Didriksen, A., Dedysh, S. N., Belova, S. E., Danilova, O. V., Svenning, M. M., Liebner, S. (2015): Methylocapsa palsarum sp. nov., a methanotrophic bacterium from a Sub-Arctic discontinuous permafrost ecosystem. - *International Journal of Systematic and Evolutionary Microbiology*, *65*, pp. 3618–3624.

DOI: http://doi.org/10.1099/ijsem.0.000465

# *Methylocapsa palsarum* sp. nov., a Methanotrophic Bacterium from a Sub-Arctic Discontinuous Permafrost Ecosystem

Svetlana N. Dedysh[1], Alena Didriksen[2], Olga V. Danilova[1], Svetlana E. Belova[1], Susanne Liebner[3]and Mette M. Svenning[2]

[1]Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow 117312, Russia

[2]UiT The Arctic University of Norway, Department of Arctic and Marine Biology, 9037 Tromsø, Norway

[3]GFZ German Research Centre for Geosciences, Section 4.5 Geomicrobiology, Telegrafenberg, 14473 Potsdam, Germany

**Author for correspondence:** Svetlana N. Dedysh. Tel: 7 (499) 135 0591. Fax: 7 (499) 135 6530. Email: dedysh@mail.ru

**Journal:** International Journal of Systematic and Evolutionary Microbiology (IJSEM).

**Contents category**: New Taxa – *Proteobacteria*.

**Running title:** *Methylocapsa palsarum* sp. nov.

The GenBank/EMBL/DDBJ accession numbers for the nearly complete 16S rRNA gene sequence and the partial *pmoA*, *mxaF* and *nifH* gene sequences of *Methylocapsa palsarum* strain NE2$^T$ are KP715289-KP715292, respectively.

**ABSTRACT**

**An aerobic methanotrophic bacterium was isolated from a collapsed palsa soil in northern Norway and designated strain NE2$^T$. Cells of this strain were Gram-negative, non-motile, non-pigmented, slightly curved thick rods that multiplied by normal cell division. They possessed a particulate methane monooxygenase enzyme (pMMO) and utilized methane and methanol. Strain NE2$^T$ grew in a wide pH range of 4.1-8.0 (optimum at 5.2-6.5) at temperatures between 6 and 32°C (optimum at 18-25°C), and was capable of atmospheric nitrogen fixation under reduced oxygen tension. The major cellular fatty acids were 18:1*ω*7*c* and 16:1*ω*7*c*; the DNA G+C content was 61.7 mol%. The isolate belonged to the family *Beijerinckiaceae* of the class *Alphaproteobacteria* and was most closely related to the facultative methanotroph *Methylocapsa aurea* KYG$^T$ (98.3% 16S rRNA gene sequence similarity and 84% PmoA sequence identity). However, strain NE2$^T$ differed from *M. aurea* KYG$^T$ by cell morphology, the absence of pigmentation, inability to grow on acetate, broader pH growth range, and higher tolerance to NaCl. We, therefore, propose a novel species, *Methylocapsa palsarum* sp. nov., for this bacterium. Strain NE2$^T$ (=LMG 28715$^T$=VKM B-2945$^T$) is the type strain.**

**Keywords:** *Methylocapsa palsarum* sp. nov., methanotrophic bacteria, Arctic ecosystems.

The genus *Methylocapsa* was originally proposed to accommodate aerobic, mildly acidophilic, encapsulated, non-motile, dinitrogen-fixing methanotrophs that possess particulate methane monooxygenase (pMMO) (Dedysh *et al*., 2002). Cells of these bacteria contain well-developed intracellular membranes (ICM) packed in parallel on one side of the cell. At present, this genus includes two species, i.e. *M. acidiphila* (Dedysh *et al*., 2002) and *M. aurea* (Dunfield *et al*., 2010). The type species of this genus, *M. acidiphila*, is represented by obligate methanotrophs that utilize only methane and methanol as growth substrates and display a unique ability to grow actively in nitrogen-free media under fully aerobic conditions (Dedysh *et al*., 2004). By contrast, members of the second described species of this genus, *M. aurea*, are facultative methanotrophs, which, in addition to methane and methanol, are also capable of growth on acetate (Dunfield *et al*., 2010).

Representatives of the genus *Methylocapsa* are typical inhabitants of acidic wetlands and soils (Dedysh *et al*., 2003; Dedysh, 2009). *Methylocapsa*-related transcripts of *pmoA* and *nifH* genes encoding pMMO and dinitrogenase reductase, respectively, were detected in a subarctic palsa peatland, suggesting that these methanotrophs are metabolically active in this ecosystem (Liebner & Svenning, 2013). Further cultivation studies resulted in isolation of a novel *Methylocapsa*-like isolate, strain NE2$^T$. Here, we characterize this novel methanotroph and determine its taxonomic position.

Strain NE2$^T$ was isolated from a sample of the moss *Sphagnum lindbergii* collected in August 2011 in northern Norway (N 69°41.116, E 29°11.752), at the transition from the sub-Arctic to the Arctic. The sampling site represented a stabilized successional stage of a previously collapsed palsa (Seppälä, 1986). Samples of the wet moss (pore water pH 4.6) were placed in 120 ml serum bottles, sealed with rubber septa, and $CH_4$ (20%, v/v) was added to the headspace using syringes equipped with disposable filters (0.22 μm). Bottles were incubated in static conditions at 23°C under LED light (PHILIPS, type NIL 130F). After 1.5 months of incubation one of the *Sphagnum* plants was transferred to a new 120 ml serum bottle containing 20 ml of

mildly acidic (pH 5.5) mineral medium M2 (Dedysh *et al*., 2002) and 20% $CH_4$ (v/v) in the headspace. Turbidity due to growth of methanotrophs was observed in the bottle after three weeks of incubation in the dark. Dilution series prepared from this enrichment culture were streaked on Whatman polycarbonate filters (Nucleopore Track-Etch Membrane with pore size 0.2 μm). The filters were left floating on a surface of the liquid medium M2 in Petri dishes and incubated in plastic containers with 20% $CH_4$ (v/v) in the headspace. Colonies that appeared on these filters after 2 months of incubation were successively re-streaked and left floating on diluted nitrate mineral salts medium (DNMS; Dunfield *et al.*, 2003). This procedure was repeated until the colonies containing morphologically uniform cells were obtained. One of these colonies was used to inoculate a serum bottle with DNMS medium and 20% $CH_4$ (v/v) in the headspace. The resulting culture, strain $NE2^T$, grew well both in M2 and DNMS media. Growth in the latter medium, however, was non-homogenous due to formation of large cell aggregates and, therefore, strain $NE2^T$ was further maintained in liquid M2 medium and transferred at 1-month intervals.

In order to identify this isolate, the 16S rRNA gene sequence of strain $NE2^T$ was determined. PCR-mediated amplification of the 16S rRNA gene was performed using primers 9f and 1492r and reaction conditions described by Weisburg *et al.* (1991). Phylogenetic analysis was carried out using the ARB program package (Ludwig *et al*., 2004). The trees were constructed using distance-based (neighbor-joining), maximum-likelihood (DNAml), and maximum-parsimony methods. The significance levels of interior branch points obtained in neighbor-joining analysis were determined by bootstrap analysis (1000 data re-samplings) using PHYLIP (Felsenstein, 1989). The comparative 16S rRNA gene sequence analysis revealed that strain $NE2^T$ belongs to the family *Beijerinckiaceae*, the class *Alphaproteobacteria*, and is most closely related to the facultative methanotroph *Methylocapsa aurea* $KYG^T$ (98.3% 16S rRNA gene similarity) (Fig. 1). Therefore, *M. aurea* $KYG^T$ was used as a reference strain in our study.

The absence of heterotrophic satellites in strain NE2$^T$was checked by phase-contrast and electron microscopy and by plating onto 1:10 diluted R2A medium (Difco). Only one cell morphotype was observed under light microscopy and no growth on diluted R2A medium was observed after 3 weeks of incubation.

Morphological observations and cell size measurements were made with an Axioplan 2 microscope and Axiovision 4.2 software (Carl Zeiss). Cells of strain NE2$^T$ were Gram-negative, non-motile, encapsulated, slightly curved thick rods, 1.0-1.2 μm in width and 1.6-2.4 μm in length (Fig. 2a). They reproduced by binary fission and occurred singly or in irregularly-shaped aggregates. The formation of rosettes was not observed. A distinctive bipolar appearance, which is characteristic of *M. aurea* KYG$^T$ (Fig. 2b) and occurs due to the presence of highly refractile intracellular granules of poly-β-hydroxybutyrate at each cell pole, was not observed for strain NE2$^T$. On agar-solidified M2 medium, strain NE2$^T$ formed small (1-2 mm in diameter), non-pigmented, round, slimy colonies. Liquid cultures displayed white turbidity. Incubation in static conditions often resulted in formation of large cell aggregates.

For scanning electron microscopy, cells of an exponentially growing culture were fixed in 2.5% glutaraldehyde in a freshly made growth medium, sedimented on poly-L-lysine coated glass, washed in phosphate buffered saline, post-fixed with 1% aqueous osmium tetroxide, dehydrated in a graded series of ethanol and critical point dried in a Balzer Union CPD 020 Critical Point Dryer (Lichtenstein). The specimen samples were mounted on aluminium stubs with silver glue, coated with gold/palladium in a Polaron Range Sputter Coater (Ringmer, UK) and scanned with a Carl Zeiss Zigma Field Emission scanning electron microscope, Electron Microscope Laboratory, UiT. The obtained image shows cells of strain NE2$^T$ clamped to each other by means of a fibrous material (Fig. 1c). For preparation of ultrathin sections, cells of the exponentially growing cultures were collected by centrifugation and pre-fixed with 1.5% (w/v) glutaraldehyde in 0.05 M cacodylate buffer (pH 6.5) for 1 h at 4ºC and then fixed with 1% (w/v) $OsO_4$ in the same buffer for 4 h at 20ºC. After dehydration in an ethanol series, the samples were

embedded into Epon 812 epoxy resin. Thin sections were cut on an LKB-4800 microtome, stained with 3% (w/v) uranyl acetate in 70% (v/v) ethanol, and then were stained with lead citrate (Reynolds, 1963) at 20 °C for 4–5 min. The specimen samples were examined with a JEM-100B transmission electron microscope at an accelerating voltage of 80 kV. Thin-sectioned cells of strain NE2$^T$ displayed well-developed intracellular membranes (ICM) packed in parallel on one side of the cell membrane (Fig. 2d). This type of ICM arrangement is typical for members of the genus *Methylocapsa* (Dedysh *et al*., 2002; Dunfield *et al*., 2010).

Physiological tests were carried out on cultures grown in liquid medium M2 with methane as the sole substrate. Growth of strain NE2$^T$was monitored by nephelometry at 410 nm using a "Specol" spectrophotometer (Carl Zeiss) for 2 weeks under a variety of conditions, including temperatures of 2-37ºC, pH values of 3.9-8.0, and NaCl concentrations of 0-3.0 % (w/v). Variations in the pH were achieved by mixing 0.1M solutions of $H_3PO_4$, $KH_2PO_4$, and $K_2HPO_4$. The following carbon sources (each at a concentration of 0.05%w/v) were examined to determine the range of substrates that can be utilized by strain NE2$^T$: methanol, ethanol, formate, formaldehyde, glucose, fructose, arabinose, lactose, sucrose, maltose, galactose, acetate, citrate, oxalate, malate, pyruvate and succinate. The capacity to utilize methanol at concentrations from 0.01 to 5% (v/v) was determined in liquid M2 medium supplemented with methanol. Nitrogen sources were tested by replacing $KNO_3$ in medium M2 with 0.05% (w/v) $(NH_4)_2SO_4$, $NaNO_2$, urea, hydroxylamine, peptone, L-serine, L-proline, L-alanine, L-asparagine and yeast extract. For assessing $N_2$-fixation capability, a nitrate-free medium was used. In all substrate utilization tests, growth was examined after 1-month of incubation and confirmed by comparison to a respective negative control.

Strain NE2$^T$ grew on methane or methanol as the sole carbon and energy sources. The specific growth rate on methane under optimal growth conditions (see below) was 0.027 $h^{-1}$ (equivalent to a doubling time of 25 h). Methanol supported growth only when used at concentrations below 0.5% (v/v); the most active growth occurred at 0.2% (v/v). Growth factors

were not required. In contrast to *M. aurea* KYG$^T$, strain NE2$^T$ was unable to grow on acetate as well as on other multicarbon ($C_n$) compounds tested. Strain NE2$^T$ grew in a wide pH range of 4.1-8.0 with an optimum at pH 5.2-6.5. This was dramatically different from *M. aurea* KYG$^T$, which grew in a very narrow pH range of 5.2-7.2 and was unable to develop at pH below 5.0. The temperature range for growth was 6-32ºC, with the optimum at 18-25ºC. Strain NE2$^T$ did not require NaCl for growth and grew well in the presence of NaCl up to 0.1% (w/v). Growth inhibition of 50% was observed in the presence of 0.3% (w/v) NaCl, and concentrations above 0.5% (w/v) completely inhibited growth. Nitrogen sources included nitrates, urea, L-proline, L-alanine, L-asparagine, peptone and yeast extract (0.05% w/v). Surprisingly, strain NE2$^T$ did not utilize ammonium salts, which makes it different from both *M. aurea* KYG$^T$ and *M. acidiphila* B2$^T$. The isolate was able to fix dinitrogen, although its growth in nitrogen-free medium was more active under micro-oxic (sealed flasks filled with liquid medium by ½ volume and with 50% air and 50% nitrogen in a headspace) than under fully oxic conditions. Partial fragment of the *nifH* gene (encoding dinitrogenase reductase) was amplified using primers and reaction conditions described by Dedysh *et al*. (2004). The *nifH* gene fragment from strain NE2$^T$ displayed highest similarity (89-90% nucleotide sequence similarity and 95% derived amino acid sequence identity) to the corresponding gene fragments from various strains of the genera *Bradyrhizobium* and *Azorhizobium*.

For cellular fatty acid analysis, strain NE2$^T$ was grown under the same growth conditions as described for *M. aurea* KYG$^T$ (Dunfield et al., 2010), i.e. in batch cultures in DNMS medium at 24ºC for 10 days. The fatty acid profiles were analyzed at the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) as described by Kämpfer & Kroppenstedt (1996). The cellular fatty acid profile of strain NE2$^T$ was similar to those of *M. aurea* KYG$^T$ and *M. acidiphila* B2$^T$ (Table 1). The major cellular fatty acid in all of these methanotrophs was 11-*cis*-octadecenoic acid (18:1$\omega$7*c*). Its content in strain NE2$^T$ (55% of the total fatty acids), however, was lower than that in both described species of

the genus *Methylocapsa* (78-82%). In addition, the contents of 16:1$\omega$7*c* and 16:0 fatty acids were significantly higher in our novel isolate than in *M. aurea* KYG$^T$ and *M. acidiphila* B2$^T$.

The DNA base composition of strain NE2$^T$ was determined by thermal denaturation using a Unicam SP1800 spectrophotometer (UK) at a heating rate of 0.5°C min$^{-1}$. The mol % G+C value was calculated according to Owen *et al.* (1969) using *Escherichia coli* K-12 (G+C 51.7 mol %) as a standard. The DNA G+C content of strain NE2$^T$ was 61.7 mol%. This is within the range of values characteristic for other members the genus *Methylocapsa* (61.4-63.1 mol%) (Table 2).

Partial fragments of the *pmoA* (active-site polypeptide of pMMO) and *mxaF* (large subunit of methanol dehydrogenase) genes were amplified from DNA of strain NE2$^T$ using the primers and the reaction conditions described by Holmes *et al*. (1995) and McDonald & Murrell (1997), respectively. Comparative analysis of the *pmoA* gene revealed strain NE2$^T$ belongs to the phylogenetic lineage defined by the genus *Methylocapsa* and displays only 79.8% nucleotide sequence similarity (or 84% derived amino acid sequence identity) to the *pmoA* gene fragment from *M. aurea* KYG$^T$ (Fig. 3). The *mxaF* gene fragment from strain NE2$^T$ was most closely related to *mxaF* from members of the genus *Methylocystis* (86-87% nucleotide sequence similarity or 94-96% derived amino acid sequence identity). Notably, the highest amino acid sequence identity (97-98%) of MxaF from strain NE2$^T$ was observed with MxaF fragments retrieved from an acidic forest soil in a course of a study on active methylotrophs identified by means of stable isotope probing (Radajewski *et al*., 2002). This suggests that strain NE2$^T$-like methanotrophs might inhabit various acidic terrestrial environments. The *mmoX* gene encoding a subunit of soluble MMO (sMMO) could not be amplified from DNA of our isolate with any of the previously described *mmoX*-targeted primers (McDonald *et al*., 1995; Miguez *et al*., 1997; Shigematsu *et al*., 1999; Auman *et al*., 2000; Vorobev *et al*., 2011). In order to confirm the absence of sMMO in strain NE2$^T$, the colorimetric naphthalene oxidation test (Graham *et al*., 1992) was performed with *Methylocystis bryophila* H2s$^T$ as the positive control. As expected,

this test was positive for sMMO-possessing *Methylocystis bryophila* H2s$^T$, but negative for strain NE2$^T$.

In summary, our novel isolate was phenotypically and genotypically distinct from the two currently described species of the genus *Methylocapsa* (Table 2). Phylogenetically, strain NE2$^T$ was most closely related to *M. aurea* KYG$^T$, but differed from it by cell morphology, the absence of pigmentation, inability to grow on acetate, broader pH growth range, and higher tolerance to NaCl. In addition, DNA–DNA hybridization experiment was performed for strain NE2$^T$ and *M. aurea* KYG$^T$ as described by De Ley *et al.* (1970), and showed only 57% homology between these methanotrophs. Although displaying similar cell morphology, pH growth range and substrate utilization pattern, our novel isolate was phylogenetically distinct from *M. acidiphila* B2$^T$ and could also be differentiated from this methanotroph by the inability to utilize ammonium salts as nitrogen sources and to grow in N-free media under fully oxic conditions. Therefore, we propose a novel species, *Methylocapsa palsarum* sp. nov., for this bacterium.

**Description of *Methylocapsa palsarum* sp. nov.**

*Methylocapsa palsarum* (pal.sa'rum. N.L. gen. pl, n. palsarum of palsa bogs).

The description is as for the genus but with the following additional traits. Cells are 1.0-1.2 μm wide and 1.6-2.4μm long. Colony color is white. Carbon sources include methane and methanol. Nitrogen sources are nitrates, urea, L-proline, L-alanine, L-asparagine, peptone and yeast extract. Fixes $N_2$ via oxygen-sensitive dinitrogenase. Optimal growth at 18-25ºC and pH 5.2-6.5. NaCl inhibits growth at a concentration of 0.5% (w/v). The type strain is strain NE2$^T$ (=LMG 28715$^T$=VKM B-2945$^T$), which was isolated from a collapsed palsa soil, northern Norway.

**ACKNOWLEDGMENTS**

This research was supported by the collaborative project between the Russian Fund of Basic Research (project No14-04-93082) and The Research Council of Norway (233645/H30), and the Program “Molecular and Cell Biology” of Russian Academy of Sciences. The study, as part of the European Science Foundation EUROCORES Programme EuroEEFG, project MECOMECON, was funded through The Research Council of Norway (201270/V40) and Tromsø Research Foundation. The authors thank N.E. Suzina for thin section analysis, E.N. Detkova for DNA G+C content analysis, and A.G. Hestnes as well as Advanced Microscopy Core Facility, Faculty of Health Sciences, UiT The Arctic University of Norway for scanning electron microscopy.

## REFERENCES


**Auman, A.J., Stolyar, S., Costello, A.M. & Lidstrom, M.E. (2000).** Molecular characterization of methanotrophic isolates from freshwater lake sediment. *Appl Environ Microbiol* **66**, 5259-5266.

**De Ley, J., Cattoir, K. & Reynaerts, A. (1970).** The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

**Dedysh, S. N., Khmelenina, V. N., Suzina, N. E., Trotsenko, Y. A., Semrau, J. D., Liesack, W. & Tiedje, J. M. (2002).***Methylocapsa acidiphila* gen. nov., sp. nov., a novel methane-oxidizing and dinitrogen-fixing acidophilic bacterium from *Sphagnum* bog. *Int J Syst Evol Microbiol* **52**, 251-261.

**Dedysh, S.N., Dunfield, P.F., Derakshani, M., Stubner, S., Heyer, J.& Liesack, W. (2003).** Differential detection of type II methanotrophic bacteria in acidic peatlands using newly developed 16S rRNA-targeted fluorescent oligonucleotide probes. *FEMS Microbiol Eco*l **43**, 299-308.

**Dedysh, S.N., Ricke, P. & Liesack, W. (2004).** NifH and NifD phylogenies: an evolutionary basis for understanding nitrogen fixation capabilities of methanotrophic bacteria. *Microbiology* **150**, 1301-1313.

**Dedysh, S.N.** (2009) Exploring methanotroph diversity in acidic northern wetlands: molecular and cultivation-based studies. *Microbiology* (translated from *Mikrobiologiya*) **78**, 655-669.

**Dunfield, P.F., Khmelenina, V.N., Suzina N.E., Trotsenko,Y.A. & Dedysh, S.N. (2003).** *Methylocella silvestris* sp. nov., a novel methanotrophic bacterium isolated from an acidic forest cambisol. *Int J Syst Evol Microbiol* **53**, 1231-1239.

**Dunfield, P.F., Belova, S.E., Vorob'ev, A.V., Cornish, S.L. & Dedysh, S.N. (2010).** *Methylocapsa aurea* sp. nov., a facultatively methanotrophic bacterium possessing a particulate methane monooxygenase. *Int J Syst Evol Microbiol* **60**, 2659-2664.

**Felsenstein, J. (1989).** PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164-166.

**Graham, D.W., Korich, D.G., LeBlanc, R.P., Sinclair, N.A. & Arnold, R.G. (1992).** Applications of a colorimetric plate assay for soluble methane monooxygenase activity. *Appl Environ Microbiol* **58**, 2231–2236.

**Holmes, A. J., Costello, A., Lidstrom, M. E. & Murrell, J. C. (1995).** Evidence that particulate methane monooxygenase and ammonium monooxygenase may be evolutionarily related. *FEMS Microbiol Lett* **132**, 203-208.

**Kämpfer, P. & Kroppenstedt, R.M. (1996).** Numerical analysis of fatty acid patterns of the coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989-1005.

**Liebner, S. &Svenning, M. (2013).** Environmental transcription of *mmoX* by methane-oxidizing *Proteobacteria* in a subarctic palsa peatland. *Appl Environ Microbiol* **79**, 701-706.

**Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G. & other authors** (**2004).** ARB: a software environment for sequence data. *Nucleic Acids Res* **32,** 1363-1371.

**McDonald, I.R., Kenna, E.M. & Murrell, J.C. (1995).** Detection of methanotrophic bacteria in environmental samples with the PCR. *Appl Environ Microbiol* **61**, 116-121.

**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.

**Miguez, C.B., Bourque, D., Sealy, J.A., Greer, C.W. & Groleau, D. (1997).** Detection and isolation of methanotrophic bacteria possessing soluble methane monooxygenase (sMMO) genes using the polymerase chain reaction (PCR). *Microb Ecol* **33**, 21-31.

**Owen, R.J., Lapage, S.P., & Hill, L.R. (1969).** Determination of base composition from melting profiles in dilute buffers. *Biopolymers* 7, 503-516.

**Radajewski, S., Webster, G., Reay, D.S., Morris, S.A., Ineson, P., Nedwell, D.B., Prosser, J.I. & Murrell, J.C. (2002).** Identification of active methylotroph populations in an acidic forest soil by stable-isotope probing. *Microbiology* **148**, 2331-2342.

**Reynolds, E.S. (1963).** The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol* **17**, 208-213.

**Seppälä, M. (1986).** The origin of palsas. *Geografiska Annaler. Series A, Physical Geography* **68**, 141-147.

**Shigematsu, T., Hanada, S., Eguchi, M., Kamagata, Y., Kanagawa, T. & Kurane, R. (1999).** Soluble methane monooxygenase gene clusters fron trichloroethylene-degrading *Methylomonas*

sp. strains and detection of methanotrophs during in situ bioremediation. *Appl Environ Microbiol* **65**, 5198-5206.

**Tamas, I., Smirnova, A.V., He, Z. & Dunfield, P.F.** (**2014).** The (d)evolution of methanotrophy in the *Beijerinckiaceae*, a comparative genomics analysis. *ISME J* **8,** 369-382.

**Vorobev, A.V., Baani, M., Doronina, N.V., Brady, A.L., Liesack, W., Dunfield, P.F. & Dedysh, S.N. (2011).** *Methyloferula stellata* gen. nov., sp. nov., an acidophilic, obligately methanotrophic bacterium possessing only a soluble methane monooxygenase. *Int J Syst Evol Microbiol* **61**, 2456-2463.

**Weisburg, W.G., Barns, S.M., Pelletier, D.A. & Lane, D.J. (1991).** 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697-703.

**Table 1.** Cellular fatty acid composition of strain NE2$^T$ in comparison to *Methylocapsa aurea* KYG$^T$ and *Methylocapsa acidiphila* B2$^T$ (the major fatty acid is shown in bold).

| **Fatty acid** | **Strain NE2$^T$** | ***Methylocapsa aurea* KYG$^T$*** | ***Methylocapsa acidiphila* B2$^T$**** |
|---|---|---|---|
| 13:1 | - | 0.8 | - |
| 14:0 | - | - | - |
| i15:0 | 0.4 | 0.3 | 0.1 |
| 15:0 | - | - | - |
| 16:1$\omega$7*c* | **30.2** | 6.3 | 4.7 |
| 16:1$\omega$7*t* | - | - | - |
| 16:1$\omega$5*c* | - | - | 0.1 |
| 16:0 | **11.7** | 5.9 | 7.3 |
| i17:1$\omega$9*c* | 0.1 | 0.2 | - |
| i17:0 | 0.8 | 0.9 | 0.6 |
| 17:1$\omega$8*c* | - | - | - |
| 17:1$\omega$7*c* | - | - | 1.0 |
| 17:0 cyclo | - | - | - |
| 17:0 | - | - | 0.1 |
| i18:0 | - | - | - |
| 18:1$\omega$9*c* | - | 0.4 | - |
| 18:1$\omega$7*c* | **55.0** | **81.5** | **78.3** |
| 18:0 | 1.1 | 0.8 | 7.6 |
| 19:0$\omega$8*c* cyclo | - | 2.5 | - |
| 19:0 | 0.4 | - | - |
| 20:0 | 0.2 | - | - |

*****Data taken from Dunfield *et al*. (2010),

******Data taken from Dedysh *et al*. (2002),

-, not detected.

**Table 2.** Characteristics that differentiate strain NE2$^T$ and other described members of the genus *Methylocapsa*, i.e. *M. aurea* (Dunfield *et al*., 2010) and *M. acidiphila* (Dedysh *et al*., 2002).

| **Characteristic** | **strain NE2$^T$** | ***M. aurea*** | ***M. acidiphila*** |
|---|---|---|---|
| Cell morphology | thick rods | bipolar curved rods | coccoids |
| Pigmentation | none | yellow | none |
| Growth on acetate | - | + | - |
| pH growth range | 4.1-8.0 | 5.2-7.2 | 4.2-7.2 |
| Optimal growth temperature, °C | 18-25 | 25-30 | 20-24 |
| Sensitivity to NaCl (%, w/v) | 0.5 | 0.3 | 0.5 |
| Use of ammonium as nitrogen source | - | + | + |
| Growth in N-free medium under fully oxic conditions | - | - | + |
| Major fatty acids | 18:1$\omega$7*c*, 16:1ω7c, 16:0 | 18:1$\omega$7*c* | 18:1$\omega$7*c* |
| DNA G+C content | 61.7 | 61.4 | 61.9* |

*****data based on genome sequence analysis (Tamas *et al*., 2014).

## FIGURE CAPTIONS

**Figure 1.** 16S rRNA gene-based neighbour-joining tree showing the phylogenetic position of strainNE2$^T$ in relation to other representatives of the *Beijerinckiaceae* and some members of the *Methylocystaceae*. Bootstrap values (1000 data re-samplings) >50% are shown. Black circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. The type I methanotrophs *Methylomicrobium album* (X72777), *Methylobacter luteus* (AF304195), *Methylomonas methanica* S1 (AF304196) and *Methylococcus capsulatus* Texas (NR_029241) were used as an outgroup (not shown).Bar, 0.05 substitutions per nucleotide position.

**Figure 2. (a, b)** Phase-contrast micrographs of cells of strain NE2$^T$ (**a**) and *M. aurea* KYG$^T$ (**b**) grown in liquid medium M2 with methane for 7 days. Bar, 5 μm. (**c**) Image of cells of strain NE2$^T$ obtained by scanning electron microscopy. Bar, 1 μm. (**d**) Electron micrograph of an ultrathin section of methane-grown cells of strain NE2$^T$. Bar, 0.5 μm.

**Figure 3.** Phylogenetic tree based on partial PmoA sequences (147 amino acid positions), showing the relationship of the sequence from strain NE2$^T$ to those of other representative methanotrophic bacteria. Bootstrap values (1000 data re-samplings) >60% are shown. Bar, 0.1 substitutions per amino acid position.

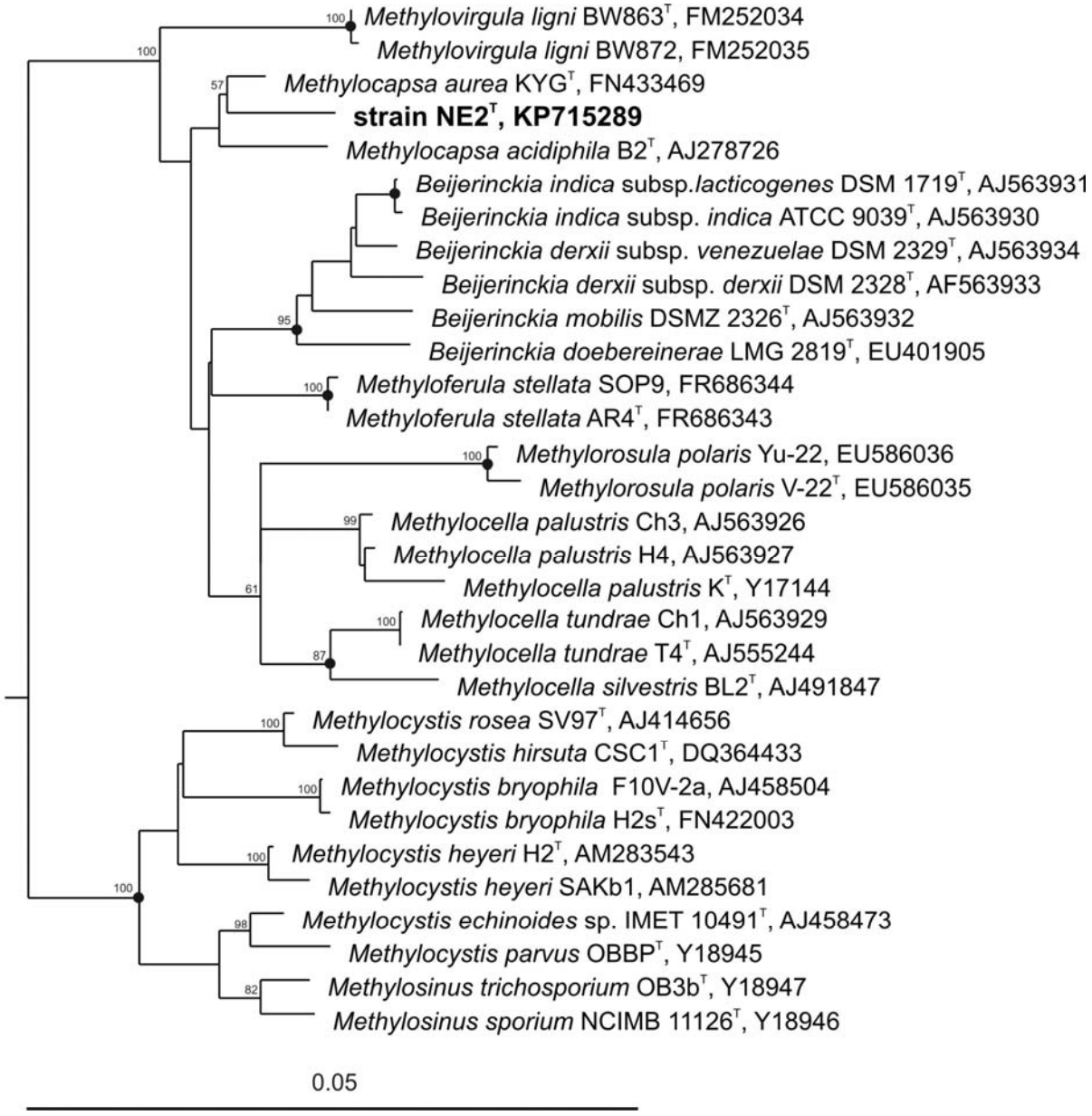
Methylovirgula ligni BW863T, FM252034
Methylovirgula ligni BW872, FM252035
Methylocapsa aurea KYGT, FN433469
strain NE2T, KP715289
Methylocapsa acidiphila B2T, AJ278726
Beijerinckia indica subsp.lacticogenes DSM 1719T, AJ563931
Beijerinckia indica subsp. indica ATCC 9039T, AJ563930
Beijerinckia derxii subsp. venezuelae DSM 2329T, AJ563934
Beijerinckia derxii subsp. derxii DSM 2328T, AF563933
Beijerinckia mobilis DSMZ 2326T, AJ563932
Beijerinckia doebereinerae LMG 2819T, EU401905
Methyloferula stellata SOP9, FR686344
Methyloferula stellata AR4T, FR686343
Methylorosula polaris Yu-22, EU586036
Methylorosula polaris V-22T, EU586035
Methylocella palustris Ch3, AJ563926
Methylocella palustris H4, AJ563927
Methylocella palustris KT, Y17144
Methylocella tundrae Ch1, AJ563929
Methylocella tundrae T4T, AJ555244
Methylocella silvestris BL2T, AJ491847
Methylocystis rosea SV97T, AJ414656
Methylocystis hirsuta CSC1T, DQ364433
Methylocystis bryophila F10V-2a, AJ458504
Methylocystis bryophila H2sT, FN422003
Methylocystis heyeri H2T, AM283543
Methylocystis heyeri SAKb1, AM285681
Methylocystis echinoides sp. IMET 10491T, AJ458473
Methylocystis parvus OBBPT, Y18945
Methylosinus trichosporium OB3bT, Y18947
Methylosinus sporium NCIMB 11126T, Y18946
100
100
57
95
100
100
99
61
100
87
100
100
100
100
98
82
0.05

**Figure 1.**

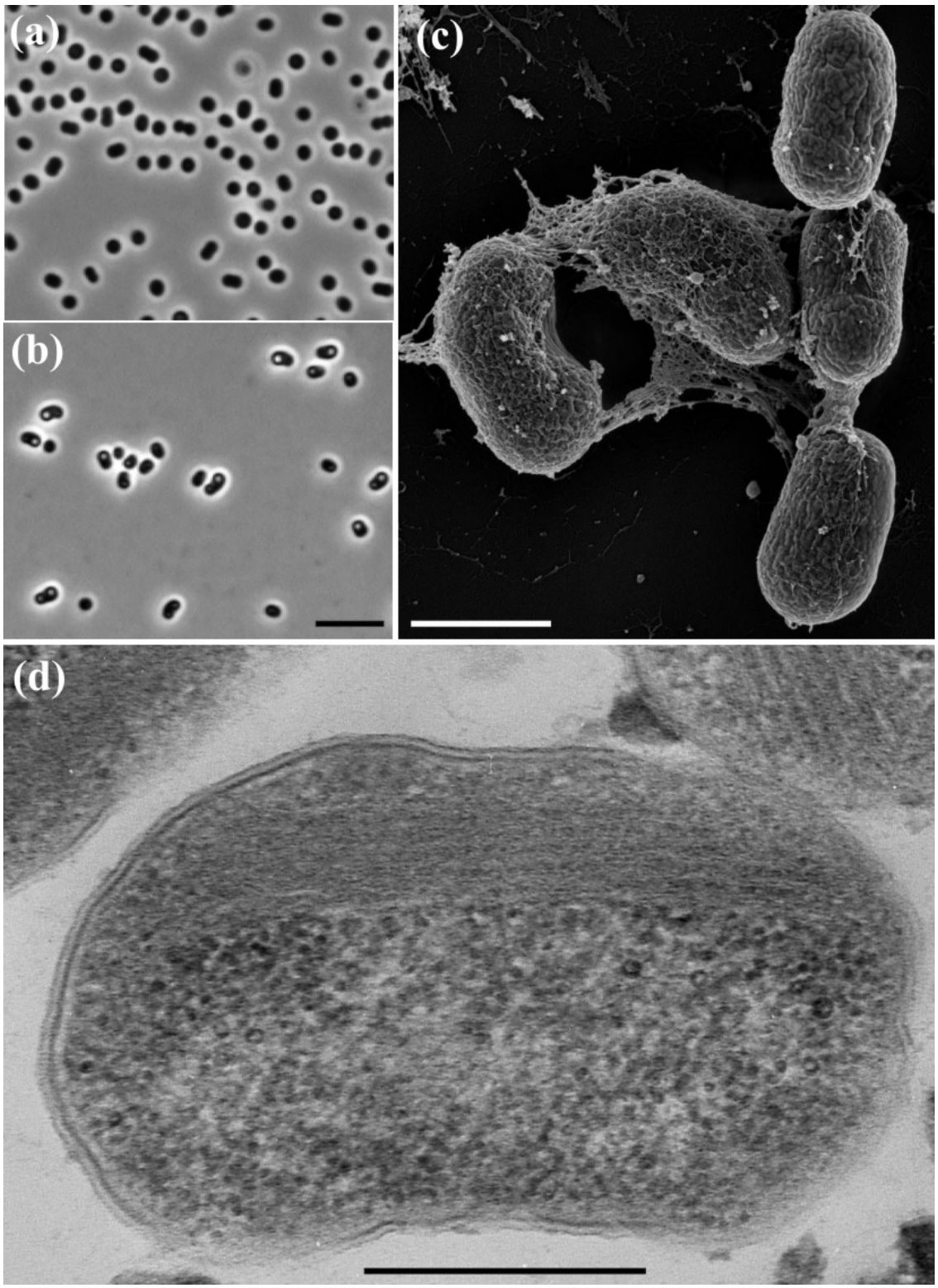


**Figure 2.**


Methylocapsa acidiphila B2T, AJ278727
Methylocapsa aurea KYGT, FN433470
strain NE2T, KP715290
Methylocystis heyeri H2T, AM283546
Methylocystis hirsuta CSC1T, DQ364434
Methylocystis roseae SV97T, AJ414657
Methylocystis sp. M, U81596
Methylocystis parvus OBBPT, U31651
Methylocystis echinoides IMET 10491T, AJ459000
Methylogaea oryzae E10T, EU359002
Methylococcus capsulatus BathT, L40804
Methylocaldum gracile 14LT, U89301
Methylohalobius crimeensis 10KiT, AJ581836
Methylothermus thermalis MYHTT, AY829010
Methylomicrobium buryatense 5BT, AF307139
Methylovulum miyakonense HT12T, AB501285
Methylobacter tundripaludum SV96T, AJ414658
Methylomonas paludis MG30T, HE801217
68
100
100
100
82
86
97
100
100
0.10

**Figure 3.**