Labrys methylaminiphilus sp. nov., a novel facultatively methylotrophic bacterium from a freshwater lake sediment

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A new bacterial isolate from a methylamine enrichment culture is described, representing a novel species of facultatively methylotrophic bacteria. The non-motile bacterium is Gram-negative, replicates by budding and does not form endospores. The isolate utilizes methylated amines, as well as a variety of monosaccharides, disaccharides, amino acids, organic acids, aromatic compounds and alcohols as substrates, but does not utilize methanol. Growth factors are not required, although yeast extract stimulates growth. The major components of the fatty acid profile are C_{18:1}ω7c, C_{19:0} cyclo and C_{16:0}. The dominant cellular phospholipids are phosphatidyl acid, phosphatidylcholine and phosphatidylethanolamine. The G + C content of the DNA is 65.7 ± 0.3 mol%. 16S rRNA gene-based phylogenetic analysis revealed that the novel isolate belongs to the *α-Proteobacteria* and is closely related to the only representative of the genus *Labrys*, *Labrys monachus* (97.4 % sequence similarity). However, the level of DNA–DNA relatedness with *L. monachus* is less than 3 %, justifying the placement of this isolate into a novel species of the genus *Labrys*. The name *Labrys methylaminiphilus* sp. nov. is proposed (type strain JLW10^T = ATCC BAA-1080^T = DSM 16812^T).

Methylotrophs are a group of bacteria capable of metabolizing compounds containing no carbon–carbon bonds as sole sources of carbon and energy. These bacteria are ubiquitous in the biosphere and have been isolated from a wide variety of natural habitats including plants, soils and freshwater and marine sediments (Anthony, 1982). Methylotrophs play an important role in biogeochemical cycling and possess a potential for use in bioremediation (Pol *et al.*, 1994; De Marco *et al.*, 2004). We are studying a specific habitat that is rich in methylotrophic activity: the sediment of Lake Washington at a depth of about 60 m (Kuivila *et al.*, 1988; Costello & Lidstrom, 1999). Until recently, research at this site has focused primarily on methanotroph populations (Auman *et al.*, 2000; Costello & Lidstrom, 1999) and no survey of broader methylotroph presence has been

conducted. This study focused on enrichment, isolation and taxonomic description of methylamine-utilizing bacteria from Lake Washington sediment. One of these isolates, a novel facultative methylotroph, is described.

Sediment samples were collected on 21 July 2003, off the RV *Clifford Barnes* from a 63 m-deep site in Lake Washington, Seattle, WA, USA (47° 38.075' N 122° 15.993' W) using a box core that allowed collection of undisturbed sediment. Sediment cores were taken to a depth of approximately 20 cm and subsectioned into layers approximately 1.5 cm thick. Samples were transported to the laboratory on ice and used immediately. One millilitre samples of the upper 1.0 cm of the sediment cores were inoculated into 250 ml flasks containing 50 ml 0.2 × salts medium (Harder *et al.*, 1973) supplemented with 0.01 % methylamine (w/v) and were incubated for 6–8 days at room temperature with shaking (125 r.p.m.). In subsequent enrichments, 10 ml of the previous enrichment culture were diluted 1 : 4 (to a total of 50 ml) in the same medium, with the methylamine concentration increased to 0.2 %. Flasks were incubated at room temperature with shaking for 3–5 days. Strain JLW10^T was obtained as a colony after the third enrichment culture was plated onto a solid medium (0.2 × salts medium solidified by 1.8 % Bacto agar, supplemented with 0.2 %

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Abbreviation: MH₄FDH, methylenetetrahydrofolate dehydrogenase.

The GenBank/EMBL/DDBJ accession number for the nearly complete sequence of the 16S rRNA gene of *Labrys methylaminiphilus* JLW10^T is AY766152.

methylamine) and incubated at room temperature for 1 week. The isolate was further purified by triplicate streaking on solid medium. The purity of the culture was monitored by microscopy and by restriction fragment length polymorphism (RFLP) analysis of 16S rRNA genes, obtained by PCR amplification of DNA from either a single colony or from 5 ml of liquid culture as described below. For routine cultivation, a methylamine-supplemented minimal medium (Harder *et al.*, 1973) was used. In addition, the following media were used: MMB medium (DSMZ, medium 628), TGY broth (Murray, 1992) and Luria-Bertani medium (Sambrook *et al.*, 1989). For long-term storage, 200 µl DMSO was added to 1.8 ml exponentially growing liquid culture and the mixed suspensions were stored at -80°C . *Labrys monachus* DSM 5896^T, obtained from the DSMZ, was used as a reference strain.

For scanning electron microscopy, cells were washed with double-distilled water and fixed in modified Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde, 8 mM CaCl_2 in 0.1 M cacodylate buffer, pH 7.4) for 2 h at 4°C . Cells were then washed three times for 5 min with double-distilled water, spotted onto plastic coverslips coated with 1% poly-L-lysine, dehydrated in a graded series of ethanol to 100% and subsequently critical-point-dried. Samples were sputter-coated with gold/palladium and viewed with a JOEL JSM 6300F scanning electron microscope at 15 kV. Cells of isolate JLW10^T were Gram-negative, non-motile, capsulated, rod-shaped bacteria, 0.7–1.0 µm wide and 1.0–1.2 µm long (Fig. 1). They appeared as single cells or in pairs. Cells reproduced by budding and did not appear to form cysts or other resting bodies. Capsule formation, observed by electron microscopy, was also visualized with India ink (Pelikan) by light microscopy.

The ability of the isolate to utilize various carbon substrates was tested in basal liquid medium in which methylamine was replaced by one of the following carbon sources: methanol, formate, dimethylamine, trimethylamine,

methylsuccinic acid, vanillin, choline, betaine, oxalic acid, sarcosine, acetate, pyruvate, glycolate, citrate, malate, succinate, glucose, maltose, fructose, sucrose, mannitol, ethanol, acetone, toluene, glycerol, serine, valine, alanine, cysteine, asparagine, arginine, aspartate, methionine, histidine, tryptone or yeast extract, at final concentrations of 0.01 and 0.1% (w/v). Methane utilization was tested using a methane/air (1:1, v/v) atmosphere.

Nitrogen sources were tested in nitrogen-free basal liquid medium (as above) in which $(\text{NH}_4)_2\text{SO}_4$ was replaced with K_2SO_4 . The following compounds were tested at a concentration of 0.1% (w/v): KNO_3 , methylamine, dimethylamine, trimethylamine, urea, guanidine, glycine, serine, valine, alanine, cysteine, asparagine, arginine, aspartate, methionine, histidine, threonine, proline, glutamate, phenylalanine, tryptophan, peptone and yeast extract. For N_2 fixation experiments, the same nitrogen-free basal liquid medium was used. Temperature optima were investigated in TGY medium. pH optima were investigated in the minimal salts medium supplemented with 0.1% methylamine. Due to the poor growth exhibited by the control strain *L. monachus* on methylamine, growth medium for *L. monachus* was supplemented by 10% TGY. $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ at a final total concentration of 0.05 M was used as the buffering system (pH range 4.0–9.6). Culture pH was monitored at the completion of each experiment, to ensure that no shifts in pH had occurred. Growth rates were determined by monitoring cell density at 600 nm using a plate reader (Bioscreen C MBR) or in bulk cultures (250 ml flasks). Specific growth rates were calculated as the mean of triplicate determinations. The optimal concentration of methylamine for growth was tested using the plate reader at the following concentrations of methylamine (w/v): 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1 and 2%. Desiccation resistance, Voges-Proskauer reaction, oxidation/fermentation catabolism test, nitrite reduction, fluorescent pigment production, urease production, indole production and gelatin hydrolysis were tested according to Smibert & Krieg (1994). Plate assays were conducted to test ability to degrade agarose and starch. Tests for cellulose and nitrocellulose degradation were conducted in liquid media, using sterile 1 cm strips of cellulose or nitrocellulose.

Colonies of isolate JLW10^T grown at 30°C for 3 days on a basal medium supplemented with methylamine were white, 1–2 mm in diameter, circular, convex, opaque and butyrous. Specific growth rates in liquid determined using the Bioscreen plate reader were 0.067 h^{-1} in minimal medium supplemented with methylamine (0.2%) and 0.24 h^{-1} in TGY medium. Comparable rates were obtained in flask experiments (data not shown). Isolate JLW10^T grew in the temperature range $10\text{--}35^{\circ}\text{C}$, with an optimum at $28\text{--}30^{\circ}\text{C}$. The isolate was resistant to desiccation under the conditions tested. Isolate JLW10^T grew over a pH range of 4.0–9.5, with an optimum at pH 5.0–7.0. The optimal concentration of methylamine for growth of JLW10^T was 0.05% and concentrations above 0.5% were toxic. While

Fig. 1. Scanning electron micrograph of JLW10^T cells. A large amount of capsular material is evident. Bar, 1 µm.

isolate JLW10^T did not require additional growth factors when grown on methylamine, the addition of yeast extract did stimulate growth. In addition to methylamine, the following substrates supported growth of isolate JLW10^T as sole sources of carbon and energy: dimethylamine, trimethylamine, methylsuccinic acid, choline, betaine, fructose, glucose, maltose, lactose, mannose, sucrose, sarcosine, succinate, pyruvate, malate, citrate, oxalate, acetate, mannitol, ethanol, acetone, glycerol and all the tested amino acids, with the exceptions of tryptophan and methionine. No growth occurred on methane, methanol, glycolic acid, vanillin, toluene, naphthalene, formaldehyde, DMSO or trichloroethylene. In addition, methylamine, dimethylamine, trimethylamine, urea, ammonium, nitrate, and all tested amino acids served as sole nitrogen sources for growth. Isolate JLW10^T did not grow without an added nitrogen source. Accordingly, no product was amplified with primers specific for the *nifH* gene (Zehr & McReynolds, 1989), which encodes the Fe-protein of nitrogenase, suggesting that the strain is unable to utilize N₂. The isolate hydrolysed agar, agarose, humic acids and cellulose, but not starch or nitrocellulose. Indole and Voges–Proskauer tests were negative. No water-soluble fluorescent pigment was produced on King B medium. Tests for oxidase and catalase were positive. Urease activity was absent. Tests for oxidation of carbohydrates were positive. Glucose was fermented, but not sucrose or lactose. No gas production was observed.

Sensitivity to antibiotics was examined by spreading cells onto TGY agar plates and placing onto them Difco discs that contained the following antibiotics (µg ml⁻¹): gentamicin (10), neomycin (30), streptomycin (10), ampicillin (10), chloramphenicol (30), erythromycin (15), kanamycin (30), nalidixic acid (30), penicillin (10) and tetracycline (30). The effect of antibiotics on cell growth was assessed after 2 weeks. Cells were resistant to kanamycin, ampicillin, erythromycin, nalidixic acid, tetracycline, chloramphenicol and penicillin, but were sensitive to streptomycin, neomycin and gentamicin.

For cell extract preparation, methylamine-grown cells were pelleted by centrifugation at 5000 r.p.m. at 4 °C, resuspended in 1 ml buffer (25 mM Tris/HCl, 10 mM EDTA, pH 7.2 or 25 mM KH₂PO₄/Na₂HPO₄, pH 7.2) and disrupted by passage through a French pressure cell at 1.2 × 10⁸ Pa. Cell extracts were centrifuged at 20 817 g for 25 min at 4 °C to remove cell debris. Hydroxypyruvate reductase and serine glyoxylate aminotransferase were assayed as described by Goodwin (1990), hexulosephosphate synthase was assayed as described by Shishkina *et al.* (1976) and phosphoribulokinase and ribulose biphosphate carboxylase/oxygenase were assayed as described by Tabita (1980). Methylene-tetrahydrofolate dehydrogenase (MH₄FDH) was assayed as described by Vorholt *et al.* (1998). Methanol dehydrogenase and methylamine dehydrogenase were assayed according to Anthony & Zatman (1965) and Eady & Large (1968), respectively. *N*-Methylglutamate synthase/lyase was assayed according to Kimura *et al.* (1995), with a

modification as follows. Formaldehyde formation was followed by coupling to NADPH production in the presence of MH₄FDH. The modified reaction mixture contained the following additional compounds: 0.1 mM NADP, 3 mM tetrahydrofolate and 1 U MH₄FDH (all from Sigma). The activity of formate dehydrogenase was measured in the following reaction mixture: 50 mM potassium phosphate buffer, pH 7.0, 1 mM NAD and 20 mM formate. The activity of formaldehyde dehydrogenase was measured in the following reaction mixture: 50 mM potassium phosphate buffer, pH 7.0, 1 mM NAD, 10 mM formaldehyde and 2 mM glutathione. Protein concentration was determined spectrophotometrically (Dawson *et al.*, 2002). The data on enzyme activity measurements are presented in Table 1. No activity was detected for methanol dehydrogenase. Accordingly, PCR amplification of the *mxrA* gene with specific primers (McDonald & Murrell, 1997) produced negative results. No methylamine dehydrogenase activity was measured; however, cell extracts possessed *N*-methylglutamate synthase/lyase activity, the key enzyme for an alternative pathway for methylamine oxidation, the *N*-methylglutamate pathway (Table 1). No activities were detected for key enzymes of either the ribulose monophosphate cycle for formaldehyde assimilation or for the Calvin–Benson–Bassham cycle, but activities of key enzymes of the serine cycle were present (Table 1). High activity of MH₄FDH was present, indicating that the tetrahydrofolate-linked pathway may be involved in formaldehyde oxidation/detoxification. One of the most common formaldehyde oxidation pathways in methylotrophs is an analogous pathway linked to tetrahydromethanopterin, the so-called ‘archaeal’ pathway (Vorholt *et al.*, 1999). We were not able to test for any enzyme activities indicative of this pathway, as tetrahydromethanopterin or its derivatives are not commercially available. However, we were able by PCR to amplify a key gene for this pathway, *fae*,

Table 1. Enzyme activities in cell extracts of isolate JLW10^T

All activity determinations were carried out in triplicate and are given as means ± SEM in nmol min⁻¹ (mg protein)⁻¹. ND, Not detectable.

Enzyme	Activity
Methanol dehydrogenase	ND
Methylamine dehydrogenase	ND
<i>N</i> -Methylglutamate synthase/lyase	82 ± 10
Hexulosephosphate synthase	ND
Hydroxypyruvate reductase	134 ± 20
Serine glyoxylate aminotransferase	188 ± 30
Ribulose-1,5-bisphosphate carboxylase/oxygenase	ND
Phosphoribulokinase	ND
Methylene-tetrahydrofolate dehydrogenase (NADP)	47 ± 5
Formaldehyde dehydrogenase (GSH)	109 ± 12
Formate dehydrogenase (NAD)	29 ± 4

encoding the formaldehyde activating enzyme (Vorholt *et al.*, 2000). NAD-linked, glutathione-dependent formaldehyde dehydrogenase and formate dehydrogenase activities were also present.

Cellular phospholipid fatty acid (PLFA) analyses were performed by Microbial Insights (<http://microbe@microbe.com/>). Lipids were recovered using the modified Bligh and Dyer method (White *et al.*, 1979). Extractions were performed using one-phase chloroform/methanol/buffer extractant. Lipids were recovered, dissolved in chloroform and fractionated on disposable silicic acid columns into

neutral, glyco- and polar lipid fractions. The polar lipid fraction was *trans*-esterified with mild alkali to recover the PLFA as methyl esters in hexane. The PLFAs were analysed by gas chromatography with peak confirmation performed by electron impact mass spectrometry (GC/MS). The phospholipids were separated by TLC on Kieselgel 60 F254 plates (Merck) using chloroform/methanol/7 M ammonium hydroxide/water (65:25:3:1) as a solvent system. Iodine stain (Dittmer & Wells, 1969), Dragendorff spray, ninhydrin reagent (Daniels *et al.*, 1994) and phosphomolybdic acid staining were used for phospholipid identification. Fatty acids found in the polar lipid fraction of isolate JLW10^T are

Table 2. Differential characteristics of novel strain JLW10^T and related budding and/or facultative methylotrophic bacteria

Taxa: 1, *Angulomicrobium* (data from Fritz *et al.*, 2004); 2, *L. monachus* (this study unless indicated); 3, JLW10^T (this study); 4, *Methylorhabdus* (Doronina *et al.*, 1995); 5, *Methylarcula* (Doronina *et al.*, 2000); 6, *Aminobacter* (Kämpfer *et al.*, 2002); 7, *Methylophila* (Doronina *et al.*, 1998); 8, '*Methylosulfonomonas*' (Holmes *et al.*, 1997). NR, Not reported; –, not detectable; –/+, variable; w, weak.

Characteristic	1	2	3	4	5	6	7	8
Morphology (flagella)	–	–*	–	–	–	+	+	+
Reproduction by:								
Budding	+	+	+	–	–	+	–	–
Division	–	–*	–	+	+	–	+	+
pH range	5.2–8.0	6.0–9.5	4.0–9.5	6.0–7.0	5.5–10	NR	5.0–9.0	6.0–7.6
Optimum pH	6.8–7.0	6.0–8.0	5.0–7.0	6.8–7.4	7.5–8.5	6.0–8.0	6.5–7.5	6.6
Temperature range (°C)	15–40	20–50	10–35	10–45	10–40	NR	NR	20–37
Optimum growth temperature (°C)	28–30	28–30	28–30	28–34	29–35	30–37	28–35	30
Catalase	+	+	+	+	+	+	–	+
Oxidase	+	+	+	+	+	+	+	–/+
Nitrate reduction	–	+	+	+	–	–	+	NR
Hydrolysis of:								
Gelatin	NR	–	W	W	NR	NR	W	NR
Starch	NR	–	–	W	NR	NR	W	NR
Agarose	NR	–	+	NR	NR	NR	NR	NR
Humic acid	NR	NR	+	NR	NR	NR	NR	NR
Utilization of:								
Methanol	–	W*	–	+	W	–	+	+
Methylamine	NR	W*	+	+	+	+	+	+
Serine cycle	NR	NR	+	+	+	NR	+	+
Calvin cycle	NR	NR	–	–	–	NR	–	–
Methylamine dehydrogenase	NR	NR	–	+	–	–	+	NR
N-Methylglutamate pathway	NR	NR	+	–	+	+	–	NR
Major phospholipids†	PG, PEA, PC, PA	PG, PEA, PA‡	PEA	PEA, PG, CL, PC	PEA, PC	NR	PEA, PC	NR
Fatty acids (% of total content)								
C _{16:0}	5.0	19.7‡	17.7	15.4	0.7–1.0	4.9	2.8–5.5	7.8–8.7
C _{18:1ω7clω9t/ω12t}	35.8	2.9‡	32.8	53.6	73.4–74.2	46.4	66.0–69.6	72.4–78.7
C _{18:0}	10.5	13.0‡	1.7	4.0	14.7–15.6	1.5	3.6–4.5	2.0–2.3
C _{19:0} cyclo	24.8	40.4‡	49.4	16.7	0.7–1.0	13.7	8.4–15.1	14.2–28
C _{20:1ω9}	11.7	11.9‡	–	0.7	NR	0.7–1.3	NR	NR
Unknown ECL 18-081/18-063	4.1	4.2‡	–	NR	NR	10.9–24.5	NR	NR
DNA G + C content (mol%)	68	68*	65.7	66.2	57–61	62–64	66–70	57

*Data from Vasil'eva & Semenov (1984).

†CL, Cardiolipin; PA, phosphatidic acid; PC, phosphatidylcholine; PEA, phosphatidylethanolamine or phosphatidyltrimethylethanolamine; PG, phosphatidylglycerol.

‡Data from Fritz *et al.* (2004).

listed in Table 2, together with fatty acids reported in the literature for two reference strains representative of budding bacteria (*Labrys* and *Angulomicrobium*) and four reference strains representative of non-pigmented facultatively methylotrophic bacteria (*Methylophila*, *Aminobacter*, *Methylarcula* and *Methylorhabdus*). The major components of the hydrolysate obtained from methylamine-grown cells of isolate JLW10^T were C_{18:1}ω7c, C_{19:0} cyclo and C_{16:0}. The major phospholipids were phosphatidyl acid, phosphatidylcholine and phosphatidylethanolamine.

For DNA extraction, cells (5 g wet weight) were resuspended in 10 ml lysis buffer [10 mM NaCl, 20 mM Tris/HCl, pH 8.0, 1 mM EDTA, 100 mg proteinase K ml⁻¹, 50 mg RNase A ml⁻¹ and 0.5 % (w/v) SDS, all from Sigma] and incubated overnight. DNA was purified by phenol/chloroform extraction and ethanol precipitation (Sambrook *et al.*, 1989). The G+C content was determined by HPLC separation as described in Tamaoka & Komagata (1984) and was found to be 65.7 ± 0.3 mol% (*n* = 3). DNA–DNA hybridization experiments were performed using the competition procedure described by Johnson (1994). DNA (1 µg) from isolate JLW10^T was labelled with [³H]dCTP (Amersham) using a nick translation kit (Roche). Competitor DNA was digested using a combination of *Pst*I and *Hind*III (NEB). DNA extracted from *L. monachus* and JLW10^T (1 mg) was immobilized onto 0.22 µm Nytran N membranes (Schleicher & Schuell) and hybridized with the labelled JLW10^T DNA (specific activity 5 × 10⁶ d.p.m. mg⁻¹), as described by Sambrook *et al.* (1989). After hybridization, filters were counted in a liquid scintillation counter (Beckman) and the percentage of hybridization was calculated as described by Johnson (1994). Three independent tests were carried out for each pair of DNA preparations. These experiments showed that

isolate JLW10^T and *L. monachus* shared only a low level of DNA–DNA relatedness (< 3 %).

The 16S rRNA gene fragment was amplified as described by Lane (1991) and cloned into the pCR 2.1 vector using the Topo-TA Cloning kit (Invitrogen). DNAs from 30 clones were digested with *Alu*I (NEB) and subjected to RFLP analysis, resolved in 2 % agarose gels. Two different RFLP patterns were observed with a frequency of approximately 50 % each. Representatives of each pattern were sequenced. DNA sequencing was carried out using the BigDye 3.1 termination sequencing kit (Applied Biosystems). Gel analyses were performed by the Department of Biochemistry Sequencing Facility at the University of Washington, using an ABI 3700 high-throughput capillary DNA analyser. Plasmids representing the two different RFLP patterns contained inserts of identical DNA, indicating that the two RFLP patterns resulted from two different orientations of the fragment within the cloning vector. For phylogenetic analyses, this sequence was aligned with reference sequences using the CLUSTAL W program (Thompson *et al.*, 1994) and the alignments were manually refined. The PHYLIP package (Felsenstein, 2003) was used to perform neighbour-joining and parsimony analyses, with 1000 bootstrap analyses performed for each method. The two analyses resulted in similar branching patterns. Based on these analyses, the novel isolate fell into the α-subclass of *Proteobacteria* and was most closely related to *L. monachus* (97.4 % similarity; Fig. 2). Sequences from both isolate JLW10^T and *L. monachus* exhibited 86–94 % similarity to those of other known members of the α-subclass of *Proteobacteria*, and both were most closely related to the genera *Methylosinus* and *Methylocystis*.

Aerobic, Gram-negative, facultatively methylotrophic bacteria (methylotrophs) comprise a very heterogeneous

Fig. 2. 16S rRNA gene-based phylogenetic tree (neighbour-joining) showing the position of strain JLW10^T in relation to methylotrophic and non-methylotrophic α-proteobacteria. Bootstrap values (%) are shown at nodes. The sequence from *Burkholderia caledonica* LMG 19076^T was used as the outgroup. Bar, 10 % sequence divergence.

group and span the α -, β - and γ -subclasses of *Proteobacteria*. Within the α -subclass, at least 12 genera are classified that include well-characterized methylotrophic species: *Hyphomicrobium*, *Methylobacterium*, *Aminobacter*, *Methylorhabdus*, *Methylarcula*, *Methylopilula*, *Xanthobacter*, *Paracoccus*, *Rhodopseudomonas*, 'Methylsulfonomonas', *Ancylobacter* and *Albibacter*. However, the ability to grow on a variety of reduced single-carbon (C_1) substrates is even more widespread, since some bacteria capable of growth on C_1 compounds have never been classified as methylotrophs. For example, C_1 growth has been reported for bacteria involved in polymer transformation, such as *Sagittula stellata* (Gonzalez *et al.*, 1997), or oligotrophic budding bacteria such as *Angulomicrobium* (Fritz *et al.*, 2004).

Isolate JLW10^T, as described here, is another representative of heterotrophic bacteria with a broad range of metabolic capacities. Even though originally isolated as a dominant culture in methylamine enrichments, this bacterium is capable of growth on a variety of organic substrates, including polymers such as agarose and humic acids. Of all characterized bacteria, isolate JLW10^T is most related to *L. monachus* (Vasil'eva & Semenov, 1984; Fritz *et al.*, 2004). Based on genotypic analysis, both bacteria are clearly separated from all known taxa and form a distinct branch within the α -subclass of *Proteobacteria*. However, the low DNA–DNA relatedness and the unique fatty acid profile of isolate JLW10^T separate it from *L. monachus*. The dominance of $C_{19:0}$ cyclo fatty acid (49.4%) is a distinctive feature of this isolate that will potentially become a signature for this bacterium at the species level. Based on comparative analysis of JLW10^T with other representative genera and species, we propose that isolate JLW10^T represents a novel species within the genus *Labrys*. The species name proposed for isolate JLW10^T, *Labrys methylaminiphilus*, reflects the propensity of this strain to grow on methylamine and the history of its enrichment and isolation.

Description of *Labrys methylaminiphilus* sp. nov.

Labrys methylaminiphilus (me.thy'la.mi.ni.phi'lus. N.L. n. *methyl* the methyl radical; N.L. n. *amini* the amine group; Gr. adj. *philos* loving; N.L. masc. adj. *methylaminiphilus* methylamine-loving, referring to the methylamine-utilizing activity of the bacterium).

Cells are Gram-negative, non-motile, non-spore-forming, capsulated, multiply by budding and are oxidase- and catalase-positive. Growth is aerobic but may be micro-aerophilic. Grows on methylamine, dimethylamine and trimethylamine as sole sources of energy and carbon. Methylamine is oxidized to formaldehyde via the N-MG pathway. Carbon is assimilated via the serine cycle. Other substrates supporting growth include a wide range of sugars, organic acids, amino acids, aromatic compounds and alcohols. Nitrogen sources include methylated amines, most amino acids, urea, peptone, ammonium salts and

nitrites. No additional growth factors are required, but yeast extract has a stimulatory effect. The major components of the fatty acid profile are $C_{18:1\omega7c}$, $C_{19:0}$ cyclo and $C_{16:0}$. The major phospholipids are phosphatidyl acid, phosphatidylcholine and phosphatidylethanolamine. The G + C content of the DNA is 65.7 ± 0.3 mol%. Cells are $0.7\text{--}1.0$ μm wide and $1.0\text{--}1.2$ μm long. The temperature range for growth is $10\text{--}35$ °C, with optimal growth at $28\text{--}30$ °C. The pH range is $4.0\text{--}9.5$, with optimal pH at $5.0\text{--}7.0$. Does not produce indole and the Voges–Proskauer test is negative. No water-soluble fluorescent pigments are produced on King B medium. Oxidase and catalase activities are present, but not urease activity.

The type strain, JLW10^T (=ATCC BAA-1080^T=DSM 16812^T), was isolated from the sediment of Lake Washington, Seattle, WA, USA.

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