Determination of the Spiral Conformation of *Aquaspirillum* spp. by Scanning Electron Microscopy of Elongated Cells Induced by Cephalixin Treatment

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The effect of the β -lactam antibiotic cephalixin on the spiral conformation of cells of *Aquaspirillum* spp. was examined by scanning electron microscopy. *A. itersonii* and *A. peregrinum*, which are known to have a left-handed spiral shape, elongated and still showed left-handed spirals in medium containing cephalixin. The spiral conformation of the elongated cells is therefore considered to represent the natural condition. The spiral conformations of *A. metamorphum* and *A. psychrophilum* grown in ordinary cultures were difficult to determine because they have short cells without a complete spiral. After cephalixin treatment, the cells of these species elongated and displayed spiral forms, right-handed in *A. metamorphum* and left-handed in *A. psychrophilum*. This elongation method may be useful for checking and determination of the spiral handedness of short spiral or curved bacteria such as vibrios.

INTRODUCTION

Members of the genus *Spirillum* as described by Krieg & Smibert (1974) have a spiral shape, a rigid cell body, polytrichous polar flagella and a unique corkscrew motion. This genus has since been divided into three genera, *Spirillum*, *Aquaspirillum* and *Oceanospirillum* (Krieg, 1984). The spiral conformation of these bacteria is considered to be important for their classification and in relation to their mechanism of motility.

Terasaki (1972) and Krieg (1976) described the spiral handedness of various spirilla as observed by light microscopy. Scanning electron microscopy has been recently used to describe the spiral conformation of 10 species of *Spirillum* (*sensu lato*) and one species of *Rhodospirillum* (Yoshii *et al.*, 1982). Scanning electron microscopy provides more accurate results than light microscopy, since it produces three-dimensional images with higher resolving power. However, the spiral handedness of three species, *Aquaspirillum* (formerly *Spirillum*) *metamorphum*, *A. (S.) psychrophilum* and '*Spirillum lunatum*', could not be determined because their short cells did not form complete spirals.

In the present study we attempted to determine the spiral conformation of these three species by scanning electron microscopy of elongated cells induced by treatment with the β -lactam antibiotic cephalixin.

METHODS

Strains. *Aquaspirillum* (formerly *Spirillum*) *metamorphum* ATCC 15280 and *A. (S.) itersonii* ATCC 12639 were obtained from Dr Terasaki, Suzugamine Women's College, Hiroshima, Japan. *A. (S.) psychrophilum* IFO 13611, '*S. lunatum*' IFO 3985 and *A. (S.) peregrinum* IFO 13617 were obtained from the Institute for Fermentation, Osaka, Japan. *Escherichia coli* W strain has been maintained in our laboratory. *A. itersonii* and *A. peregrinum* were used as positive controls known to have a left-handed spiral (Yoshii *et al.*, 1982) and *E. coli* was used as a negative control.

Culture methods. Nutrient broth or nutrient agar (Nissui, Tokyo, Japan) was used for growth of *E. coli*, and a modified nutrient broth or agar (5 g polypeptone and 3 g meat extract l⁻¹, pH 7.0) was used for the spirilla. Each organism was cultured on agar slopes at its optimal temperature. *A. psychrophilum* was incubated at 25 °C for 3 d, the other spirilla at 30 °C, and *E. coli* at 37 °C for 2 d. The cultures were transferred into liquid medium (1.2×10^8 to 5.0×10^8 organisms ml⁻¹), incubated for 6 h at the optimal temperature and used as an inoculum.

Antibiotic. Cephalixin (Shionogi-Lilly Co.) was prepared by reconstitution with distilled water and filter sterilized by passage through 0.45 µm filters.

Elongation treatment with cephalixin. Serial dilutions of cephalixin (0.1 to 250 µg ml⁻¹) were prepared in fresh broth and 2 ml portions were placed in test tubes. Each tube was inoculated with 2 ml of the appropriate culture and incubated for 24 h at its optimal temperature. Tubes containing 2 ml fresh cephalixin-free medium inoculated and incubated in the same way were used as controls. Tubes which contained elongated cells when examined by dark-field light microscopy were selected and examined by scanning electron microscopy.

Scanning electron microscopy. Specimens were prepared as follows. The cultures were washed three times in saline solution by centrifugation at 1500 g for 10 min at 5 °C and treated by the double fixation method with 2% (v/v) glutaraldehyde/1% (w/v) osmium tetroxide (Ryter & Kellenberger, 1958). The fixed material was washed twice in distilled water and dehydrated with increasing concentrations of acetone prior to critical-point drying in carbon dioxide. The dried cells on glass slides or small sheets of filter paper were coated with gold/palladium alloy in an evaporator and observed by scanning electron microscopy (JEOL JSM-F7, 13 kV).

Spiral handedness. Spiral handedness was determined on printed photographs, carefully prepared so as not to make mirror images. The scanning electron microscope used made real images and not mirror images as described by Yoshii (1978). Right- (or left-) spiral handedness was defined as rotation clockwise (or counter-clockwise) while moving along the spiral away from the observer (Yoshii, 1978; Carleton *et al.*, 1979).

RESULTS AND DISCUSSION

Five spirilla and one strain of *E. coli* were observed with or without cephalixin treatment by scanning electron microscopy.

In the absence of cephalixin, *A. itersonii* and *A. peregrinum* showed a left-handed spiral form (Fig. 1a) as previously described (Yoshii *et al.*, 1982). After cephalixin treatment, over 80% of the cells of both these species formed long filaments, but kept their native left-handed spiral forms at antibiotic concentrations of 0.5 to 2.5 µg ml⁻¹ (*A. itersonii*) and 25 to 125 µg ml⁻¹ (*A. peregrinum*) (Fig. 1b). No other marked changes in their morphology were observed at these concentrations. Although a change in handedness has been reported for a helical mutant of *Bacillus subtilis* when grown in different media (Mendelson, 1978), this was not observed as a result of the treatment in the present study. *E. coli* cells grown in ordinary culture were rod shaped, and although 20 to 30% of the population elongated when treated with between 25 and 125 µg cephalixin ml⁻¹, the elongated cells did not display any spiral forms (Fig. 2). These results demonstrate that these bacteria retain the original features of their spirality even in cephalixin-induced filaments. Thus the spiral handedness of the elongated cells is considered to represent the natural condition.

A. metamorphum, *A. psychrophilum* and '*S. lunatum*' grown in cephalixin-free medium showed rod or short curved shapes with an incomplete spiral (Fig. 3a, c), and their spiral handedness could not be directly determined. After cephalixin treatment (0.05 to 0.5 µg ml⁻¹), 40 to 50% of *A. metamorphum* cells become elongated; some organisms appeared damaged and others showed no change in their morphology. All the cells which elongated and showed complete spirals exhibited right-handed ones (Fig. 3b). Most *A. psychrophilum* cells exposed to 50 to 125 µg cephalixin ml⁻¹ elongated and also showed complete left-handed spiral forms (Fig. 3d). The handedness of '*S. lunatum*' could not be determined, because it did not elongate and did not form a complete spiral at any concentration of cephalixin between 0.05 and 125 µg ml⁻¹.

Terasaki (1972) described both *A. psychrophilum* and *A. metamorphum* as having a right-handed spiral. The right-handed conformation of *A. metamorphum* is confirmed in the present study. However, we found *A. psychrophilum* to have a left-handed spiral. The reasons for this difference in results are unknown. We consider that although spiral handedness is a stable characteristic, a change in handedness might occur during long-term maintenance, and that the results from scanning electron microscopy after cephalixin-induced elongation are probably more accurate.

Fig. 1. Scanning electron micrographs of *A. peregrinum*. (a) Cells grown in cephalixin-free medium, showing a typical left-handed spiral shape. (b) Cells treated with 125 µg cephalixin ml⁻¹. Large numbers of elongated cells are observed, which display a left-handed spiral. No right-handed spiral forms were observed. Bars, 5 µm.

Fig. 2. Scanning electron micrograph of an elongated cell of *E. coli* treated with 125 µg cephalixin ml⁻¹. Bar, 10 µm.

Fig. 3. (a) Scanning electron micrograph of *A. metamorphum* grown in cephalixin-free medium. Rod-shaped or curved cells, whose spiral handedness was unclear, were observed. Bar, 1 µm. (b) Scanning electron micrograph of *A. metamorphum* treated with 0.05 µg cephalixin ml⁻¹. A few elongated cells with a right-handed spiral can be seen. Bar, 10 µm. (c) Scanning electron micrograph of *A. psychrophilum* grown in cephalixin-free medium. Short cells with incomplete spirals, whose spiral handedness was unclear, were observed. Bar, 1 µm. (d) Scanning electron micrograph of *A. psychrophilum* treated with 125 µg cephalixin ml⁻¹. Several extremely elongated cells with a left-handed spiral can be seen. Bar, 10 µm.

The methods described may also be useful for checking and determination of the spiral handedness of other short spiral or curved bacteria such as vibrios.

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