Methylobacterium populi sp. nov., a novel aerobic, pink-pigmented, facultatively methylotrophic, methane-utilizing bacterium isolated from poplar trees (Populus deltoides × nigra DN34)

Benoit Van Aken,1 Caroline M. Peres,2† Sharon Lafferty Doty,3 Jong Moon Yoon1 and Jerald L. Schnoor1

1Department of Civil and Environmental Engineering, University of Iowa, 4105 Seamans Center, Iowa City, IA 52242, USA
2Department of Microbiology, University of Iowa, 3-432 Bowen Science Building, Iowa City, IA 52242, USA
3Department of Biochemistry, Box 357350, University of Washington, Seattle, WA 98195, USA

A pink-pigmented, aerobic, facultatively methylotrophic bacterium, strain BJ001T, was isolated from internal poplar tissues (Populus deltoides × nigra DN34) and identified as a member of the genus Methylobacterium. Phylogenetic analyses showed that strain BJ001T is related to Methylobacterium thiocyanatum, Methylobacterium extorquens, Methylobacterium zatmanii and Methylobacterium rhodesianum. However, strain BJ001T differed from these species in its carbon-source utilization pattern, particularly its use of methane as the sole source of carbon and energy, an ability that is shared with only one other member of the genus, Methylobacterium organophilum. In addition, strain BJ001T is the only member of the genus Methylobacterium to be described as an endophyte of poplar trees. On the basis of its physiological, genotypic and ecological properties, the isolate is proposed as a member of a novel species of the genus Methylobacterium, Methylobacterium populi sp. nov. (type strain, BJ001T = ATCC BAA-705T = NCIMB 13946T).

Species of the genus Methylobacterium are strictly aerobic, facultatively methylotrophic, Gram-negative, rod-shaped bacteria that are able to grow on one-carbon compounds (e.g. methanol or methylamine), as well as on a variety of C2, C3 and C4 substrates (Green, 1992). Only the type species, Methylobacterium organophilum, has been shown to use methane as the sole source of carbon and energy (Patt et al., 1976). The genus Methylobacterium belongs to the α2 subclass of the Proteobacteria and currently consists of 14 species with validly published names (Heumann, 1962; Ito & Iizuka, 1971; Kouno & Ozaki, 1975; Patt et al., 1976; Rock et al., 1976; Austin & Goodfellow, 1979; Green & Bousfield, 1983; Urakami & Komagata, 1984; Bousfield & Green, 1985; Green et al., 1988; Urakami et al., 1993; Wood et al., 1998; Doronina et al., 2000, 2002; McDonald et al., 2001; Sy et al., 2001). Members of the genus Methylobacterium are distributed in a wide variety of natural and man-made environments, including soil, air, dust, fresh- and marine water and sediments, water supplies, bathrooms, air-conditioning systems and masonry (Hiraishi et al., 1995; Trotsenko et al., 2001). Some species have been described as opportunistic human pathogens (Truant et al., 1998; Hornei et al., 1999). In addition, methylotrophic bacteria are frequently associated with terrestrial and aquatic plants, where they colonize roots and leaf surfaces (Austin et al., 1978; Yoshimura, 1982; Corpe & Rheem, 1989; Trotsenko et al., 2001; Lidstrom & Chistoserdova, 2002). The association of Methylobacterium species with plants seems to rely on a symbiotic relationship between the bacterium and the plant host. Plants produce methanol...
(representing nearly 50% of the total volatile atmospheric organic carbon), which is toxic and is used by \textit{Methylobacterium} species as the sole source of carbon and energy (i.e. the methanol cycle; Trotsenko et al., 2001). In response, \textit{Methylobacterium} species produce phytohormones (cytokinins and auxins), which are known to stimulate plant growth (Ivanova et al., 2001; Koenig et al., 2002), fix atmospheric nitrogen (Sy et al., 2001) or help plants to fight pathogens (Holland & Polacco, 1994). Bacteria are often pink to red, due to the presence of carotenoids, and are referred to as pink-pigmented facultative methylo trophs. Members of the genus \textit{Methylobacterium} are highly resistant to dehydration, freezing, chlorine, UV and ionizing radiation and elevated temperatures (Trotsenko et al., 2001). \textit{Methylobacterium} species are known to metabolize a range of toxic organic chemicals, such as methyl chloride (McDonald et al., 2001), methyl bromide (Goodwin et al., 2001), methyl iodide (Schaefer & Oremland, 1999), dichloromethane (Doronina et al., 2000), ethylated sulfur-containing compounds (de Zwart et al., 1996), methylated amines (Trotsenko et al., 2001), methyl tert-butyl ether (Mo et al., 1997) and cyanate and thiocyanate (Wood et al., 1998). In this paper, the formal taxonomic description of a novel \textit{Methylobacterium} strain, BJ001\textsuperscript{T}, isolated from poplar tissues and able to use methane as the sole source of carbon and energy, is reported.

\textit{Methylobacterium} sp. strain BJ001\textsuperscript{T} was isolated from poplar plantlets and from tissue cultures (\textit{Populus deltoides} × \textit{nigra} DN34) that were developed initially from surface-sterilized explants and maintained under axenic conditions (Van Aken & Schnoor, 2002). Images of tissue cultures containing strain BJ001\textsuperscript{T} are available in Supplementary Fig. A in IJSEM Online. Poplar plantlets were surface-sterilized before bacterial isolation. The isolated bacterium was maintained routinely on Luria–Bertani (LB) medium, sterilized before bacterial isolation. The isolated bacterium was critical point-dried, mounted on stubs, sputter-coated with gold/palladium and visualized by using a Hitachi S-4000 SEM equipped with a field-emission electron source. Carbon-source utilization tests were performed by using a standard protocol described by Green & Bousfield (1982). Cellular fatty acids were extracted according to Bligh & Dyer (1959), purified on Sephadex beads (Amersham Biosciences) and analysed by GC-mass spectroscopy (Gerhardt et al., 1994). DNA G + C content was determined by HPLC analysis of individual nucleosides, resulting from DNA hydrolysis and deporphorylation (Mesbah et al., 1989). DNA manipulations were carried out according to standard protocols (Ausubel et al., 1999; Sambrook & Russell, 2000). 16S and 16S–23S intergenic spacer (IGS) rDNA analyses were performed by PCR amplification using the following primers: 27f (positions 11–27 of bacterial 16S rDNA, \textit{Escherichia coli} numbering), 1522r (positions 1492–1522), 926f (positions 901–926) and 115r/23S (positions 97–115 of bacterial 23S rDNA, \textit{E. coli} numbering) (Hurek et al., 1997; Tan et al., 2001). PCR conditions were as described by Tan et al. (2001). PCR products were cloned in a pGEM vector (Promega) and submitted to the University of Iowa DNA Core (Iowa City, IA) for sequencing. Determined rDNA and reference sequences from GenBank were aligned by using CLUSTAL\_W multiple alignment and BIOEDIT (version 5.0.9) software. The tree topology was inferred by the parsimony method (heuristic search) using PAUP (version 4.0) software (Sinauer Associates). DNA–DNA hybridization was carried out according to Doronina et al. (2002), using a method based on that of Denhardt (1966). Unlabelled, denatured DNA of \textit{Methylobacterium} species was immobilized on nitrocellulose membranes (Bio-Rad) (Ausubel et al., 1999; Sambrook & Russell, 2000). Reference DNA from strain BJ001\textsuperscript{T} was labelled with deoxy\textsuperscript{[3]}\textsuperscript{[2]}\textsuperscript{H}cytidine 5’-triphosphate (57 Ci mmol\textsuperscript{-1} = 2.1 × 10\textsuperscript{10} MBq mmol\textsuperscript{-1}) by using a Nick Translation kit (N5500; Amersham Biosciences). After labelling, reference DNA exhibited a specific activity of 1.47 × 10\textsuperscript{10} Bq µg\textsuperscript{-1} and a mean size of 400–600 bp (as determined on 2.0% agarose gel). Labelled DNA was denatured at 100°C for 10 min. Hybridization was performed according to Denhardt (1966), with a ratio of labelled to immobilized DNA of 1:100 (Doronina et al., 2002). Low-stringency (pre)-hybridization solution consisted of 30% (v/v) formamide, 1× SSC, 5× Denhardt’s solution, 1.0% SDS and 100 µg denatured salmon sperm DNA ml\textsuperscript{-1}. Radioactivity retained on the membrane was determined by liquid scintillation counting.

The bacterium isolated from poplar tissues, strain BJ001\textsuperscript{T}, was identified by phylogenetic analysis as a member of the genus \textit{Methylobacterium} (Fig. 1). Members of this genus are known to colonize the rhizosphere and phyllosphere of a variety of plant species (Austin et al., 1978; Yoshimura, 1982; Corpe & Rheem, 1989; Holland & Polacco, 1994; Trotsenko et al., 2001). However, this is the first report of an endophytic association with a poplar tree (\textit{Populus} sp.). The isolate had the following characteristics of the genus \textit{Methylobacterium} (Green, 1992). Cells were rod-shaped (0.8–1.0 × 1.0–10.0 µm), frequently branched and occurred singly or in rosettes (Fig. 2). They exhibited polar growth and were motile by a single polar or lateral flagellum. Photomicrographs of strain BJ001\textsuperscript{T} are available in Supplementary Figs B and C in IJSEM Online. Cells stained Gram-negative and colonies were pink to red. Cells were strictly aerobic and catalase- and oxidase-positive (Gerhardt et al., 1994). Due to the chemotaxonomic homogeneity of the genus \textit{Methylobacterium}, phylogenetic analyses constitute a critical tool for species identification (Green & Bousfield, 1982; Doronina et al., 2002). According to 16S rDNA sequences, the closest relatives of strain BJ001\textsuperscript{T} were \textit{Methylobacterium thiocyanatum}, \textit{Methylobacterium
Methylobacterium populi sp. nov.

Methylobacterium populi T (AY251818)
- Methylobacterium thiocyanatum DSM 11400T (U59016)
- Methylobacterium rhodesianum JCM 2810 (D32230)
- Methylobacterium extorquens ATCC 14718 (AF293375)
- Methylobacterium chloromethanum VKM B-2223T (AF198624)
- Methylobacterium lutanum VKM B-2219T (AY009403)
- Methylobacterium suamine VKM B-2238T (AY009404)
- Methylobacterium dichloromethanum VKM B-2191T (AF227128)
- Methylobacterium rhodinum JCM 2811T (D32229)
- Methylobacterium organophilum JCM 2833T (D32226)
- Methylobacterium mesophilum JCM 2829T (A3400919)
- Methylobacterium fujisawaense DSM 5980T (A3225011)
- Methylobacterium radiotolerans JCM 2831T (D322573)
- Methylobacterium nodulans ORS2960T (D222783)
- Rhodopseudomonas palustris ATCC 17001T (D253212)
- Agrobacterium tumefaciens NCPPB 2437T (D14500)
- Escherichia coli 582 (J01859)

Fig. 1. Phylogenetic tree based on 16S rDNA sequences of members of the genus Methylobacterium and other representatives of the Proteobacteria, showing the location of strain BJ001T. GenBank accession numbers are provided in parentheses. Distances reflect the number of pairwise character differences.

exotorquens, Methylobacterium zatmanii and Methylobacterium rhodesianum, with 99-3, 99-1, 98-6 and 98-5% 16S rDNA sequence similarity, respectively, corresponding to the interspecies separation level of the genus Methylobacterium (94-2–99-4%; Doronina et al., 2002). On the basis of 16S–23S IGS rDNA sequences, strain BJ001T shared 78-7–82-1% similarity with M. extorquens (GenBank accession nos AF293375 and AF338180) and 66-5% similarity with the type species, M. organophilum (GenBank accession no. AF338181). The 16S and 16S–23S IGS rDNA sequence of strain BJ001T and sequence similarity matrices are available in Supplementary Tables A and B in IJSEM Online. Levels of DNA relatedness between strain BJ001T and its closest relatives, as determined by DNA–DNA hybridization, were 15–59%, which indicates that strain BJ001T can be separated from other members of the genus Methylobacterium (Table 1). Phenotypic differences between Methylobacterium species are limited and often rely on utilization of carbon and energy sources (Green, 1992). Like other members of the genus, strain BJ001T grew on C1 substrates, such as methanol, methylamine, formate and formaldehyde. In addition, strain BJ001T utilized methane, an ability that is shared with only one other species of the genus, M. organophilum (Patt et al., 1976). Strain BJ001T may play an important ecological role by consuming methane, the greenhouse effect of which is 20 times higher than that of carbon dioxide (Trotsenko et al., 2001). Strain BJ001T differed from its closest relatives (i.e. M. thiocyanatum, M. extorquens, M. zatmanii and M. rhodesianum) in several other carbon-source utilization features (summarized in Table 2). M. thiocyanatum grows on glucose, arabinose, glutamate, citrate, cyanate and thiocyanate, M. zatmanii grows on trimethylamine and M. rhodesianum grows on dimethylamine, none of which support growth of strain BJ001T. On the other hand, M. extorquens does not use fructose, M. zatmanii does not use betaine and M. rhodesianum does not use tartrate, all of which are substrates for strain BJ001T (Rock et al., 1976; Urakami & Komagata, 1984; Green, 1992; Wood et al., 1998). Fructose, the first hexose synthesized by plant photosynthesis, was by far the best carbon substrate for strain BJ001T, which may be related to its association with Populus trees. Tables of carbon- and nitrogen-source utilization and enzymic reactions of BJ001T are available as Supplementary Tables D, E and F in IJSEM Online.

Strain BJ001T has been shown to mineralize the toxic explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and B in IJSEM Online. Levels of DNA relatedness between strain BJ001T and its closest relatives, as determined by DNA–DNA hybridization, were 15–59%, which indicates that strain BJ001T can be separated from other members of the genus Methylobacterium (Table 1). Phenotypic differences between Methylobacterium species are limited and often rely on utilization of carbon and energy sources (Green, 1992). Like other members of the genus, strain BJ001T grew on C1 substrates, such as methanol, methylamine, formate and formaldehyde. In addition, strain BJ001T utilized methane, an ability that is shared with only one other species of the genus, M. organophilum (Patt et al., 1976). Strain BJ001T may play an important ecological role by consuming methane, the greenhouse effect of which is 20 times higher than that of carbon dioxide (Trotsenko et al., 2001). Strain BJ001T differed from its closest relatives (i.e. M. thiocyanatum, M. extorquens, M. zatmanii and M. rhodesianum) in several other carbon-source utilization features (summarized in Table 2). M. thiocyanatum grows on glucose, arabinose, glutamate, citrate, cyanate and thiocyanate, M. zatmanii grows on trimethylamine and M. rhodesianum grows on dimethylamine, none of which support growth of strain BJ001T. On the other hand, M. extorquens does not use fructose, M. zatmanii does not use betaine and M. rhodesianum does not use tartrate, all of which are substrates for strain BJ001T (Rock et al., 1976; Urakami & Komagata, 1984; Green, 1992; Wood et al., 1998). Fructose, the first hexose synthesized by plant photosynthesis, was by far the best carbon substrate for strain BJ001T, which may be related to its association with Populus trees. Tables of carbon- and nitrogen-source utilization and enzymic reactions of BJ001T are available as Supplementary Tables D, E and F in IJSEM Online.

Table 1. DNA–DNA hybridization values between Methylobacterium populi BJ001T and its closest relatives

<table>
<thead>
<tr>
<th>Species</th>
<th>DNA–DNA hybridization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylobacterium populi BJ001T</td>
<td>100</td>
</tr>
<tr>
<td>Methylobacterium thiocyanatum DSM 11400T</td>
<td>47</td>
</tr>
<tr>
<td>Methylobacterium extorquens ATCC 14718</td>
<td>59</td>
</tr>
<tr>
<td>Methylobacterium zatmanii JCM 2810</td>
<td>34</td>
</tr>
<tr>
<td>Methylobacterium rhodesianum JCM 2810</td>
<td>25</td>
</tr>
<tr>
<td>Methylobacterium suamine VKM B-2238T</td>
<td>33</td>
</tr>
<tr>
<td>Methylobacterium lusitanum VKM B-2239T</td>
<td>15</td>
</tr>
<tr>
<td>Methylobacterium organophilum JCM 2833T</td>
<td>28</td>
</tr>
<tr>
<td>Agrobacterium tumefaciens NCPPB 2437T</td>
<td>7</td>
</tr>
</tbody>
</table>

The hybridization procedure was carried out according to the method of Denhardt (1966).

Fig. 2. Scanning electron micrograph of strain BJ001T isolated from Populus deltoides × nigra DN34. Bar, 5-0 μm.
BJ001T is proposed as the type strain of a novel source utilization pattern (including the use of methane) on the basis of its 16S and 16S–23S IGS rDNA sequence or in situ species may be suited for natural attenuation bacterium spread distribution in a diversity of environments, be regarded as a diagnostic test. According to their wide- been tested among other members of the genus and cannot be regarded as a biospecies. M. extorquens (Wood 11, M. rhodesianum (Rock et al., 1976); 12, M. rhodanum (Heumann, 1962); 13, M. suoniense (Doronina et al., 2002); 14, M. thiocyanatum (Wood et al., 1998); 15, M. zatmanii (Rock et al., 1976). +, Growth; −, no growth; V, variable; w, weak growth; ND, not determined.

Table 2. Differential carbon-substrate utilization among Methylobacterium species

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>W</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Fucose</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>W</td>
<td>−</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Aspartate/L-glutamate</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Citrate</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Sebacate</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Acetate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Betaine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Tartrate</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>V</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>V</td>
<td>−</td>
</tr>
<tr>
<td>Ethanol</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Methane</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Methyamine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dimethylamine</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Cyanate</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Nutrient agar</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>V</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
</tbody>
</table>

which contaminate numerous military sites in the USA, to CO2 (data not shown). Whereas this extends the list of organic pollutants that are potentially biodegraded by Methylobacterium species, this particular ability has not been tested among other members of the genus and cannot be regarded as a diagnostic test. According to their widespread distribution in a diversity of environments, Methylobacterium species may be suited for natural attenuation or in situ bioremediation (including phytoremediation) of polluted sites.

On the basis of its 16S and 16S–23S rDNA sequence similarity data, DNA–DNA hybridization values, carbon-source utilization pattern (including the use of methane) and endophytic association with poplar trees, strain BJ001T is proposed as the type strain of a novel Methylobacterium species, with the name Methylobacterium populi sp. nov.

Description of Methylobacterium populi sp. nov.

Methylobacterium populi (po’pu.li. L. gen. n. populi of poplar).

Cells are aerobic, Gram-negative, asporogenous rods (0.8–1.0 × 1.0–10.0 μm) that occur singly, in pairs or in rosettes. Cells are motile by one single polar or lateral flagellum. Colonies are pink to red, slow-growing and 0.1–0.2 mm in diameter after 4 days at 28 °C on LB or nutrient agar (NA) plates. The pink pigment is water-insoluble and has absorption maxima at 390, 473, 505 and 534 nm in chloroform/methanol (1:1). Positive for the following enzymic reactions: catalase, oxidase, alkaline phosphatase, esterases (C4 and C6), valine arylamidase, z-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Carbon sources utilized are D-fructose, glycerol, methanol, ethanol, formate, acetate, succinate, lactate, tartrate, pyruvate, fumarate, salicylate, formaldehyde, methyamine, methane and betaine. Grows on LB and NA plates at 28 °C. Does not use D- or L-arabinose, D-fucose, D-galactose, D-glucose, D-lactose, D-mannose, D-xylose, sucrose, propan-2-ol, n-butanol, inositol, mannitol, sorbitol, L-aspartate, L-glutamate, glycine, citrate, sebacate, dimethylamine, trimethylamine, chloromethane, dichloromethane, cyanate or thiocyanate. Nitrogen sources utilized are ammonium, nitrate, L-alanine, L-aspartate, L-glutamate, L-glutamine, glycine, L-tryptophan and methyamine. Cellular fatty acids are: hexadecanoate (palmitic acid, C16:0), 64±0.4% (n = 3); cis-9-octadecenoate (oleic acid, C18:1ω9c), 81±0.1% (n = 3); and octadecanoate (stearic acid, C18:0ω0), 11±0.3% (n = 3). Optimal pH for growth is 7.0; does not grow at pH 4.0 or 9.0. Optimal temperature for growth is 20–30 °C; does not grow at 15 or 40 °C. Does not grow in the presence of 2-0% NaCl. DNA G+C content is 70±0.3 mol% (n = 3).
The type strain, BJ001T (=ATCC BAA-705=NCIMB 13946T), was isolated from internal poplar tissues (Populus deltoides × nigra DN34) obtained from Hramoor Nursery (Manistee, MI, USA).

Acknowledgements

This is a contribution of the W. M. Keck Phytotechnology Laboratory at the University of Iowa, supported by a gift from the W. M. Keck Foundation. We thank SERDP (Strategic Environmental Research and Development Program award number 02 CU13-17) for financial support. We acknowledge C. S. Harwood (University of Iowa, IA, USA) and M. E. Lidstrom (University of Washington, WA, USA) for relevant discussion.

References


Populus nigra ATCC BAA-705 = NCIMB 13946T, was isolated from internal poplar tissues (Populus deltoides × nigra DN34) obtained from Hramoor Nursery (Manistee, MI, USA).


