NOTES

Microtubule in *Azotobacter vinelandii* Strain O

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There are numerous reports in the literature of microtubules occurring in plant and animal cells and protozoans. More recently, a microtubule has been observed in the bacterium *Proteus mirabilis* (W. van Iterson, J. M. F. Hoeniger, and E. N. van Zanten, *J. Cell Biol.* **32**:1, 1967). We wish to report the presence of microtubules in another bacterium, *Azotobacter vinelandii* strain O. This structure was first observed in negatively stained preparations of the *Azotobacter* R₃ fraction which contains the membrane-bound particulate enzyme complexes that carry out electron transport function (P. Jurtshuk, P. R. Aston, and L. Old, *J. Bacteriol.* **93**:1069, 1967). The presence of this microtubule also can be demonstrated in whole cells which have been either negatively stained or fixed and sectioned.

Whole cells were prepared for negative staining and sectioning by the procedure essentially described by J. M. F. Hoeniger, W. van Iterson, and E. N. van Zanten (*J. Cell Biol.* **31**:603, 1966). Negative stains of whole cells and of the R₃ fraction were prepared with 2% (w/v) potassium phosphotungstate. These samples were placed on carbon-coated, Formvar-reinforced grids. Specimens for sectioning were fixed in 0.5% glutaraldehyde for 1 hr at 4°C, followed by an overnight fixation at 4°C in Kellenberger's osmium fixative (E. Kellenberger, A. Ryter, and J. Sechaud, *J. Biophys. Biochem. Cytol.* **4**:671, 1958). Specimens were then dehydrated in a graded alcohol series, embedded in a plastic mixture of 70% dodecenyl succinic anhydride, 20% Araldite 6005 (A. P. Cargille), and 10% Epon 812 (Shell Chemical Co., San Francisco, Calif.), and polymerized at 80°C for 48 hr. Sections were cut on a Porter-Blum MT-2 microtome with a diamond knife and were poststained with lead citrate (E. S. Reynolds, *J. Cell Biol.* **17**:208, 1963). All preparations were examined with a Hitachi HS-7S electron microscope.

Microtubules, which have been described structurally as straight "dense-walled" cylinders, are observed readily in whole cells of *A. vine-*

landii. These cells were partially emptied of their cytoplasm content by treatment with penicillin and were resuspended in negative stain (Fig. 1).

The diameter of a microtubule released from the disrupted cell was calculated from a negatively stained preparation (Fig. 2) and found to be approximately 235 Å. The diameter of the core measured approximately 100 Å. In several instances, "cog-wheel" structures (Fig. 2) were seen in positions that suggested possible breakage from one end of the microtubule. The overall diameter of this cog-wheel structure measured 250 Å, with a central core measuring 100 Å. Twelve prominent cogs can be seen. Although this gear-like structure may be unique for the microtubule, it is somewhat reminiscent of the 13 circular subunits observed in a transverse section of a tubule in the juniper root cell (M. C. Ledbetter and K. R. Porter, *Science* **144**:872, 1964). Ten subfilaments also have been observed in the constituent fibrils (or microtubules) of rat sperm tails (D. C. Pease, *J. Cell Biol.* **18**:313, 1963). The wall of the microtubule isolated from nucleated red-blood cells of salamander consisted of 12 to 14 longitudinal subunits in a circular arrangement (J. G. Gall, *J. Cell Biol.* **31**:639, 1966).

The length of microtubules observed to date has varied, and lengths as long as 1 μ have been observed. The broken microtubule structure is readily observed in Fig. 3. The structural appearance of a microtubule is distinguished readily from flagella (Fig. 1 and 3) in a negatively stained preparation. Longitudinally sectioned microtubules (Fig. 4) in fixed cells exhibited a diameter of approximately 215 Å. Figure 5 shows a thin section in which intracellular microtubules have been cross-sectioned. The diameter of these tubules is 190 Å. The dimensions of the *Azotobacter* microtubule described in this communication are within the range reported for *Proteus mirabilis* by van Iterson and co-workers.

As yet, no known function has been proven for this microstructure, although it is believed to

FIG. 1-2. Negatively stained preparation with 2% potassium phosphotungstate.

FIG. 1. Whole cell of *Azotobacter vinelandii* after penicillin treatment. MT, microtubules (several are visible in this region); F, flagella. $\times 49,000$.

FIG. 2. Isolated microtubule exhibiting core filled with stain. Note cog-wheel structure. $\times 244,000$.

FIG. 3. Negatively stained preparation with 2% potassium phosphotungstate. Fragmented microtubule (MT) contrasted with flagella (F). $\times 93,000$.

FIG. 5. Thin section of penicillin-treated cell. Whole cell showing two microtubules (MT) in cross section having dimensions corresponding to those observed in the longitudinally sectioned whole cell. $\times 185,000$.

FIG. 4. Thin section of penicillin-treated cell. Longitudinally sectioned whole cell. Note microtubules (MT). $\times 122,000$.

be involved in intracellular protoplasmic movements. It has been postulated, also, that microtubules may play a role in support function where they serve as elastic cytoskeletal elements, and that energy released at their surfaces is translated by some unknown mechanism into cytoplasmic movements (K. R. Porter and L. G.

Tilney, *Science* **150**:382, 1965; L. G. Tilney and K. R. Porter, *Protoplasma* **50**:317, 1966).

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