

Taxonomy of the Lyme Disease Spirochetes

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Morphology, physiology, and DNA nucleotide composition of Lyme disease spirochetes, *Borrelia*, *Treponema*, and *Leptospira* were compared. Morphologically, Lyme disease spirochetes resemble *Borrelia*. They lack cytoplasmic tubules present in *Treponema*, and have more than one periplasmic flagellum per cell end and lack the tight coiling which are characteristic of *Leptospira*. Lyme disease spirochetes are also similar to *Borrelia* in being microaerophilic, catalase-negative bacteria. They utilize carbohydrates such as glucose as their major carbon and energy sources and produce lactic acid. Long-chain fatty acids are not degraded but are incorporated unaltered into cellular lipids. The diamino amino acid present in the peptidoglycan is ornithine. The mole % guanine plus cytosine values for Lyme disease spirochete DNA were 27.3-30.5 percent. These values are similar to the 28.0-30.5 percent for the *Borrelia* but differed from the values of 35.3-53 percent for *Treponema* and *Leptospira*. DNA reannealing studies demonstrated that Lyme disease spirochetes represent a new species of *Borrelia*, exhibiting a 31-59 percent DNA homology with the three species of North American borreliae. In addition, these studies showed that the three Lyme disease spirochetes comprise a single species with DNA homologies ranging from 76-100 percent. The three North American borreliae also constitute a single species, displaying DNA homologies of 75-95 percent. Lyme disease spirochetes and *Borrelia* exhibited little or no DNA homology (0-2 percent) with the *Treponema* or *Leptospira*. Plasmids were present in the three Lyme disease spirochetes and the three North American borreliae.

Spirochetes are helically shaped bacteria with a distinguishing anatomy and locomotion. The order Spirochaetales consists of two families and five genera (Table 1) which is indicative of the heterogeneity in physiology and habitat that exists among these morphologically similar bacteria [1]. The genera *Treponema*, *Borrelia*, and *Leptospira* contain spirochetes pathogenic for humans and animals.

Spirochetes share the following morphological features: (1) a multilayered outer envelope (OE) or outer membrane that surrounds the protoplasmic cylinder; (2) the protoplasmic cylinder, which consists of the peptidoglycan layer, cytoplasmic membrane, and the enclosed cytoplasmic contents; and (3) the periplasmic flagella (axial filaments or axial fibrils) which are positioned between the outer envelope and the protoplasmic cylinder. The periplasmic flagella are attached subterminally at each end of the protoplasmic cylinder with the unattached part extended toward the opposite cell end (Fig. 1).

The etiological agent of Lyme disease possesses the aforementioned basic morphological features of spirochetes (Fig. 2). Our electron microscopic studies were conducted on the human spinal fluid isolate [2]. The outer envelope is elastic and

TABLE 1
Classification of Spirochetes

Order	Spirochaetales
Family	Spirochaetaceae
Genus	<i>Cristispira</i>
	<i>Spirochaeta</i>
	<i>Treponema</i>
	<i>Borrelia</i>
Family	Leptospiraceae
Genus	<i>Leptospira</i>

frequently separates from the protoplasmic cylinder, forming blebs when the cells are exposed to adverse conditions. The Lyme disease spirochetes are similar to the *Treponema* and *Borrelia*, which assume spherical forms when exposed to hypotonic conditions due to the separation of the outer envelope from the protoplasmic cylinder. An intact outer envelope is required for spirochete viability and the conversion of the cells to spherical forms renders them non-viable. The outer envelope of these spherical forms can be readily solubilized by a low concentration (0.7 mM) of the detergent, sodium dodecyl sulfate. The solubilized treponemal outer envelope can be reaggregated by removal of the sodium dodecyl sulfate and the subsequent addition of divalent or trivalent cations [3]. Divalent cations are not required for the reaggregation of *Leptospira* outer envelope [4]. Although the outer envelope of spirochetes may resemble the outer envelope of gram-negative bacteria such as *Escherichia coli*, classical endotoxin has not been detected in the *Leptospira*, *Treponema*, or *Borrelia* [5,6].

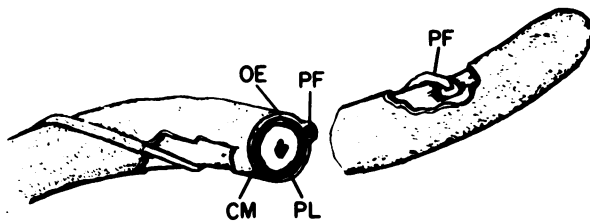


FIG. 1. Diagram of spirochete cell structure: OE = outer envelope, PF = periplasmic flagellum, CM = cytoplasmic membrane, PL = peptidoglycan layer.

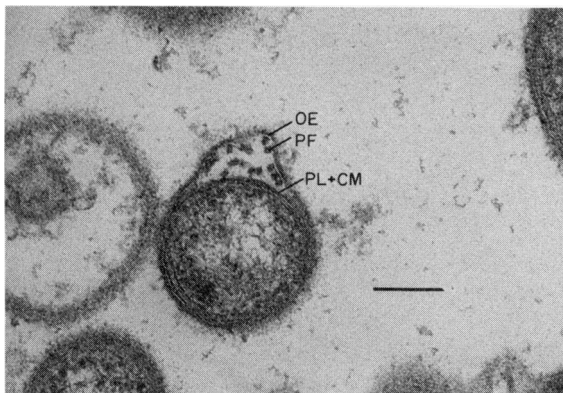


FIG. 2. Electron micrograph of cross section of Lyme disease spirochete (human spinal fluid isolate): OE = outer envelope, PF = periplasmic flagella. PL + CM = peptidoglycan layer plus cytoplasmic membrane (bar = 0.1 μ m).

The periplasmic flagella are the cell structures responsible for the unique flexing type of motility that is characteristic of spirochetes. Spirochetes exhibit maximal velocities in viscous environments. This is probably due to the location of the periplasmic flagella, since the motility of bacteria with external flagella is decreased under these conditions. The number of periplasmic flagella per cell end varies among the spirochetes. The number of periplasmic flagella per cell end for some of the spirochetes are: *Treponema* pathogenic for humans, three; nonpathogenic treponemes, one to eight; *Borrelia*, 15–20; and *Leptospira*, one. The Lyme disease spirochetes appear to have seven periplasmic flagella per cell end. These structures are most accurately enumerated at their points of insertion into the protoplasmic cylinder. It is difficult to determine the number of periplasmic flagella using cross sections of these cells, since these structures overlap. With the exception of *Leptospira*, periplasmic flagella also overlap in the other spirochetes.

The peptidoglycan diamino amino acid of the Lyme disease spirochetes is ornithine. The diamino amino acid present in the peptidoglycan of *Treponema* and *Borrelia* is also ornithine, whereas the cell wall of *Leptospira* contains α,ϵ -diaminopimelic acid [5]. These spirochetes are resistant to the action of lysozyme due to an intact outer envelope. Once the integrity of the outer envelope is destroyed, lysozyme can enter the cell and degrade the peptidoglycan layer.

The cytoplasmic tubules which lie next to the inner layer of the cytoplasmic membrane are present in the *Treponema* but are absent in the *Leptospira* and *Borrelia*, as well as the Lyme disease spirochetes.

Spirochetes are helically shaped bacteria and the helical conformation varies among the spirochetes [7]. The *Leptospira*, *Borrelia turicatae*, and the avirulent *Treponema*, *T. denticola*, *T. refringens*, and *T. phagedenis*, have right-handed helices (coil clockwise). In contrast, the virulent *Treponema*, *T. pallidum* and *T. paraluis-cuniculi*, and some of the oral spirochetes in subgingival plaque have left-handed helices (coil counterclockwise). Hayes et al. reported that the Lyme disease spirochetes freshly isolated from *Ixodes dammini* displayed left-handed coiling and a bacteriophage was associated with these cells. Upon subsequent subcultures of the isolate, the cells converted to right-handed coiling and the bacteriophage was no longer detectable [8]. We have found that the Lyme disease spirochetes isolated from human spinal fluid [2] have the left-handed helical conformation (Fig. 3). This helical conformation appears to be a stable identifying characteristic since all the cells examined had left-handed coiling and these cells have been routinely transferred in culture media for over six months.

The Lyme disease spirochetes apparently have nutritional requirements similar to those of the *Borrelia* since they can be cultivated in a modified Kelly's *Borrelia* medium [9,10]. The utilization of radio-labeled glucose, fatty acids, and amino acids by these spirochetes was studied to verify this similarity (Table 2). Glucose is required for the growth of *Borrelia*. If glucose is deleted from the medium, growth ceases after several transfers. The role of glucose as a major substrate was confirmed with the glucose incorporation study. The utilization of uniformly labeled ^{14}C -glucose was similar for the Lyme disease spirochete and *B. hermsii*, with label appearing in all of the cell fractions. The relatively large amount of label in the lipid (ether-ethanol) fraction is due to its incorporation into the water-soluble backbone of the complex lipids but not into the fatty acid acyl chains of these lipids [11]. Fatty acids are required for the growth of the *Borrelia* [12] and they are incorporated unaltered into cellular lipids, suggesting the spirochete is incapable of either chain elongation of β -oxidation of long-chain fatty acids [11]. The incorporation of



FIG. 3. Scanning electron micrograph of the Lyme disease spirochete isolated from human spinal fluid. Note left-handed (counter-clockwise) coiling of cell (bar = 0.5 μm).

TABLE 2
Incorporation of Various Substrates by Lyme Disease Spirochetes and *Borrelia hermsii*

Labeled Compound	Distribution of Radioactivity in Cell Fractions: % of Total in Cell							
	Lyme Disease Spirochete				<i>Borrelia hermsii</i>			
	Cold PCA-Soluble	Ether Ethanol-Soluble	Hot PCA-Soluble	Residue (Protein)	Cold PCA-Soluble	Ether Ethanol-Soluble	Hot PCA-Soluble	Residue (Protein)
Glucose	9.6	65.1	22.1	4.2	2.6	74.8	20.7	1.9
Myristic acid	2.8	95.4	0.1	1.8	0.2	98.1	0.1	1.7
Palmitic acid	4.6	92.6	0.9	1.9	0.3	92.7	0.1	7.0
Alanine	11.5	7.9	0	80.6	0	0	0	100.0
Arginine	2.4	0	14.0	83.6	8.8	0.6	0	90.6
Aspartic acid	0	3.9	0	96.1	22.6	0	0	77.4
Glutamic acid	0	0	2.4	87.6	23.4	0	0	76.6
Glycine	5.3	0	12.1	82.6	0.5	0	0	99.5
Isoleucine	0	0	0	100.0	0	0	0	100.0
Leucine	11.1	0	0	88.9	0	0	0	100.0
Lysine	0	0	4.0	96.0	1.8	0	0	98.2
Phenylalanine	2.9	3.6	2.8	90.7	0	0	0	100.0
Proline	0	0	0	100.0	0	0	0	100.0
Serine	4.5	0	0	95.5	0.6	0	0	99.4
Threonine	6.2	0	0	93.8	2.0	0	0	98.0
Tyrosine	2.4	9.2	0	88.4	1.5	1.8	0	96.7
Valine	0	0	0	100.0	4.5	0	2.5	93.1

TABLE 3
Major Metabolic End Products of Spirochetes

<i>Borrelia hermsii</i> Lyme disease spirochete	Lactic acid
<i>I. dammini</i> isolate	Lactic acid
<i>Treponema</i>	Heterofermentive
<i>Leptospira</i>	Acetic acid

myristic and palmitic acids was almost entirely (93–98 percent) restricted to the lipid fraction of the Lyme disease spirochete and *B. hermsii*. Thus, these spirochetes are similar in their incorporation of fatty acids into cellular lipids and their inability to degrade these compounds. Amino acids do not serve as an energy source for the Lyme disease spirochete or *B. hermsii*. We found that 72–100 percent of the radioactivity of the 14 amino acids studied was incorporated into the cellular protein and that little or no label was present in the lipid or nucleic acid fractions (Table 2). The utilization of glucose as the major energy source, rather than amino acids, was confirmed by analyzing the metabolic end products of these spirochetes (Table 3). Both the Lyme disease spirochete and *B. hermsii* [12] are homofermentative with lactic acid as their metabolic end product. This is in contrast to the *Leptospira*, which produce acetic acid, and the *Treponema*, which are heterofermentative [13]. The oxygen requirements of the Lyme disease spirochetes also resemble those of the *Borrelia* as well as the *Leptospira*. Metronidazole, a drug that inhibits the growth of anaerobic organisms, did not interfere with the growth of the aerobic *Leptospira*, *Borrelia*, or the Lyme disease spirochete at concentrations as high as 100 $\mu\text{g/ml}$ (Table 4). In contrast, the growth of the anaerobic *Treponema* was inhibited by as little as 1 $\mu\text{g/ml}$ of the drug.

Lyme disease spirochetes were found to lack the hydrogen peroxide degrading enzymes, catalase. This observation explains why the Lyme disease spirochete (human spinal fluid isolate) is rapidly killed by polymorphonuclear neutrophils from patients with chronic granulomatous disease [14]. These neutrophils are defective in the production of hydrogen peroxide, which participates in the intracellular killing of microorganisms. Catalase-negative organisms are killed by these defective neutrophils because they produce the hydrogen peroxide required for the microbicidal event. *Borrelia* are also catalase-negative [12].

A summary of phenotypic characteristics of Lyme disease spirochetes, *Borrelia*, *Treponema*, and *Leptospira* is given in Table 5. On the basis of this information, the

TABLE 4
Metronidazole (100 $\mu\text{g/ml}$) Sensitivity
of Spirochetes

Organism	Growth
<i>Leptospira interrogans</i>	
Serovar <i>canicola</i>	+
<i>Treponema denticola</i>	–
<i>Borrelia hermsii</i>	+
Lyme disease spirochete	
<i>I. dammini</i> isolate	+

TABLE 5
Phenotypic Characteristics of Spirochetes

	Lyme Disease Spirochetes	<i>Borrelia</i>	<i>Treponema</i>	<i>Leptospira</i>
Cell diameter	0.18–0.25 μm	0.2–0.5 μm	0.1–0.4 μm	0.1 μm
Cell length	4–30 μm	3–20 μm	5–20 μm	4–20 μm
Number of periplasmic flagella per cell end	7	15–20	1–8	1
Overlapping of periplasmic flagella at cell center	Present	Present	Present	Present
Cytoplasmic tubules	Absent	Absent	Present	Absent
Diamino amino acid present in peptidoglycan	Ornithine	Ornithine	Ornithine	α,ϵ -diaminopemelic acid
Conditions for conversion to spherical forms	Hypotonic	Hypotonic	Hypotonic	Hypertonic
Oxygen requirements	Microaerophilic	Microaerophilic	Anaerobic	Aerobic
Catalase production	Absent	Absent	Absent	Present
Major carbon and energy source	Carbohydrate	Carbohydrate	Carbohydrate and/or amino acids	Long-chain fatty acids
Long-chain fatty acid requirement	Present	Present	Present	Present
Capacity to degrade long-chain fatty acids	Absent	Absent	Absent	Present
Metabolic end products	Lactic acid	Lactic acid	Several	Acetic acid
Tick-transmitted	+	+	–	–

Lyme disease spirochetes more closely resemble the *Borrelia* than the *Treponema* or *Leptospira*.

The most definitive means for identifying an organism and establishing its relationship to other organisms is by determining similarities in nuclear DNA nucleotides. The first step in this approach is analyzing the mole % guanine plus cytosine (G + C) of the DNA. A direct relationship exists between genome size and moles % G + C. As shown in Table 6, the mole % G + C of the three Lyme disease spirochetes' DNA ranges from 27.3 to 30.5, suggesting they are very similar and possess a small genome. The moles % G + C of the Lyme disease spirochetes are in the same range as the three North American borreliae but differ from the higher moles % G + C values of the *Treponema* and *Leptospira* (Table 6). The relationships among the spirochetes suggested by the mole % G + C were confirmed by DNA homology studies [15]. When radiolabeled DNA from the *I. dammini* spirochete was used as a probe, DNA homologies of 100 percent with the *I. ricinus* spirochete and 76 percent with the human spinal fluid isolate were observed (Table 7), indicating that these three spirochetes are of the same species. This is of interest, since the spirochetes represent isolates from the U.S. and Europe. In addition, the 37, 46, and 59 percent homology manifested with *B. parkeri*, *B. turicatae*, and *B. hermsii*, respectively, demonstrated that the Lyme disease spirochetes constituted a new species of *Borrelia* (Table 7). These results were verified using radiolabeled *B. hermsii* DNA as the probe (Table 8). Homologies at the species level were detected with the *Borrelia* and at the genus level were observed with the Lyme disease

TABLE 6
Moles % G + C of Spirochetal DNAs^a

Organism	Tm°C ^c	Moles % G + C ^b
<i>Ixodes dammini</i> spirochete	81.8°	30.5
<i>Ixodes ricinus</i> spirochete	80.9°	28.3
Human cerebrospinal fluid isolate	80.5°	27.3
<i>Borrelia hermsii</i>	81.8°	30.5
<i>Borrelia turicatae</i>	81.5°	29.8
<i>Borrelia parkeri</i>	80.8°	28.0
<i>Treponema vincentii</i>	88.5°	46.8
<i>Treponema scoliodontum</i>	84.0°	36.0
<i>Treponema pallidum</i> (Nichols)	83.5–91.0°	35–53
<i>Leptospira interrogans</i>	84–86°	35–40
<i>Leptospira biflexa</i>	85.3°	39

^aSee [15].

^bMoles % G + C was calculated from thermal denaturation studies using the formula: Moles % G + C = (Tm - 69.3) 2.44. Results are the average of three experiments.

^cTm°C = Temperature corresponding to the midpoint in the rise of optical density during the thermal denaturation of DNA

spirochetes (Table 8). As previously suggested by the mole % G + C data, no significant DNA homology was detected between the *I. dammini* spirochete or *B. hermsii* with the *Treponema* and *Leptospira* studied (Tables 7 and 8).

Plasmid DNA was detected in the three Lyme disease spirochetes and the three North American borreliae [15]. The *I. ricinus* spirochete, the human cerebrospinal fluid isolate, and the *Borrelia* contained a single plasmid, whereas the *I. dammini* spirochete contained two plasmids. Lyme disease in the U.S. may be accompanied by arthritis while in Europe the extracutaneous symptoms tend to be neurological. Since the *I. dammini* spirochete and the *I. ricinus* spirochete appear to be genetically homologous, the differences in extracutaneous symptoms observed may be plasmid-

TABLE 7
DNA Homologies Between Lyme Disease Spirochetes and Other Spirochetes^a
Probe: *Ixodes dammini* spirochete DNA

Organism	RBR ^b , 60°C
<i>Ixodes dammini</i> spirochete	100
<i>Ixodes ricinus</i> spirochete	100
Human cerebrospinal fluid isolate	76
<i>Borrelia hermsii</i>	59
<i>Borrelia turicatae</i>	46
<i>Borrelia parkeri</i>	37
<i>Treponema phagedenis</i>	2
<i>Treponema denticola</i>	2
<i>Leptospira interrogans</i> serovar <i>hardjo</i>	1
<i>Leptospira biflexa</i> serovar <i>patoc</i>	1

^aSee [15].

^bRBR = Relative binding ratio

$$RBR = \frac{100 \times \text{cpm heterologous DNA bound to filters} - \text{background cpm}}{\text{cpm homologous DNA bound to filters} - \text{background cpm}}$$

Results are the average of two experiments.

TABLE 8
DNA Homology Between *Borrelia hermsii* and Other Spirochetal DNAs*

Organism	RBR ^b , 60°C
<i>Borrelia hermsii</i>	100
<i>Borrelia turicatae</i>	86
<i>Borrelia parkeri</i>	77
<i>Ixodes dammini</i> spirochete	58
<i>Ixodes ricinus</i> spirochete	44
Human cerebrospinal fluid isolate	31
<i>Treponema denticola</i>	0
<i>Treponema vincentii</i>	0
<i>Treponema scoliodontum</i>	0
<i>Leptospira interrogans</i> serovar <i>hardjo</i>	0
<i>Leptospira interrogans</i> serovar <i>canicola</i>	0
<i>Leptospira biflexa</i> serovar <i>patoc</i>	0

*See [15].

^bRBR = Relative binding ratio

$$\text{RBR} = \frac{100 \times \text{cpm heterologous DNA bound to filters} - \text{background cpm}}{\text{cpm homologous DNA bound to filters} - \text{background cpm}}$$

Results are the average of two experiments.

mediated properties of these spirochetes. *Treponema pallidum* is the only other pathogenic spirochete reported to contain plasmids [16].

ADDENDUM

A number of Lyme disease spirochetes have been isolated from humans, animals, and ticks. Research on these spirochetes would be facilitated if investigators studied a few representative organisms whose history is well documented. In addition, these representative spirochetes should be placed in international depositories such as the American Type Culture Collection (ATCC). At this symposium five Lyme disease isolates were recommended as representative isolates. They are: the human spinal fluid, skin, and blood isolates reported by Steere et al. [2]; the *I. dammini* spirochete isolated by Burgdorfer et al. [17], and the *I. ricinus* spirochete isolated by Barbour et al. [10]. The *I. dammini* and *I. ricinus* spirochetes have been deposited at ATCC and assigned numbers 35210 and 35211, respectively. The three human isolates will be deposited shortly. Also, these spirochetes are available from our laboratory and from the Rocky Mountain Laboratories, Hamilton, Montana, for those that encounter difficulty in obtaining them from international depositories.

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