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NOTES

Helical Conformation of *Treponema pallidum* (Nichols Strain), *Treponema paraluis-cuniculi*, *Treponema denticola*, *Borrelia turicatae*, and Unidentified Oral Spirochetes

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Borrelia turicatae (mouse virulent) and *Treponema denticola*, a small oral treponeme, formed right-handed helices as determined by scanning electron microscopy. *Treponema pallidum* (Nichols strain), *Treponema paraluis-cuniculi*, and two unidentified oral spirochetes displayed left-handed helices.

The order *Spirochaetales* consists of a heterogenous group of procaryotic organisms. However, the spirochetes do share the following characteristics: helical shape, flexible cells, flagella located beneath an outer envelope, and a unique type of motility. The helix handedness of the spirochetes received little attention until recently. The helical conformation of the aerobic spirochete, *Leptospira*, was found to be right-handed by several research groups (2, 8, 17). The nonpathogenic, anaerobic spirochetes *Treponema refringens* (15, 18) and *Treponema phagedenis* (7, 8) also possess right-handed helices. Sequeira (15) examined the handedness of the pathogenic treponemes *T. pallidum*, the causative agent of syphilis, and *T. pertenue*, the etiological agent of yaws. Based on his observations with the dark-field microscope, he concluded that living *T. pallidum* is a flat wave form, and not a spiral, and that *T. pertenue* was similar in form to *T. pallidum*. Cox also conducted a dark-field examination of *T. pallidum* and verified the observations of Sequeira (3). However, recent scanning electron microscopic examinations of *T. pallidum* have established that spiral forms of this spirochete exist (4, 5).

Many spiral plants are known with the direction of the spiral being a species-dependent characteristic (9). By analogy, helix handedness in spirochetes might be expected to be a stable identifying characteristic as well as a tool for studying their evolution. In fact, the helix handednesses of *Leptospira* (2, 17) and the spiral bacteria *Aquaspirillum* and *Oceanospirillum* (10, 16) are considered to be genetically stable.

The present study describes an examination of the helical conformations (clockwise coiling being right-handed and counterclockwise being left-handed) of *T. pallidum* (Nichols strain), *Treponema paraluis-cuniculi*, *Treponema denticola*, *Borrelia turicatae*, and unidentified oral spirochetes by scanning electron microscopy. These coilings are compared with those previously determined for other *Treponema* species and *Leptospira*.

Specimens for scanning electron microscopy were prepared as described by Carleton et al. (2). Image inversion of the handedness did not occur, since the Polaroid photographs used were real images. This was verified by photographing a gold wire with a left-handed helix.

T. pallidum (Nichols strain), the etiological agent of syphilis, was examined with the scanning electron microscope. This spirochete was maintained in rabbits as previously described (1). Of the forty cells which were studied in detail, approximately 75% had helical conformations. The remaining cells lacked coiling and appeared to be in the planar wave form. All the helical forms of *T. pallidum* were left-handed (Fig. 1 and 2). The left-handed conformation of *T. pallidium* cells was readily discerned when compared with that of *Leptospira interrogans* serovar Canicola strain Moulton, which had a right-handed helix (Fig. 3). This *Leptospira* strain was cultivated in a Tween-80-albumin medium (6). *T. paraluis-cuniculi*, a rabbit pathogen of low virulence, was also examined for handedness. This organism was obtained from testicular tissue of a naturally infected rabbit. Ten cells were carefully examined. All cells were in helical conformations and were exclusively left-handed helices. In contrast to these two noncultivable, pathogenic treponemes, the avirulent, cultivable *Treponema* species *T. phagedenis* (7, 8) and *T. refringens* (15, 18) are reported to have right-handed helices. The genetic studies of *Treponema* by Miao and Fieldsteel

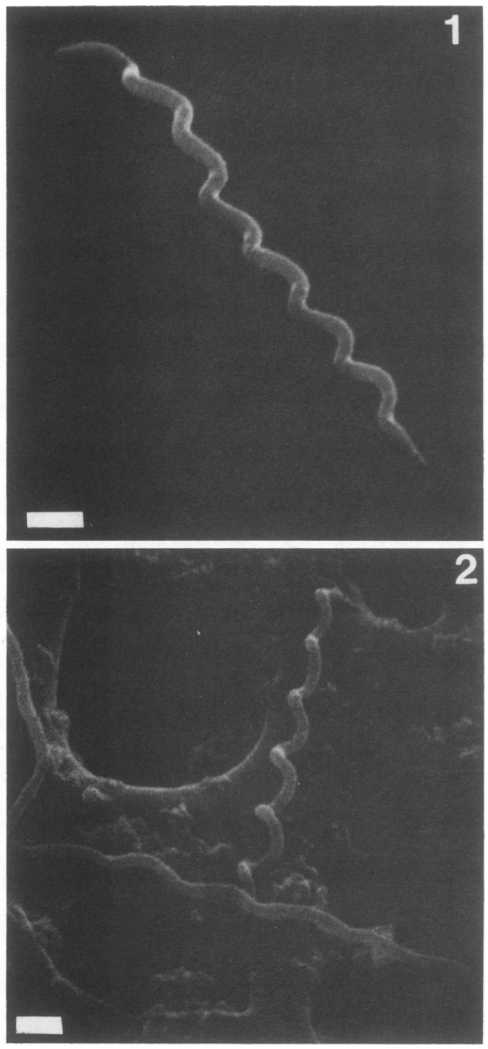

FIG. 1 and 2. *Scanning electron micrographs of T. pallidum, Nichols strain. Bar represents 0.5 μm.*

(13, 14) have shown that at least three genetically distinct groups exist. The noncultivable, pathogenic *T. pallidum* and *T. pertenue* constitute one group, and biotypes of the cultivable, nonpathogenic species *T. phagedenis* and *T. refringens* represent the other two genetic groups. The possibility exists that the morphological feature of left-handed helices can be associated with a genetically distinct group of *Treponema* species, the pathogenic treponemes. Members of the other two genetic groups within *Treponema* that have been examined for helical conformation displayed right-handedness. Studies with additional members of these genetic groups will be necessary to substantiate this proposition.

A study of subgingival plaque obtained from a case of adult periodontitis revealed a large number of spirochetes of various sizes. Examination of a group of spirochetes attached to what appeared to be a leukocyte showed two spirochetes with left-handed helical conformation (Fig. 4). The other oral spirochetes associated

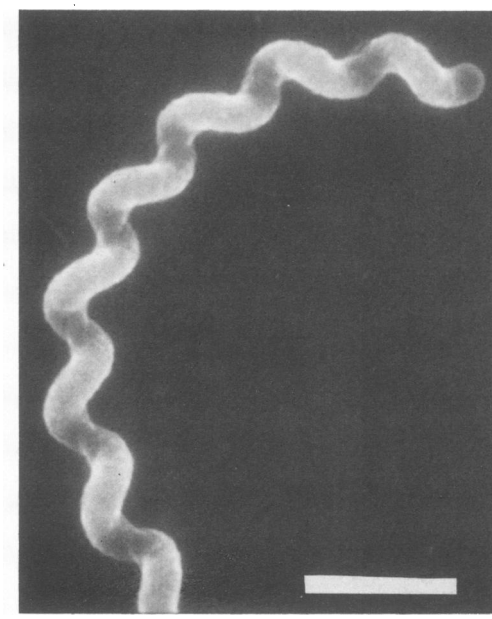

FIG. 3. *Scanning electron micrograph of L. interrogans serovar Canicola strain Moulton. Bar represents 0.5 μm.*

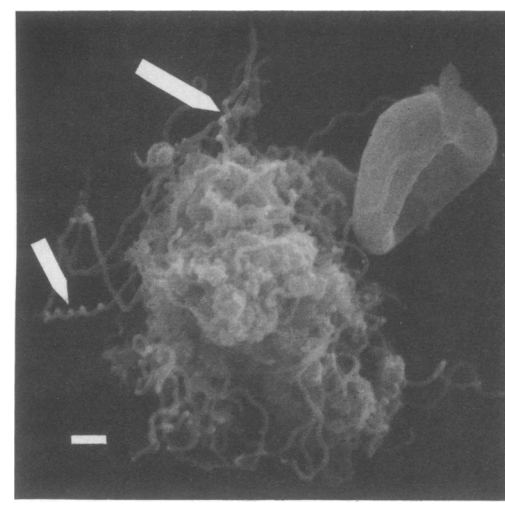

FIG. 4. *Scanning electron micrograph of unidentified oral spirochetes. Spirochetes with left-handed helical conformations are indicated with arrows. Bar represents 1.0 μm.*

with the leukocyte did not display obvious coiling and may have been in a flat wave form, as was observed with preparations of *T. pallidum*. Alternatively, this lack of coiling may have been an artifact introduced during the processing of the specimen. *T. denticola*, a small, nonpathogenic oral spirochete which was maintained under anaerobic conditions in prereduced medium (11), was examined and found to have a right-handed helical conformation (Fig. 5). The oral spirochetes are presently divided into three groups based on cell diameter and number of flagella. Most of the small spirochetes, such as *T. denticola*, can be cultivated with relative ease. Only a few of the intermediate-size oral spirochetes and none of the large-size spirochetes have been cultivated, and these are the spirochetes thought to be involved in periodontal disease (12). The two left-handed oral spirochetes in Fig. 4 appear to be of the intermediate size.

B. turicatae, one of the etiological agents of tick-borne relapsing fevers, was examined for type of helical conformation. The organisms were obtained from the blood of a spirochetemic mouse. *B. turicatae* was found to possess a right-handed helix (Fig. 6), as do the pathogenic and nonpathogenic *Leptospira* species (2, 8, 17) and the nonpathogenic, cultivable *Treponema* species (7, 8, 15, 18).

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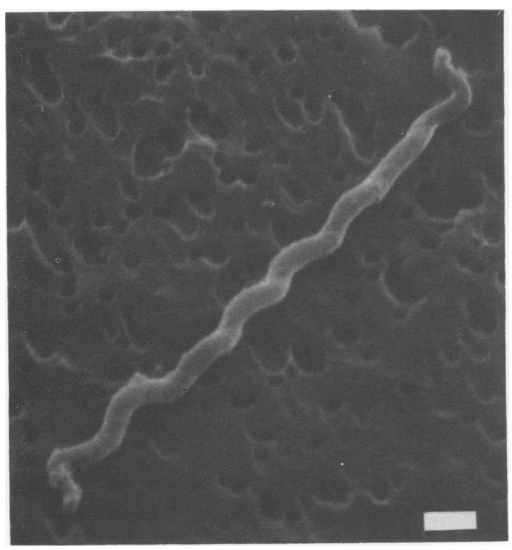

FIG. 5. *Scanning electron micrograph of T. denticola. Bar represents 0.5 μm.*

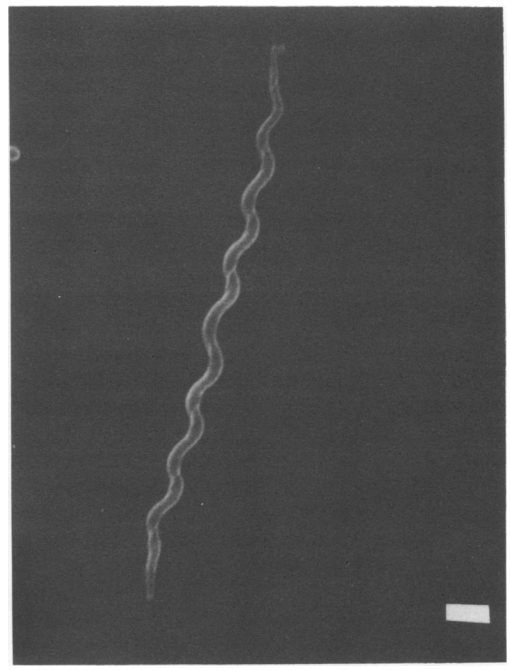

FIG. 6. *Scanning electron micrograph of B. turicatae. Bar represents 0.5 μm.*

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