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Morphological and Ecological Characteristics of *Spirochaeta plicatilis*

R. P. Blakemore and E. Canale-Parola

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01002, U.S.A.

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Summary. Two types of large spirochetes, differing in size, and in coiling and motility patterns, were observed in sulfide-containing mud collected from a brackish marsh. The spirochetes measured 50—250 μm in length and 0.5—0.75 μm in diameter. One type was identified as *Spirochaeta plicatilis* Ehrenberg, inasmuch as its general characteristics corresponded to those described for this species by other authors. The second type of large spirochete was designated "large spirochete type B". Both types possessed ultrastructural features typical of spirochetes, e.g. outer sheath, axial fibrils and protoplasmic cylinder. The axial fibrils were arranged in a bundle and, in type B spirochetes, were inserted in a row near each end of the cell. The outermost layer of type B cells, interpreted to be the outer sheath or a main component of it, appeared to consist of an array of fibril-like elements which transversely circumscribed the cell. Transverse septa were observed in *S. plicatilis*, but not in type B spirochetes. In the mud samples both types of spirochetes were present near the mud-water interface within mats of sulfur-containing *Beggiatoa* trichomes, together with a variety of other microorganisms. A striking increase in the number of large spirochetes consistently occurred during lysis of the *Beggiatoa* trichomes, at low levels of H_2S. The large spirochetes were attracted to the lysing trichomes and grouped about them. Under strictly anaerobic conditions *S. plicatilis* remained motile for 8 days, and type B for 25 days. Experiments involving the use of chemical and biological indicators showed that *S. plicatilis* is either an anaerobe which can tolerate low O_2 tensions, or a microaerophile able to metabolize and possibly grow in the absence of O_2. Type B spirochetes behaved as strict anaerobes.

Spirochaeta plicatilis was first described in 1835 by Ehrenberg who noted the coiled shape, the exceptionally large size, and vigorous motility of this bacterium (Ehrenberg, 1835, 1838). Despite this early description, the organism has never been isolated in pure culture. Zuelzer (1911) reported the presence of *S. plicatilis* in fresh-water and marine, sulfide-containing environments where it occurred together with *Lamprocystis*, *Beggiatoa*, *Oscillatoria*, and other microorganisms. The latter author examined *S. plicatilis* by light microscopy and extended Ehrenberg's morphological observations. Dyar (1947) isolated an organism which was identified as *S. plicatilis*, but later was shown to be a species of *Saprospira* (Lewin, 1962; Starr and Skerman, 1965). In spite of the scarce information available on *S. plicatilis*, this organism is recognized as the

type species of the genus *Spirochaeta* (Breed *et al.*, 1957) in accordance with the International Code of Nomenclature of Bacteria (1966), a situation which generates considerable taxonomic uncertainty with regard to other spirochetes presently assigned to this genus (Hespell and Canale-Parola, 1970b).

Spirochetes are defined as helical or wavy bacteria possessing axial fibrils inserted near the ends of the cells. The axial fibrils are wound together with the protoplasmic cylinder which consists of the cytoplasmic and nuclear regions bound by the cell membrane and cell wall. The protoplasmic cylinder, as well as the axial fibrils, are enclosed by a multilayered membrane called "outer sheath" or "outer cell envelope". Except for the coiled shape, none of the above-mentioned structural features had been conclusively demonstrated in *S. plicatilis* at the time the present investigation was initiated. Thus, it was not apparent whether the type species of the genus *Spirochaeta* conformed to the modern definition of a spirochete.

We have investigated various aspects of the fine structure, ecology and physiology of *S. plicatilis*. The purpose of our work was to acquire information that would provide us with a greater understanding of the biology of this unusual bacterium, and would help in clarifying the taxonomic status of *S. plicatilis* and other species of *Spirochaeta*. Since our attempts at isolating *S. plicatilis* in pure culture have not yet been successful, the investigation was conducted by studying this organism as it occurred in enrichments and in natural environments.

Materials and Methods

Collection and Maintenance of Mud Samples. Black, sulfide-rich mud containing large spirochetes was collected at Woods Hole, Massachusetts, from a marshy area subject to regular tidal flooding from a sea-water pond. A small fresh-water stream, which reversed its flow at high tide, contributed water to the marsh. The mud samples were kept at room temperature for up to two years, in covered glass jars. Shortly after collection, each jar contained approximately 500—1000 ml of mud under 3—5 cm of marsh water. Although during storage the mud surface was always covered by a layer of water, the amount of liquid above the mud decreased due to evaporation, and, consequently, the salinity of the samples increased. As described below, large spirochetes developed in relatively large numbers in these mud "enrichments".

Sealed Wet Mount Preparations. In sealed wet mount preparations containing 0.05 ml of mud enrichment material, the large spirochetes increased in number during incubation (1—5 weeks) at room temperature. The wet mount enrichments were sealed with paraffin (1 v melted paraffin wax: 3 v paraffin oil).

Light Microscopy. Cell numbers were estimated by direct microscopic (phase-contrast) count at 120× magnification. Photomicrographs were taken through a Zeiss GFL phase-contrast microscope equipped with a Leitz Mikas camera attachment and series M1 Leica camera body. Kodak Plus-X or Tri-X film (35 mm) was used.

Electron Microscopy. The large spirochetes were physically separated from the mud of enrichments prior to placing them on electron microscope grids (formvar-coated, 100 mesh, carbon-reinforced copper). Separation of type B spirochetes (see Results) was accomplished by suspending enrichment material from sealed wet mount preparations in 2—3 ml of 0.01 M potassium phosphate buffer, pH 7. To fix the cells 0.1 ml of 2% (v/v) glutaraldehyde was added. After allowing the coarse debris to sediment, the supernatant liquid was centrifuged ($8{,}000 \times g$, 15 min) and the pellet transferred onto grids. *S. plicatilis* cells (see Results), which readily adhered to glass surfaces, were allowed to attach to glass slides by placing drops of mud from "enrichments" into the depressions of agglutination slides (one drop/depression), and incubating for 5 min with intermittent agitation. After the debris was gently washed off the slides with distilled water or 0.01 M potassium phosphate buffer (pH 7.4), the spirochetes which remained attached to the glass were individually transposed to electron microscope grids by means of a pipette with a fine (200 μm dia.) tip. Fixation, when used, was accomplished by adding 0.1 ml of 2% glutaraldehyde (in sea water) to enrichment material in each slide depression. After 20 min, the fixative was removed by washing with distilled water.

The location of the large spirochetes on the grids was determined by phase-contrast microscopy. The spirochetes on the grids were stained with 0.25% (w/v) phosphotungstic acid (pH 6.8) or with 1% (w/v) ammonium molybdate in 2% (w/v) ammonium acetate buffer (pH 5.5) by conventional procedures (Canale-Parola *et al.*, 1967). Specimens were examined in a Phillips EM 200 electron microscope equipped with a 30 μm objective aperture and operating at 60 kv. Images were recorded on 35 mm Kodak Fine Grain Release Positive film.

Ecological and Physiological Studies. Salinities were determined as per cent NaCl using a Model PM-4 Barnstead Purity Meter.

Sulfide was assayed colorimetrically by the method of Fogo and Popowsky (1949), or detected by means of lead acetate test strips.

All incubations were at room temperature.

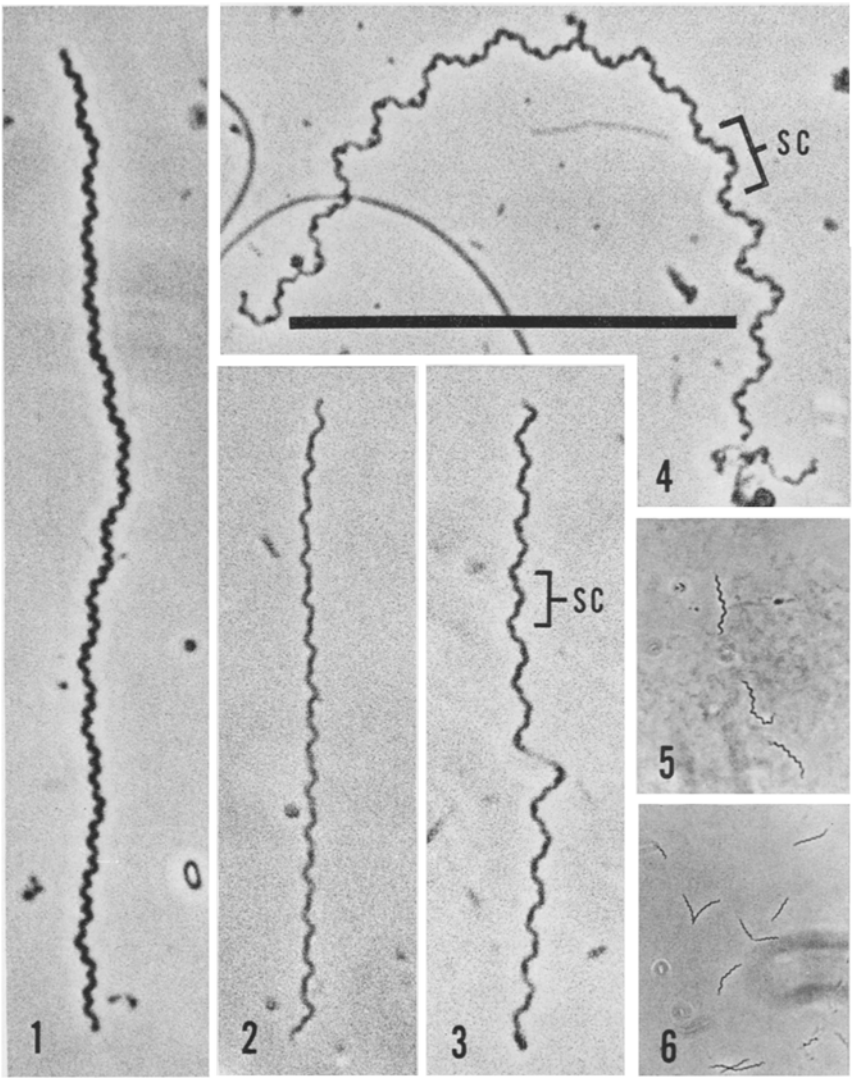
Methylene blue and resazurin (4.6×10^{-2} to 7.5×10^{-2} M, final conc.) were added to enrichment material sealed with paraffin in wet mount preparations. In these preparations a 2×22 mm filter paper strip was placed between the slide and coverslip in order to observe the rate and extent of dye reduction.

Results

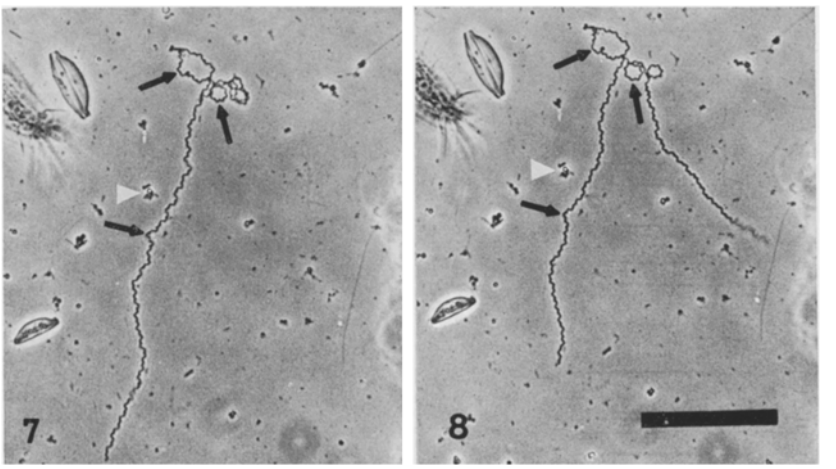
Morphology and Motility

Two types of large spirochetes, differing in morphology and other characteristics, were observed in the mud samples. Because the two types were infrequently observed in the same mud sample, it was possible to study each type in the absence of the other. As discussed below, one of the two types of large spirochetes exhibited characteristics identical to those described by Ehrenberg (1835, 1838) and Zuelzer (1911) for *Spirochaeta plicatilis*, and henceforth will be referred to as *S. plicatilis*. The second type was designated "large spirochete type B".

S. plicatilis (Figs. 1 and 4) generally ranged in length from 80 to 250 μm and was 0.75 μm thick. Large spirochete type B (Figs. 2 and 3) was shorter (50—150 μm in length) and thinner (approximately 0.5 μm in dia.) than *S. plicatilis*.



Figs. 1—6. Phase-contrast photomicrographs of living spirochetes. Wet mount preparations. *S. plicatilis* in extended position (Fig. 1) is longer and thicker than type B spirochetes (Fig. 2). Type B spirochetes (Fig. 3) and *S. plicatilis* (Fig. 4) often contract to form secondary coils (*SC*). Periodic swellings along the length of the cell (Figs. 3 and 4) are optical artifacts. The size of the large spirochetes is compared with that of other *Spirochaetales* such as *S. litoralis* (Fig. 5) and *Treponema denticola* (Fig. 6). Bar, 100 nm. Figs. 1—6 are at the same magnification

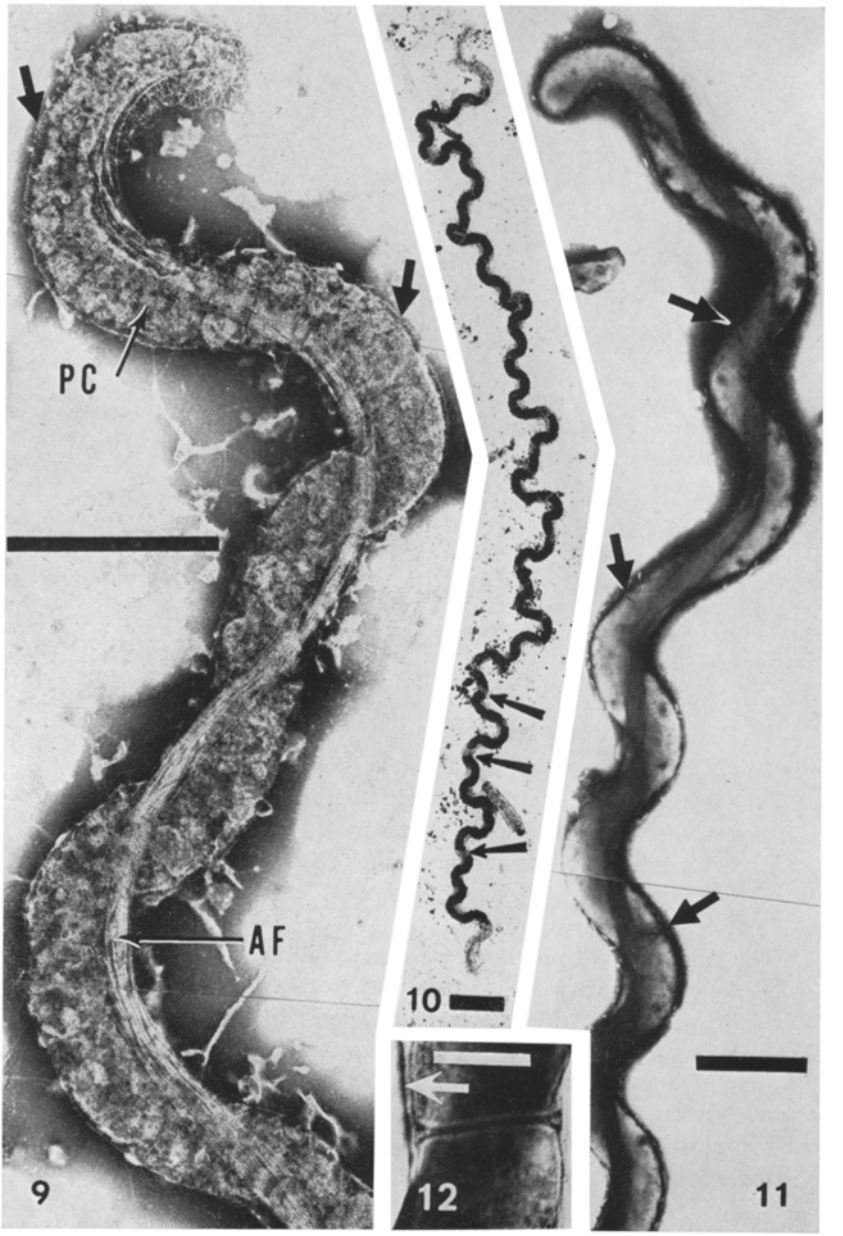


Figs. 7 and 8. Phase-contrast photomicrographs of *S. plicatilis*. Wet mount preparation. The photographs were recorded several seconds apart and illustrate "creeping" translation of a spirochete in contact with a glass surface. Black arrows indicate reference points to show that, despite translation, the cell has maintained a fixed pattern of coils. The absence of slippage is evidenced by relating some point on the cell to an external marker (white arrowhead). Although the forward-reverse direction of translation alternated every few seconds, a net displacement of the cell toward the right has occurred. Bar, 50 μm

The coiling of type B spirochetes was looser than that of *S. plicatilis* and the cells had shallow primary coils within deeper regular secondary coils even when in an extended (uncontracted) position (Fig. 2).

Both types were conspicuously larger than other known spirochetes (Figs. 1—6), with the possible exception of *Cristispira* which is comparable in size.

The two types of spirochetes translated bi-directionally and displayed secondary coils (Figs. 3 and 4) resulting from contraction of the cells. *S. plicatilis* was often observed to translate in a "creeping" fashion upon solid surfaces. This pattern of motility is illustrated in Figs. 7 and 8. Although the spirochete shown in these figures had translated for approximately 30 μm upon the surface of the coverslip during the few seconds elapsed between photographs, its coiling pattern remained virtually unchanged. The spirochete moved forward through a fixed pattern of coils, essentially without slippage. [Slippage is defined by Jahn and Landman (1965) as the failure of the rear coils to follow the path of the anterior end.] Although *S. plicatilis* cells frequently moved in this "creeping" fashion, they also translated without being in contact with solid surfaces. During the latter type of motility the cells displayed



Figs. 9—12

rapid rotational movements and wide waves travelled along the length of the organism. In addition, cells of *S. plicatilis* exhibited a diversity of flexing, looping and darting movements.

Type B organisms were also vigorously motile and flexible. Nonetheless, their motility pattern differed from that of *S. plicatilis*. Characteristically, type B cells assumed a continuous spinning motion which resulted in an alternating forward and reverse translation according to the direction of rotation. During vigorous rotational movements, the ends of these cells assumed a hooked or curled shape which was lost as the cells decreased the rate of spin. Spirochetes of type B did not adhere to glass surfaces for more than a few seconds at a time, but translated, rotated and flexed freely in the liquid environment.

The longest *S. plicatilis* specimens were recovered from enrichments with declining populations of large spirochetes. When long (250 μm), motile specimens of this type were touched with the tip of glass microneedles, they suddenly fragmented into four or more units. Each of these units retained full motility and normal coiling.

Ultrastructure

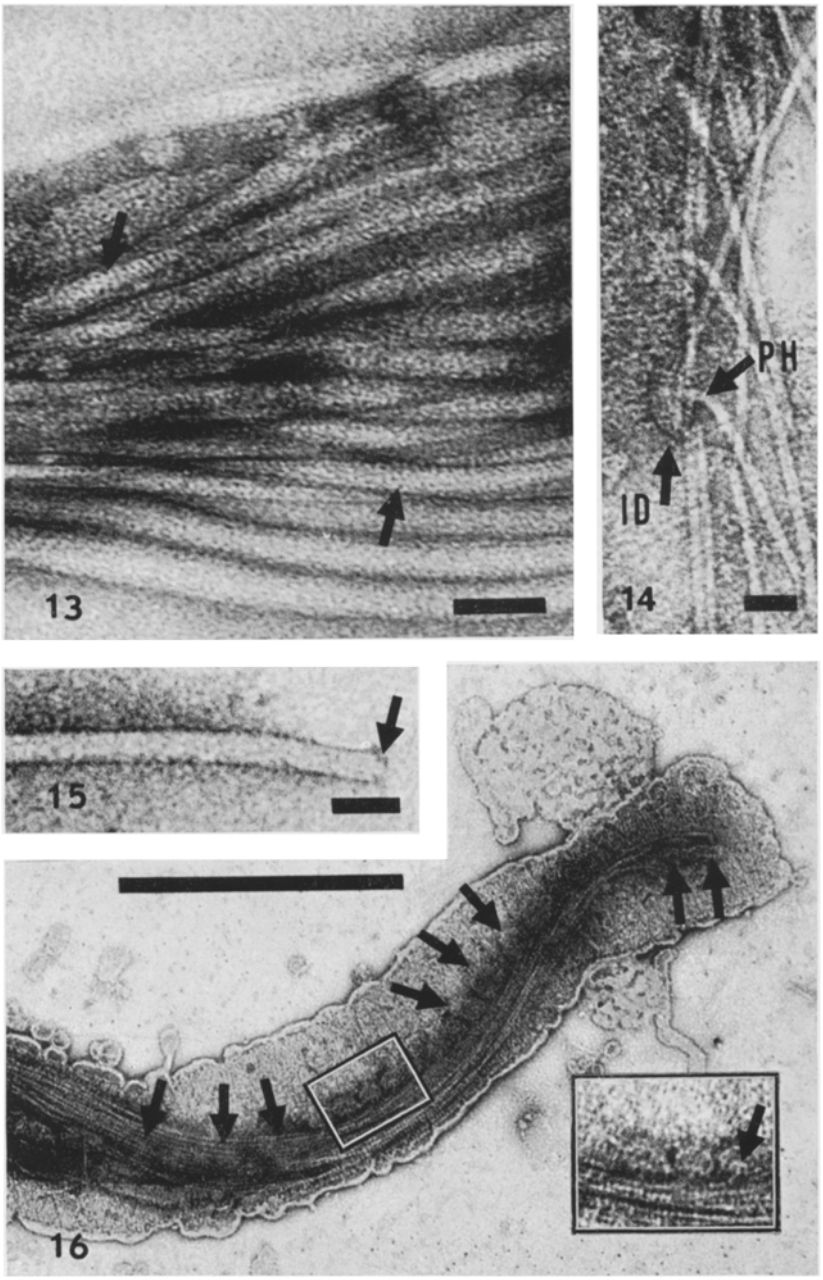
S. plicatilis and type B cells possessed ultrastructural features typical of spirochetes, e.g., outer sheath, axial fibrils and protoplasmic cylinder (Figs. 9—12). The axial fibrils were present as a bundle winding about the coiled protoplasmic cylinder (Figs. 9—11, 19). However, the two types of spirochetes differed with regard to the insertion pattern of their axial fibrils. The insertion points of type B cells were arranged in

Fig. 9. Electron micrograph of one end of a spirochete of type B. The bundle of axial fibrils (*AF*) winds about the protoplasmic cylinder (*PC*). Both of these structures are enclosed by an outer sheath of transversely arranged fibrils (unlabeled arrows). Unfixed cell, negatively stained with phosphotungstate. Bar, 1 μm

Fig. 10. Electron micrograph of a type B spirochete. A bundle of axial fibrils (arrows) is wound about the cell. The cell has retained its basic coiling pattern (see Figs. 2 and 3) consisting of secondary coils superimposed upon primary coils. The cell ends are less electron dense than the remainder of the cell, probably because the cytoplasm has contracted upon drying. Unfixed cell, stained with phosphotungstate. Bar, 2 μm

Fig. 11. Electron micrograph of one end of a *S. plicatilis* cell. Arrows indicate locations of septa which were observed at evenly spaced intervals along the length of cells of this type. Glutaraldehyde fixed cell, washed with buffer, negatively stained with phosphotungstate. Bar, 2 μm

Fig. 12. Electron micrograph of septum region in a *S. plicatilis* cell treated as described in the legend of Fig. 11. The white arrow points to the outer sheath. Bar, 0.5 μm



Figs. 13—16

a single row near each end of the organism (Fig. 16). As many as 30 axial fibril insertions were observed at each end of type B cells. In type B organisms, the ends of the cells often appeared less electron-opaque than the cytoplasm which seemed to have contracted away from the cell ends (Fig. 10). This permitted closer observation of the axial fibril insertion points, as compared to *S. plicatilis* cells in which the cell ends apparently remained intact (Fig. 11).

In contrast to axial fibrils of type B cells, the axial fibrils of *S. plicatilis* appeared to insert in subterminal clusters. Detailed fine structure of this region, however, was not resolved by the techniques of electron microscopy employed. *S. plicatilis* cells apparently contained fewer axial fibrils than type B cells, probably from 18—20 inserted at each end of the organism.

The axial fibrils of *S. plicatilis* and of type B organisms were similar in ultrastructure and size to those of other spirochetes (Holt and Canale-Parola, 1968; Jackson and Black, 1971; Joseph and Canale-Parola, 1972; Nauman *et al.*, 1969). The fibrils possessed a proximal hook associated with an insertion disc(s) (Fig. 14 and inset of Fig. 16). Broken fibrils occasionally displayed a core (10 nm in dia.) which protruded from a non-striated sheath (Fig. 15). The fibrils measured from 15—20 nm in diameter and showed a granular substructure (Fig. 13). Striated tubular structures (Holt and Canale-Parola, 1968; Jackson and Black, 1971; Joseph and Canale-Parola, 1972; Nauman *et al.*, 1969) were not observed. The maximum diameter of the axial fibril bundle ranged between 0.13 and 0.18 μm depending upon the cell type and preparative treatment.

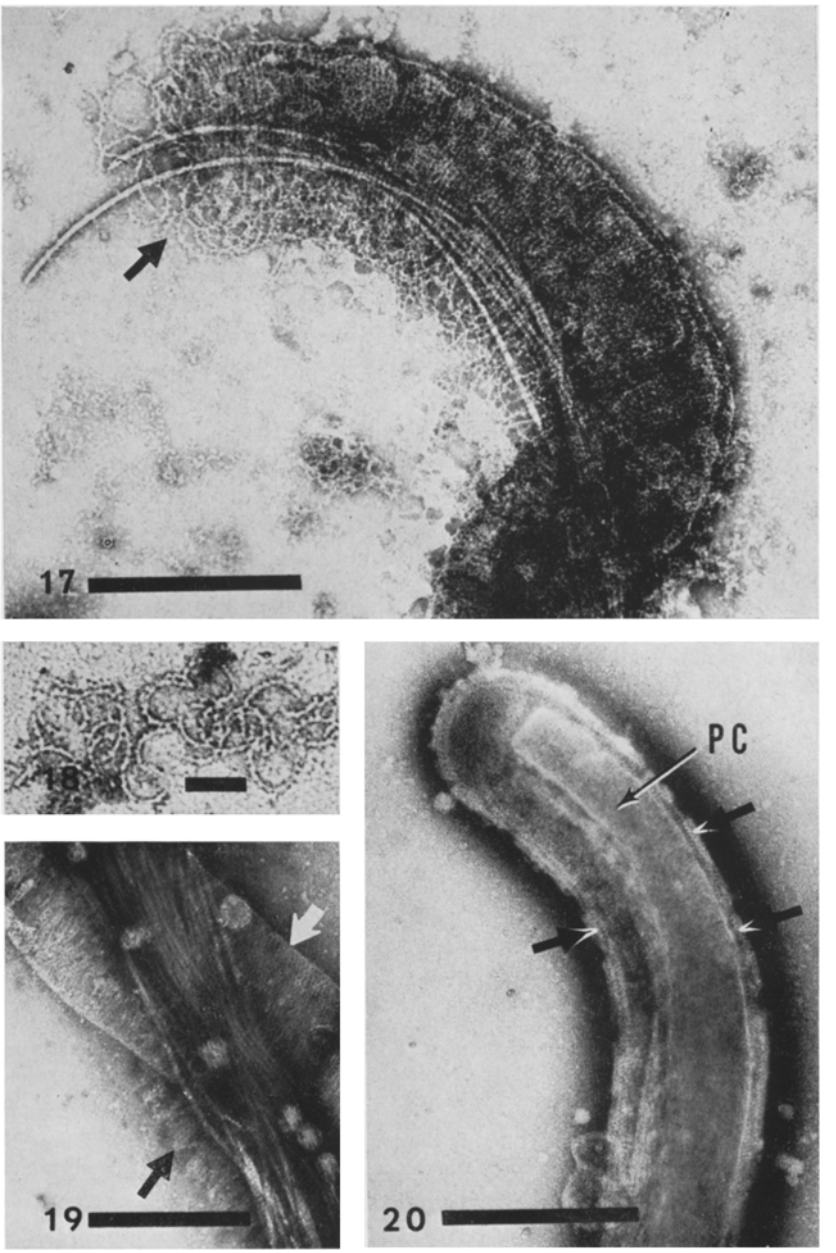
The outer sheath of *S. plicatilis* cells showed no readily apparent substructure, and behaved similarly to that of other spirochetes with

Fig. 13. Electron micrograph of a portion of an axial fibril bundle in *S. plicatilis*. Note the granular substructure (arrows). Unfixed cell, osmotically shocked with 0.2 M sucrose. Negatively stained with ammonium molybdate. Bar, 50 nm

Fig. 14. Electron micrograph of proximal hooks (*PH*) and disc-like insertion structures (*ID*) of axial fibrils in a type B spirochete. Unfixed cell washed with distilled water, negatively stained with ammonium molybdate. Bar, 100 nm

Fig. 15. Electron micrograph showing the broken end of an axial fibril from *S. plicatilis*. The core (arrow) protrudes from the non-striated sheath. Treated and stained as the preparation in Fig. 13. Bar, 50 nm

Fig. 16. Electron micrograph of one end of a type B spirochete. The insertion points of the axial fibrils are arranged in a row. The arrows indicate some of the insertion points. The inset is an enlargement (1.7 ×) of the area enclosed by the rectangle. The arrow within the inset points to an insertion disc. Unfixed cell, negatively stained with ammonium molybdate. Bar, 1 μm



Figs. 17—20

respect to its lack of stability during negative staining (Holt and Canale-Parola, 1968).

The outermost layer of type B cells, which we interpret to be the outer sheath or a main component of it, differed noticeably from the outer sheath of other spirochetes whose ultrastructure has been studied. In unfixed, stained or unstained cells this layer had become partially disrupted and appeared to consist of an array of fibril-like elements which transversely circumscribed the cell and surrounded both the axial fibrils and the protoplasmic cylinder (Figs. 17—19). The fibril-like elements were most noticeable at the ends of the cell, where they had become disarranged, forming loops and coils (Fig. 9). In addition to the fibrillar array, the intact outer sheath may include other elements which might have been destroyed in preparation. In fixed, negatively stained cells the outer sheath apparently retained its integrity, but the fibrillar array was still discernible (Fig. 20).

Regularly spaced transverse septa were observed throughout the length of *S. plicatilis* cells. These partitions were seen in fixed or unfixed cells which were subsequently washed and negatively stained (Figs. 11 and 12). Septa were not seen in similarly treated type B cells.

Ecological and Physiological Observations

Mats of *Beggiatoa* trichomes developed near the mud-water interface of many of the mud samples during the first 2 or 3 weeks after collection. Most of these sulfur-containing trichomes measured from 12—20 μm in diameter, a size range indicative of *B. arachnoidea* and *B. mirabilis*. Purple sulfur bacteria, *Thiospira*, *Oscillatoria*, *Spirulina*, diatoms, small

Fig. 17. Electron micrograph of part of a type B spirochete. Disruption of the cell caused by preparative procedures reveals the fibrillar nature of the outer sheath (arrow). Unfixed cell, negatively stained with phosphotungstate. Bar, 0.5 μm

Fig. 18. Electron micrograph of fibrillar elements of the outer sheath which have been displaced from a type B spirochete treated as described in the legend of Fig. 17. Note the smooth concave surface and convoluted convex surface of the coils. Bar, 100 nm

Fig. 19. Electron micrograph of a portion of a type B spirochete near one of its ends. The fibrillar elements of the outer sheath form a tight array (white arrow) which has become partially disrupted (black arrow). The bundle of axial fibrils is visible. Unstained cell. Bar, 0.5 μm

Fig. 20. Electron micrograph of one end of a type B spirochete fixed with glutaraldehyde. The outer sheath appears to be intact. Portions of the fibrillar array are visible (unlabeled arrows). *PC* protoplasmic cylinder or cytoplasm. Compare to the unfixed cell in Fig. 17. Stained with ammonium molybdate. Bar, 0.5 μm

spirochetes and a variety of other microorganisms were also present. *S. plicatilis* or spirochetes of type B were occasionally found in samplings of the mud surface during this early stage of an ecological succession occurring in the mud samples. The large spirochetes were most commonly present within tangled masses of *Beggiatoa* trichomes, and sometimes were seen "creeping" or otherwise moving on the surface of trichomes. After 14—20 additional weeks the supply of sulfide generated by biological activity in the mud had become low, as indicated by the disappearance of most of the sulfur inclusions in the *Beggiatoa* trichomes and in the purple sulfur bacteria. Gradual lysis of the *Beggiatoa* trichomes and, at the same time, a striking increase in the number of large spirochetes were observed microscopically. Maximum numbers of either *S. plicatilis* or spirochetes of type B (approximately 10^3 cells/ml surface mud) were present during the early stages of *Beggiatoa* lysis (Fig. 21). When maximum numbers of large spirochetes were present, the supernatant liquid above the mud generally had a pH between 7 and 8, and contained less than 5 mg per liter of soluble sulfide and between 2.7 and 3.7% NaCl. Eventually the uppermost layer of the mud changed from a black to a rusty color, suggesting the occurrence of surface oxidation. The above-mentioned observations showed that it was possible to enrich for the large spirochetes simply by allowing the mud to undergo a prolonged ecological succession. Enrichments in which mats of *Beggiatoa* trichomes did not develop never yielded large numbers of *S. plicatilis* or of type B spirochetes.

After the lysis of most of the *Beggiatoa* trichomes, the number of large spirochetes in the mud enrichments had drastically decreased. A second bloom of *Beggiatoa* trichomes could be obtained upon continued incubation of the mud enrichments, after replacing the supernatant liquid with aged sea water through which H_2S was bubbled for 10—15 sec. During the lysis and disappearance of this second *Beggiatoa* population,

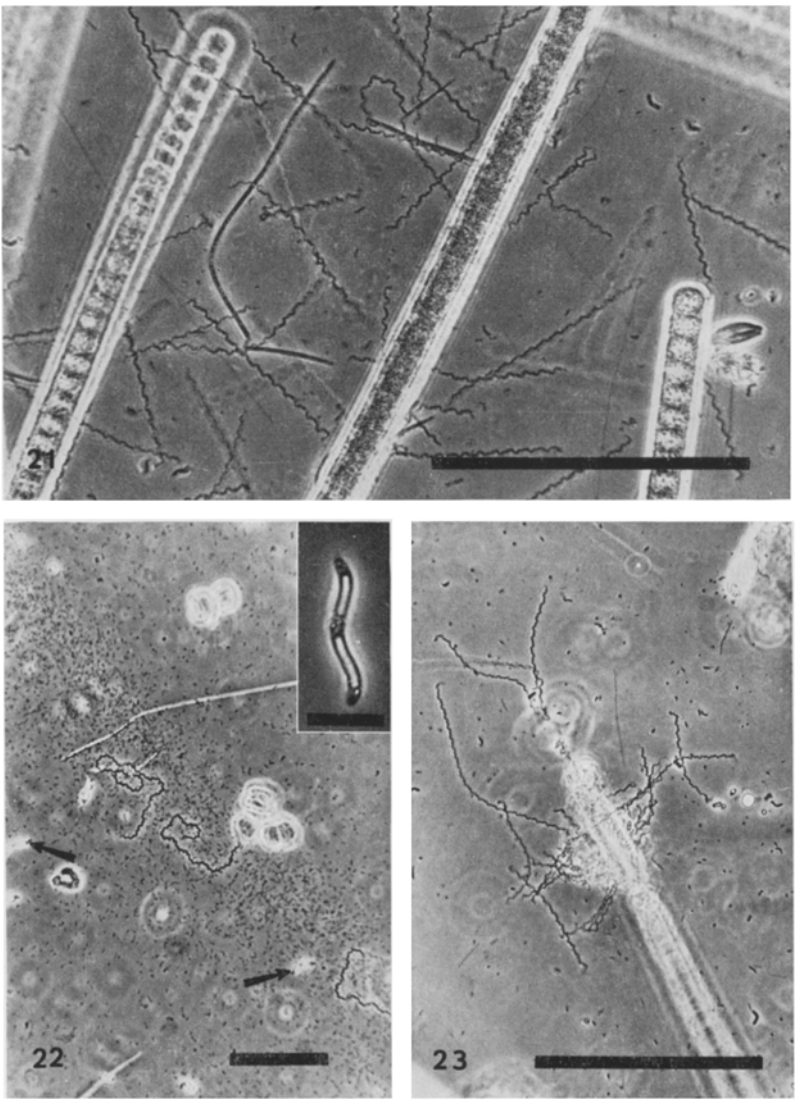
Fig. 21. Phase-contrast photomicrograph of type B spirochetes together with *Beggiatoa* trichomes. Wet mount preparation. Bar, 100 μm

Fig. 22. Phase-contrast photomicrograph of *S. plicatilis* associated with a band of microaerophilic vibrios and spirilla. The band runs diagonally from upper left to lower right in the figure. Arrows point to, and inset shows the unusual colorless sulfur bacterium *Thiospira bipunctata (Spirillum bipunctatum)* (Molisch, 1912), which was common is *S. plicatilis* enrichments. Paraffin-sealed wet mount preparation incubated in the dark, in air. Bars, 50 μm (figure), 10 μm (inset)

Fig. 23. Phase-contrast photomicrograph of type B spirochetes which have aggregated about a lysing *Beggiatoa* trichome. Paraffin-sealed wet mount preparation incubated in the dark. Bar, 100 μm

relatively large numbers of *S. plicatilis* or type B spirochetes were again observed in the mud.

The apparent association of large spirochetes with *Beggiatoa* was confirmed by observing the latter two kinds of organisms in paraffin-sealed wet mount preparations. During prolonged (1—5 weeks) incuba-



Figs. 21—23

tion of these preparations, containing spirochetes of type B and *Beggiatoa* trichomes from mud enrichments, the trichomes eventually showed a progressive lysis, often beginning at one end of the filament. The large spirochetes were attracted to these lysing areas and grouped about them (Fig. 23). The number of large spirochetes increased dramatically as the trichomes lysed. This type of enrichment was used to obtain spirochetes of type B for electron microscopy.

In paraffin-sealed wet mount preparations of material from the mud enrichments, a band of microaerophilic vibrios and spirilla frequently developed near the periphery of the coverslip several mm from its edge. In these preparations, decolorization of redox indicators took place in the central regions (within 4 h with methylene blue and 12 h with resazurin), but not in the areas near the edge of the coverslip. This indicated that O_2 diffused through the paraffin seal and that an O_2 gradient was present in the liquid beneath the coverslip. Although cells of *S. plicatilis* were present in the central regions of the preparations, and most often in the band of microaerophilic bacteria (Fig. 22), they avoided the areas of higher redox potential external to the band and near the edge of the coverslip. Spirochetes of type B were observed in the central regions, but generally not in the band or in the peripheral regions outside the band. Washed cells of the obligately anaerobic *Spirochaeta litoralis* (Hespell and Canale-Parola, 1970b), when added to the paraffin-sealed wet mount preparations, behaved in a manner similar to spirochetes of type B.

When the paraffin-sealed wet mount preparations were stored in the dark under strictly anaerobic conditions (Bray dishes, or gas displacement jars), the band of microaerophilic bacteria did not form and the large spirochetes were distributed randomly in the liquid under the coverslip. Under these anaerobic conditions *S. plicatilis* cells remained motile for 8 days, and type B spirochetes for up to 25 days. When similar preparations were stored in air, in the dark, both types of spirochetes displayed motility for periods up to 2 months.

Discussion

The overall morphology, the size range, and the flexing and creeping movements of the *S. plicatilis* cells present in our mud samples were identical with those described for this organism by Ehrenberg (1835, 1838) and Zuelzer (1911). Zuelzer's drawings, depicting the varied shapes of *S. plicatilis* cells during movement as well as their primary and secondary coiling (Zuelzer, 1911, Textfigs. 1 and 2), faithfully illustrate these same characteristics in the *S. plicatilis* cells we observed. Furthermore, the large spirochetes we describe in this report were present, like

the *S. plicatilis* cells studied by Zuelzer (1911), in H_2S-containing natural environments and in association with *Beggiatoa* trichomes. Finally, it is interesting to note that Zuelzer (1911) found that long specimens of *S. plicatilis*, when subjected to slight pressure, fragmented into units. As previously mentioned, we detected the same behavior in *S. plicatilis* present in our mud samples. In conclusion, the close correspondence in characteristics indicates that Ehrenberg's and Zuelzer's *S. plicatilis* and the large spirochetes we observed, and to which we have referred by the latter name, are the same organism.

A spirochete slightly thinner (0.5 μm) and somewhat shorter (up to 200 μm) than *S. plicatilis* was observed by Zuelzer (1911) in a marine environment. Zuelzer regarded the thinner spirochete as a subspecies of *S. plicatilis* and named it *S. plicatilis marina*, even though she expressed hesitation in using the environment in which she found it and slight morphological differences as taxonomic criteria. Type B spirochetes resemble *S. plicatilis marina* in size and overall morphology, and may indeed be the same organism.

The work reported in this paper demonstrates that *S. plicatilis* possesses ultrastructural features typical of spirochetes, e.g., axial fibrils wound around a coiled protoplasmic cylinder, and an outer sheath. Attempts to show the presence of axial fibrils in *S. plicatilis* by light microscopy of stained preparations have been reported (Zuelzer, 1911). However, results obtained by the latter experimental approach are not conclusive since the procedure causes appearance of artifacts interpretable as axial fibrils in helical bacteria which do not possess these structures, e.g., *Spirillum*, *Saprospira* (Dyar, 1947). We did not detect axial fibrils in living cells of *S. plicatilis* examined by bright-field, phase-contrast, dark-field, or Nomarski differential interference-contrast microscopy.

The presence of cross walls in *S. plicatilis* may explain the observation that long specimens of these organisms, when touched with a glass needle, fragment into motile units. Possibly, as the spirochetes grow in length, cross walls are formed and, in later growth stages, axial fibrils develop on either side of each cross wall. The outer sheath is probably the last to divide, as has been observed in other spirochetes (Listgarten and Socransky, 1964). A mechanical disturbance may cause these chains of spirochetes to fragment suddenly into individual cells. On the other hand, it is equally possible that *S. plicatilis* grows as a multicellular spirochete, with axial fibrils inserted only at one end of each terminal cell. The long specimens observed by light microscopy may consist of chains of these multicellular spirochetes, and each unit formed upon fragmentation may be a multicellular spirochete.

The association of *S. plicatilis* with *Beggiatoa* trichomes is interpretable in terms of a chemotactic response of the large spirochetes toward lytic materials and/or metabolic products released by the sulfur bacteria. The spirochetes may utilize these materials as substrates for growth. H_2S-containing environments are favorable for the development of anaerobic free-living spirochetes (Canale-Parola *et al.*, 1968; Hespell and Canale-Parola, 1970b). The natural occurrence of *Beggiatoa* in these environments probably provides the spirochetes with a continuous source of nutrients. Anaerobic respiration resulting in reduction of sulfate to sulfide has not been detected in spirochetes that were tested (Hespell and Canale-Parola, 1970a).

Both *S. plicatilis* and type B spirochetes retained their motility for many days under anaerobic conditions. However, this does not necessarily indicate that the large spirochetes are able to grow in the absence of O_2.

S. plicatilis cells were attracted to bands of microaerophilic bacteria and to the anaerobic regions of sealed wet mount preparations. Their association with the bands may be a result of taxis toward low O_2 tensions, or of chemotaxis toward lytic or metabolic products released by the amassed microaerophiles. Apparently *S. plicatilis* is either an anaerobe which can tolerate low O_2 tensions, or a microaerophile which is also able to metabolize and possibly grow in the absence of O_2.

Type B spirochetes remained motile for as long as 25 days in the absence of O_2. Their response to O_2 gradients, which was identical to that of the strictly anaerobic *S. litoralis*, indicates that type B spirochetes are anaerobes.

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Dr. E. Canale-Parola
Department of Microbiology
University of Massachusetts
Amherst, Massachusetts 01002, U.S.A.