Methylophilaceae

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A novel obligate methylamine utilizer (strain JLW8T), isolated from Lake Washington sediment, was characterized taxonomically. The isolate was an aerobic, Gram-negative bacterium. Cells were rod-shaped and motile by means of a single flagellum. Reproduction was by binary fission and no resting bodies were formed. Growth was observed within a pH range of 5–8, with optimum growth at pH 7.5. It utilized methylamine as a single source of energy, carbon and nitrogen. Methylamine was oxidized via methylamine dehydrogenase and formaldehyde was assimilated via the ribulose monophosphate cycle. The cellular fatty acid profile was dominated by C16 : 0 and the major phospholipid was phosphatidylethanolamine. The DNA G + C content was 54 mol%. 16S rRNA gene sequence analysis indicated that the new isolate was closely related (97–98 % similarity) to a broad group of sequences from uncultured or uncharacterized Betaproteobacteria, but only distantly related (93–96 % similarity) to known methylotrophs of the family Methylophilaceae. Strain JLW8T (= ATCC BAA-1282T = DSM 17540T) is proposed as the type strain of a novel species in a new genus within the family Methylophilaceae, Methylophilaceae, Methytenera mobilis gen. nov., sp. nov.

Abbreviation: RuMP, ribulose monophosphate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JLW8T is DQ287786.
Transmission electron microscopy was performed as described previously (Kalyuzhnaya et al., 2005). Negatively stained preparations and thin sections were viewed using a 1200 Ex II transmission electron microscope (JEOL) at an operating voltage of 80 kV. The strain was routinely grown in the basal salts medium supplemented with 0-1 or 0-2 % methylamine. Cells were stored in the same basal salts medium supplemented with 10 % DMSO, at −80 °C. 16S rRNA gene sequences were aligned using the CLUSTALW program (Higgins et al., 1996). Phylogenetic analysis was carried out using the PHYLIP package (Felsenstein, 2003). The distance method was employed, and 100 bootstrap analyses were performed.

Colonies of strain JLW8T were cream to light brown and 1–2 mm in diameter when grown at 30 °C for 4–7 days. No pigmentation was observed when cells were grown in liquid culture. Cells did not form aggregates in liquid culture. Microscopy revealed that cells were rod-shaped, 0.6–1.2 × 0.3–0.4 μm, occurred singly, were motile by means of a single polar flagellum (Fig. 1) and had a Gram-negative cell wall structure. Some cells possessed cell wall extrusions, or ‘prostheca’-like structures. Cells reproduced by binary fission and did not form resting bodies. Strain JLW8T utilized only methylamine as a growth substrate, but not methanol, formate, dimethylamine, trimethylamine, organic acids, sugars, amino acids, C2-C6 alcohols or methane. No growth occurred on TGY, LB or Nutrient media. The optimal concentration of methylamine required for growth was tested using a Bioscreen C (MBR) plate reader at the following concentrations (w/v): 0, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1 and 2 %. Growth occurred at 0-01–0.5 % methylamine with an optimum at 0.05–0.1 %. The specific growth rate in liquid basal medium supplemented with 0.1 % methylamine was 0.134 h⁻¹. Strain JLW8T grew in the temperature range 10–34 °C, and a pH range 5–8.5, with optimal growth occurring at 30 °C and a pH of 7.5. Elimination of ammonium from the basal mineral medium did not affect growth, indicating that methylamine could serve as a nitrogen source. However, replacing ammonium salts with nitrate salts (0.1 %) resulted in growth inhibition. The test for nitrate reduction was negative. Because strain JLW8T was restricted to growth using methylamine as a single source of carbon, we were not able to verify its ability to grow without a nitrogen source. However, a PCR amplification test using primers specific for the nifH gene (Zehr & McReynolds, 1989) was negative, suggesting that the strain was unable to utilize N2. No growth was observed at concentrations of NaCl above 0.1 %, SDS concentrations above 0.001 % or H2O2 concentrations above 0.00003 %. Tests for oxidase and catalase were positive. Urease activity was not detected. Cells were not resistant to desiccation, heating to 70 °C for 5 min or to 60 °C for 10 min, and growth was observed after heating to 60 °C for 5 min.

Sensitivity to antibiotics was examined in liquid cultures. The following antibiotics were tested (μg ml⁻¹): ampicillin (30), chloramphenicol (10), gramicidin (10), kanamycin (30), nalidixic acid (30), penicillin (30), tetracycline (10), rifampicin (30), rifomycin (10) and streptomycin (10). The effect of antibiotics on cell growth was assessed after 1 week. No growth was observed in the presence of gramicidin, kanamycin or tetracycline, whereas chloramphenicol, ampicillin, nalidixic acid and streptomycin inhibited growth.

Activities of key enzymes for methylotrophy were measured in cell-free extracts of strain JLW8T, as described by Kalyuzhnaya et al. (2005). Tests for methylamine dehydrogenase were positive [23 ± 5 nmol min⁻¹ (mg protein)⁻¹; n = 3], suggesting its role in methylamine oxidation. Tests
for methanol dehydrogenase activity were negative. Accordingly, no PCR product with primers specific for the mxaF gene (McDonald & Murrell, 1997) was obtained. Combined activities of the key enzymes of the ribulose monophosphate (RuMP) cycle, hexulose phosphate synthase and 6-phospho-3-hexuliosomerase, were detected \[13 \pm 3 \text{ nmol min}^{-1} (\text{mg protein})^{-1}; n = 3\], indicating that the RuMP cycle was operational, whereas no activities of key enzymes of the serine cycle, hydroxypyruvate reductase and serine-glyoxylate aminotransferase, were detected. No activities of formaldehyde dehydrogenase (measured with or without glutathione) or formate dehydrogenase (measured with NAD) were detected.

Cellular phospholipid fatty acid analysis was performed by Microbial Insights (http://www.microbe.com). The fatty acid profile was dominated by C16 : 1\(\text{v7c}\) (66 %) and C16 : 0 (32 %), which is typical of the known obligate or restricted facultative methylotrophs within the Betaproteobacteria. However, strain JLW8\(^T\) did not contain the C16 : 1\(\text{v7t}\) isomer found so far in all representatives of the Methylophilaceae.

The major phospholipid, as detected by TLC (Findlay & Evans, 1987), was phosphatidylethanolamine (> 80 % of the total phospholipid fraction).

The G + C content of the genomic DNA was 54·3 ± 0·3 mol% \((n = 3)\). Analysis of a nearly complete sequence of the 16S rRNA gene indicated that strain JLW8\(^T\) was only distantly related to known methylotrophic bacteria of the family Methylophilaceae, sharing 94·3–95·6 % similarity with representatives of the genus Methylophilus and 93·1–94·8 % similarity with representatives of the genera Methylovorus and Methylobacillus. However, it was more closely related (97–98 % similarity) to environmental 16S rRNA gene sequences and to the sequence of the uncharacterized strain HTCC349 isolated from a trichloroethene- and dichloroethene-contaminated aquifer (Connon et al., 2005). Phylogenetic analysis revealed that the 16S rRNA gene sequence of strain JLW8\(^T\) grouped with environmental sequences and the sequence of strain HTCC349, and that these were separated from the sequences of species representing the genera Methylobacillus, Methylovorus and Methylophilus (Fig. 2).

All representatives of the Methylophilaceae described thus far have been reported to be obligate or restricted facultative methylotrophs capable of utilizing methanol as a sole source of carbon and energy and possessing methanol dehydrogenase activity (Jenkins & Jones, 1987; Doronina et al., 2004, 2005a). Strain JLW8\(^T\) is the first example of a representative of the Methylophilaceae lacking the ability to grow on methanol and restricted to methylamine as a single C\(_1\) substrate. Based on this feature as well as other distinctive features such as the lack of C16 : 1\(\text{v7t}\) fatty acid (Table 1) and low level of 16S rRNA gene sequence similarity with members of the genera Methylobacillus, Methylovorus

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**Fig. 2.** Phylogenetic tree showing the relationship of strain JLW8\(^T\) to representatives of the Methylophilaceae and to other members of the Betaproteobacteria, based on 16S rRNA gene sequences. Filled circles indicate bootstrap support over 95 %, shaded circles bootstrap support over 70 % and open circles bootstrap support over 50 %. Strain collection accession numbers (where available) and GenBank accession numbers (in parentheses) are shown. Bar, 10 % sequence divergence.
and Methylotrivorus, strain JLW8T is proposed as the type strain of a novel species in a new genus within the family Methylphilaceae, Methylotenera mobilis gen. nov., sp. nov.

16S rRNA gene sequences closely related to that of strain JLW8T have been amplified from a variety of environments, such as wastewater treatment plants, contaminated aquifers, mining waste and sedimentary rocks (based on descriptions such as wastewater treatment plants, contaminated aquifers, mining waste and sedimentary rocks), pointing to the important function of this group of organisms.

### Description of Methylotenera mobilis sp. nov.

Methylotenera mobilis (mo’bi. lis. L. fem. adj. mobilis motile).

General characteristics are as for the genus. Obligate methylamine utilizer. Grows at pH 5–8.5, with an optimum at pH 7.5. Temperature optimum is 30 °C. No cell aggregation in liquid medium. Cells are 0.6–1.2 × 0.3–0.4 μm in size and occur singly. Cells are not resistant to desiccation or heating (70 °C for 5 min).

The type strain, JLW8T (= ATCC BAA-1282T = DSM 17540T), was isolated from freshwater Lake Washington (USA).

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### References


