Biology of Budding Bacteria

III. Fine Structure of *Rhodomicrobium* and *Hyphomicrobium* spp.

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Received for publication 30 September 1964

**ABSTRACT**

Conti, S. F. (Dartmouth Medical School, Hanover, N.H.), and Peter Hirsch. Biology of budding bacteria. III. Fine structure of *Rhodomicrobium* and *Hyphomicrobium* spp. J. Bacteriol., 89:503-512, 1965.—The ultrastructure of 14 strains of hyphomicrobia, and of *Rhodomicrobium vannielii*, was investigated by means of electron microscopy of thin sections. The majority of the strains of hyphomicrobia possessed a well-developed internal membrane system, which appeared to be derived by invagination from the cytoplasmic membrane. The subcellular organization of the hyphomicrobia and *R. vannielii* was investigated.

*Hyphomicrobium vulgare* and *Rhodomicrobium vannielii*, the sole representatives of their genera (Bergey's *Manual*), characteristically multiply by formation of a daughter cell at the end of a hypha (filament). This mode of cell division clearly sets them apart from other true bacteria. Although *H. vulgare* and *R. vannielii* appear to be morphologically similar, they are physiologically quite distinct. *H. vulgare*, and recently isolated *Hyphomicrobium* strains, are aerobic organotrophs, whereas *R. vannielii* is an anaerobic photoorganotroph. Their morphological similarity has led to the suggestion that *H. vulgare* is the colorless counterpart of *R. vannielii* (van Niel, 1954).

The study of Boatman and Douglas (1961) has established some of the principal ultrastructural features of *R. vannielii*; the reproductive mechanism has also been described (Murray and Douglas, 1950). In an attempt to elucidate further and to compare the subcellular organization of budding bacteria, the fine structure of two strains of *H. vulgare*, some recently isolated strains of hyphomicrobia (Zavarain, 1960; Hirsch and Conti, 1964a, b), and *R. vannielii*, was examined.

**MATERIALS AND METHODS**

A total of 14 strains of hyphomicrobia were investigated. *H. vulgare* strain ZV-580 was obtained from G. A. Zavarain; recent isolates of hyphomicrobia, in addition to those previously described (Hirsch and Conti, 1964a), were also studied. *R. vannielii* was obtained from H. C. Douglas.

The hyphomicrobia were grown at 30 C in minimal medium "337" (Hirsch and Conti, 1964b) with 0.05 m methylamine as the carbon source and (NH₄)₂SO₄ as the nitrogen source. Cultures, grown with shaking or stirring, were collected by centrifugation at various stages of growth, and were prepared for electron microscopy by the procedures of Ryter and Kellenberger (1958). *R. vannielii* was grown as described by Morita and Conti (1964), with an incident light intensity of 300 ft-c; 0.18% sodium lactate was substituted for sodium acetate in the culture medium. Cells were embedded in either Vestopal W or Epon 812, sectioned with an LKB Ultratome, stained with lead hydroxide by the procedure of Millonig (1961), and examined with an Akashi TRS-80 or Phillips EM-200 electron microscope.

**RESULTS**

In agreement with the observations of Boatman and Douglas (1961), the subcellular structure of *R. vannielii* was found to be characterized by the presence of a peripheral lamellar membrane system. The cytoplasm, cytoplasmic membrane, and cell wall are continuous with the hyphal portion of the cell; structures resembling cross walls (Fig. 2) were observed within the hyphae. A cross section (Fig. 1) of a representative cell illustrates that the membranes are concentrically arranged at the periphery of the cell; in some regions they are closely apposed to one another, thus giving the appearance of being paired membranes. In addition to the membranes at the periphery of the cell, other intracytoplasmic membranes were observed (Fig. 2). The possible continuity of the cytoplasmic membrane, peripheral membranes, and intracytoplasmic membranes, all approximately 90 A wide, could not be

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FIG. 1. Cross section through a *Rhodobacter vanniellii* cell, illustrating the structure of the peripheral membrane (PM) system. × 115,500.

FIG. 2. Longitudinal section of a cell in which the peripheral membrane system is not well developed; there are indications of continuity of the cytoplasmic membrane with the peripheral membranes (PM). Note (arrow) the presence of a structure resembling a cross wall in the hypha. *N* = nucleus; *CW* = cell wall. × 69,000.
determined with certainty. Although the peripheral membranes appeared to be continuous with one another when observed in cross sections, longitudinal sections generally revealed that the membrane system is usually open at one or both ends of the cell, as shown by Boatman and Douglas (1961). In some instances, however, median longitudinal sections showed (Fig. 4) that the peripheral membranes are not open; they seem to enclose completely the nuclear material. Cells in which the peripheral membranes are absent or only poorly developed were also observed (Fig. 3).

On the basis of their fine structure, the hyphomicrobia could be divided into two groups: those which possessed, and those which lacked, a distinctive intracellular membrane system. The two strains of *H. vulgare* (NQ-321 and Mev-333), and the ZV-580 strain obtained from Zavarzin, did not appear to have a distinct intracellular membrane system under our experimental conditions. An extensive membrane system was observed in all other strains of hyphomicrobia examined.

The structure of the strains lacking internal membranes is illustrated in Fig. 5 and 8. As in most bacteria, the fibrillar nucleoplasmic regions and ribosomal particles constituted the principal internal elements of the cell; poly-β-hydroxybutyrate granules were also quite prominent in some cells. The cell wall, cytoplasmic membrane, and cytoplasm of the cell and filament were continuous. In contrast with *R. vannielii*, cross walls were not observed within the hypha.

The appearance of strain ZV-580 was quite distinctive in the electron micrographs. Rosettes, characterizedly formed in growing cultures (Zavarzin, 1960), were also observed in the thin sections. Figure 8 illustrates the appearance of a rosette; the adhesion of the cells appears to be due to the presence of a holdfast material. In one instance, cells apparently in the process of conjugation were observed (Fig. 6).

All other strains of hyphomicrobia possessed an extensive intracellular membrane system (Fig. 7, 9-14). In sections, these membranes (approximately 90 Å wide) appeared to form spherical to ellipsoidal vesicles or tubelike invaginations derived from the cytoplasmic membrane. In rare instances, they formed a peripheral lamellar system similar to that of *R. vannielii* (Fig. 7 and 11). In many cells a definite connection of the intracytoplasmic membranes to the cytoplasmic membrane could be observed (Fig. 10-14). Where this continuity was distinct, the transparent central area of the vesicle appeared to open into the area between the cell membrane and cell wall. The vesicles were present throughout the cytoplasm, although median sections indicated that they were most abundant in the peripheral region of the rod portion of the cell. Occasionally, vesicles could be observed within the hypha (Fig. 9, 12, 14a). The dimensions of the cytoplasmic membrane, and of the membranes surrounding the vesicles, were identical. They appeared as two dense parallel lines, enclosing a less-dense region, with a total thickness of approximately 90 Å.

The localized complex membranous infoldings observed in a number of bacteria (Murray, 1963), and recently shown to be present in the gram-negative stalked bacteria of the genera *Caulobacter* and *Aiticococcus* (Poindexter and Cohen-Bazire, J. Cell Biol., in press), were not observed.

**DISCUSSION**

Our observations on the fine structure of *R. vannielii* are in close agreement with the results of Boatman and Douglas (1961). These investigators concluded that the components of the internal membrane system are folded so as to form a hollow laminated ellipsoid that is open at one or both ends of the cell. Our micrographs confirmed this conclusion, although in some cells it appeared that the peripheral membrane system completely encloses the nuclear region. Since an opening in the membranes must be necessary for migration of nuclear material into the hypha, disruption and reformation of the membrane system may occur in some cells during cell division.

Although a peripheral membrane system is quite prominent in most cells of *R. vannielii*, it appears to be poorly developed or missing from others. These observations may have been due to the heterogeneity of the cell population of the culture. Actively growing cultures contained motile, flagellated "swarmer" cells, mature cells with hyphae, and daughter cells (buds at the ends of hyphae) in various stages of development; in sections these cell types could not be distinguished from one another with any degree of certainty. Since structures resembling cross walls are formed while the daughter cells are still attached (thus resulting in complete independence of both cells), we can presume that the daughter cells at this stage must contain bacteriochlorophyll and lamellae. It may well be that the cells with a poorly developed membrane system represent daughter cells in early stages of growth, whereas the older cells contain the most prominent lamellae. Since lamellae were not
Figs. 3-7.
observed within hyphae, we further suggest that
the lamellae arise de novo in the daughter cells,
the most probable site of formation being the
cytoplasmic membrane.

Many questions regarding the structure and
mode of division of *R. vaniieli* are as yet un-
answered. Although Boatman and Douglas
(1961) demonstrated the localization of bacterio-
chlorophyll in membranes, it cannot be concluded
that all membranes (including the cytoplasmic
membrane) contain bacteriochlorophyll and
possess the capacity to carry out photosynthetic
electron transport; the site and mechanism of
formation of these membranes is also not estab-
lished. The process by which nuclear material is
transmitted along the hypha to the bud is still
obscure, although the behavior of chromatic
material during cell division has been described
(Murray and Douglas, 1950). Studies of the
development of swarmers cells, by light and elec-
tron microscopy, may give further insight into
these problems.

Most hyphomicrobia can be placed in the
-growing list of bacteria which possess mem-
branous intrusions into the cytoplasm (Murray,
1963). The vesicles which are observed appear
to result from sections through tubelike invagina-
tions from the cytoplasmic membrane, the con-
tinuities with the cell membrane being obscured
by the plane of the section. Some of the vesicles,
however, appear to be interconnected and
physically separated from the cytoplasmic
membrane. The intracellular appearance of some of the
hyphomicrobial cells (Fig. 12 and 13) is strikingly
similar to that of photosynthetic bacteria, e.g.,
*Rhodospirillum rubrum* (Cohen-Bazire and Kunisawa,

It is difficult to interpret the absence of a
distinct intracytoplasmic membrane system in
three of the strains of hyphomicrobia examined
(ZV-580, NQ-521, and Mev-533). The internal
membrane system may be entirely absent; it
may be absent or poorly developed at the particu-
lar stage of growth studied, or it may not be
readily visible because of lack of contrast or
inadequate specimen preparation. The latter
possibilities seem improbable, because the proce-
dures employed revealed internal membranes in
the other strains. A most intriguing possibility is
that the capacity of these three strains to form
the intracellular membranes has been lost or
repressed in some fashion. In such a case, a com-
parison of these strains (e.g., cytochromes, respiratory
capacity, etc.) with those hypho-
microbia possessing the membrane system might
give some insight as to the function of the intra-
cytoplasmic membranes.

The ultrastructure of the membrane-deficient
strains throughout the various stages of growth
is being followed. The effect of various environ-
mental conditions (e.g., oxygen tension) is also
under study, because it has been established that
the internal structure of both photosynthetic
(Cohen-Bazire and Kunisawa, 1963) and non-
photosynthetic (Vanderwinkel and Murray, 1962)
bacteria is drastically affected by the conditions
of growth.

The subcellular organization and appearance of
*R. vaniieli* and most hyphomicrobia give
additional support to the view that the form and
appearance of the membranous infoldings can
vary quite widely in procaryotic cells. The form
assumed by the infoldings gives no indication as
to whether they are concerned with a photo-
synthetic or respiratory function.

The results of this study lend further support to
the suggestion of van Niel (1954) that *H.
vulgar* (and other hyphomicrobia) can be con-
sidered as a colorless counterpart of *R. vaniieli*.

**Acknowledgments**

This investigation was supported by Public
Health Service research career program award
1-K3-GM-8716 to the senior author, by research
grant GB-2387 from the National Science Founda-
tion, and by research grant GM-08565 from the
U.S. Public Health Service.

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**Fig. 3.** Cross section of *Rhodomicrobium vaniieli* cell, illustrating the presence of intracytoplasmic
membranes (im) in addition to those at the periphery. Note the area (arrow) which appears to be comprised
of a mass of intertwined membranes. X 64,000.

**Fig. 4.** Longitudinal section through a cell, showing the continuity of the peripheral membranes (PM)
neat the hyphal region of the cell. X 43,000.

**Fig. 5.** Hyphomicrobium vulgar* NQ-521, illustrating the general appearance of cells of strains which
appear to be lacking a well-defined internal membrane system. H = hypha; r = ribosomes. X 58,000.

**Fig. 6.** Hyphomicrobium strain ZV-580 cells which appear to be in the process of conjugation. Note the
continuity of the cell wall, cell membrane, and nuclear area. X 41,500.

**Fig. 7.** Hyphomicrobium strain D-824; cross section through a cell in which the internal membranes (im)
appear as peripheral lamellae. Compare the appearance of this cell to that of *R. vaniieli* in *Fig. 3.* X 44,000.
**Fig. 8.** Hyphomicrobium strain ZV-580: a section through a rosette. The component cells appear to adhere by means of a holdfast material (hf). Note the absence of a well-developed internal membrane system. X 84,000.

**Fig. 9.** Hyphomicrobium strain B-522; longitudinal section illustrating the general features of the hyphomicrobial cell. The cell wall and cytoplasmic membrane of the rod portion of the cell and hypha (H) are continuous. The major structural elements of the cell are: cell wall (CW), cytoplasmic membrane (CM), internal membranes (im), nucleoplasm (N), ribosomes (r), and a poly-ß-hydroxybutyrate granule (PHB). Note the membrane-bounded vesicle within the hypha. X 80,000.
Fig. 10. *Hyphomicrobiurn* strain H-526; a longitudinal section of a cell with a well-developed internal membrane (im) system. Note the distinct vesicles (V), and tubelike structures which appear to be formed by invagination of the cytoplasmic membrane (CM). The material enclosed within the membranes is less electron-dense than the cytoplasm. N = nucleoplasm; CW = cell wall; PHB = poly-β-hydroxybutyrate granule. × 60,000.

Fig. 11. *Hyphomicrobiurn* strain H-526; section of two cells, illustrating the appearance of the internal membranes (im). The membranes in the cell at the left appear to be in the form of lamellae, whereas they appear as vesicles in the cell at the right. Note the distinct invagination (in) of the cell membrane (cm) into the cytoplasm. × 147,000.
FIG. 12. *Hyphomicrobium* strain H-526; longitudinal section showing the presence of numerous vesicles (v) within the cell and hypha. The interconnection of the vesicles (arrow), and invagination (in) of the cytoplasmic membrane (cm) can be readily observed. The relationship of the hypha to the rod portion of the cell is also apparent. × 77,000.

FIG. 13. *Hyphomicrobium* strain H-526; longitudinal section of a cell containing numerous well-defined membrane-bounded vesicles; the vesicles closely resemble the "chromatophores" of photosynthetic bacteria. Note the invaginations (in) of the cytoplasmic membrane. × 97,600.
Fig. 14. Hyphomicrobium strain M-552; serial sections of a cell, illustrating the connection of the cytoplasmic membrane to the internal membranes (in); some of the vesicles (arrow) also appear to be interconnected. The membrane-bounded region of the vesicles has the same electron density and appearance as the area between the cell wall and cytoplasmic membrane. X 94,000.
LITERATURE CITED


