HELICAL FINE STRUCTURE OF FLAGELLA OF A MOTILE DIPHTHEROID

MORTIMER P. STARR AND ROBLEY C. WILLIAMS

University of California: Department of Bacteriology, Davis, California; and Virus Laboratory, Berkeley, California

Received for publication October 30, 1951

Studies on the nature of the bacterial flagella have received considerable impetus from the hypothesis advanced by Pijper (1947) that flagella are not the locomotor organs of bacteria, but are merely "mucous twirls" trailing from the cell surface. A considerable body of criticism has arisen (Houwink and van Iterson, 1950) challenging Pijper's viewpoint. As a result, numerous contributions concerning flagellar chemistry, physics, and morphology have been presented (Weibull, 1950) which, although not entirely sufficient to resolve the basic controversy, have advanced our knowledge concerning flagella. The observations reported here are presented with no intention of deciding between the two views, but only as a noncommittal contribution to the subject of flagellar fine structure.

PRESENT KNOWLEDGE ON FINE STRUCTURE OF FLAGELLA

Very little appears to be known about the fine structure of bacterial flagella. In view of the small width of flagella, demonstration of a possible fine structure requires the use of electron microscopy at higher than average resolution. Houwink and van Iterson (1950) state that in their "best flagella pictures faint indications are to be found for a cross structure in these organs"; however, these workers are not certain of the reality of this fine structure. Knaysi (1951) refers to flagella as "structureless fibers". De Robertis and Franchi (1951) claim to have demonstrated a helical structure in the flagella of Bacillus brevis when trypsin digested, but their published micrographs seem to show principally a nodular structure on the flagellar surface. In some cases there appears to be an intimation of helical structure, but the quality of the micrographs as reproduced is not sufficiently high to preclude the possibility that this apparent structure had been introduced by imperfect microscopic imagery.

MATERIAL STUDIED

Our interest in this subject stems from an examination of various bacteria, which, while otherwise similar to the ordinarily nonmotile corynebacteria, are motile and possess flagella. Because the arrangement of these flagella might shed some light on the anomalous taxonomic position of these microbes (Starr and Pirone, 1942; Clark and Carr, 1951), routine electron micrographs of these organisms were prepared. In the case of one of these bacteria, a helical fine structure of the flagella was noted, which led to a more detailed electron microscopic examination. The culture used is an undescribed motile diphtheroid,
Figure 1. Low magnification micrograph of some Congo diphtheroid bacteria and associated flagella. The magnification is too low to permit any detail of flagellar structure to be seen. X 10,000.

Figure 2. Portion of a “ghost” of a Congo diphtheroid bacterium, and some adjacent flagella. The long flagellum at right angles to the edge of the bacterium shows a fine helical structure. The flagellum parallel to the edge of the bacterium is oriented to the direction of shadowing in a way such as to obscure the helical structure. X 50,000.
Figure 3. High magnification micrographs exhibiting the helical fine structure of flagellar material of the Congo diphtheroid bacterium. The structure is that of a left-handed, triple-threaded helix, with a diameter of 19 mµ and an axial periodicity of 50 mµ. X 100,000.
isolated from human hemoculture in the Belgian Congo by Dr. R. de Vignat, and sent to us by Dr. E. V. Morse of the University of Wisconsin.

Cultures of the Congo diphtheroid bacterium were grown on BBL "eugonagar" (trypticase soy agar with cystine and glucose) at 37 C for 18 to 24 hours. When examined with a light microscope in a wet mount, almost all the cells showed a high degree of motility. The preparations for electron microscopy were made from a water suspension of the bacteria. The suspension was either sprayed (Backus and Williams, 1950) as microdroplets upon the microscope specimen films, or it was applied as a gross drop in the usual manner. Reference particles of polystyrene latex (Backus and Williams, 1949) were added to the preparation in order to establish precisely the angle of shadow-casting and the direction of the shadowing beam with respect to the flagellar axis. The shadowing element was uranium.

**OBSERVATIONS**

Figure 1 is a low magnification micrograph of the Congo diphtheroid bacterium, with adjacent flagella. As is the case with most electron micrographs prepared from bacteria that have been suspended in distilled water, there is no way of being certain that the flagella seen actually are attached to, or originated from, an adjacent bacterium.

Figure 2 shows a portion of a bacterium and some adjacent flagella at a moderate magnification. Some of the flagella shown here can be seen to have a surface structure of a helical nature. It is found that most of the flagella have this structure in the several preparations examined on five separate occasions over a period of eight months.

Figure 3 shows individual flagella at high magnification. The helical structure is plainly seen, and some of its details are these: (1) the diameter of the flagella is 19 mμ; (2) the helix has the form of a triple-threaded screw; (3) the direction of the helical turns is invariably that of a left-handed screw; (4) the distance along the flagellar axis for one complete period (or turn) is about 50 mμ; (5) except for cases of obvious kinking, the length of a full period is approximately constant along one flagellum, and also from one flagellum to another.

**DISCUSSION**

The first point that arises is the reality of the phenomenon. Do the helices seen on the micrographs represent helices in the flagella as prepared for micrography? It can be demonstrated easily that a type of "pseudo-helix" appears on micrographs of nodulated filaments if the electron image has moved during the micrography. In such cases, the direction of the "pseudo-helical" turns (that is, left-handed or right-handed) would then be expected to vary from one photographic exposure to another, depending upon the direction of the random drift of the image. Also, in micrographs showing this "pseudo-helical" structure, the background detail would appear to have a linear structure. The invariable left-handed orientation of the helical structure in our micrographs and the absence of any linear structure in the background, as shown in figure 3, rule out the possibility of this type of artifact.
Some confusion can arise easily in attempting to infer the direction of a helical structure in the object from observations of micrographs, owing to possible left to right perversion of the image during the photography (Weibull, 1950). For example, a photographic transparency of a left-handed helix will show a right-handed helix if one turns the transparency over and looks through the back.

It appears that the observed helical structure is not likely to be a result of mechanical intertwisting of smaller fibrils, inasmuch as no partially frayed flagella, nor fibrils of smaller diameter, are ever seen in the micrographs. We believe that failure to observe such smaller fibrils is not due to limitations in the resolving power of the microscope.

At the present time, the Congo diphtheroid appears to be the only organism to show this highly organized flagellar fine structure; relatively few bacteria, however, have been photographed with sufficiently high resolution to demonstrate such a structure, if it exists. As far as is known to us, no natural protein fibrils have ever been reported to exhibit a helical structure of anything like the smallness of scale of the flagella described here. Even this fineness of structure is not to be confused with the scale of size of the protein helices postulated by Pauling et al. (1951), since the diameter of our flagellar helices is about 40 times that of their polypeptide configuration. On the other hand, this flagellar fine structure appears, superficially at least, to be a distinctly different property from the flagellar structure studied by Weibull (1950), which involves a simple "spiral" with a period of about 2 \( \mu \). The helical fine structure found by us for the flagella of the Congo diphtheroid is about one-fiftieth the scale of such gross flagellar forms. For reasons mentioned previously, a comparison with the structure reported in trypsin-treated flagella by de Robertis and Franchi (1951) is not possible at this time.

SUMMARY

A helical fine structure is demonstrated in the flagella of a motile diphtheroid bacterium. This structure appears as a left-handed, triple-threaded helix of 50 \( \mu \) axial period. Evidence for the reality of this structure and its relationship to other reported flagellar structures are discussed.

REFERENCES


