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CHAPTER 3.1.13

Methylobacterium

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Introduction

The genus *Methylobacterium* is composed of a variety of pink-pigmented facultatively methylotrophic (PPFM) bacteria, which can grow on one-carbon compounds such as formate, formaldehyde and methanol as sole source of carbon and energy as well as on a wide range of multicarbon growth substrates. Most, but not all, strains can grow on nutrient agar and some can grow on methylated amines. Only one strain has been reported to utilize methane as sole carbon source.

Taxonomy

The first *Methylobacterium* strain to be described in the literature was isolated by Bassalik (1913) from earthworm contents and named *Bacillus extorquens*. Although they are common soil and environmental organisms, the PPFM bacteria were not isolated and studied extensively until the 1960s and 1970s, when interest in the study of the one-carbon assimilation pathways common to methylotrophic organisms and in the commercial applicability of these organisms first began.

Prior to 1960, the taxonomy of many of the isolates now assigned to *Methylobacterium* was still uncertain. Although regarded as Gram-negative, these organisms often stained Gram-variable. This Gram-variability, coupled with their morphological properties (mainly rods, which are occasionally branched and exhibit polar growth), has contributed to much of the confusion surrounding their checkered taxonomic history.

For example, Bhat and Barker (1948) assigned the *B. extorquens* isolate of Bassalik to the genus *Vibrio* as *V. extorquens*. Krasil'nikov (1959) and Bassalik et al. (1960) subsequently transferred the same species to *Pseudomonas* and *Flavobacterium*, respectively, before it temporarily came to rest, in the 8th edition of *Bergey's Manual of Determinative Bacteriology*, as *P. extorquens* incertae sedis (Doudoroff and Palleroni, 1974).

In a study of amine-utilizing bacteria, den Dooren de Jong (1927) described the pink, methylamine-utilizing species *Protaminobacter rubrum*, which De Vries and Derx (1953) later found to be very similar to organisms they had isolated from leaf nodules and leaf surfaces. On the grounds that all the organisms were Gram-negative, motile, branching rods, De Vries and Derx (1953) grouped *P. rubrum* with their isolates in the genus *Mycoplana* as *M. rubra*.

Peel and Quayle (1961), studying C-1 assimilatory pathways in methylotrophs, noted similarities between their own isolates, *Pseudomonas* AM1, and various other methylotrophic bacteria, including *V. extorquens*, *Protaminobacter ruber* (spelling amended, Breed et al., 1957) and *Pseudomonas methanica* isolated by Dworkin and Foster (1956). Peel and Quayle questioned the justification for classifying these organisms in different genera.

An early, but limited, taxonomic study by Stocks and McCleskey (1964) compared *V. extorquens*, *Protaminobacter ruber*, *Pseudomonas* AM1, and Harrington and Kallio's (1960) strain of *Pseudomonas methanica* with their own isolates and concluded that all were very similar and should be regarded as strains of *V. extorquens*, with the reservation that *Vibrio* might not prove to be the most suitable generic location.

In the more recent studies, Kouno and Ozaki (1975) isolated 59 different PPFM isolates from a variety of soil and water samples, and Austin et al. (1978) studied isolates from the phylloplane of *Lolium perenne*. In both these studies, there was agreement that the taxonomy of these organisms was obscure and needed further examination. Subsequently, Austin and Goodfellow (1979) concluded that their isolates were sufficiently different from *Protaminobacter ruber*, *Pseudomonas rhodos* (Heumann, 1962) and *P.* AM1 to merit placement in a new species, which they named *Pseudomonas mesophilica*.

Patt et al. (1974) isolated the first reported PPFM strain able to utilize methane and created

a new genus *Methylobacterium* to accommodate it. The new species *M. organophilum* was proposed for this single strain. Unfortunately, the genus description was based on the detailed examination of only one strain (*M. organophilum* xx), which has since lost its ability to utilize methane (R. S. Hanson, personal communication).

In a detailed taxonomic study, Green and Bousfield (1982) found *M. organophilum* strain xx to be phenotypically very similar to many of the strains of PPFM discussed previously, none of which could utilize methane. All of the 149 strains examined, using 140 biochemical, physiological, and morphological features, including *M. organophilum*, fell into either of two related (≥70% similarity) clusters, which were well separated from other facultative methylotrophs and nonmethylotrophic reference strains. As a result of this work, Green and Bousfield (1982) suggested that all the PPFM bacteria constituted a distinct taxon, which could be excluded from most of the genera to which they had previously been assigned. The genus *Methylobacterium* was chosen to accommodate this taxon (Green and Bousfield, 1981).

However, as the description of *Methylobacterium* (Patt et al., 1976) excluded organisms that could not utilize methane, an emended genus description was proposed (Green and Bousfield, 1983) that would allow the inclusion of all PPFM strains (whether they utilized methane or not), thus removing methane assimilation as an essential feature of the genus. At the same time, the description of *Methylobacterium* was more tightly circumscribed to prevent the genus from being used as a "dumping ground" for every facultative methane utilizer subsequently isolated, irrespective of its taxonomic relatedness to the PPFM bacteria.

As a result of this proposal the emended genus *Methylobacterium*, in addition to the type species *M. organophilum*, now contained three other validly named species: *Pseudomonas rhodos* (Heumann, 1962), renamed *M. rhodinum*; *Pseudomonas mesophilica* (Austin and Goodfellow, 1979), renamed *M. mesophilicum*; and *Pseudomonas radiora* (Ito and Iizuka, 1971), renamed *M. radiotolerans*.

Despite the overall similarities of the PPFM organisms and their recognition as a distinct taxon, there nevertheless remained doubts about the internal heterogeneity of the group (Green and Bousfield, 1983; Urakami and Komagata, 1981). This doubt was reinforced when subsequent work on representative strains of PPFM (Hood et al., 1987; Hood et al., 1988) involving DNA-DNA similarities and electrophoretic comparison of total soluble proteins revealed four major and several minor similarity (homology) groups. As a result, three new species of *Methylobacterium* were proposed (*M. rhodesianum*, *M. zatmanii* and *M. fujisawaense*) and several other probable centers of variation within the genus were recognized. Other workers have since described four new species. In 1993, Urakami et al. (1993) described a new species *Methylobacterium aminovorans*, which is involved in the biodegradation of tetramethylammonium hydroxide (TMAH) and *N*,*N*-dimethylformamide (DMF). In 1998, a strain that tolerates high (≥50 mM) thiocyanate and cyanate and is capable of utilizing these compounds as sole nitrogen source was isolated and described. This new species was named *Methylobacterium thiocyanatum* (Wood et al., 1998). The two most recently proposed species can grow on chlorinated methyl compounds as sole carbon and energy source. *M. chloromethanicum* (McDonald et al., 2001) and *M. dichloromethanicum* (Doronina et al., 2000) can utilise chloromethane (methyl chloride) and dichloromethane, respectively.

Two additional non-pink pigmented facultative methylotrophs claimed to be able to utilize methane as sole source of carbon and energy and to belong to the genus *Methylobacterium* ("*M. ethanolicum*" and "*M. hypolimneticum*"; see Lynch et al., 1980) have since been shown to be taxonomically unrelated to the PPFM (P. N. Green, unpublished observations).

Thus, twelve validated species of the genus *Methylobacterium* presently exist—the eleven discussed above plus *M. extorquens*, the type strain of which was recovered in one of the major DNA similarity (homology) groups and had already been described by Urakami and Komagata (1984) as *Protomonas extorquens*. As *Methylobacterium* was shown to have nomenclatural priority over *Protomonas* (Bousfield and Green, 1985), *P. extorquens* was subsequently transferred to the genus *Methylobacterium*.

Habitat

Members of the genus *Methylobacterium* are ubiquitous in nature and are thus found in a variety of habitats (Green and Bousfield, 1981; Green and Bousfield, 1983), including soil, dust, freshwater, lake sediments, leaf surfaces and nodules, rice grains, air, and hospital environments, and in various products and processes, e.g., as contaminants in pharmaceutical preparations such as face creams. As a common airborne organism, PPFM can occur in a wide variety of commercial processes where growth conditions are favorable, including various fermentation processes. Our identification service has also had isolates from pure water users such as silicon chip manufacturers.

The PPFM bacteria are strict aerobes and can be isolated from almost any freshwater environment where some dissolved oxygen exists; from tap water systems (Grfi and Bauer, 1973), where they produce pink ropy masses of growth; and from stratified lake systems (Hanson, 1980), where they occupy a special niche. The methane-oxidizing PPFM strains of *Methylobacterium organophilum* were isolated from the metalimnion of Lake Mendota, USA. Only in this stratified layer during the summer months, where methane was available in the presence of reduced oxygen tensions, did aerobic methane oxidation take place. Indeed, because of their ability to metabolize various breakdown products present in plant detritis such as methanol, methylamine, various other methylated compounds, and (in some cases) methane, the PPFM bacteria may play an important ecological role in the carbon cycle in nature. In addition, their ability to resist a certain degree of desiccation and to scavenge trace amounts of nitrogen and carbon (P. N. Green, unpublished observations) make them well suited for survival in stressful environments.

One such environment in which high numbers of PPFM bacteria are often found is roadside dust. Using selective media such as methanol salts agar (see Isolation), information has been obtained which suggests a correlation between levels of vehicular traffic and numbers of PPFM bacteria.

The association of *Methylobacterium* spp. with plants has been studied by various workers. In a numerical taxonomic study of phylloplane bacteria isolated from *Lolium perenne*, Austin and Goodfellow (1978) found pink chromogens (PPFM organisms) to be one of the major phenotypes isolated. Yoshimura (1982) found similar organisms in his study of pine-forest phylloplane bacteria and showed that their numbers varied quite dramatically with the seasons and with the accompanying environmental conditions.

After M. E. Rhodes-Roberts (personal communication) had isolated a PPFM strain (*Mycoplana rubra* NCIB 10409) from the sterilized leaf nodules of *Psychotria mucronata* and Corpe and Basile (1982) reported associations of similar organisms with lower plants, it became apparent that extra- and/or intracellular symbiotic or mutualistic associations may exist between plants and some strains of *Methylobacterium*. Although Corpe and Basile (1982) produced evidence to suggest that the PPFM bacteria present on mosses and liverworts may produce growth-stimulatory substances for these lower plants, there is presently no further evidence to support the theory of a symbiotic association. Also, radiotracer studies using $^{14}CO_2$ failed to demonstrate the uptake of labeled metabolites present in leaf exudates by a known *Methylobacterium* strain inoculated onto the surface of young *Vicia faba* plants (P. N. Green, unpublished observations). Thus, there remains the possibility that populations of PPFM attached to dust or soil particles are deposited on plant surfaces by the wind, and their numbers on leaf surfaces merely reflect the population size in the surrounding environment or as dictated by climatic conditions.

Because they are common airborne organisms, *Methylobacterium* spp. are also found as occasional contaminants in a variety of systems: domestic water supplies (mentioned above), media chills (refrigerated rooms on cabinets in which bacteriological culture media is stored prior to use), pharmaceutical products, fermentation vessels (especially in association with other C-1 compound-utilizing bacteria), and hospital environments (Gilardi and Faur, 1984). In the last case, they may pose a threat as opportunistic pathogens to seriously ill patients.

Isolation and Cultivation

Because of the ability of *Methylobacterium* spp. to grow on methanol as sole carbon and energy source and because of their characteristic pigmentation, these organisms are relatively easy to isolate. The following methanol mineral salts (MMS) medium is a suitable selective medium for *Methylobacterium*.

Methanol Mineral Salts Medium

The following are added per liter:

K_2HPO_4	1.20 g
KH_2PO_4	0.62 g
$CaCl_2 \cdot 6H_2O$	0.05 g
$MgSO_4 \cdot 7H_2O$	0.20 g
NaCl	0.10 g
$FeCl_3 \cdot 6H_2O$	1.0 mg
$(NH_4)_2SO_4$	0.5 µg
$CuSO_4 \cdot 5H_2O$	5.0 µg
$MnSO_4 \cdot 5H_2O$	10.0 µg
$Na_2MoO_4 \cdot 5H_2O$	10.0 µg
H_3BO_3	10.0 µg
$ZnSO_4 \cdot 7H_2O$	70.0 µg
$CoCl_2 \cdot 6H_2O$	5.0 µg

The MMS medium is sterilized by autoclaving at 121°C for 20 min and cooled to 50°C. A filter-sterilized vitamin solution (Colby and Zatman, 1973) is added if required, along with 0.1–0.2% (v/v) sterile methanol. The pH of the medium is adjusted to pH 7.0. Solidified media (MMS agar) are prepared by the addition of 1.5–2% Oxoid (purified) agar before autoclaving. No PPFM strain isolated to date has been shown to require vitamins or other added growth factors.

Although *Methylobacterium* strains can grow between 5° and 37°C, all grow well at 30°C, and

thus 25–30°C can be used for all isolation and subsequent growth experiments. These organisms are fairly slow growers, often taking 2–3 days at 30°C to produce clearly visible colonies or confluent growth and often taking more than 7 days for colonies to reach their maximum size of 1–3 mm in diameter. Growth is sometimes more luxuriant, with a deeper pigmentation, on Glycerol-Peptone (GP) agar.

Glycerol-Peptone Agar

The following are added per liter:

Agar	15 g
Glycerol	10.0 g
Peptone (Difco)	10.0 g

The pH is adjusted to pH 7.0

Although this medium is useful for subculturing stocks of pure *Methylobacterium* spp., it is less suitable for enrichment than the MMS medium, as other rapidly growing heterotrophs present in the sample can overgrow the PPFM bacteria. Certain antibiotics (see Identification) can also be considered for use in selective media, as can individual carbon sources for use in isolating specific groups or species of *Methylobacterium*. A summary of the properties of various species and isolates is given in Table 1.

If MMS agar is used as a selective medium, the vast majority of the pink colonies that reach diameters of more than 1 mm will be strains of PPFM organisms. Pink methylotrophic yeasts are not uncommon, but bacterial pink methylotrophs other than *Methylobacterium* species are rare.

Growth of PPFM organisms in liquid media is almost always characterized by a surface ring and/or thin pellicle, indicative of their aerobic nature.

When attempting to isolate PPFM strains from leaf surfaces, a leaf impression technique, using one of the above media, is recommended. Homogenization of whole leaves or embedding leaves in molten agar are alternatives, although they are not as successful as the impression technique.

Table 1. Substrate[a] utilized as sole carbon source to differentiate strains of *Methylobacterium*.

Species	D-Glucose	D-Fucose	D-Xylose	L-Arabinose	Fructose	L-Aspartate/L-Glutamate	Citrate	Sebaceate[b]	Acetate	Betaine	Methylamine	Trimethylamine	Methane	TMAH	DMF	Chloromethane	Dichloromethane	Growth on peptone-rich nutrient agar[c]
M. zatmanii	–	–	–	–	+	–	–	–	+	–	+	V	–	–	–	nd	nd	+
M. extorquens	–	–	–	–	–	V	–	–	+	+	+	–	–	–	–	nd	nd	V
M. rhodesianum	–	–	–	–	+	V	–	–	+	+	+	–	–	–	–	nd	nd	+
M. rhodinum	V	–	–	–	+	+	+	–	+	+	+	–	–	–	–	nd	nd	+
M. aminovorans	–	–	–	–	+	+	–	–	+	+	+	+	–	+	+	nd	nd	+
M. organophilum	+	–	–	–	+	–	–	–	+	–	+	+	V	–	–	nd	nd	+
M. chloromethanicum[f]	″	nd	″	″	″	″	″	nd	nd	″	+	″	″	nd	″	–	nd	″
M. dichloromethanicum[g]	–	–	–	–	+	+	–	–	+	+	+	–	–	nd	–	nd	+	+
M. thiocyanatum[d]	+	nd	nd	V	+	+[e]	+	nd	+	nd	+	–	nd	nd	nd	nd	nd	+
M. radiotolerans	+	+	+	+	–	+	+	+	+	+	–	–	–	–	–	nd	nd	+
M. fujisawaense	V	+	+	+	V	+	+	+	V	–	–	–	–	–	–	nd	nd	+
M. mesophilicum	V	+	+	+	–	+	+	V	–	–	–	–	–	–	–	nd	nd	–

Symbols: +, utilized as substrate; –, not utilized; V, variable result; and nd, no data.

Abbreviations: TMAH, tetramethylammonium hydroxide; DMF, *N*,*N*-dimethylformamide.

[a]Owing to the slow growth of some strains on certain substrates, carbon utilization tests were read after 14 days of incubation at 30°C (Green and Bousfield, 1982). Doubtful results were checked by twice subculturing in liquid medium.

[b]Most strains which utilize sebacate can also utilize pimelate, suberate, azelate and adipate.

[c]Nutrient agar e.g. Oxoid cm55.

[d]Taken from Wood et al. (1998).

[e]Tested for glutamate only.

[f]Taken from McDonald et al. (2001).

[g]Taken from Doronina et al. (2000).

If fungal contamination of samples from particular habitats is a problem when attempting to isolate strains of PPFM, 20 μg/ml of cycloheximide can be added to the medium.

Isolation and cultivation of PPFM bacteria involving single carbon susbstrates should be carried out on an appropriate salts basal medium (e.g. MMS where the methanol is replaced by the test substrate). For most substrates a final concentration of 0.1% is satisfactory. (For those strains utilising dichloromethane, see Doronina et al. 2000. Gaseous compounds methane and chloromethane are usually provided as head space gases in the proportions 50 : 50 methane : air and 2 : 98 chloromethane : air.)

Identification

All *Methylobacterium* strains are rods (0.8–1.0 × 1.0–8.0 mm), which occur singly or occasionally in rosettes (Patt et al., 1974; Heumann, 1962). They are often branched or pleomorphic, especially in older stationary-phase cultures (Fig. 1a). There is some evidence to suggest that they exhibit polar growth or a budding morphology (L. B. Perry, unpublished observations). All strains are motile by a single polar, subpolar or lateral flagellum, although some strains are not vigorously motile. Cells often contain large sudanophilic inclusions (poly-β-hydroxybutyrate) and sometimes also volutin granules (Fig. 1b). They are Gram-negative, although many strains stain as Gram-variable. Representative strains have a multilayered cell wall structure and the type of citrate synthase (Green and Bousfield, 1982) characteristic of Gram-negative bacteria. Most strains grow slowly and some not at all on nutrient agar. After 7 days of incubation at 30°C, colonies on GP agar are 1 to 3 mm in diameter and pale pink to bright orange-red, whereas colonies on MMS agar are a more uniform pale pink. The pigment is nondiffusible, is nonfluorescent, and probably is a carotenoid (Downs and Harrison, 1974; Ito and Iizuko, 1971). In static liquid media, most strains form a pink surface ring and/or pellicle.

All strains are strict aerobes and are catalase and oxidase (often weakly) positive. They are chemoorganotrophs and facultative methylotrophs, capable of growth on a variety of C-1 compounds. All grow on formaldehyde (often at micromolar concentrations), formate, and methanol; some grow on methylated amines. Only one species (*M. organophilum*) is reported to have utilized methane as sole carbon and energy source, but the ability has since been lost by the type (and only) strain. This organism's ability to assimilate methane was thought to be plasmid borne and easily lost if cultures were not maintained on an inorganic medium in a methane atmosphere (R. S. Hanson, personal communication). Representative PPFM strains have been reported to assimilate C-1 compounds via the

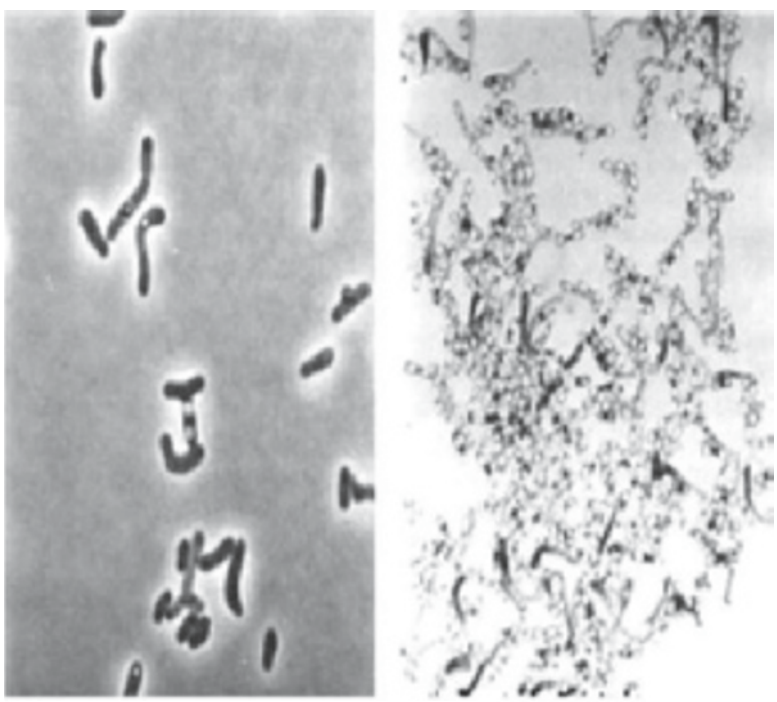

Fig. 1.

isocitrate lyase deficient (icl^-)$^-$serine pathway (Bellion and Spain, 1976; Quayle, 1972) and to have a complete tricarboxylic acid cycle when they are grown on complex organic substrates. The serine pathway is the major route for assimilating 1-carbon compounds such as methanol and methylated amines in *Methylobacterium* spp. Carbon is assimilated into the cell via formaldehyde and is incorporated via an icl^- variant of the pathway described by Quayle.

The optimum growth temperature for all *Methylobacterium* strains is in the range 25 to 30°C. Some strains will grow at 15°C or less, and some will at or above 37°C. Although growth is optimal around neutrality, some strains can grow at pH 4 and some at pH 10. Growth factors are not required by any strain although calcium pantothenate (Urakami et al., 1993) has been shown to stimulate the growth of some strains, and most strains do not degrade or hydrolyze casein, starch, gelatin, cellulose, lecithin or DNA. Urease is produced by all strains, and some strains have weak lipolytic activity. The enzymes β-galactosidase, L-ornithine decarboxylase, L-lysine decarboxylase and L-arginine dihydrolase are not produced. Indole (except for *M. thiocyanatum*) and hydrogen sulfide (H_2S) are also not produced. The methyl red and Voges-Proskauer tests are negative, although some strains reduce nitrate to nitrite.

Most strains are sensitive to the chemotherapeutic agents kanamycin, gentamycin, albamycin T, streptomycin, framycetin and especially the tetracyclines, whereas most are resistant to cephalothin, nalidixic acid, penicillin, bacitracin, carbenicillin, colistin sulfate, polymyxin B and nitrofurantoin.

Species within the genus *Methylobacterium* are differentiated mainly by the pattern of compounds they utilize as sole carbon and energy source (see Table 1). However, care should be taken to standardize such tests because they are notoriously difficult to duplicate between laboratories. All carbon utilization tests shown in Table 1 were carried out as described by Green and Bousfield (1982), who used faintly turbid suspensions of cells (which had been thrice washed in sterile saline) to inoculate media. All tests were read only after 14 days of incubation at 30°C, and growth was compared to a negative control containing no added carbon source. This long incubation time was necessary to allow for slow growth on certain compounds. Doubtful results should always be checked by twice subculturing in liquid media.

The following compounds were used by most (≥95%) strains of *Methylobacterium*: glycerol, malonate, succinate, fumarate, α-ketoglutarate, DL-lactate, DL-malate, acetate, pyruvate, propylene glycol, ethanol, methanol and formate. Some strains (see Green and Bousfield, 1982, and Table 1) can also utilize L-arabinose, D-xylose, D-fucose, D-glucose, D-galactose, D-fructose, L-aspartate, L-glutamate, adipate, sebacate, D-tartrate, citrate, citraconate, saccharate, monomethylamine, trimethylamine, trimethylamine-*N*-oxide, ethanolamine, butylamine, dimethylglycine, betaine, tetramethylammonium chloride, *N*-N-dimethylformamide, chloromethane and dichloromethane. None of strains appear to use any of the disaccharides or sugar alcohols examined (Green and Bousfield, 1982) (except for glycerol) or any of the following as sole carbon and energy source: propionate, DL-arginine, L-valine, glycine, geraniol, tryptamine, histamine, putrescine, *m*-hydroxybenzoate, testosterone, sarcosine, phenol, thiourea, tetramethylurea, hexane or benzene. Ammonia, nitrate and urea can serve as nitrogen sources. *Methylobacterium thiocyanatum* can utilize cyanate and thiocyanate as sole source of nitrogen for growth (Wood et al., 1998).

The fatty acid composition of PPFM cells is comprised largely (around 70–90%) of $C_{18:1}$ mono-unsaturated straight-chain acids, and the major isoprenoid quinone components are ubiquinones with 10 isoprene units (Urakami and Komagata, 1979; Urakami and Komagata, 1986). Representative strains have been shown to contain 3-hydroxy $C_{14:0}$ as the principal hydroxy fatty acid (Urakami and Komagata, 1987; Urakami et al., 1993), in addition to small amounts of $C_{16:1}$ and $C_{19:0}$ cyclopropane acids. Similarly, representative strains were shown to contain large amounts of cardiolipin (diphosphatidylglycerol), phosphatidylethanolamine and phosphatidylcholine, and a small amount of phosphatidylglycerol in their phospholipids (Urakami et al., 1993) and to contain bacterial hopanoids or sterols (Urakami and Komagat, 1986; Knani et al., 1994). The DNA base composition is 68.0–72.4 mol% G+C (Hood et al., 1987; Urakami et al., 1993).

DNA-DNA similarity studies (Hood et al., 1987) and electrophoretic comparison of total soluble proteins (Hood et al., 1987; Urakami et al., 1993) from representative strains have demonstrated the existence of a number of similarity (homology) groups within the genus *Methylobacterium*, several of which have been subsequently proposed as new species (Green and Bousfield, 1988; Urakami et al., 1993; Wood et al., 1998), which can be distinguished phenotypically (see Table 1). Genotypic studies (Wilfrum et al., 1986; Tsuji et al., 1990; Bratina et al., 1992) have confirmed that strains belonging to the genus *Methylobacterium* belong to a single, if somewhat heterogeneous, taxon that is clearly distinguished from other methylotrophic and non-methylotrophic genera.

Sato (1978), Sato and Shimizu (1979), and Nishimura et al. (1981) have shown that strains of *Methylobacterium* can form bacteriochlorophyll *a* under specific cultural conditions, thus suggesting a common link in their ancestry with the phototrophs.

Recent DNA-rRNA similarity studies by Dreyfus et al. (1988) have placed *Methylobacterium* in the rRNA superfamily IV of De Ley (1978), along with other members of the *Agrobacterium-Rhizobium* complex.

Preservation

All members of the genus *Methylobacterium* survive freeze drying or lyophilization. These organisms can also be cryopreserved in their liquid growth medium supplemented with a suitable cryoprotectant (e.g., 10–15% [v/v] glycerol).

Applications

Although *Methylobacterium* strains have potential for the production of single-cell protein from methanol, their bioconversion ratios (cell mass formed: substrate consumed) are inferior to those of other methylotrophs. Thus, no immediate future is seen for these organisms in this capacity. However, PPFM strains have been used in fermentation processes for the manufacture of various coenzymes (coenzyme Q_{10}); amino acids (L-lysine, L-tyrosine, L-phenylalanine and L-glutamic acid); and vitamins (vitamin B_{12}) and as a source of poly-β-hydroxybutyrate (Stirling and Dalton, 1985; Hou, 1984). Their carotenoid pigment, which has been tested as a colorant in the food industry, may also have commercial applications.

The ease with which PPFM strains can be isolated from environmental samples and their (albeit tentative) link with vehicular emissions suggest possible uses for these organisms as environmental pollution indicators. In particular, the ability of some strains to grow in the presence of particulate exhaust material (soot) is interesting. The evidence that a number of these organisms can grow on some of the polycyclic aromatic hydrocarbons and long-chain aliphatic hydrocarbons contained in exhaust emissions (P. N. Green, unpublished observations) suggests a possible role for these bacteria as biological monitors of vehicular pollution.

In addition, several PPFM strains (Ito and Iizuka, 1971) have exhibited resistance to gamma-ray irradiation 10 to 40 times higher than that tolerated by several other Gram-negative bacteria examined and in a similar resistance range to that tolerated by *Deinococcus (Micrococcus) radiodurans* under certain test conditions. This resistance, coupled with their easily identifiable pigmented colonies, may make some PPFM strains suitable candidates for irradiation-quality-control monitoring in the food and packaging industries.

The ability of *M. thiocyanatum* (and probably other *Methylobacterium* strains) to tolerate and degrade relatively high (≥50 mM) levels of cyanate and thiocyanate may have uses in the bioremediation of thiocyanate wastes from various manufacturing processes. Similarly, a use for relevant species in the biological treatment of industrial effluents (e.g. dicloromethane in wastewaters) remains a possibility awaiting further study.

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