Taxonomic implications of reproductive mechanisms of *Hyphomicrobium*-facies and *Pedomicrobium*-facies of a pleomorphic budding bacterium

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Slide cultures of *Hyphomicrobium* T37 were observed microscopically. The organism behaved as classical *Hyphomicrobium* by budding from a polar hypha, and as *Pedomicrobium* when irregular colonies were produced by three processes: production of several hyphae from the cell, branching of hyphae, and sessile budding. The criteria for delimiting the genus *Pedomicrobium* are discussed in the light of these facts.

INTRODUCTION

After the description of *Hyphomicrobium* (Stutzer et Hartleb, 1898) several investigators demonstrated its unique reproduction by budding a flagellated swarmer from a hypha (Kingma Boltjes, 1936; Mevius, 1953; Zavarzin, 1960). Murray and Douglas (1950) showed that the colonial, photosynthetic bacterium *Rhodomicrobium* reproduced in the same manner. A morphologically similar bacterium (*Pedomicrobium*) was detected in manganese deposits (Aristovskaya, 1961). *Hyphomicrobium* may be pleomorphic and form colonies resembling *Pedomicrobium* (Hirsch and Conti, 1964; Tyler and Marshall, 1967).

Reproduction in *Hyphomicrobium* has been investigated by slide culture and in this paper the criteria for the genus *Pedomicrobium* are discussed.

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MATERIALS AND METHODS

Hyphomicrobium strain T37 was isolated from manganese deposits in Tasmania (Tyler and Marshall, 1967). Mineral medium 337 (Hirsch and Conti, 1964) was used with C_1 sources methanol (M), methylamine hydrochloride (MH) and both together (MHM) as detailed in Bauld, Tyler and Marshall (1971).

A layer of medium was poured on a glass slide and inoculated with T37 cells taken from a 3–5 day slope (22 C). A Teflon membrane was placed over the agar (Noller and Durham, 1968), bubbles removed by smoothing and the membrane sealed to the slide with wax. The slide culture was incubated at room temperature (19–23 C) on the microscope.

Photomicrographs were taken with 63 ×, or 100 ×, Neofluar phase contrast objectives (Carl Zeiss, Oberkochen). Electron-microscopy was as in Bauld et al. (1971).

RESULTS

The classical reproductive process of *Hyphomicrobium* was repeatedly demonstrated. Fig. 1 shows that swarmers produce a slender hypha at one pole. A distal swelling occurs, after which no further hyphal elongation takes place. When the swelling approximately equals the mother cell in size, detachment of the daughter cell occurs.

Fig. 2 shows that many buds may be produced by a hypha. Failure to swim away results from lack of water between agar and membrane, or from lack of flagella.

In contrast to this *Hyphomicrobium*-facies, many cells grew in the *Pedomicrobium* manner, producing cellular – and colonial – pleomorphy (Bauld et al.,

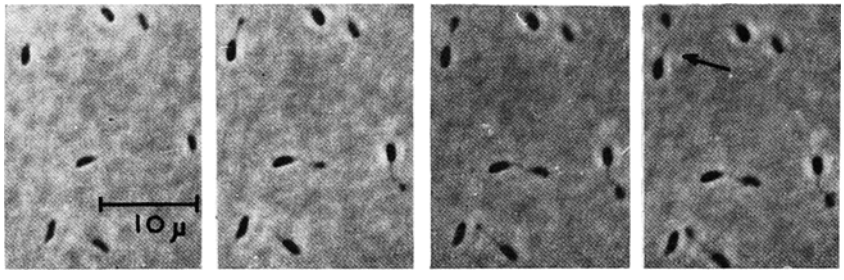

Fig. 1. Reproduction of single cells of *Hyphomicrobium* T37 on MH medium at 20 C, showing classical *Hyphomicrobium-facies*. 0, 16.5, 21.5, 24.5, hr. Swarmer release occurred after 24.5 hr (arrowed). 1,250 ×.

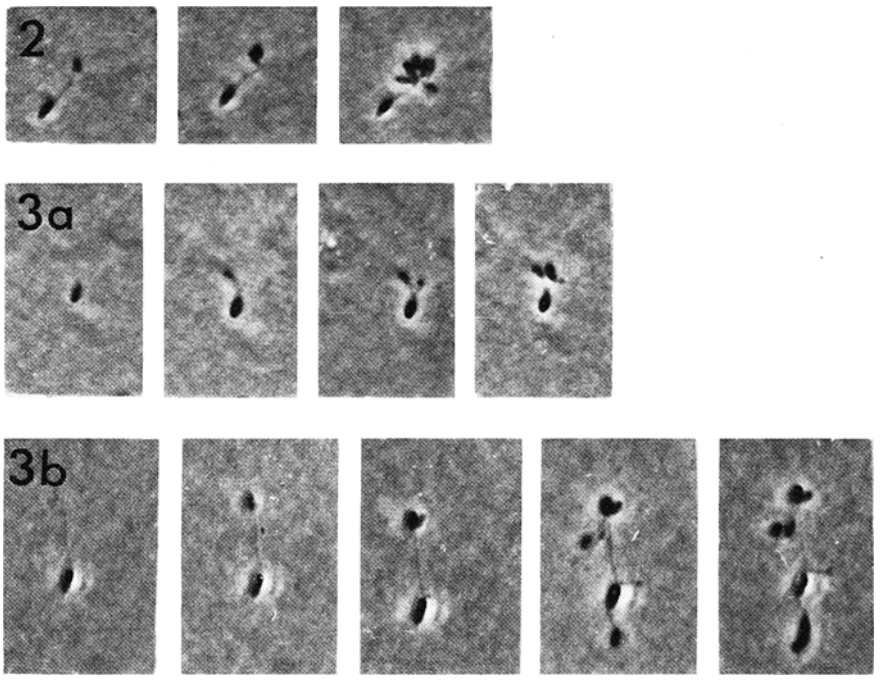

Fig. 2. T37, *Hyphomicrobium-facies*. Multiple budding of mother cell at 21 C on methanol medium. 0,4, 16 hr. 1,500 ×.
Fig. 3. T37 *Pedomicrobium-facies*. Production of several hyphae by one cell.
a. Successive production of 3 hyphae from one pole. 0, 12, 16, 20 hr. 1,500 ×.
b. Production of several hyphae, followed by budding, leads to colony formation. 0, 8, 12, 20, 24 hr. 1,500 ×.

1971). Fig. 3 shows early stages in development of colonial pleomorphy by successive formation of hyphae at one pole (Fig. 3a). Development of the first bud was complete before a second hypha commenced. After 20 hr a third bud was produced but its point of origin is not clear.

Fig. 3b demonstrates the formation of colonial and cellular pleomorphy. After 8 hr the mother produced a bud from pole A. After 12 hr, a second bud was produced from this hypha. Between 12 and 20 hr the mother cell generated two hyphae, one at each pole. The hypha at pole B quickly produced a large bud whereas the hypha at pole A had still not budded eight hr after formation.

A sequence of colony formation is shown in Fig. 4a. A bud formed on a short hypha enlarged until it approximately equalled the mother cell (4–12 hr) and then elongated, a process interpreted as sessile budding (Bauld et al., 1971). After 20 hr sessile budding occurred at the opposite pole of the mother, the bud so formed rapidly budding from a short hypha. After 28 hr, the first formed bud

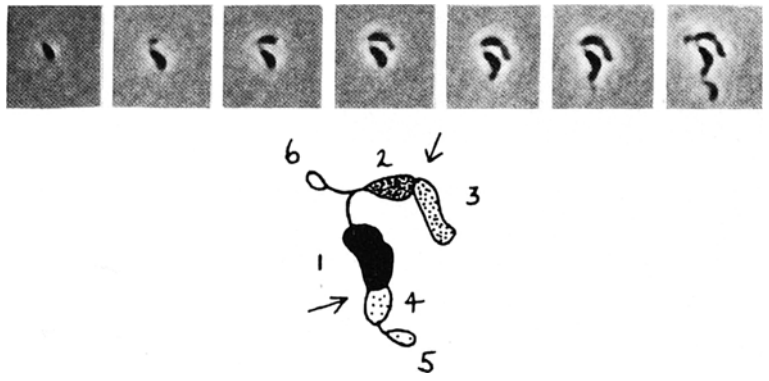

Fig. 4. T37, *Pedomicrobium*-facies, formation of colony.
a. Development of colony during 28 hr of incubation. 0, 4, 12, 16, 20, 24, 28 hr. 1,250 ×.
b. Diagram of the sequence of budding. Sessile budding occurs at two points (arrowed).

produced a second daughter. This sequence is shown diagrammatically in Fig. 4b.

Sessile budding is not confined to the *Pedomicrobium*-facies. New strains of *Hyphomicrobium* isolated from manganese deposits in Tasmania carried out classical reproduction by sessile budding (Fig. 5). Electron microscopy showed that very short hyphae are present (Fig. 6).

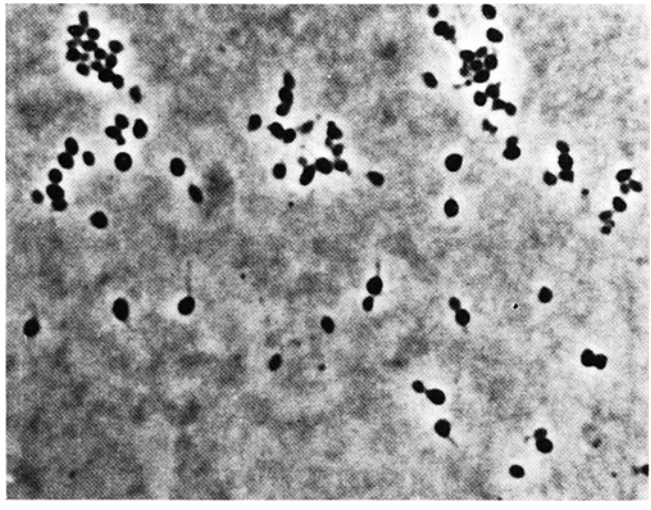

Fig. 5. *Hyphomicrobium* strain T61 showing prolific budding on minute hyphae (sessile budding). 1,500 ×.

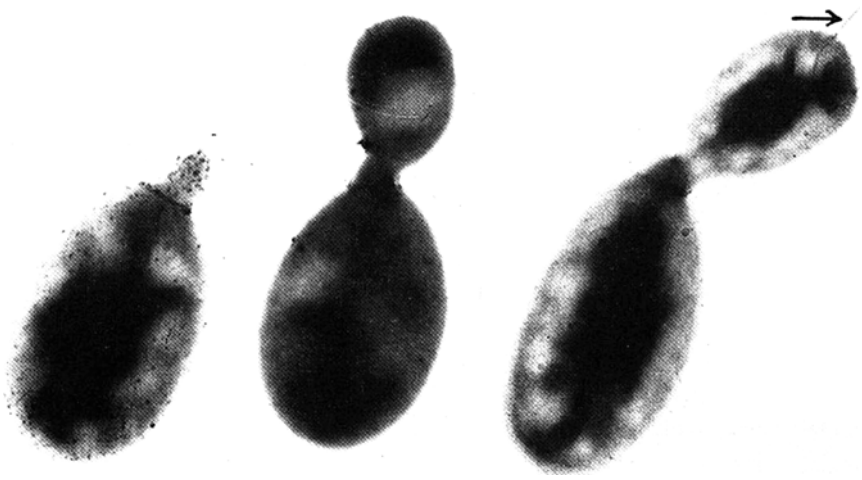

Fig. 6. Classical reproductive sequence of *Hyphomicrobium* in a strain with minute hyphae. A flagellum appears to be present on the mature daughter cell (arrow). Negative-stained. 20,000 ×.

DISCUSSION

Since *Hyphomicrobium* is aerobic we had to use Teflon membranes to cover our slides. This allowed gas exchange while preventing water loss, but impaired resolution. Since budding and hyphal extension occurs in several planes, a 63 × objective was used to allow adequate depth of focus and field of view. The amount of water between agar and membrane proved critical; too much caused currents during focussing, too little prevented buds swimming from their parents.

The classical picture of reproduction in *Hyphomicrobium* is production of flagellated swarmers on long, polar hyphae produced by mother cells (Kingma Boltjes, 1936; Mevius, 1953; Zavarin, 1960). Daughter cells lose their flagella, produce hyphae and repeat the process. Mother cells are presumed to produce further buds on the same hyphae. This has been shown clearly in T37 in slide culture. Rosette formation, a characteristic of Zavarin's strain of *Hyphomicrobium* (Zavarin, 1960), is rare in T37.

Duchow and Douglas (1949) recognised the similarity between reproduction of *Rhodomicrobium* and *Hyphomicrobium*. In *Rhodomicrobium*, however, daughter cells remain attached to parent hyphae, generate further hyphae from distal poles and so produce regular, branching colonies. Release of daughter cells is not obligatory for *Hyphomicrobium* and a colonial habit may develop as in *Rhodomicrobium* (Hirsch and Conti, 1964 (Fig. 27); Hirsch, 1968*a* (Fig. 14);

Tyler and Marshall, 1967), the factors dictating retention (colony formation) or release being unknown. The colonial tendency is marked in *Hyphomicrobium* T37.

Bauld et al. (1971) showed that the solitary or colonial habit depended on carbon source, and produced at will dendroid colonies or simple budding. This variable morphology has been confirmed by slide culture. Dendroid colonies of T37 are formed in three ways: production of several hyphae from a cell, branching of hyphae, and sessile budding. The first and second methods have been well documented (Fig. 4; Tyler and Marshall, 1967; Bauld et al., 1971). Hyphal formation is not essential for budding in *Hyphomicrobium* (Hirsch and Conti, 1964), sessile budding occurring during simple budding (Figs. 6, 7) and during formation of colonies (Fig. 5). Some forms of cellular pleomorphy, such as long sausages (Tyler and Marshall, 1967, Fig. 10), may result from continuous sessile budding, broad cytoplasmic connection being maintained between daughter and mother cells. Extension of this principle reconciles dichotomous lobing (Bauld et al., 1971) with the process of budding.

Aristovskaya (1961) isolated a manganese-oxidizing bacterium (*Pedomicrobium*) forming dendroid colonies similar to *Rhodomicrobium*, and Zavarzin (1961) suggested relationship between *Hyphomicrobium*, *Pedomicrobium* and *Rhodomicrobium*. However, Tyler and Marshall (1967) regarded *Pedomicrobium* as but one facies of a pleomorphic *Hyphomicrobium*, a contention supported by Bauld et al. (1971). Hirsch (1968*b*) disagrees, claiming that in *Pedomicrobium* hyphae grow out from one to several points whereas in *Hyphomicrobium* hyphae grow from the poles only. This distinction cannot be applied to T37. We regard cells with several hyphae as multipolar, the several lobes of parent cells resulting from repeated, dichotomous sessile budding. Further, our organism behaves as typical *Hyphomicrobium* or as *Pedomicrobium* depending on conditions. With methanol as carbon source the *Hyphomicrobium*-facies predominates while with methylamine the *Pedomicrobium*-facies is at least partially displayed (Bauld et al., 1971). With methanol and methylamine together the *Pedomicrobium*-facies predominates. One of Hirsch's own strains of *Hyphomicrobium* exhibits cellular and colonial pleomorphy when grown in this way (Hirsch and Conti, 1964, Fig. 20). The *Pedomicrobium*-facies of T37 is best developed when the organism oxidizes manganese (Tyler and Marshall, 1967); the original description of *Pedomicrobium* (Aristovskaya, 1961) was of cells from a manganese-encrusted colony. The variable morphology of T37 questions the credibility of the genus *Pedomicrobium* as depicted by Aristovskaya (1961) and interpreted by Hirsch (1968*b*). The pleomorphic tendency in most Hyphomicrobiales (Hirsch and Conti, 1964; Johnson and Weisrock, 1969; Pongratz, 1957; Shah and Bhat, 1968) signals caution in adopting morphological features as generic criteria.

This pleomorphy has philosophical significance in bacterial taxonomy, beset as it is by the same problems (Mandel, 1969) plaguing taxonomy of other apomicts (Tyler, 1970). Without a genetic concept of species, nomenclatural taxa are assembled in hierarchical schemes. Unfortunately, hierarchical values of taxonomic characters vary in different groups of bacteria – criteria sufficient to separate "genera" in one group may separate only "species" in another (Leifson, 1966). These phenetic taxa are catalogue numbers, and so long as catalogues are sufficient, all is well. However, when codified by international laws catalogue numbers assume respectability and come to exist in men's minds as biological realities. Hierarchical catalogues may even be interpreted phyletically.

In the present case we have no evidence that the number of hyphae per cell is somehow diagnostic at generic or even specific level. Contrarily, we have evidence that forms taken by our organism are expressions of pleomorphy. By analogy with *Rhodomicrobium* (where a generic criterion is anaerobic photosynthesis) we could equally well use manganese – or iron – oxidation as the diagnostic feature of *Pedomicrobium*. Unfortunately, in culture this is as inconstant as the number of hyphae per cell.

It is humane not to burden the nomenclature without good reason, and so we describe our organism as the taxon *Hyphomicrobium* T 37. Inasmuch as its range of morphology spans two described taxa we use the terms *Hyphomicrobium*-facies and *Pedomicrobium*-facies as informal descriptive terms for the extremes of the range. If omega taxonomic studies reveal considerable genetic distance between *Hyphomicrobium* and "*Pedomicrobium*" we shall be happy to be proved wrong.

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