Fine Structure of *Thiobacillus novellus*

By M. KOCUR, T. MARTINEC AND K. MAZANEC

*Czechoslovak Collection of Microorganisms, and Department of Histology
and Embryology, J. E. Purkyně University, Brno, Czechoslovakia*

(Accepted for publication 22 January 1968)

SUMMARY

The study of ultrathin sections of *Thiobacillus novellus* has shown that it has a structure similar to that of *T. thiooxidans* and other Gram-negative bacteria. The cell wall showed a multilayered structure and varied in thickness between 250 and 350 Å. The cytoplasmic membrane and 70 Å thick. On certain media a capsule was formed. The cytoplasm contained ribosomes and polymetaphosphate granules. The structure of the nuclear material was similar to that observed in other bacteria.

INTRODUCTION

Thiobacillus novellus Starkey 1935 represents an exception among species of the genus *Thiobacillus*. While the other species of the genus are both motile and obligate autotrophic *T. novellus* is non-motile and facultative autotrophic.

Sijderius (1946) was of the opinion that *Thiobacillus novellus* was identical with *Micrococcus denitrificans*. The mentioned species, however, differ both in their ultrastructure (Kocur, Martinec & Mazanec, 1968) and in their % GC values in the deoxyribonucleic acid (Boháček, Kocur & Martinec, 1965). Therefore they cannot be considered as identical.

The purpose of this paper was to give a description of the fine structure of *Thiobacillus novellus* and compare it with the structures of similar organisms already described by other authors.

METHODS

Organism. The strain *Thiobacillus novellus* CCM 1077 (ATCC 8093) used in the present study came from the Czechoslovak Collection of Microorganisms, J. E. Purkyně University, Brno. The cells were grown on two different media: on the medium for autotrophic growth of *T. novellus* recommended by Santer, Boyer & Santer (1959) and on the medium of Chang & Morris (1962).

Electron microscopy. After 24 hr cultivation at 30°, the cells were flushed with saline, washed three times and centrifuged for 5 min. After 2 hr fixation with 1% solution of OsO₄ in phosphate buffer at pH 7.3 according to Millonig (1962), the cells were flushed with appropriate buffer and refixed with 10% neutral formol at 4° for 1 hr. After having been washed with saline the cells were centrifuged and embedded in 1.3% agar and dehydrated with alcohol. The dehydrated blocks were embedded in Epon 812 according to the prescription of Luft (1961).

Ultrathin sections made with an ultramicrotome, type Tesla Bs478, were stained for 20 min with a 2% solution of uranylacetate (pH 5) and for 10 min with lead citrate according to Reynolds (1963). The sections were studied and photographed in an electron microscope, type Tesla Bs413 A.

RESULTS

In the same preparation of *Thiobacillus novellus* two types of cells were observed: short rods, measuring 0.8 to $1 \times 1.7 \mu$ and longer ones, the size of which was $1 \times 3.5 \mu$. As can be seen from Pl. 1, fig. 1, 2 the cell wall (CW) has a multilayered structure characteristic of Gram-negative bacteria and its total thickness ranges from 250 to 350 Å (see Pl. 3, fig. 5, 6).

On certain cultivating media a capsule was formed. When cultivated in the medium of Chang & Morris (1962) the cell wall of *Thiobacillus novellus* was covered with a mucous layer the thickness of which ranged from 700 to 1000 Å (Pl. 3, fig. 5 and 6). But when cultivated in the medium of Santer *et al.* (1959) it did not form a capsule (Pl. 1, fig. 1, 2; Pl. 2, fig. 3, 4; Pl. 3, fig. 7, 8).

The cytoplasmic membrane is a typical unit membrane 80 Å in width and corresponds by its structure and thickness to other Gram-negative bacteria. It can best be seen in Pl. 2, fig. 4. The cytoplasm is of granular structure and contains: (i) electron-dense particles—ribosomes (R); (ii) inclusions (P). No mesosomes were observed.

There occur frequently light areas in *Thiobacillus novellus*, looking like vacuoles situated either in the end (Pl. 1, fig. 1, 2) or in the central part of the organism (Pl. 3, fig. 5). These areas are separated from the cytoplasm by a membrane.

The nuclear material of *Thiobacillus novellus* has a typical filamentous structure of highly varied course. It is clearly distinguishable from the granular cytoplasm by its light colour.

Division in *Thiobacillus novellus* proceeds in the same way as in other Gram-negative bacteria (Conti & Gettner, 1962; Mahoney & Edwards, 1966). Different stages of division are shown in Pl. 3, fig. 7, 8. Plate 3, fig. 7, shows the early stage of division—the cytoplasmic membrane and the cell wall begin to invaginate into cytoplasm. As can be seen from Pl. 3, fig. 8, each daughter cell has its own continuous cytoplasmic membrane (see arrows), while the cell wall is common to both cells.

DISCUSSION

The results obtained in the present work showed that the cell-wall structure of *Thiobacillus novellus* is similar to that of other Gram-negative bacteria (Mahoney & Edwards, 1966; Remsen & Lundgren, 1966). At present, the fine structure of only a single species of the genus *Thiobacillus*, *T. thiooxidans*, is known (Mahoney & Edwards, 1966). The comparison of its electron micrographs with those of *T. novellus* showed that these organisms have a similar cell structure. No differences in the cell-wall structure explaining the different physiology of the facultative autotrophic *T. novellus* and the strict autotrophic *T. thiooxidans* were observed. *T. novellus* differs from *T. thiooxidans* only in its greater size, in non-flagellated cells which can be encapsulated under certain conditions.

The internal structure of both organisms is similar. We found in *Thiobacillus novellus* large electron-dense bodies similar to those observed by Mahoney & Edwards

(1966) in *T. thiooxidans*. These osmiophilic bodies are most probably composed of polymetaphosphate. As for the light areas occurring in *T. novellus*, these are considered to be artifacts produced by plasmolysis as they are situated between the cell wall and the cytoplasmic membrane. It is known that plasmolysis can occur in various stages of preparation of the blocks for ultrathin sections.

REFERENCES

- BOHÁČEK, J., KOCUR, M. & MARTINEC, T. (1965). Deoxyribonucleic acid base composition and taxonomy of the genus *Micrococcus*. *Publ. Fac. Sci. Univ. J. E. Purkyně, Brno, K* **35**, 318.
- CHANG, J. P. & MORRIS, J. G. (1962). Studies on the utilization of nitrate by *Micrococcus denitrificans*. *J. gen. Microbiol.* **29**, 301.
- CONTI, S. F. & GETTNER, M. E. (1962). Electron microscopy of cellular division in *Escherichia coli*. *J. Bact.* **83**, 544.
- KOCUR, M., MARTINEC, T. & MAZANEC, K. (1968). Fine structure of *Micrococcus denitrificans* and *Micrococcus halodenitrificans* in relation to their taxonomy. *Antonie van Leeuwenhoek* **34**, 19.
- LUFT, J. L. (1961). Improvements in epoxy resin embedding methods. *J. biophys. biochem. Cytol.* **9**, 409.
- MAHONEY, R. P. & EDWARDS, M. R. (1966). Fine structure of *Thiobacillus thiooxidans*. *J. Bact.* **92**, 487.
- MILLONIG, G. (1962). Further observation on a phosphate buffer for osmium solution in fixation. In Breese, S. S., Jun. (ed.), *Electron Microscopy, 5th int. Congr., Philadelphia, Pa.* p. P-8. New York and London: Academic Press.
- REMSEN, CH. & LUNDGREN, D. G. (1966). Electron microscopy of the cell envelope of *Ferrobacillus ferrooxidans* prepared by freeze-etching and chemical fixation techniques. *J. Bact.* **92**, 1765.
- REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208.
- SANTER, M., BOYER, J. & SANTER, U. (1959). *Thiobacillus novellus*. I. Growth on organic and inorganic media. *J. Bact.* **78**, 197.
- STARKEY, R. L. (1935). Isolation of some bacteria which oxidize thiosulphate. *Soil Sci.* **39**, 197.
- SIJDERIUS, R. (1946). *Heterotrophe bacteriën, die thiosulfaat oxydeeren*. Thesis, University of Amsterdam, The Netherlands.

EXPLANATION OF PLATES

PLATE 1

Fig. 1, 2. Longitudinal section showing cell wall (CW), cytoplasmic membrane (CM), nuclear material (N), vacuole-like area (V). Fig. 1, $\times 95,000$; fig. 2, $\times 75,000$.

PLATE 2

Fig. 3, 4. Longitudinal and cross-sections showing cell wall (CW), cytoplasmic membrane (CM), nuclear material (N) and ribosomes (R). Fig. 3, $\times 75,000$; fig. 4, $\times 125,000$.

PLATE 3

Fig. 5, 6. Portion of cells showing capsules (CA), cell wall (CW), cytoplasmic membrane (CM), polymetaphosphate granule (P) and vacuole-like area (V). Fig. 5, $\times 85,000$; fig. 6 $\times 85,000$.

Fig. 7, 8. Portion of cells showing various stages of cell division. Cell wall (CW), cytoplasmic membrane (CM). Fig. 7, $\times 75,000$; fig. 8, $\times 75,000$.