Motility and Flagellation of Cellulomonads

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The flagellation and motility of seven cellulomonads (*Cellulomonas biazotea* ATCC 486^T, [T = type strain], *Cellulomonas fimi* ATCC 484^T, *Cellulomonas flavigena* ATCC 482^T, *Cellulomonas gelida* ATCC 488^T, "*Cellulomonas subalbus*" ATCC 489, *Cellulomonas cellasea* ATCC 487^T, and *Cellulomonas uda* ATCC 491^T) were examined by light microscopy and electron microscopy. Cells of all seven strains possessed polar multitrichous flagella, and a few cells possessed polar monotrichous flagella.

The genus *Cellulomonas* Bergey et al. (1) was based originally on a single, physiological property, the ability to hydrolyze cellulose. This genus included such varied types of bacteria as nonsporeforming, gram-negative, rod-shaped, polarly flagellated species, peritrichous, gram-variable, rod-shaped organisms now placed in the genus *Pseudomonas*, and the coryneform group of bacteria. Clark (4–7) revised the criteria for species differentiation in *Cellulomonas*, and this revision was used in *Bergey's Manual of Determinative Bacteriology*, 7th ed. (3). In *Bergey's Manual*, 8th ed., Kieddie (8) recognized only a single species. However, the reduction of *Cellulomonas* to a single species has been challenged (2, 10).

Previously, motility and type of flagellation were used in differentiating the cellulomonads (6). Kellerman et al. (9) described *Cellulomonas biazotea*, *Cellulomonas cellasea*, and *Cellulomonas gelida* as having one to three peritrichous flagella. Clark and Carr (7) reported one to four flagella for *Cellulomonas fimi* and *C. gelida*; these flagella were arranged in polar or lateral positions. Yamada and Komagata (11) reported motility in *Cellulomonas* species and noted that the flagellation of these organisms was lateral with one, two, or more flagella. Stackebrandt and Kandler (10) observed motility in some of the *Cellulomonas* strains which they examined but did not determine the type of flagellation. The purpose of this study was to determine the type of flagellation of representative species of the genus *Cellulomonas* in order to provide accurate morphological descriptions of members of this genus.

The following bacterial species were obtained from the American Type Culture Collection, Rockville, Md.: *C. biazotea* ATCC 486^T, (T = type strain), *C. cellasea* ATCC 487^T, *C. fimi* ATCC 484^T, *Cellulomonas flavigena* ATCC 482^T, *C. gelida* ATCC 488^T, "*Cellulomonas subalbus*" ATCC 489, and *Cellulomonas uda* ATCC 491^T.

Bacteria from 18-, 24-, and 48-h cultures were harvested by centrifugation and suspended in physiological saline for 25 min at 35°C before examination. The cultures were grown in medium adjusted to pH 7.0 and containing 5 g of tryptone, (Difco Laboratories), 5 g of yeast extract (BBL Microbiology Systems), 5 g of K₂HPO₄, 0.5 g of glucose, and 1,000 ml of distilled water. The bacteria were negatively stained with a filter-sterilized 2% phosphotungstic acid solution (pH 7.3), and they were examined with an Hitachi model HU-8 transmission electron microscope.

Motile cells were observed by phase-contrast microscopic examination of hanging drop suspensions from cultures of all seven strains. The percentage of motile cells varied greatly. The cultures of *C. biazotea*, *C. fimi*, and *C. uda* contained relatively few motile cells. Stackebrandt and Kandler (10)

reported that cells of "*C. subalbus*" DSM 20110 were motile only when glucose was omitted from the medium and that *C. biazotea* lost its motility when distilled water was used in the medium.

Clearly attached polar multitrichous flagella were demonstrated by electron microscopy of all strains. A few cells had single polar flagella. Representative results for four of the seven strains examined are shown in Fig. 1. No differences in location of attachment or number of flagella among species were found. The number of flagella per cell varied from one to four, with two to four being the most common. The flagella were apparently nonsheathed and had typical basal hooks. However, the resolution of the electron micrographs did not allow the structure of either the hook or the flagellum to be determined.

In this study seven *Cellulomonas* strains were examined for motility and the point of flagellum attachment by electron microscopy. My evidence indicates that the majority of cells have polar multitrichous flagella and that this should be the correct morphological description for members of this genus. Lateral flagella with clear evidence of attachment were not observed.

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LITERATURE CITED

1. Bergey, D. H., R. S. Breed, R. W. Hammer, F.-C. Harrison, and F. M. Huntoon. 1923. *Bergey's manual of determinative bacteriology*, 1st ed. The Williams & Wilkins Co., Baltimore, Md.
2. Braden, A. R., and D. W. Thayer. 1976. Seriological study of *Cellulomonas*. *Int. J. Syst. Bacteriol.* 26:123–126.
3. Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. *Bergey's manual of determinative bacteriology*, 7th ed. The Williams & Wilkins Co., Baltimore, Md.
4. Clark, F. E. 1951. The generic classification of certain cellulolytic bacteria. *Soil Sci. Soc. Am. Proc.* 15:180–182.
5. Clark, F. E. 1952. The generic classification of the soil coryneform bacteria. *Int. Bull. Bacteriol. Nomencl. Taxon.* 2:45–46.
6. Clark, F. E. 1953. Criteria suitable for species differentiation in *Cellulomonas* and a revision of the genus. *Int. Bull. Bacteriol. Nomencl. Taxon.* 3:179–199.
7. Clark, F. E., and P. H. Carr. 1951. Motility and flagellation of the soil corynebacteria. *J. Bacteriol.* 62:1–6.
8. Kieddie, R. M. 1974. *Cellulomonas*, p. 629–631. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore, Md.
9. Kellerman, K. F., I. G. McBeth, F. M. Scales, and N. R. Smith.

FIG. 1. Electron micrographs of typical negatively stained *Cellulomonas* cells. Each micrograph is at the same magnification. (a) *C. cellasea*. (b) *C. biazotea*. (c) *C. cellasea*. (d) "*C. subalbus*."

1913. Identification and classification of cellulose-dissolving bacteria. *Centralbl. Bakteriol. Abt. 2* 39:502-522.
10. **Stackebrandt, E., and O. Kandler.** 1979. Taxonomy of the genus *Cellulomonas*, based on phenotypic characters and deoxyribonucleic acid-deoxyribonucleic acid homology, and proposal of

- seven neotype strains. *Int. J. Syst. Bacteriol.* 29:273-282.
11. **Yamada, K., and K. Komagata.** 1972. Taxonomic studies on coryneform bacteria. IV. Morphological, cultural, biochemical, and physiological characteristics. *J. Gen. Appl. Microbiol.* 18:399-416.