

## Appearance of Straight Flagellar Filaments in the Presence of *p*-Fluorophenylalanine in *Pseudomonas aeruginosa*

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In the presence of *p*-fluorophenylalanine, a normally flagellated strain of *Pseudomonas aeruginosa* produced straight flagellar filaments at the distal ends of preexisting flagella, indicating polar growth on its flagella.

It was reported by Kerridge (5) that *Salmonella typhimurium* grown in the presence of an amino acid analogue, *p*-fluorophenylalanine (FPA), produced curly flagella with a wavelength approximately half that of normal ones, and it was assumed that incorporation of this amino acid analogue into flagellin resulted in the change of flagellar morphology (6, 8). This phenomenon was applied by Iino (3) for the examination of the polarity of flagellar growth. Addition of FPA to a culture of *S. typhimurium* resulted in production of curly flagellar filaments at the tips of preexisting normal ones.

In the present experiment, the effect of FPA on the flagellar morphology of *Pseudomonas aeruginosa* was investigated. In this organism, FPA has been found to be incorporated into the cells (9, 10).

The strain used was *P. aeruginosa* PJ106 (Ade<sup>-</sup>, Leu<sup>-</sup>, Str<sup>r</sup>, FP<sup>+</sup>), which was a derivative of strain PTO (2). Five milliliters of the culture, grown at 37°C with gentle shaking in 50 ml of synthetic medium [1.4% K<sub>2</sub>HPO<sub>4</sub>, 0.6% KH<sub>2</sub>PO<sub>4</sub>, 0.4% sodium citrate, 0.2% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> · 7H<sub>2</sub>O, 0.04% adenine sulfate, 0.02% L-leucine, and 0.4% glucose in distilled water], was transferred at the exponential growth phase (4 × 10<sup>8</sup> cells/ml) to 50 ml of prewarmed synthetic medium containing 1.0 mg of FPA per ml (FPA-containing medium), and incubation was continued. At various times after the start of incubation, 2.5-ml samples were removed and fixed immediately in 5% (vol/vol) formalin, washed with distilled water by centrifugation (1,000 × *g* for 15 min) and negatively stained with 0.5% sodium phosphotungstate (pH 6.8) on copper grids coated with polyvinyl Formvar film. The samples were dried in vacuo and examined in a JEM-7A electron microscope at 80 kV. Photographs were taken at a magnification of ×5,000. As a standard for magnification, polystyrene latex particles (diameter, 0.7900 ± 0.004 μm, Dow Chemical Co.) were photographed simultaneously. Bacterial numbers in

liquid media were counted in an Elma bacterial counting chamber. Flagellar length was expressed as normal wave units: 1 unit equaled 1.8 μm, equivalent to the average contour length of a normal wave.

During incubation, cells multiplied to 1.5 times in 20 min. Thereafter, probably because of inhibition by FPA, their growth rate declined remarkably and no significant increase in either cell number and cell mass was detected. Extensive filamentation of the cells that had been described for FPA-treated *Escherichia coli* (7) was not observed under the present experimental condition. Until 10 min after the start of incubation, the observed flagella were all normal (Fig. 1a). After 15 min, straight filaments, instead of curly ones which had been observed on FPA-treated *S. typhimurium*, and heteromorphous ones consisting of normal and straight parts, were detected on both formalin-fixed and unfixed samples (Fig. 1b and c). Observations under phase-contrast microscopy revealed that the fraction of the cells that were motile remained at about 70% throughout the incubation.

Samples at 0, 20, and 40 min were analyzed in detail. Numbers of randomly photographed cells were 337 at 0 min, 438 at 20 min, and 270 at 40 min. In all of the heteromorphous flagella, whose observed numbers totaled 65 at 20 min and 71 at 40 min, a straight section was present at the distal end of each flagellum. Average numbers of flagella per cell were 0.8 at 0 min, and 0.7 at both 20 and 40 min. As the multiplications of cell numbers at 20 and 40 min were 1.5 times the numbers at 0 min, the total number of flagella at 20 and 40 min was calculated to be 1.3 times the total number at zero time. The normal, wavy sections of flagella increased in length during the first 20 min of incubation, but not thereafter (Fig. 2a). Increase in the total number of entirely straight flagella also occurred during the first 20 min. These straight flagella were inferred to be synthesized by non-

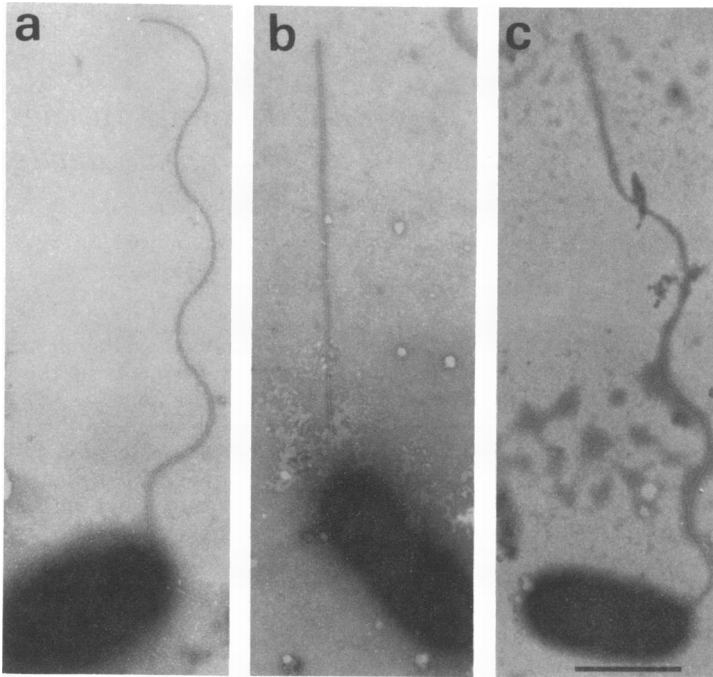


FIG. 1. Normally flagellated cell of *P. aeruginosa* PJ106 (a), and cells carrying a straight flagellum (b) and a heteromorphous one (c), detected after incubation in FPA-containing medium for 20 min. Negatively stained with phosphotungstate. Scale bar represents 0.1  $\mu\text{m}$ .

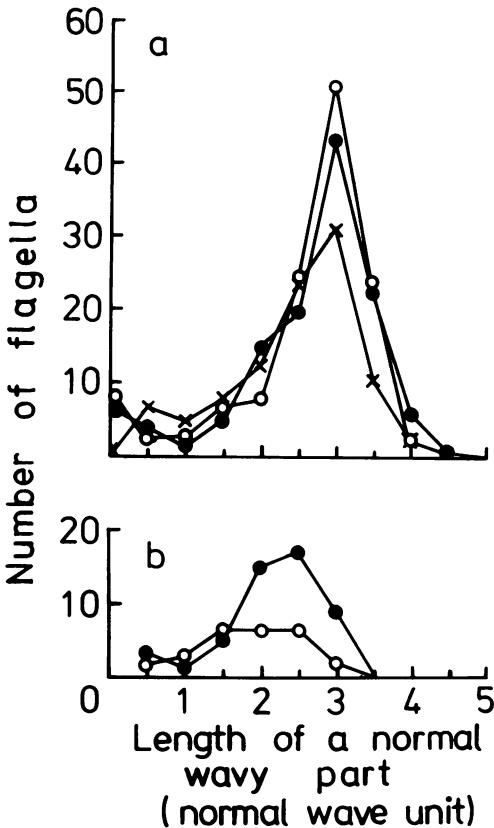


FIG. 2. Length distribution of the normal, wavy sections of flagella of *P. aeruginosa* PJ106 cells grown in FPA-containing medium (a). Total numbers of flagella examined were 270 at 0 min, 278 at 20 min, and 168 at 40 min. The fraction whose length of a normal, wavy section equaled zero corresponds to straight flagella. In the figure, total number at 0 min was taken as 100. Numbers at 20 and 40 min were adjusted by multiplying the percentage of each fraction by the ratio of total number of flagella to zero time. Thus, the total numbers of flagella at both 20 and 40 min were taken as 130. Length distribution of the normal, wavy sections of heteromorphous flagella (b). The fractions of heteromorphous flagella in (a) were plotted. Flagellar length was expressed with normal wave units: 1 unit equaled 1.8  $\mu\text{m}$ . Symbols:  $\times$ , 0 min;  $\circ$ , 20 min;  $\bullet$ , 40 min.

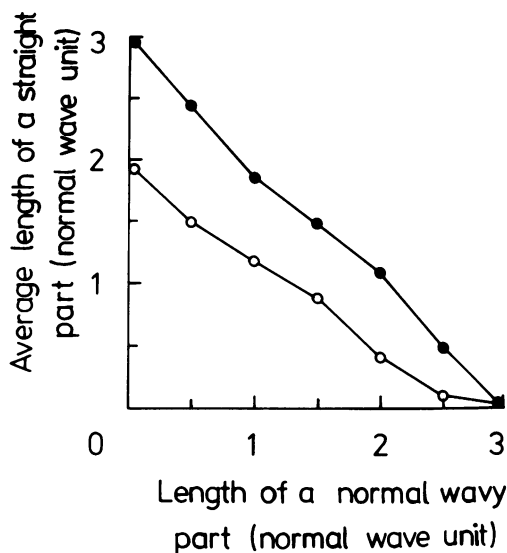


FIG. 3. Average length (normal wave units) of a straight section of a flagellum with different lengths of normal waves in *P. aeruginosa* Pj106 grown in FPA-containing medium for 20 min (○) and 40 min (●).

flagellate bacteria during the first 20 min. During the second 20 min, probably because of the inhibitory effects of FPA on the flagellar-synthesizing apparatus, as described by Kerridge (5), no increase in the total number was detected. Both the number of heteromorphous flagella carrying straight sections at their tips and the length of a straight section of a flagellum increased during the incubation, showing the production of straight filaments at the distal

end of preexisting normal ones and the elongation of those straight filaments. The length of a straight section of a flagellum was shorter as the length of its normal, wavy section was longer, and reached zero for 3 to 3.5 normal wave units (Fig. 3).

These results indicate polar growth of *Pseudomonas* flagella, with a decline in growth rate with increase in length, conforming with the mode of flagellar growth previously reported for *S. typhimurium* and *Bacillus subtilis* (1, 3, 4).

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