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Methylotenera mobilis gen. nov., sp. nov., an obligately methylamine-utilizing bacterium within the family *Methylophilaceae*

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A novel obligate methylamine utilizer (strain JLW8^T), 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 5–8·5, 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 $C_{16:0}\omega7c$ and $C_{16:0}$ 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 JLW8^T (=ATCC BAA-1282^T=DSM 17540^T) is proposed as the type strain of a novel species in a new genus within the family *Methylophilaceae*, *Methylotenera mobilis* gen. nov., sp. nov.

Methylotrophs are microbes capable of utilizing single C_1 compounds as sole sources of energy and carbon. Methylotrophic ability is especially widespread within the *Proteobacteria*, encompassing the alpha, beta and gamma subdivisions (Anthony, 1982; Hanson & Hanson, 1996). Methylotrophs of the *Alphaproteobacteria* include four genera of obligate and restricted facultative methanotrophs (*Methylosinus*, *Methylocystis*, *Methylocapsa* and *Methylocella*) and a broad group (over ten genera) of facultatively methylotrophic bacteria (*Methylobacterium*, *Methylopila*, *Hyphomicrobium*, etc.). Methylotrophs of the *Gammaproteobacteria* are represented by nine genera of obligately methanotrophic bacteria (*Methylomonas*, *Methylococcus*, *Methylothermus*, *Methylomicrobium*, *Methylosphaera*, *Methylobacter*, *Methylocaldum*, *Methylohalobius* and *Methylosarcina*) and three genera of non-methanotrophic methylotrophic bacteria (*Methylophaga*, *Marinobacterium* and *Pseudomonas*). Betaproteobacterial methylotrophs are represented by three genera (*Methylobacillus*, *Methylophilus* and *Methylovorus*) within the family *Methylophilaceae* and so far by a single genus (*Methylibium*) within the *Burkholderiales* (Nakatsu *et al.*, 2006). 16S rRNA gene sequences with close similarity to those of methylotrophs of the family *Methylophilaceae* have been detected in a variety of ecosystems; a few representative strains have been cultured but not formally characterized (Connon & Giovannoni, 2002; Lueders *et al.*, 2004; De Marco *et al.*, 2004; Fuchs *et al.*, 2005; Gich *et al.*, 2005). In this study we describe a novel betaproteobacterial obligate methylotroph isolated from lake sediment. Based on 16S rRNA gene sequence analysis, this strain is shown to be more closely related to uncultured or unidentified *Betaproteobacteria* than to known betaproteobacterial methylotrophs. We propose that this new isolate represents a novel species of a new genus within the family *Methylophilaceae*.

Strain JLW8^T was isolated from sediment recovered from Lake Washington, Washington State, USA, after enrichment in a basal salts medium (Harder *et al.*, 1973) diluted five-fold and supplemented with 0·1 % methylamine as described previously (Miller *et al.*, 2005). The purity of the culture was monitored by microscopy, by 16S rRNA gene sequence amplification and analysis, and by testing the ability to grow on tryptone–glucose–yeast extract (TGY; Murray, 1992), Luria–Bertani (LB; Sambrook *et al.*, 1989) or Nutrient (Difco) media. Characterization of the ultrastructural, phenotypic and genotypic properties of strain JLW8^T was performed as described previously (Miller *et al.*, 2005).

Abbreviation: RuMP, ribulose monophosphate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JLW8^T is DQ287786.

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Transmission election microscopy was performed as described previously (Kalyuzhnaya *et al.*, 2005). Negatively stained preparations and thin sections were viewed by 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 JLW8^T 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 JLW8^T utilized only methylamine as a growth substrate, but not methanol, formate, dimethylamine, trimethylamine, organic acids, sugars, amino acids, C_2–C_6 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 JLW8^T 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 JLW8^T 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 N_2. No growth was observed at concentrations of NaCl above 0·1 %, SDS concentrations above 0·001 % or H_2O_2 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.

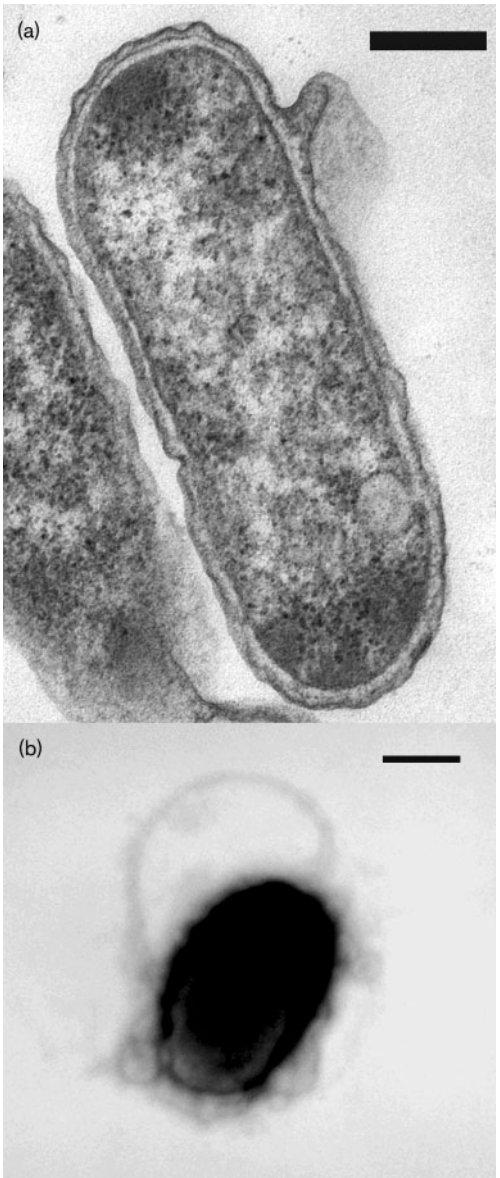

Fig. 1. Electron micrographs of thin-sectioned cells of strain JLW8^T showing details of the cell wall (a) and a negatively stained cell (b). Bars, 0·2 μm.

Activities of key enzymes for methylotrophy were measured in cell-free extracts of strain JLW8^T, 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

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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-hexuloisomerase, were detected [13 ± 3 nmol min^{-1} (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 $C_{16:1}\omega7c$ (66 %) and $C_{16:0}$ (32 %), which is typical of the known obligate or restricted facultative methylotrophs within the *Betaproteobacteria*. However, strain JLW8^T did not contain the $C_{16:1}\omega7t$ 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 $54{\cdot}3 \pm 0{\cdot}3$ 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 $C_{16:1}\omega7t$ fatty acid (Table 1) and low level of 16S rRNA gene sequence similarity with members of the genera *Methylophilus*, *Methylobacillus*

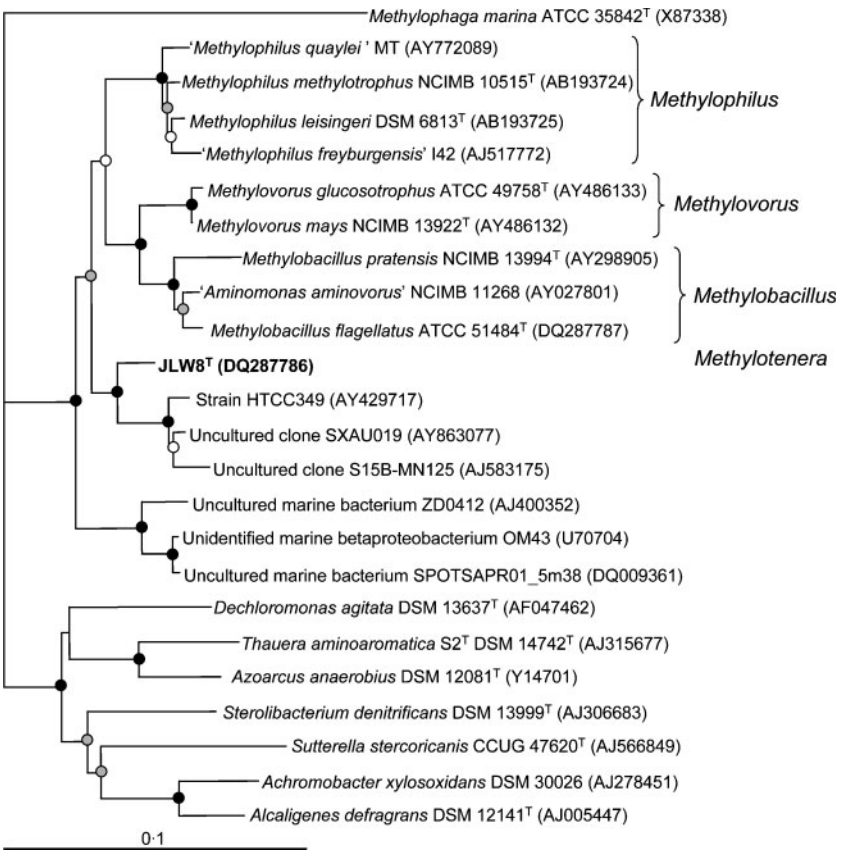

Fig. 2. Phylogenetic tree showing the relationship of strain JLW8^T to representatives of the *Methylophilaceae* and to other members of the *Betaproteobacteria*, based on 16S rRNA gene sequences. Filled circles indicate bootstrap support over 95 %, shaded circles bootstrap support over 70 % and open circles bootstrap support over 50 %. Strain collection accession numbers (where available) and GenBank accession numbers (in parentheses) are shown. Bar, 10 % sequence divergence.

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Table 1. Differential characteristics of strain JLW8^T and related methylotrophic bacteria of the family *Methylophilaceae*

Taxa: 1, strain JLW8^T; 2, *Methylophilus* (data from Ikemoto *et al.*, 1978; Doronina *et al.*, 2005b); 3, *Methylovorus* (Doronina *et al.*, 2005a; Govorukhina & Trotsenko, 1991); 4, *Methylobacillus* (Govorukhina & Trotsenko, 1991; Govorukhina *et al.*, 1987; Doronina *et al.*, 2004). +, Positive; +/−, variable; −, negative.

Characteristic	1	2	3	4
Flagellation	+	+/−	+	+
Growth using:				
Methanol	−	+	+	+
Methylamine	+	+/−	+/−	+/−
Glucose	−	+	−	+/−
Optimum growth temperature (°C)	30	25–37	35–40	29–42
Optimum pH	7·5	6·5–7·5	7·0–7·5	6·5–7·5
Methanol dehydrogenase	−	+	+	+
Methylamine dehydrogenase	+	+/−	+/−	+/−
RuMP pathway	+	+	+	+
Fatty acid content (%):				
$C_{16:0}$	32	32–48	47–49	41–43
$C_{16:1}\omega7c$	66	46–53	32–35	32–36
$C_{16:1}\omega7t$	0	4–12	2–3	3–8
$C_{18:0}$	0·3	0·6–4	0·6–0·7	0·7–1·2
$C_{18:1}\omega7c$	0·7	0·7–2	1–2	5
DNA G+C content (mol%)	54	50–53	56–58	53–62

and *Methylovorus*, strain JLW8^T is proposed as the type strain of a novel species in a new genus within the family *Methylophilaceae*, *Methylotenera mobilis* gen. nov., sp. nov.

16S rRNA gene sequences closely related to that of strain JLW8^T have been amplified from a variety of environments, such as wastewater treatment plants, contaminated aquifers, mining waste and sedimentary rocks (based on descriptions of the sequence sources in the NCBI database), pointing to the widespread distribution and potential ecologically important function of this group of organisms.

Description of *Methylotenera* gen. nov.

Methylotenera (Me.thy.lo.ten′er.a. Gr. n. *methyl* the methyl group; L. fem. adj. *tenera* delicate; N.L. fem. n. *Methylotenera* delicate methyl-utilizing organism).

Gram-negative rods, motile by means of a single flagellum. Do not form resting bodies. Multiply by binary fission. Do not grow in TGY, LB or Nutrient media. Utilize methylamine as a single source of carbon, energy and nitrogen, but do not utilize methanol. Tests for urease and nitrate reduction are negative. Tests for catalase and oxidase are positive. Nitrates inhibit growth. Oxidize methylamine via methylamine dehydrogenase and assimilate C_1 units via the RuMP pathway. Major fatty acids are $C_{16:1}\omega7c$ and $C_{16:0}$. The major phospholipid is phosphatidylethanolamine. The G+C content of the DNA is 54·3 mol%. The type species is *Methylotenera mobilis*.

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, JLW8^T (=ATCC BAA-1282^T=DSM 17540^T), was isolated from freshwater Lake Washington (USA).

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References

Anthony, C. (1982). *The Biochemistry of Methylotrophs.* London: Academic Press.

Connon, S. A. & Giovannoni, S. J. (2002). High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. *Appl Environ Microbiol* **68**, 3878–3885.

Connon, S. A., Tovanabootr, A., Dolan, M., Vergin, K., Giovannoni, S. J. & Semprini, L. (2005). Bacterial community composition determined by culture-independent and -dependent methods during propane-stimulated bioremediation in trichloroethene-contaminated groundwater. *Environ Microbiol* **7**, 165–178.

De Marco, P., Pacheco, C. C., Figueiredo, A. R. & Moradas-Ferreira, P. (2004). Novel pollutant-resistant methylotrophic bacteria for use in bioremediation. *FEMS Microbiol Lett* **234**, 75–80.

Doronina, N. V., Trotsenko, Y. A., Kolganova, T. V., Tourova, T. P. & Salkinoja-Salonen, M. S. (2004). *Methylobacillus pratensis* sp. nov., a novel non-pigmented, aerobic, obligately methylotrophic bacterium isolated from meadow grass. *Int J Syst Evol Microbiol* **54**, 1453–1457.

Doronina, N. V., Ivanova, E. G. & Trotsenko, Y. A. (2005a). Phylogenetic position and emended description of the genus *Methylovorus*. *Int J Syst Evol Microbiol* **55**, 903–906.

Doronina, N. V., Ivanova, E. G., Trotsenko, Y. A., Pshenichnikova, A., Kalinina, E. & Shvets, V. (2005b). *Methylophilus quaylei* sp. nov., a new aerobic obligately methylotrophic bacterium. *Syst Appl Microbiol* **28**, 303–309.

Felsenstein, J. (2003). *Inferring Phylogenies.* Sunderland, MA: Sinauer Associates.

Findlay, J. B. C. & Evans, W. H. (1987). *Biological Membranes: A Practical Approach.* Washington, DC: IRL Press.

Fuchs, B. M., Woebken, D., Zubkov, M. V., Burkill, P. & Amann, R. (2005). Molecular identification of picoplankton populations in contrasting waters of the Arabian Sea. *Aquat Microb Ecol* **39**, 145–157.

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Gich, F., Schubert, K., Bruns, A., Hoffelner, H. & Overmann, J. (2005). Specific detection, isolation, and characterization of selected, previously uncultured members of the freshwater bacterioplankton community. *Appl Environ Microbiol* **71**, 5908–5919.

Govorukhina, N. I. & Trotsenko, Y. A. (1991). *Methylovorus*, a new genus of restricted facultatively methylotrophic bacteria. *Int J Syst Bacteriol* **41**, 158–162.

Govorukhina, N. I., Kletsova, L. V., Tsygankov, Y. D., Trotsenko, Y. A. & Netrusov, A. I. (1987). Characteristics of a new obligate methylotrophous bacterium. *Mikrobiologiia* **56**, 849–854.

Hanson, R. S. & Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiol Rev* **60**, 437–471.

Harder, W., Attwood, M. & Quayele, J. R. (1973). Methanol assimilation by *Hyphomicrobium* spp. *J Gen Microbiol* **78**, 155–163.

Higgins, D. G., Thompson, J. D. & Gibson, T. J. (1996). Using CLUSTAL for multiple sequence alignments. *Methods Enzymol* **266**, 383–402.

Ikemoto, S., Katoh, K. & Komagata, K. (1978). Cellular fatty acid composition in methanol-utilizing bacteria. *J Gen Appl Microbiol* **24**, 41–49.

Jenkins, O. & Jones, D. (1987). Taxonomic studies on some Gram-negative methylotrophic bacteria. *J Gen Microbiol* **133**, 453–473.

Kalyuzhnaya, M. G., Stolyar, S. M., Auman, A. J., Lara, J. C., Lidstrom, M. E. & Chistoserdova, L. (2005). *Methylosarcina lacus* sp. nov., a methanotroph from Lake Washington, Seattle, USA, and emended description of the genus *Methylosarcina*. *Int J Syst Evol Microbiol* **55**, 2345–2350.

Lueders, T., Wagner, B., Claus, P. & Friedrich, M. W. (2004). Stable isotope probing of rRNA and DNA reveals a dynamic methylotroph community and trophic interactions with fungi and protozoa in oxic rice field soil. *Environ Microbiol* **6**, 60–72.

McDonald, I. R. & Murrell, J. C. (1997). The methanol dehydrogenase structural gene *mxaF* and its use as a functional gene probe for methanotrophs and methylotrophs. *Appl Environ Microbiol* **63**, 3218–3224.

Miller, J. A., Kalyuzhnaya, M. G., Noyes, E., Lara, J. C., Lidstrom, M. E. & Chistoserdova, L. (2005). *Labrys methylaminiphilus* sp. nov., a novel facultatively methylotrophic bacterium from a freshwater lake sediment. *Int J Syst Evol Microbiol* **55**, 1247–1253.

Murray, R. G. E. (1992). The family *Deinococcaceae*. In *The Prokaryotes*, 2nd edn, pp. 3732–3744. Edited by A. Balow, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.

Nakatsu, C. H., Hristova, K., Hanada, S., Meng, X.-Y., Hanson, J. R., Scow, K. M. & Kamagata, Y. (2006). *Methylibium petroleiphilum* gen. nov., sp. nov., a novel methyl *tert*-butyl ether-degrading methylotroph of the *Betaproteobacteria*. *Int J Syst Evol Microbiol* **56**, 983–989.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Zehr, J. P. & McReynolds, L. A. (1989). Use of degenerate oligonucleotides for amplification of the *nifH* gene from the marine cyanobacterium *Trichodesmium thiebautii*. *Appl Environ Microbiol* **55**, 2522–2526.

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