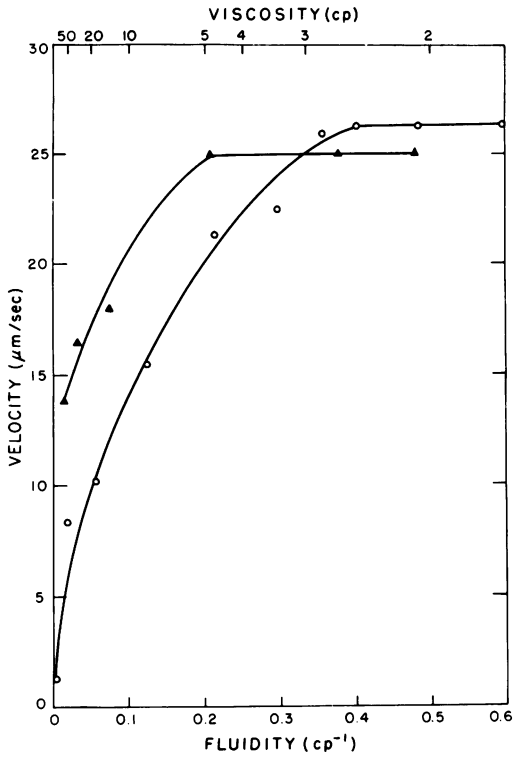

FIG. 6. *Effect of viscosity on the average velocity of S. aurantia M1. PVP* (○) *and methyl cellulose* (▲) *were the viscous agents.*

PVP solutions were more uniform than those of methyl cellulose, which often appeared to contain small gel-like particles. For this reason PVP was used as the viscous agent in all subsequent experiments.

S. aurantia strains J4T and J4L as well as *S. halophila* P1 behaved in a fashion similar to *S. aurantia* M1. As the viscosity of the suspending buffer was increased above 2 to 4 cp, the average velocity of the cells decreased. However, at slightly elevated viscosities the average velocity of *S. halophila* P1 was enhanced (Fig. 2). An enhancement of velocity at slightly elevated viscosities was also exhibited by Hel⁻ Mot⁺ mutants of *S. halophila*. This type of response has been reported for a variety of flagellated bacteria (14, 16).

The effect of viscosity on the motility of *S. aurantia* and *S. halophila* strains was markedly different from the effect of viscosity on the motility of *L. interrogans* B16. Kaiser and Doetsch (12) reported that the velocity, as well as the percentage of translating cells of this leptospire, increased until the viscosity of the suspending solution exceeded 300 cp. Our observations on the velocity of *L. interrogans* B16 were consistent with those of Kaiser and Doetsch (12), inasmuch as we found no decrease in the average velocity as the viscosity was increased to 500 cp. However, the techniques we used (see Materials and Methods) did not allow us to measure accurately the velocity of *L. interrogans* B16 at high viscosities. The increase in light scattering caused by the high concentrations of PVP made it difficult to see clearly the slender leptospiral cells. At elevated viscosities the leptospiral cells changed in shape, exhibiting wide waves or coils superimposed on their tight coils.

The percentage of *L. interrogans* cells that exhibited translational motility was dependent upon the length of time the cells remained in the microscope observation chambers. Thus, when cells were incubated in the observation chambers for 5 min, and then observed microscopically, the results obtained were similar to those reported by Kaiser and Doetsch (12). After this 5-min period 15 to 20% of the cells exhibited translational motility in the absence of a viscous agent, as compared to 50 to 60% in the presence of 10% PVP (250 cp). However, during the first 3 min after the cell suspensions were placed in the observation chambers, approximately 80% of the cells exhibited translational motility, even in the absence of PVP.

With the exception of the leptospires, maximum velocities for all motile spirochetes tested were at 1 to 2 cp. The parental strain *S. halophila* P1 exhibited a maximum velocity of 16 μm/s at 2 cp (Table 2). In comparison, at this same viscosity, the Hel⁻ Mot⁺ mutants of *S.*

TABLE 2. *Effect of viscosity on motility of spirochetes*

Strain	MIV(cp)	Maximum velocity (μm/s)	Viscosity at which maximum velocity occurred (cp)
S. halophila P1	1,000	16	2
S. halophila R532	70	9	2
S. halophila R51	120	7	2
S. aurantia J4T	1,000 (1,000)[a]	21 (21)	1
S. aurantia J4L	600 (600)	14 (21)	1
S. aurantia M1	300 (300)	28 (26)	1-2
L. interrogans (*biflexa*) B16	—[b]	30	1-500[c]

[a] Parentheses indicate experiments were performed using cells suspended in fresh GTY medium (7) rather than in chemotaxis buffer (see Materials and Methods).

[b] An MIV for *L. interrogans* could not be determined since the average velocity did not change measurably over the range of viscosities tested.

[c] The highest viscosity at which velocity measurements of *L. interrogans* were obtained was 500 cp (see Results).

halophila had velocities approximately one-half that of the parental strain (Table 2). When suspended in growth medium, at low viscosities, the loosely coiled strains of *S. aurantia* had maximum velocities equal to, or greater than, the tightly coiled *S. aurantia* J4T (Table 2).

MIV. The minimum viscosity at which spirochetes lost translational motility (MIV) was determined by using PVP as the viscous agent. At this viscosity the strains tested not only lost translational motility, but also all other types of movement (e.g., flexing, undulating) were also absent. In all cases the cellular paralysis at the MIV (or at higher viscosities tested) could be reversed, and normal motility observed, by diluting the PVP solution with the appropriate buffer. Thus, the concentrations of PVP used to immobilize the cells did not damage the motility system in the spirochetes.

The MIV for the tightly coiled *S. halophila* P1 or *S. aurantia* J4T was approximately 1,000 cp (Table 2). MIV values for the loosely coiled *S. aurantia* strains J4L and M1 were 600 and 300 cp, respectively (Table 2). MIV values for two *S. halophila* Hel$^-$ Mot$^+$ mutants were 70 and 120 cp (Table 2), approximately one-tenth the MIV for the wild-type strain P1. The MIV for flagellated bacteria, such as *E. coli* and *Spirillum serpens*, was 60 cp (E. P. Greenberg and E. Canale-Parola, in preparation).

Migration of growing cells of *S. halophila* in agar gels. Measurements of the rate of migration of *S. halophila* P1 and *S. halophila* Hel$^-$ Mot$^+$ mutants yielded information consistent with the experiments described above. In gels of low agar concentrations (e.g., 0.25% wt/vol) the migration rate of *S. halophila* P1 was over twice as great as the migration rate of any of the Hel$^-$ Mot$^+$ mutants (Fig. 7). Furthermore, strain P1 migrated through 2% agar gels, whereas Hel$^-$ Mot$^+$ strains did not migrate through gels containing more than 1% agar (Fig. 7). With regard to these experiments, it should be cautioned that perhaps chemotaxis as well as motility was being measured. The growth rates of strain P1 and the Hel$^-$ Mot$^+$ mutants in ISM broth were similar.

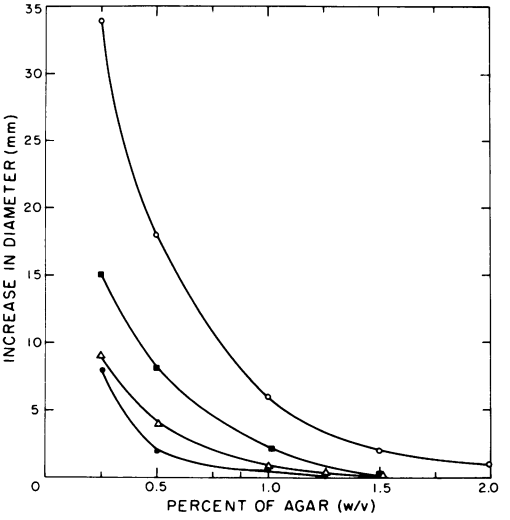

FIG. 7. *Effect of agar concentration on migration of growth rings of S. halophila strains P1 (○), R51 (■), R532 (△), and R752 (●). Each point indicates the total increase in ring diameter after 96 h at 37°C.*

DISCUSSION

The findings reported in this paper, as well as the work of Kaiser and Doetsch (12) with leptospires, indicate that spirochetes maintain translational motility in environments of relatively high viscosity. Flagellated bacteria, such as *E. coli, P. aeruginosa,* and *Spirillum serpens* are immobilized at substantially lower viscosities (E. P. Greenberg and E. Canale-Parola, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, I42, p. 161). In addition, we report that motile mutant spirochetes which have little or no coiling, as compared to their parental strain, lack the ability to move in viscous environments through which the parental strain swims readily (Table 2). Furthermore, loosely coiled strains of *S. aurantia* were immobilized at considerably lower viscosities than the tightly coiled *S. aurantia* J4T (Table 2). This evidence indicates that a correlation exists between the cell coiling of spirochetes and their ability to move through viscous environments. Among the spirochetes we investigated, this ability was greater in the more extensively coiled strains.

The Hel$^-$ mutants of *S. halophila* exhibited substantially lower maximum velocities than the parental strain (Table 2). It is possible that the characteristic coiling of *S. halophila* is required for most efficient translational motion of this spirochete. When cells were suspended in GTY medium (7) the maximum velocities of the loosely coiled strains of *S. aurantia* were equal to, or greater than, the maximum velocity of the tightly coiled strain J4T (Table 2). This observation suggests that tighter coiling is not directly related to velocity at the lower viscosities tested. However, this question needs further investigation inasmuch as our results with *S. aurantia* may reflect strain variation in some other facet of the motility mechanisms of these spirochetes.

At slightly elevated viscosities (2 cp) the average velocity of *S. halophila* was enhanced

(Fig. 2, Table 2) in a fashion similar to that reported for a variety of flagellated bacteria (14, 16). Schneider and Doetsch (14) suggested that, in flagellated bacteria, the viscosity of the medium controls the conformation of the flagellar helix and thus affects the propulsive efficiency. Possibly the conformation of *S. halophila* cells is subtly changed by the viscosity of the medium and this results in greater efficiency of motility at 2 cp. As previously mentioned, the shape of leptospires does in fact change in viscous solutions, as well as in agar gels (21) or on solid surfaces (5). Under such conditions, wide waves are superimposed on the narrow primary coils of the leptospiral cell.

It should be noted that the effect of viscosity on *S. aurantia* M1 varied slightly when methyl cellulose was used, rather than PVP, as the viscous agent (Fig. 6). Thus, the MIV values reported here (Table 2) should not be considered absolute. On the contrary, it should be expected that spirochetes are immobilized at slightly different viscosities depending on the nature of the viscous environment.

As suggested by Kaiser and Doetsch (12), the ability to move through viscous environments may confer to spirochetes important ecological advantages. For example, motility and chemotactic responses may be retained by free-living spirochetes inhabiting viscous mud or sediments. In their natural habitats within the body of animals, host-associated spirochetes may swim readily through viscous materials such as gingival crevice fluids or mucosal fluids. This may be a significant feature in symbiotic associations between spirochetes and animals.

The spirochetes we tested move through viscosities equivalent to those reported for human cell membranes (13, 15). Should pathogenic spirochetes also have this property, they may be capable of swimming through mammalian cell membranes into cells of the infected host. Thus, the observation that spirochetes migrate through viscous environments provides an interpretation for findings indicating that pathogenic spirochetes, such as *Treponema pallidum*, are present within fibroblasts and epithelial cells of infected humans (19, 20).

The peptidoglycan of spirochetes serves to maintain the helical configuration of these organisms (10, 11). Therefore, it is probable that certain chemical differences exist between the peptidoglycans of Hel^- mutants of *S. halophila* and the wild-type Hel^+ strain. An understanding of these differences may reveal the chemical basis for the helical nature of *S. halophila*.

Although our studies indicated that the cell coiling of spirochetes plays an important role in their movement through environments of relatively high viscosity, this type of movement is not a property of all motile coiled bacteria. Coiled or helical flagellated bacteria, such as *Spirillum serpens*, are immobilized at viscosities as low as those that immobilize flagellated bacteria of other cell shapes (E. P. Greenberg and E. Canale-Parola, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, I42, p. 161). Kaiser and Doetsch (12) suggested that viscous environments may have a damping action on bacterial flagella, thus affecting the efficiency of translational motion. It is possible, as suggested by Berg (1), that the axial fibrils participate in cell propulsion by rotating within the spirochetal cell in the region enclosed between the protoplasmic cylinder and the outer sheath. Thus, viscous environments, which affect the action of bacterial flagella, would not directly interfere with the functioning of spirochetal axial fibrils because of the endocellular location of these organelles.

It should be noted that the ability to move through viscous environments is not restricted to spirochetes. Strength et al. (18) reported that a flagellated gram-negative rod, which upon isolation grew in viscous flocs, was able to swim in solutions of relatively high viscosity. This flagellated bacterium may have developed a specialized type of motility that allows it to locomote in its environment (18).

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