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Helix Handedness of *Leptospira interrogans* as Determined by Scanning Electron Microscopy

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Representative serovars and strains of the seven genetic groups of *Leptospira interrogans*, and two previously studied serovars, were all found to form exclusively right-handed helices as determined by scanning electron microscopy. No change in handedness occurred in cells grown in a minimal medium (Tween-80 albumin) compared to cells grown in a rich medium (rabbit serum). The right-handedness of the organisms was related to the evolution, cell wall structure, and the mechanism of motility of *L. interrogans*.

Although *Leptospira interrogans* is recognized as the only species in the genus *Leptospira* (24), its members demonstrate wide genetic diversity. The genus is divided into three major complexes and subdivided into seven minor groups (4). Based on DNA hybridization results, little or no relatedness is found between representative serovars and strains of the three complexes. However, because significant genetic diversity occurs between serovars and strains within two of the complexes, the genus is further subdivided into a total of seven minor groups.

L. interrogans has been shown by electron microscopy to have a helical morphology (1, 9, 10, 12, 18-21). The present study attempts to determine the helix handedness (right-handed [clockwise], or left-handed [counterclockwise]) of representative serovars and strains of the seven genetic groups for two reasons. First, the type of helix handedness of *L. interrogans* may be an important means for classification. In *Aquaspirillum* and *Oceanospirillum* helix handedness is a stable genetic characteristic (15, 22). Thus *A. serpens* has a right-handed helix, and *O. pusillum* has a left-handed helix (15). Because representatives of the seven genetic groups will likely have one or the other helix handedness, one identifying characteristic of a complex or group could be the type of helix.

Second, determining the handedness of *L. interrogans* will yield important information concerning its motility. Recent results indicate that the axial filaments are involved in *L. interrogans* motility (5; D. B. Bromley and N. W. Charon, Abstr. Annu. Meet. Am. Soc. Microbiol., J4, p. 182, 1977). Two basic movements are proposed which propel the organisms: (i) roll of the cell cylinder about the axial filaments, and (ii) rotation of the axial filaments leading to a flexing of the cell cylinder (3, 7). For the cell to translate, most notably in a gel-like medium when rolling of the cylinder is believed to be the chief means of thrust, the roll should be clockwise if the helix is right-handed, and counterclockwise if it is left-handed (3). By determining the helix handedness of *L. interrogans*, we should be able to suggest the direction of cylinder roll during translation.

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MATERIALS AND METHODS

Organisms and culture methods. The serovars and strains used represent the seven genetic groups as determined by Brendle et al. and those studied by Czekalowski (4, 10). *L. interrogans* serovars *patoc* Patoc 1, *bataviae* Van Tienen, *javanica* Veldrat Bataviae 46, and *ranarum* Iowa City Frog were obtained from the Communicable Disease Center, Atlanta, Ga. Serovars *illini* 3055, *codice* CDC, *icterohaemorrhagiae* SC 1157, and *canicola* Hond Utrecht IV were obtained from Russell C. Johnson, University of Minnesota, Minneapolis. Turtle strain A-183 (serovar undetermined) was obtained from Lyle Hanson, University of Illinois, Urbana.

The organisms were grown and maintained in the Ellinghausen and McCullough Tween-80 bovine serum albumin (Scientific Protein Laboratories, Waunakee, Wis.) medium modified by Johnson and Harris (EMJH medium), or in rabbit serum medium (RS medium, 13). Glycerol was not included in the EMJH medium. Growth was monitored with a Coleman model 7 photonephelometer (Coleman Instruments, Oak Brook, Ill.). Readings on the photonephelometer have been previously correlated to total cell count and viable count (T. Auran, M. S. thesis, University of Minnesota, Minneapolis, 1968; 7).

Scanning electron microscopy. A 10-ml volume of an aerated culture containing logarithmic-phase cells at a density between 1.5×10^8 and 5.0×10^8 cells per ml was harvested by centrifugation at $17,500 \times g$ for 15 min at 5°C. The supernatant fluid was removed, and 5 ml of a fixative buffer containing 1% glutaraldehyde was added to the pelleted cells. This fixative buffer contained, in addition to the glutaraldehyde, 1% sodium cacodylate (pH 7.4), in 3.75% sucrose. The fixative buffer was 317 mosM as determined by freezing point depression. The cells were fixed between 15 min to 1 h at 20°C, centrifuged as before, and dehydrated with increasing concentrations of ethanol. Amyl acetate was substituted for the ethanol prior to critical point drying in carbon dioxide. The specimens were coated with gold-palladium 60/40 alloy preceded by carbon coating, and they were viewed on a Cambridge Sterioscan S4-10 scanning electron microscope with a lanthanum hexaboride gun at a 45° tilt and 20 kV accelerating voltage. Photographs were taken directly using Polaroid film type 55 P/N.

Helix handedness. A right- (or left)-handed helix is defined as one which rotates clockwise (or counterclockwise) while moving along the helix away from an observer. To characterize the handedness of the spirochetes, cells were photographed and then compared to a right-handed nichrome wire also viewed and photographed in the scanning electron microscope. Results are expressed as the total number of cells with right-handed helices divided by the total number examined for a given serovar or strain.

RESULTS

Representative serovars and strains of the seven genetic groups of *L. interrogans* were grown in EMJH medium, fixed with glutaraldehyde, and prepared for scanning electron microscopy. At ×16,000 the handedness of the spirochetes was readily discernible (as represented by serovar *patoc* Patoc 1 in Fig. 1). No image inversion of the handedness occurred during magnification and photography as determined using a known right-handed helix. Between 12 and 73 cells were examined of each serovar or strain, and their handedness was determined (Table 1). All the cells of each serovar or strain were found to form exclusively right-handed helices (Table 1).

We examined the handedness of two other serovars of *L. interrogans.* Czekalowski found serovar *icterohaemorrhagiae* Wijnberg, serovar *canicola* Hond Utrecht IV, and the *leeds* strain to be left-handed, using transmission electron microscopy (9, 10). Because the seven serovars and strains reportedly represent the major genetic groups of *L. interrogans,* we determined the handedness of our laboratory cultures of serovars *icterohaemorrhagiae* SC 1157 and *canicola* Hond Utrecht IV. Serovar *icterohaemorrhagiae* SC 1157 is a different strain but is the same serovar as *icterohaemorrhagiae* Wijnberg, and *canicola* Hond Utrecht IV is the same strain and serovar as the one studied by Czekalowski (10). Scanning electron microscopy revealed that both were right-handed when grown in EMJH medium (Table 2).

The handedness of serovars *icterohaemorrhagiae* SC 1157 and *canicola* Hond Utrecht IV was examined after growth in a different medium. Certain helical-shaped mutants of *Bacillus subtilis* were recently shown to have different handedness when grown in a rich growth medium as opposed to a minimal enriched growth medium (17). Although not stated by Czekalowski, he presumably used a rabbit serum (RS) medium for growth of *L. interrogans,* as this medium was used by him in other electron microscopic studies (11). Conceivably, serovars *icterohaemorrhagiae* SC 1157 and *canicola* Hond Utrecht IV could form left-handed cells when grown in RS medium. To test this possibility, both serovars were serially passed six times in RS medium after growth in EMJH medium. They were then examined under the scanning electron microscope. As can be seen in Table 2, no change in handedness occurred after growth in RS medium.

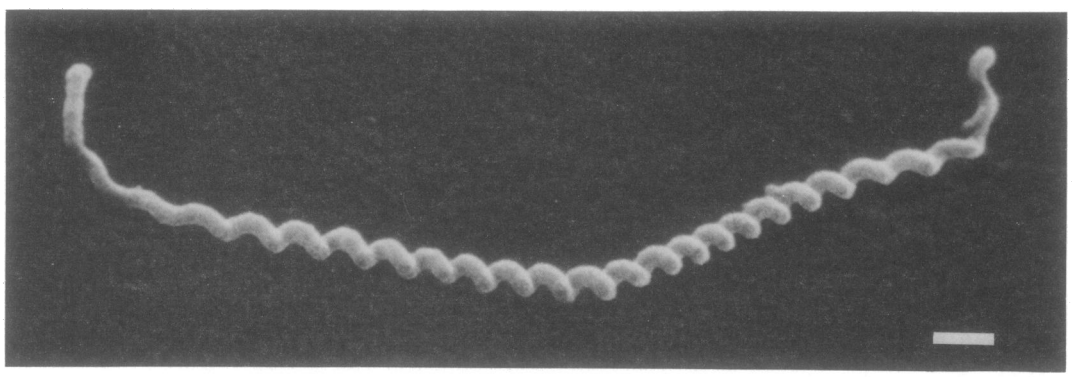

FIG. 1. *Scanning electron micrograph of serovar patoc Patoc 1. Bar represents 0.5 μm.*

TABLE 1. *Helix handedness of representatives of the seven genetic groups of L. interrogans grown in EMJH medium*

Serovar or strain	No. of right-handed cells/no. examined cells
patoc Patoc 1	73/73
Strain A-183	57/57
codice CDC	34/34
ranarum Iowa City Frog	25/25
javanica Veldrat Bataviae 46	12/12
bataviae Van Tienen	28/28
illini 3055	34/34

TABLE 2. *Helix handedness of L. interrogans grown in EMJH and RS media*

Serovar	No. of right-handed cells/no. examined cells	
	EMJH-grown cells	RS-grown cells
icterohaemorrhagiae SC 1157	16/16	74/74
canicola Hond Utrecht IV	14/14	19/19

DISCUSSION

Serovars and strains of *L. interrogans* representing the seven genetic groups were found to have right-handed helices as determined by scanning electron microscopy. Because the handedness of between 12 and 73 cells was determined for each serovar and strain, if any left-handed cells were present, they constituted a small percentage (less than 2% in some cases) of the total number of a serovar or strain. These results are in contrast to those of Czekalowski who found two serovars and one strain to be left-handed (9, 10). While this manuscript was in preparation, Kayser and Adrian reported similar results to those reported here with respect to the right-handedness of selected serovars of *L. interrogans* (14).

Although the basis for the difference between our and Kayser's and Adrian's results and those of Czekalowski is presently unknown, a number of explanations are possible. First, as Kayser and Adrian suggest, a reversal of handedness of the image of *L. interrogans* could have occurred during photography or during electron microscopy (14). By inverting the specimen in the transmission electron microscope, they show that a right-handed organism will appear left-handed. Second, *L. interrogans* could conceivably form left-handed helices in the medium Czekalowski used to grow the organisms. Changes in handedness have been observed in certain helical mutants of *B. subtilis* when grown in different media (17). However, we were unable to detect such a handedness change when employing the two standard *L. interrogans* media.

We can only speculate at this time why these spirochetes of similar dimensions but of wide genetic diversity are all right-handed. One possibility is that all *L. interrogans* evolved from the same protospirochete (6), or from the same protoleptospire. Note that *L. interrogans* varies considerably not only with respect to genetic relatedness as determined by DNA hybridization, but also with respect to guanine-plus-cytosine content (4). In light of this hypothesis, it would be interesting to determine the handedness of other spirochetes. Preliminary studies by Kayser and Adrian suggest that certain treponemes are also right-handed (14).

Another possibility for their uniform right-handedness may be related to the cell wall structure itself. For example, the molecules which determine the rigidity and cell shape of *L. interrogans* conceivably could only form right-handed helices. Although little is known about the cell wall structure of *L. interrogans*, lysozyme studies suggest that cell wall rigidity resides in the peptidoglycan (1). Recently, both left- and right-handed helices have been found in mutants of *B. subtilis* and also in wild-type *B. subtilis* grown under certain conditions (16, 17, 23). This change of cell shape has been attributed to a possible helical substructure organization of cell wall components (16, 17). We wonder if *L. interrogans* will also form left-handed helices.

Finally, the right-handedness of *L. interrogans* may be related to the motility of *L. interrogans*; specifically, it may be that left-handed *L. interrogans* are nonmotile. Recent results with nonmotile mutants of *L. interrogans* serovar *illini* 3055 indicate that the axial filaments are involved in motility. Moreover, the shape of the axial filaments has been shown to be important for *L. interrogans* motility (5; Bromley and Charon, Abstr. Annu. Meet. Am. Soc. Microbiol., J4, p. 182, 1977). Conceivably, the axial filaments of all seven genetic groups could have a similar shape. Berg et al. propose that the axial filaments and the cell cyclinder interact with one another for motility (3, 7). Thus, given a specific shape of the axial filament, the cell cylinder may necessarily have to conform to a particular shape for motility.

The information gained from this communication will aid in distinguishing between two of the models of spirochete motility (2, 3, 7). According to the model of Berg et al., the cell cylinder should roll clockwise during transla-

tional motion (3). One prediction of the model states that latex beads attached to the organism should also rotate in the same direction (clockwise) as the roll of the cell cylinder (3). On the other hand, according to another model proposed by Berg, the beads should rotate in the opposite direction as the roll of the cylinder (counterclockwise; 2).

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ADDENDUM

In confirmation of the results reported here, Z. Yoshii (Proc. Jpn. Acad. **54B**:200–205, 1978) recently found that four other serovars, along with serovar *canicola* Hond Utrecht IV, form right-handed helices.

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