NOTES

Straight Mutants of Spirillum volutans Can Swim

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Nonhelical mutant cells of Spirillum volutans ATCC 19554 can swim as fast as the helical cells. Consequently, a helical cell shape is not required for motility of this species, and the function of the polar flagellar fascicles is not merely to cause rotation, and therefore translocation, of the corkscrew-shaped cell.

The motility of the large, microaerophilic bacterium Spirillum volutans has been thought to be caused by a different mechanism than that of other bacteria. The organism possesses bipolar flagellar fascicles, each of which consists of a large number of individual flagella. The cells swim in straight lines and reverse direction frequently. There is no anterior and posterior end of the cell; upon reversal of direction the fore fascicle becomes the aft fascicle and vice versa (2, 3). As first described by Metzner (3), the fore fascicle appears to rotate in the form of a wide bell opened toward the back, whereas the aft fascicle seems to rotate in the form of a wide goblet opened away from the end of the cell. According to Metzner, the mechanical effect of the flagella is to cause the cell body to rotate in a direction opposite to that of the flagella. The rotation of the corkscrew-shaped cell was believed to be the major factor causing translocation, and the function of the flagella was believed to be merely that of causing the helical cell body to rotate. This theory has been supported by hydrodynamic analysis (1); however, Winet and Keller (6) have demonstrated that the aft flagellar bundles rotate in a helical fashion just as other bacterial flagella do, propagating a helical (or at least three-dimensional) wave. They have, therefore, envisioned the aft fascicle as directly providing the propulsive force for the organism. Recent studies by Swan (5) of cells possessing a flagellar fascicle at only one pole, yet which can reverse their flagellar orientation and direction of swimming, indicate that the fore fascicle can also propel the cells; however, it is possible that the cells might have had bipolar flagella with only one or a few flagella occurring at the pole that seemed to lack a visible fascicle by phase-contrast microscopy.

If the Metzner concept is true, then a straight cell should not be able to swim. Our recent report (4) on the methodology for obtaining colonies of S. volutans ATCC 19554 on agar media suggested that it might be possible to obtain various mutants of this organism for the first time. An aerotolerant mutant capable of forming colonies under an air atmosphere was obtained by a sequential selection method. The wild type cannot ordinarily grow under oxygen levels greater than 12%; however, when 0.1 ml of a 24-h-old culture in casein hydrolysate-succinate-salts broth (4) was spread on plates of casein hydrolysate-succinate-salts medium solidified with 1.3% carrageenan (type 1, Sigma Chemical Co., St. Louis, Mo.) and supplemented with 0.005% potassium metabisulfite, a colony would occasionally grow under 14% oxygen. Such a colony gave rise to a population that could now grow well routinely under 14% oxygen. By repeating the selection process with 16, 18, 20, and 21% oxygen, a mutant was finally obtained that could grow well routinely under an air atmosphere. Microscopic examination of this mutant revealed that approximately 50% of the cells were straight, even when cultured in broth or semisolid media; 25% of the cells were slightly curved and the remainder were as helical as the wild type. The occurrence of a large proportion of straight cells suggested that the mutant could be used to test the Metzner concept of motility.

Both the wild type and the mutant were maintained in 100-ml portions of casein hydrolysate-succinate-salts broth (lacking bisulfite) contained in 250-ml cotton-stoppered flasks and incubated at 30°C on a reciprocal shaking machine (80 oscillations min⁻¹). Cultures were transferred daily, using 1.0 ml of each previous

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culture (or 5.0 ml in the case of the mutant strain) as inoculum for the next culture. Cells were observed by dark-field microscopy at ×100 and videotaped by means of a Hitachi model GP-4DU camera and a JVC model HR-6700U VHS recorder. Cell velocities were determined by playing the tape at 1/16 of its original speed and measuring the swimming distances of individual cells on a calibrated screen. Only rapidly swimming cells were measured. From Table 1 it is evident that wild-type cells, helical cells of the mutant strain, and straight cells of the mutant strain were all capable of swimming at nearly the same speed. The mutant cells could reverse their direction of swimming in a manner similar to that of the wild-type cells. By dark-field microscopy and also by electron microscopy (Fig. 1) the configuration of the flagellar fascicles appeared identical to that of the wild-type cells. Electron microscopy also indicated that at least some of the flagellar bundles of both the mutant and the wild-type strains were not merely crescent-shaped but rather had a sinusoidal wave, although they generally had less than one complete wave (Fig. 1). Thus, the fascicles are probably helically curved like those of other bacteria, although the wavelength is unusually long (ca. 15.5 μm).

The results obtained with our straight mutant indicate that the Metzner concept of the mechanism of motility in *S. volutans* is not correct. A helical cell shape is not required for the motility of this bacterium, and the flagellar fascicles provide propulsion regardless of whether the cell body is helical or straight.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean velocity (μm/s)</th>
<th>Median velocity (μm/s)</th>
<th>Range (μm/s)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (helical)</td>
<td>125</td>
<td>130</td>
<td>71–200</td>
<td>28</td>
</tr>
<tr>
<td>Mutant (straight cells)</td>
<td>117</td>
<td>110</td>
<td>71–200</td>
<td>30</td>
</tr>
<tr>
<td>Mutant (helical cells)</td>
<td>154</td>
<td>140</td>
<td>80–260</td>
<td>46</td>
</tr>
</tbody>
</table>

* Thirty cells of each type were measured.

**TABLE 1.** Velocities of cells of the wild-type and mutant strains of *S. volutans*.

**FIG. 1.** Electron micrographs of *S. volutans* showing the polar flagellar fascicles, some of which exhibit a sinusoidal wave. (A) Wild-type cells. (B) Straight mutant cells. Shadowed with tungsten oxide. Bar represents 5 μm.

**LITERATURE CITED**