JOURNAL OF BACTERIOLOGY, Oct., 1965
Vol. 90, No. 4

Electron Microscopy of Bundled Flagella of the Curly Mutant of *Salmonella abortivoequina*

MICHIKO MITANI AND TETSUO IINO

Department of Microbial Genetics, National Institute of Genetics, Mishima, Japan

Received for publication 22 May 1965

ABSTRACT

MITANI, MICHIKO (National Institute of Genetics, Mishima, Japan), AND TETSUO IINO. Electron microscopy of bundled flagella of the curly mutant of *Salmonella abortivoequina*. J. Bacteriol. 90:1096–1101. 1965.—The arrangement of flagella was observed by dark-field and electron microscopy in three strains of *Salmonella abortivoequina*, namely, normal flagellar, curly flagellar, and paralyzed curly flagellar strains. With dark-field microscopy, bundled flagella could be seen in 5 to 10% of actively moving normal or curly mutant cells. Under the electron microscope, a great many bundled flagella were observed in the curly mutant strain, but in the normal strain most of the flagella were dissociated or the bundles were rather loose and irregular. Normal flagella seem to separate easily during the process of preparation, but not the curly ones. Single flagella were found to run parallel with each other and to form a bundle consisting of five or more flagella; the bundle was spirally gyrating, with the characteristic flagellar wave. It is thought that the bundle observed with the electron microscope corresponds to that observed under the dark-field microscope. Further, the marked decrease of bundle formation in the paralyzed curly mutant cells suggests that bundle formation is not caused by curly flagellar structure per se, but corresponds to the mode of locomotion of peritrichously flagellated bacteria.

Flagella play an important role in the locomotion of bacteria. The study of their shape in a moving bacterium promises to provide a valuable means for the clarification of the method of bacterial locomotion. The shape and behavior of flagella have been observed under the dark-field microscope by Pijper (1957), who reported that a peritrichously flagellated bacterium swimming in a liquid medium appears as a bright particle trailing the spirally twisted bundle and moving forward by its gyrating motion. On the other hand, peritrichous flagella observed in stained preparations under optical and electron microscopes are separated from each other, being distributed around the bacterial body (Houwink and van Iterson, 1950; Leifson, 1960). Occasionally, bundled flagella are found in peritrichously flagellated strains under the electron microscope. Because they are irregular, it is hard to reconstruct their conformation in the moving bacteria. Therefore, the relation between the bundled flagella as observed under an electron microscope and under a dark-field microscope has remained an open question.

During the course of an investigation of mutations concerning the shape of flagella, a curly mutant of *Salmonella abortivoequina* was found to preserve well the bundle formation observed in a moving bacterium under a dark-field microscope. The present paper presents a detailed description of bundled flagella observed in a peritrichously flagellated strain under an electron microscope.

MATERIALS AND METHODS

The three strains of *S. abortivoequina* used were: SL23, a phase 2 stable strain with normal peritrichous flagella; a curly flagellar mutant, SJ30, originating from SL23 (Iino, 1962); and a paralyzed mutant, SJ706, obtained from SJ30. Cells of the curly strain rotate and show a tendency to aggregate with each other in broth; the paralyzed mutant cannot move at all, and its cells do not aggregate.

The medium for the cultivation of bacteria consisted of 0.15% beef extract (Difco), 0.15% yeast extract (Difco), 0.5% Kyokuto peptone, 0.1% glucose, 0.35% NaCl, 0.36% K_2HPO_4, and 0.32% KH_2PO_4.

For microscopy, cells inoculated into 10 ml of medium and incubated for 2 hr (with aeration) at 37 C were used.

Dark-field microscopy was by the procedure of Pijper and Abraham (1954); bacterial samples were prepared by mixing a drop of culture fluid with the same amount of saline containing 0.5% methylcellulose (Junsei Pure Chemicals & Co., Ltd.). Manicure enamel was used for sealing the cover slides. The microscope used was a 7-v Ernst Leitz

Wetzlar 457210, in combination with a Chiyoda immersion objective (× 90) with iris and a Zeiss eyepiece (× 10).

For electron microscopy, cultures were centrifuged at 1,200 × *g* for 20 min, and the pellets were suspended in distilled water. The flagella were fixed either in 1% phosphotungstate (PTA) or in the vapor of 1% OsO_4 for 10 min. Small drops of the suspension were placed on collodion supporting films and were dried immediately in a small chamber by removing the internal air with a vacuum pump. For negative staining by PTA, the fixation was made before transferring the cells to the supporting films. Samples without fixation or fixed with OsO_4 vapor were shadowed with chromium. The electron microscope was a JEM T6S (Japan Electron Optics Laboratory Co., Ltd.), with a single condenser system and accelerating voltage of 60 kv.

RESULTS

Dark-field microscopy. Most of the cells were motile either in normal or curly mutant cultures when observed immediately after mounting them on slides. But the number of motile bacteria decreased gradually with the passage of time. Bundled flagella were observed in 5 to 10% of actively moving cells; they were blurred and had the appearance of a smooth straight tail as reported by Pijper (1957). But the shape of bundled flagella could be clearly observed in slowly moving or resting cells, showing a wavelength of 2.13 μ and an amplitude of 0.48 μ in normal cells and 0.99 μ and 0.36 μ in the curly mutant (Fig. 1). These values correspond to the characteristic wavelength and amplitude of normal and curly mutant cells, respectively (Pijper and Abraham, 1954; Leifson, Carhart, and Fulton, 1955).

Electron microscopy. A great many bundled flagella were observed under the electron microscope in the curly strain, but most of the flagella in the normal strain were dissociated (Fig. 2). The wavelength and amplitude of the bundle were approximately the same as those of a single flagellum. Proportions of the cells with bundled flagella to the observed total were compared between curly and normal cells (Table 1). In the curly mutant, the frequency of bundled flagella was a little larger in samples rapidly dried in vacuo than in those that were slowly dried without vacuum.

Bundled flagella were also found in normal cells, but far less frequently than in those with curly flagella; they were rather loose and irregular. Further, the frequency of bundled flagella was not affected by different fixation and drying procedures (PTA or OsO_4 fixation, rapid or slow drying). Normal flagella seem to dissociate easily during the preparation process.

The details of bundled flagella of the curly mutant were observed in chromium-shadowed samples (Fig. 3). Each single flagellum of a bundle was spirally coiled; they were not arranged side by side to form an agglomerate as indicated by previous workers (Houwink and van Iterson, 1950; Weibull, 1960). Though the coiled flagella were flattened out on the supporting films by PTA staining or OsO_4 fixation, the micrographs obtained from nonfixed and immediately dried specimens (Fig. 3) showed a three-dimensional structure. This structure was also seen in dark-field micrographs (Fig. 1); bright and faint parts were alternately arranged on the spiral of a bundle. The bundle observed with the electron microscope may correspond to that observed with the dark-field microscope.

Since moving curly mutant cells retain their flagellar shape, it might be thought that, in the paralyzed curly mutant, the number of bundled flagella would be reduced. To test this, paralyzed curly mutant strain SJ706 cells having more than five flagella were counted (Table 1). The average number of flagella was 8.1 per bacterium. Compared with the motile curly mutant, there were relatively few bundled flagella. A comparable decrease in the number of bundled flagella was noted in paralyzed curly cells under the dark-field

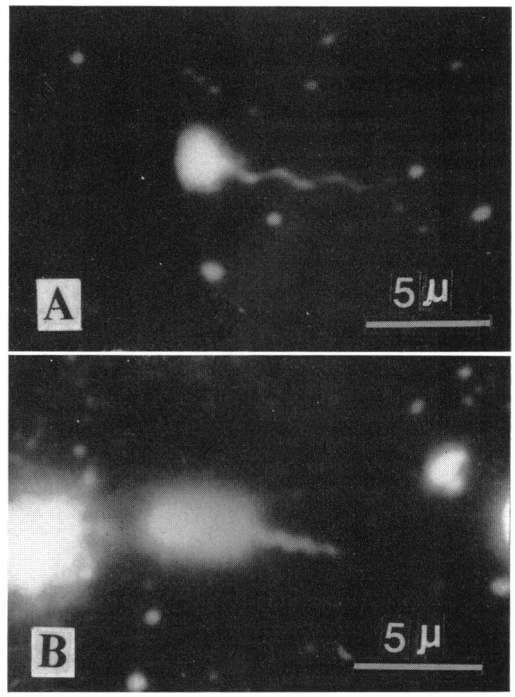

FIG. 1. *Dark-field micrographs of Salmonella abortivoequina. (A) Normal flagellar strain. (B) Curly flagellar strain.*

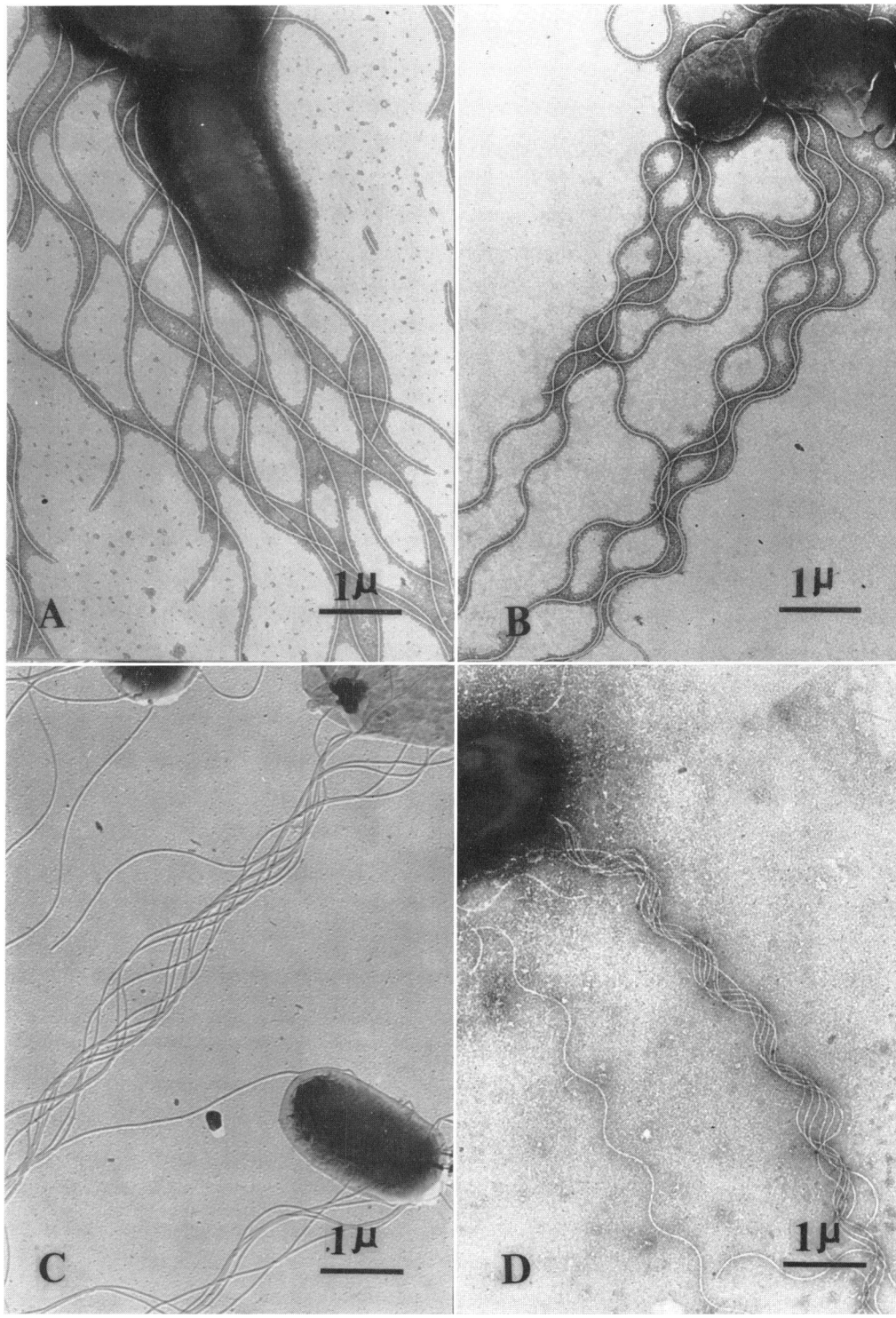

FIG. 2. *Electron micrographs of Salmonella abortivoequina. (A) Dissociated flagella of normal strain, negatively stained by PTA. (B) Dissociated flagella of curly mutant, negatively stained by PTA. (C) Bundled flagella of normal strain, shadowed by chromium. (D) Bundled flagella of curly mutant, negatively stained by PTA.*

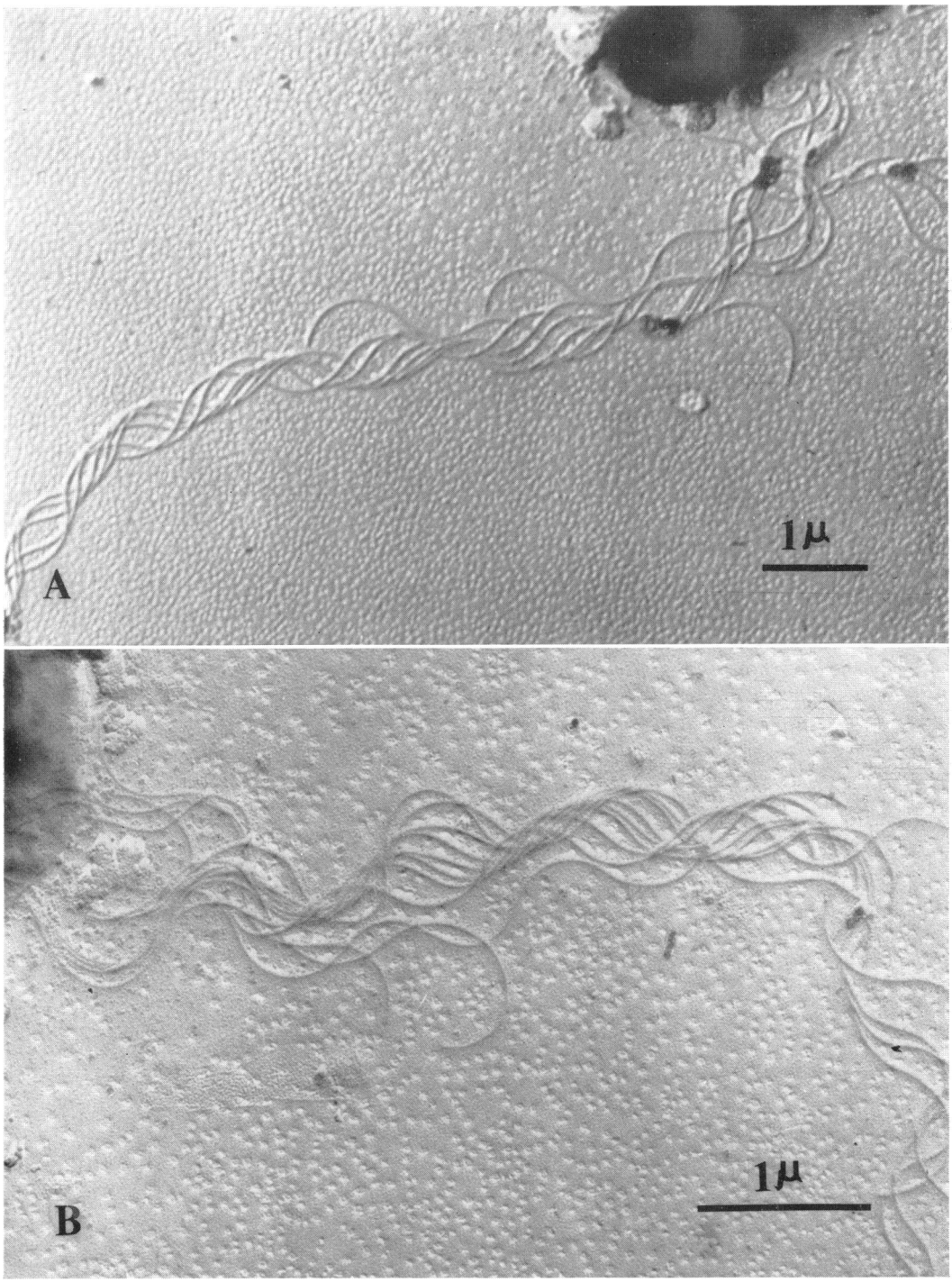

FIG. 3. *Electron micrographs of the bundled flagella of the curly flagellar mutant, shadowed by chromium.*

TABLE 1. *Number of bundled and dissociated flagella of three strains in relation to their flagellar characters (observed under the electron microscope by negative staining)*

Strain	Type of flagella	Type of motility	Wave-length	Bundled flagella		Dissociated flagella	
				Rapid drying	Slow drying	Rapid drying	Slow drying
			μ				
SL23	Normal	Translation	2.4	2 (0.7%)	8 (3.1%)	257 (99.3%)	253 (96.9%)
SJ30	Curly	Rotation	1.2	108 (97.3%)	100 (83.3%)	3 (2.7%)	20 (16.7%)
SJ706	Curly	Paralysis	1.2	11 (14.1%)		78 (85.9%)	

microscope. This indicates that the low frequency of bundled flagella observed under the electron microscope is not due to decreased preservability of the bundles. The observed reduction in bundle formation, in comparison with the motile curly mutant, supports our belief that bacterial motility is accompanied by bundle formation.

DISCUSSION

It has been noted by several investigators that the flagella of a peritrichously flagellated bacterium form a bundle when the cell is swimming (Pijper, 1957). However, in living cells, the bundled flagella are visible only under a dark-field microscope with strong illumination. Even under dark-field observation, the thickness of a flagellum is beyond the resolving power of an optical microscope. Therefore, the bundle appears as a single spiral, and the arrangement of the elementary flagella is not accessible to observation in swimming bacteria. On the other hand, electron micrographs of dried peritrichously flagellated bacteria disclose the details for each flagellum, but they do not show bundles comparable with those in living bacteria; flagella observed in electron micrographs are usually loosely distributed around the cells and, even if bundles are visible, they are quite distorted (Houwink and van Iterson, 1950).

In the present investigation, regularly coiled bundles of flagella were observed under the electron microscope in motile cells with curly flagella. In a parallel observation of normal motile bacteria, bundles were very scarce and their conformation was irregular. Under the dark-field microscope, bundled flagella were demonstrated to occur at about the same frequency in both types. From these results, it is concluded that the flagellar bundles of curly flagellar cells are not altered during preparation of samples for electron microscopy, but in the normal cells the flagella easily separate from each other. The failure to demonstrate bundled flagella comparable with those of swimming bacteria in previous electron microscopic studies may be due to the fact that the studies were mostly confined to bacteria with normal flagella.

From the electron micrographs of the curly flagellar cells, the conformation of the bundled flagella of a swimming bacterium is concluded to be as follows: single flagella are parallel with each other and form a bundle consisting of five or more flagella; the bundles gyrate spirally, with the characteristic flagellar wave. It is worth noting that, among the electron micrographs of bacterial flagella presented by Houwink and van Iterson (1950), one clear bundle of curly flagella of *Agrobacterium radiobacter* can be seen in which the flagella are coiled around each other, although the helix of the bundle is distorted.

It has already been argued that flagella possess a tendency to unite into bundles (Stocker, 1956; Houwink and van Iterson, 1950; Weibull, 1960). Weibull (1960) presented a fine picture of an agglomerate of detached flagella appearing as a tress. The curly flagellar mutant, SJ30, differs from the normal flagellar strain, SL23, at a single site of the flagellin structural gene (Iino, 1962). The increased preservability of the bundles in the curly strain may be primarily attributed to a configurational change in the flagellin monomer.

In contrast to the curly strain, bundle formation of flagella is markedly decreased in its paralyzed mutant. By genetic analysis of the paralyzed mutant, it was shown that paralysis of the strain was caused by the mutation of a motility gene, *mot* (Mitani and Iino, *unpublished data*). The biochemical role of the *mot* on bacterial motility has not been clarified as yet. However, it is known that *mot* is genetically distinct from the flagellin structural gene, *H1* and *H2*, and the paralysis caused by mutation of *mot* was presumed to be attributed not to the structural alteration of flagella but to a defect in the locomotion mechanism (Beighton, Porter, and Stocker, 1958; Enomoto, 1962). Therefore, the marked decrease in bundle formation in the paralyzed curly mutant cells may indicate that the forma-

tion of bundles is not caused by curly flagellar structure per se but reflects the mode of locomotion of peritrichously flagellated bacteria.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant AI-02872 from the National Institute of Allergy and Infectious Diseases, and by a grant from the Toyo Rayon Foundation for the Promotion of Science and Technology.

LITERATURE CITED

BEIGHTON, E., A. M. PORTER, AND B. A. D. STOCKER. 1958. X-ray and related studies of the flagella of non-motile bacteria. Biochim. Biophys. Acta **29**:8–13.

ENOMOTO, M. 1962. Grouping of paralyzed mutants in Salmonella. Ann. Rept. Natl. Inst. Genetics (Japan) **13**:75–76.

HOUWINK, A. L., AND W. VAN ITERSON. 1950. Electron microscopical observations on bacterial cytology. II. A study on flagellation. Biochim. Biophys. Acta **5**:10–44.

IINO, T. 1962. Curly flagellar mutants in Salmonella. J. Gen. Microbiol. **27**:167–175.

LEIFSON, E. 1960. Atlas of bacterial flagellation. Academic Press, Inc., New York.

LEIFSON, E., S. R. CARHART, AND M. FULTON. 1955. Morphological characteristics of flagella of Proteus and related bacteria. J. Bacteriol. **69**:73–82.

PIJPER, A. 1957. Bacterial flagella and motility. Ergeb. Mikrobiol. Immunitätsforsch. Exptl. Therap. **30**:37–95.

PIJPER, A., AND G. ABRAHAM. 1954. Wavelength of bacterial flagella. J. Gen. Microbiol. **10**:452–456.

STOCKER, B. A. D. 1956. Bacterial flagella: morphology, constitution and inheritance. Symp. Soc. Gen. Microbiol. **6**:19–40.

WEIBULL, C. 1960. Movement, p. 152–205. *In* I. C. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. 1. Academic Press, Inc., New York.