Pleomorphy in Stalked, Budding Bacteria

P. A. TYLER and K. C. MARSHALL

Department of Botany, and Department of Agricultural Science, University of Tasmania, Hobart, Tasmania, Australia

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An investigation of hyphomicrobia from manganese deposits and various freshwater habitats revealed an astonishing degree of pleomorphy in the group. A range of variation spanning two described genera (Hyphomicrobium and Pedomicrobium) was induced by varying culture conditions and was further observed in natural environments. It is suggested that Pedomicrobium is an invalid genus.

In the 1957 edition of Bergey's Manual, only four genera of budding bacteria are included, namely, Hyphomicrobium, Rhodomicrobium, Pasteuria, and Blastocaulus. Since that time, several new types have been described and named, principally by Soviet writers. Zavarzin (16) recognized nine genera and proposed a tentative scheme of classification. However, subsequent work has shown some of these organisms to be invalid or artifacts. Thus, Sorokin (12) found that "organisms" described as the new class Krassilikoviae by Kriss and Mitkevich (8) were in reality the colloblasts of Ctenophore tentacles damaged by the sampling equipment. Again, the exact nature of some other described species is uncertain. The organism named Metallogenium symbioticum by Zavarzin (17) is enigmatic; even its dimensions pose problems in cellular organization. Aristovskaya's (1) isolated two stalked, budding bacteria which were capable of oxidizing iron or manganese, and proposed the new genus Pedomicrobium.

Two genera of stalked, budding bacteria, Hyphomicrobium and Rhodomicrobium, are better documented. H. vulgare Stutzer et Hartleb has been widely studied both morphologically and physiologically (4–7, 9, 15). R. vanniellii is an anaerobic, photosynthetic bacterium similar in morphology to the aerobic Hyphomicrobium, and it also reproduces by budding (2, 3, 10, 14). Hirsch and Conti (5) found that Hyphomicrobium exhibits considerable morphological variation, dependent in part on cultural conditions. The relationship of hyphomicrobia to other bacteria has been reviewed by Starr and Skerman (13).

Hyphomicrobia were found to be responsible for deposition of manganese in hydroelectric pipelines in Tasmania. These hyphomicrobia showed considerable pleomorphy, and in pure culture exhibited variation ranging from the classical Hyphomicrobium to forms indistinguishable from Aristovskaya's Pedomicrobium. A detailed description of manganese oxidation in pipelines has been presented elsewhere (Tyler and Marshall, Antonie van Leeuwenhoek J. Microbiol. Serol., in press).

Materials and Methods

Sources of hyphomicrobia. Hyphomicrobia were isolated as pure colonies by grinding between glass plates the wet manganese deposits from pipelines and plating out in a dilution series on various media. Observations also were made on hyphomicrobia in the natural manganese deposits, on plankton from various lakes, and in several types of mucilages and jellies produced by different species of colonial diatoms, blue-green algae, Chlorococcales, and "protozoa.

Media. The following media were used both for initial platings and for maintenance of cultures: "337 medium" as described by Hirsch and Conti (5) but with MnSO₄·4H₂O at 0.02%; "337M medium" with methanol vapor as carbon source; "337MH medium" with 0.4% methyamine hydrochloride as carbon source; "PC medium," a modification of a medium of Pringsheim (11) containing yeast extract at 0.005%, MnSO₄·4H₂O at 0.002%. All media were made up with distilled water and 2% Oxoid agar. With all 337 media, phosphate buffers were autoclaved separately and added aseptically to the cooled medium.

Observation and photography. Cells from cultures and natural sources were examined by phase-contrast microscopy. Where necessary, manganese was dissolved with 5% oxalic acid. For stained preparations, cells were suspended in water or, in some instances, the agar was removed by leaching with hot water. Staining was by Ziehl-Neelsen's method for carbol fuchsin. Photographs were taken on Ilford FP3 film by use of either microelectronic flash or tungsten illumination. Development was in Agfa Rodinal at a dilution of 1:15 for 8 min at 20 C.

Results

Observation of manganese deposits. Examination of manganese deposits revealed a variety of microorganisms, the most common type being a
stalked bacterium closely resembling *Hyphomicrobium*. Large numbers ramified throughout the matrix of the deposit and careful focussing showed that the stalks repeatedly branch, forming a network. The cell is generally regular, having the typical pear shape of *Hyphomicrobium*.

**Isolation of hyphomicrobia.** Small, brown-black colonies appeared on PC medium after 4 to 6 weeks of incubation. The colonies had a dark amorphous center of oxidized manganese, from the edge of which hyphomicrobia radiated (Fig. 1). The same organisms grew on all 337 media but did not oxidize manganese. As on the PC medium, stalked cells radiated from the edge of the colony (Fig. 2). The ability to oxidize manganese of PC medium was often lost on transfer.

**Pleomorphy.** Pleomorphy in the hyphomicrobial community was characterized by the ability of individual cells to change shape and form different structures. This was particularly evident in the PC medium, where cells could be seen to undergo a series of changes leading to the formation of branched stalks, as well as the production of new buds. This pleomorphy was further enhanced by the use of phase-contrast microscopy, which allowed for the observation of living cells in detail.

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**Fig. 1.** (top) Colony form of *Hyphomicrobium T37*, showing dark center of oxidized manganese with cells radiating from the edge. Unstained colony photographed in situ after 15 days of growth in PC medium (phase contrast).

**Fig. 2.** (bottom) *Hyphomicrobium T37*, undisturbed surface colony on 337 medium, showing classical morphology with pear-shaped cells and unbranched stalks. Phase contrast of living cells. Unless stated otherwise, the scale in all figures is as shown in Fig. 11, and all cells are from stationary-phase colonies.

**Fig. 3.** (top) *Hyphomicrobium T37* on surface of 337MH medium, showing classical morphology and (arrow) a bizarre cell with regular buds. Phase contrast of living cells.

**Fig. 4.** (bottom) *Hyphomicrobium* in an algal jelly, showing regular pear-shaped cells with sparingly branched stalks. Phase contrast of Formalin-fixed cells.
crobia observed here takes two forms: firstly, a range of branching of the stalks and, secondly, a bizarre, often contorted cell shape. This is observed in natural manganese deposits, algal jellies, and plankton, and in laboratory cultures of isolates, including *Hyphomicrobium* strain T37.

Classical pear-shaped cells with unbranched stalks were characteristic of 337 medium (Fig. 2) and often were observed on other media among bizarre cells (Fig. 3). Similar forms were observed in a variety of natural habitats, though in algal jellies there is a tendency for the stalks to branch (Fig. 4, 5).

A range of bizarre cell shapes may be accompanied by varying degrees of branching of the stalks. Figures 6 and 7 show portions of colonies where pleomorphy is evident, both in the bizarre cell shape and in the extreme reticulation of the stalks. This form resembles closely Aristovskaya's illustrations of *Pedomicrobium* (1). Observations by phase-contrast microscopy suggest that anastomoses may form between some stalks. However, this requires confirmation by electron microscopy. On PC and 337MH media, cells usually were pleomorphic (Fig. 8–11), with a tendency to become giant, lobed, and swollen with refractile granules of poly-β-hydroxybutyrate (5,
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Fig. 10. Hyphomicrobium T37, showing extremely bizarre cell forms. The sausage-shaped cell is swollen at intervals with granules of poly-β-hydroxybutyrate. Live cells at edge of colony, in situ in 337MH medium. Phase contrast.

Fig. 11. Hyphomicrobium T37, showing very bizarre cell forms, with bizarre cells often bearing classical, pear-shaped buds. Camera lucida drawings of live cells in situ in 337MH medium.

6). Extremely bizarre forms are difficult to recognize as hyphomicrobia (Fig. 10 and 11). Bizarre cells often bore regular pear-shaped buds at the ends of stalks (Fig. 3 and 11). Sometimes a motile swarmer, which has remained attached, may be seen towing its bizarre parent.

Discussion

Hyphomicrobium is usually envisaged as a pear-shaped cell reproducing by the production of a bud at the end of a long, unbranched stalk. Variation from this classical morphology has recently been recorded (5, 15). The present investigation confirmed that this tendency towards pleomorphy is widespread both in natural environments and in pure culture. The illustrations presented show morphological forms ranging from the classical hyphomicrobial shape, through types with regular cells but reticulate stalks, to cells showing bizarre shapes with or without a reticulate stalk system.

Bizarre cell shape, at least in many cases, ap-
pears to be a consequence of the production of several stalks at different points on the cell. A narrowing bulge occurs in the direction in which each stalk arises (Fig. 6–9, 11). In contrast to this, regular cell shapes are found when multiple branching of stalks occurs at the point of origin (Fig. 8). In many cells, however, giantism and bizarre shape appear to be related to accumulation of large poly-β-hydroxybutyrate reserves (Fig. 10). This phenomenon has also been reported by Hirsch and Conti (5, 6). The stalk has always been regarded as an essential part of the reproductive process, and its role in the production of motile buds has been amply demonstrated (7, 9, 15). In this context, branching of the stalks should increase the capacity for bud formation.

Hirsch and Conti (5) suggested a complete re-evaluation of the budding bacteria in view of the wide variety of morphological forms encountered. In the present investigation, budding bacteria in pure culture and in natural environments displayed a whole range of morphological types, from the classical *Hyphomicrobium* to the types described by Aristovskaya (1) as *Pedomicrobium* spp. Aristovskaya noted a morphological relationship between *Pedomicrobium* and the anaerobic, photosynthetic *Rhodomicrobium*, but she did not comment on the possibility of relationships with *Hyphomicrobium*. Because of the fact that a pure culture (*Hyphomicrobium* T37) exhibited the complete range of structure mentioned above, we feel that the genus *Pedomicrobium* is invalid and that it should be regarded as a form of *Hyphomicrobium*. In view of the considerable pleomorphy demonstrated here, a cautious approach to erecting new genera of budding bacteria may save future confusion.

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**Literature Cited**