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Emendation of *Methylobacterium* Patt, Cole, and Hanson 1976; *Methylobacterium rhodinum* (Heumann 1962) comb. nov. corrig.; *Methylobacterium radiotolerans* (Ito and Iizuka 1971) comb. nov. corrig.; and *Methylobacterium mesophilicum* (Austin and Goodfellow 1979) comb. nov.

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The present definition of the genus *Methylobacterium* excludes organisms which do not utilize methane facultatively. However, reports have indicated that cultures of the type strain of the type species, *Methylobacterium organophilum*, can lose this feature easily. Furthermore, studies have shown that *M. organophilum* is phenotypically highly similar to the methane-nonutilizing, pink-pigmented, facultatively methylotrophic bacteria and that the latter should be excluded from the various genera to which they have been assigned previously. Therefore, in accordance with an earlier suggestion, we propose that the description of the genus *Methylobacterium* be emended to permit the inclusion of methane-nonutilizing organisms which are otherwise highly similar to the type species. We further propose that all of the pink-pigmented, facultatively methylotrophic bacteria, which include the species *Pseudomonas rhodos*, *Pseudomonas radiora*, and *Pseudomonas mesophilica*, be transferred to the genus *Methylobacterium*.

The genus *Methylobacterium* Patt, Cole, and Hanson 1976 (Approved Lists 1980) was proposed (16) to accommodate certain newly isolated gram-negative bacteria that have the ability to utilize methane, as well as more complex organic compounds, as carbon and energy sources (15). This genus contains only one validly named species (the type), *Methylobacterium organophilum* (16), although two species that have not been validated, "*Methylobacterium ethanolicum*" and "*Methylobacterium hypolimneticum*," have also been described (12). The present description of *Methylobacterium* (16) is based on a single strain of *M. organophilum* (strain xx [= NCIB 11278 = ATCC 27886]). In a recent numerical taxonomic study (6) we found this strain to be phenotypically highly similar to many methane-nonutilizing, pink-pigmented, facultatively methylotrophic bacteria (PPFMs), exemplified by *Pseudomonas* sp. strain AM1 of Peel and Quayle (17). The 149 PPFMs we examined by using 140 biochemical, physiological, and morphological features fell into two clusters. Each of these clusters had an internal similarity of about 80% when we used the simple matching coefficient and unweighted average linkage algorithms (19), and they were joined together at the 70% similarity level. *M. organophilum* NCIB 11278 was recovered in the larger cluster and showed a similarity of >95% compared with some of its neighbors. In view of these results, we expressed the opinion that to classify *M. organophilum* in a genus separate from all of the other PPFMs solely on the basis of methane utilization was unjustified. This opinion was reinforced by the finding that neither the National Collection of Industrial Bacteria strain nor the American Type Culture Collection strain of *M. organophilum* was any longer able to grow on methane, thus rendering them virtually indistinguishable (phenotypically) from many other PPFMs.

We also suggested that the PPFMs constituted a distinct taxon which could be excluded from most of the genera to which they had been assigned. We had previously reviewed in detail the somewhat checkered taxonomic history of the PPFMs (5); at different times these organisms had been assigned individually or collectively to the genera *Bacillus*, *Flavobacterium*, *Chromobacterium*, *Corynebacterium*, *Vibrio*, *Protaminobacter*, *Mycoplana*, *Pseudomonas*, and *Methylobacterium*. We pointed out that current concepts of the first five of these genera by definition could not accommodate the PPFMs. The genus *Protaminobacter* was no longer recognized, and our argument for the exclusion of the PPFMs from the genera *Mycoplana* and *Pseudomonas* was based largely on our own numerical taxonomic study (6). The two

PPFM clusters formed a phenon which was sharply differentiated from clusters containing the type strains of the type species of *Pseudomonas* (percent similarity, 55%) and *Mycoplana* (percent similarity, 62%). Thus, we came to the conclusion, admittedly on largely phenotypic grounds, that the only existing genus to which the PPFMs might belong was *Methylobacterium*, especially since the type species of this genus appeared to be a typical PPFM. However, the description of *Methylobacterium* excluded methane-nonutilizing organisms; since cultures of the type strain of the type species had lost the ability to utilize methane, the logical course seemed to be to redefine the genus so that this property ceased to be an invariable feature. Recent evidence that methane utilization in *M. organophilum* may be plasmid borne (7; R. S. Hanson, personal communication; M. L. O'Connor, personal communication) further supports this approach.

Although the present definition of *Methylobacterium* is too restrictive in excluding methane-nonutilizing organisms which are otherwise very like the type strain, at the same time it is too permissive in that almost any facultative methanotroph can be admitted to the genus, regardless of overall similarity to the type strain. The purpose of the present paper is twofold. First, we emend the description of the genus *Methylobacterium* to remove the anomalies described above. Second, we propose the transfer of the PPFMs to the emended genus *Methylobacterium*.

Currently, there are three validly named species of PPFM in addition to *M. organophilum*; these are *Pseudomonas rhodos* Heumann 1962 (8) (Approved Lists 1980), *Pseudomonas mesophilica* Austin and Goodfellow 1979 (1) (Approved Lists 1980), and *Pseudomonas radiora* Ito and Iizuka 1971 (9) (Approved Lists 1980). In our previous study, *M. organophilum* and *P. rhodos* fell into one of the two PPFM clusters, and *P. mesophilica* fell into the other. *P. radiora* was not included in our earlier study, but subsequently we found the type strain (NBIC 10815) to have the characteristics of the *P. mesophilica* cluster (Green, unpublished data). Despite the high phenotypic similarities within each cluster, there is evidence to suggest that there may be heterogeneity in other respects. The spread of guanine-plus-cytosine values in each cluster is about 6 to 7 mol% (6), and deoxyribonucleic acid-deoxyribonucleic acid homology between *M. organophilum* and *Pseudomonas* sp. strain AM1 is fairly low (45 to 48%) (8), although both of these organisms belong to the same phenetic cluster. Significant differences between these organisms in the induction and suppression patterns of 1-C pathway enzymes have also been reported (13, 14). Furthermore, Urakami and Komagata (20) believe that on the basis of electrophoretic studies, there may be four groups of PPFMs. Further work is clearly needed before strains of PPFMs can be allocated to new species with any confidence. For this reason we propose simply to transfer to the genus *Methylobacterium* the three PPFM species presently excluded from it. Thus, we propose the following changes. (i) *Pseudomonas rhodos* (Heumann 1962) should become *Methylobacterium rhodinum* (Heumann 1962) comb. nov. corrig. (ro.di'num. Gr. adj. *rhodinos* rosy; N. L. neu. adj. *rhodinum* rosy, because of its pink color); this species is described in references 6 and 8, and the type strain is strain ATCC 14821 (= NCIB 9421). (ii) *Pseudomonas mesophilica* (Austin and Goodfellow 1979) should become *Methylobacterium mesophilicum* (Austin and Goodfellow 1979) comb. nov. (me.so.phi'li.cum. Gr. neu. n. *meson* the middle; Gr. adj. *philikos* friendly; N. L. neu. adj. *mesophilicum* friendly to the middle, because of its preference for moderate temperatures; this species is described in references 1 and 6, and the type strain is strain ATCC 29983 (= NCIB 11561). And (iii) *Pseudomonas radiora* (Ito and Iizuka 1971) should become *Methylobacterium radiotolerans* (Ito and Iizuka 1971) comb. nov. corrig. (ra.di.o.to'ler.ans. Eng. prefix *radio* pertaining to radiation; L. part. adj. *tolerans* tolerating; N. L. part. adj. *radiotolerans* tolerating radiation, because of its high resistance to gamma radiation); this species is described in references 6 and 9, and the type strain is strain ATCC 27329 (= NCIB 10815).

Phenotypic evidence suggests that there may be a case for amalgamating some of these species, but because of the uncertainties mentioned above, we feel that to do this or to propose new species would be premature. In view of this, therefore, we make no attempt at this stage to assign any strains other than the type strains to the species of *Methylobacterium*. We suggest that for the time being, the many PPFM strains not yet allocated to particular species simply be regarded as *Methylobacterium* strains awaiting specific assignment.

The emended description of the genus *Methylobacterium*, based on the work of Green and Bousfield (6), except where stated otherwise, is given below.

Methylobacterium Patt, Cole, and Hanson 1976 (Approved Lists 1980) emend. Rods 0.8 to 1.0 by 1.0 to 8.0 μm, occurring singly or occasionally in rosettes (8, 15); occasionally branched and pleomorphic (11). Motile by single polar, subpolar or lateral flagella, although some strains are not vigorously motile. Cells often contain large sudanophilic inclusions and sometimes volutin granules. Gram negative, although

many strains are gram variable; representative strains have the multilayered cell wall structure and the type of citrate synthase (6) characteristic of gram-negative bacteria. Most strains grow slowly and some do not grow at all on nutrient agar. Colonies on glycerol-peptone agar are ≤1 to 3 mm in diameter and pale pink to bright orange red; colonies on methanol-salts agar are a more uniform pale pink. The pigment is insoluble and probably carotenoid (4, 9). In static liquid media strains grow as a pink surface ring or pellicle. Strictly aerobic; catalase and oxidase (often weakly) positive. Chemoorganotrophs, facultative methylotrophs, and occasionally facultative methanotrophs (15). The ability of some strains to utilize methane as a sole source of carbon and energy is easily lost if strains are not maintained on an inorganic medium in a methane atmosphere (Hanson, personal communication). Representative strains have been reported to assimilate 1-C compounds via the homoisocitrate pathway (10) (icl^- pathway [2, 18]) and to have a complete tricarboxylic acid cycle when they are grown on complex organic substrates (3, 15).

Members of the genus have been isolated from soil, dust, freshwater, lake sediments, leaf surfaces and nodules, rice grains, air, and hospital environments.

The optimum growth temperature is in the range from 25 to 30°C, and the deoxyribonucleic acid base composition is in the range from 60 to 70 mol% guanine plus cytosine. The type species is *M. organophilum* Patt, Cole, and Hanson 1976 (Approved Lists 1980).

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