Methylosula polaris gen. nov., sp. nov., an aerobic, facultatively methylotrophic psychrotolerant bacterium from tundra wetland soil

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Three strains of Gram-negative, aerobic, motile bacteria with bipolar flagella were isolated from acidic tundra wetland soils near the city of Vorkuta and from the Chukotka and Yugorsky Peninsulas and designated strains V-022^T, Ch-022 and Ju-022. The cells were rod-shaped, 0.5–0.6 µm in width and 1.3–4.5 µm in length and reproduced by irregular fission. These bacteria were facultative methylotrophs that used methanol, methylamines and a wide range of other sources of carbon and energy such as sugars and polysaccharides, ethanol and amino acids. The isolates used the Calvin–Benson pathway for the assimilation of one-carbon compounds and were unable to fix nitrogen. The new strains were moderately acidophilic and psychrotolerant, capable of growth over a pH range of 4.0 to 7.8, with optimum growth at pH 5.5–6.0. Growth occurred between 4 and 30 °C (optimum 20–25 °C). The principal phospholipid fatty acid was C_{18:1}ω7c. The DNA G + C content of strain V-022^T was 65.2 mol%. Analysis of the 16S rRNA gene sequences revealed that all three isolates V-022^T, Ch-022 and Yu-022 exhibited almost identical 16S rRNA gene sequences (99.9% gene sequence similarity) and formed a new lineage within the class *Alphaproteobacteria*. The name *Methylosula polaris* is suggested to accommodate this new genus and novel species with strain V-022^T (=DSM 22001^T=VKM V-2485^T) as the type strain of the type species.

Aerobic methylotrophic bacteria using one-carbon compounds, such as methane, methanol and methylated amines, are widespread in natural environments, being found in a variety of aquatic and terrestrial habitats (Anthony, 1982; Bowman *et al.*, 1997; Sorokin *et al.*, 2007). Many of the known strains of methylotrophic bacteria are obligately methylotrophic and use compounds containing no carbon–carbon bonds (Lidstrom, 2006; Bowman *et al.*, 1997; Bodrossy *et al.*, 1997). However, a variety of organisms are known that can grow facultatively either on multicarbon compounds or on one carbon compounds (Doronina *et al.*, 1998; Lidstrom, 2006; Dedysh *et al.*, 2005a). Traditionally, the major function of aerobic methylotrophs (methanotrophs)

was believed to be that they form the principal biological sink for methane in the global methane cycle (Senum & Gaffney, 1985). Recently it has been revealed that these bacteria thrive in a strong association with plants, which are a methanol emission source of global importance; plant-borne methanol makes up to 40–46% of the total volatile carbon in the atmosphere (MacDonald & Fall, 1993). A possible explanation of such plant–bacterial associations is the participation of the latter in the ‘methanol cycle’ (Trotsenko *et al.*, 2001). Methylotrophic bacteria have also been observed in hypersaline lakes where the total flux of methylated compounds is enhanced by their formation as degradation products from osmolytes (Sorokin *et al.*, 2007). Aerobic methylotrophs could also form associations with methanotrophic bacteria and utilize the products of methane oxidation as their source of carbon and energy (Trotsenko & Khmelenina, 2002). The present study describes three novel strains that are psychrotolerant, moderately acidophilic and facultatively

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains V-022^T, Ch-022 and Yu-022 are EU586035–EU586037. The accession numbers for the *mxhF* sequences of strains V-022^T and Yu-022 are EU586038 and EU586039, respectively.

methylophilic bacteria that were isolated from methane-oxidizing communities of soil from the polar tundra of the Vorkuta region and of the Chukotka and Yugorsky Peninsulas of Russia.

Strains were isolated from acidophilic, methanotrophic enrichments obtained from *Sphagnum* peat of three tundra wetland sites of Northern Russia (Berestovskaya *et al.*, 2002). Cell suspensions of these enrichments were spread onto medium 2A (Berestovskaya *et al.*, 2002) agar plates, containing 2 % (w/v) agar (Difco). The medium was amended with 0.5 % methanol as the sole carbon source and both amended and normal plates were placed into glass jars in which the atmosphere was adjusted to a 30:70 methane/air ratio. The plates were incubated at 10 °C. As colonies became visible, they were successively restreaked onto fresh plates with the same solid medium until a culture with uniform colonies was obtained. The isolates from Vorkuta and the Chukotka and Yugorsky Peninsulas were designated, correspondingly, V-022^T, Ch-022 and Yu-022. The same medium (in both liquid and solid forms) was used for further physiological examination of the methylophilic obtained.

Cell morphology and cell life cycle, as well as culture purity, were examined by using phase-contrast and electron microscopy. In the latter case, the samples were stained with 1 % (w/v) uranyl acetate. The procedure for the preparation of the ultrathin sections used in this work has been described elsewhere (Vasilyeva *et al.*, 2006). The samples were examined with a JEM-100C transmission electron microscope.

Growth of isolates in liquid medium was monitored by spectrophotometry by measuring the turbidity at 600 nm for 2 weeks, with methanol as the sole growth substrate. Growth was examined with various temperatures (4–38 °C), pH values (4.0–8.0) and NaCl concentrations [0.01–2.50 % (w/v)]. Variations in the pH level were achieved by mixing 0.05 M solutions of Na₂HPO₄ and KH₂PO₄ to create media with the same ionic strength. To determine the range of potential growth substrates for strains V-022^T, Ch-022 and Yu-022, the following carbon sources, at a concentration of 0.5 % (w/v), were tested in liquid medium 2A: methane, methanol, mono-, di-, and trimethylamines; the monosaccharides D-ribose, D-arabinose, D-xylose, lyxose, D-glucose, rhamnose, D-fructose, D-mannose and D-galactose; the disaccharides maltose, lactose, sucrose and trehalose; the trisaccharide raffinose; the polysaccharides starch and dextrin, sugar alcohols, glycerol, dulcitol, D-sorbitol, D-mannitol and inosine; the alcohols ethanol and butanol; the amino acids asparagine, L-glutamate, L-arginine, L-proline, isoleucine, phenylalanine, cysteine, serine, histidine, and methionine; the sodium salts of the organic acids pyruvate, lactate, succinate, malate, formate, acetate, propionate and butyrate; and yeast extract, Casamino acids and pectin. Autotrophic growth with hydrogen as the electron donor was studied at the optimal pH and temperature using 500 ml serum bottles with 100 ml medium under an atmosphere

containing 20 % CO₂, 30 % air and 50 % H₂. Growth was examined after 14 days of incubation.

For fatty acid analysis, cells were grown on liquid medium 2A with methanol and harvested in the late exponential growth phase. The analysis was performed by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany).

In order to establish the routes of primary C₁ metabolism in strain V-022^T, enzyme activities were determined in extracts of cell growth on methylamine. For the preparation of cell-free extracts, the biomass was harvested from cells at the exponential growth phase and washed twice with distilled H₂O. Concentrated cell suspensions prepared with 50 mM Tris/HCl buffer pH 7.0 were disrupted (6 × 1 min) in an ultrasonic disintegrator (model 150 W; MSE) at 4 °C. The supernatant obtained by centrifugation of the cell homogenate at 30 000 g for 1 h was used as a cell-free extract.

In order to measure the activities of methylamine dehydrogenase EC 1.4.99.3 (Eady & Large, 1968), formaldehyde dehydrogenase (NAD/GSH-linked) EC 1.2.1.1, formate dehydrogenase EC 1.2.1.2, hydroxypyruvate reductase EC 1.1.1.25, 3-hexulosephosphatesynthase and ribulosebiphosphate carboxylase EC 4.1.1.39 (Loginova & Trotsenko, 1979), enzyme assays were performed at 30 °C in cell-free extracts, according to the methods given in the papers cited above.

Spectrophotometric assays were performed with a Specord UV VIS machine and radioactivity was counted in a liquid scintillation spectrometer (SL-30; Intertechnique).

Enzyme activities were expressed as nmol of substrate transformed or of the product formed in 1 min per mg of protein. Protein concentration was determined by the method of Lowry (Lowry *et al.*, 1951) using BSA as a reference.

The isolation of DNA, subsequent determination of the G+C content and DNA–DNA hybridization experiments were performed according to the methods of Marmur (1961) and De Ley *et al.* (1970). The nearly complete 16S rRNA gene was obtained using general bacterial primers 11F–1492R according to standard protocols. The amplification of *mxhA*, the gene coding for methanol dehydrogenase, was performed with primer sets developed by McDonald & Murrell (1997) and Dedysh *et al.* (2005b) according to the corresponding protocols. The amplification of *pmoA* and *pmoF* genes, encoding different forms of particulate methane monooxygenase, was performed with the primer set *pmoA* f189–*pmoA* r682 developed by Holmes *et al.* (1995) and a modified set with *pmoF*: 5'-GCGGATT-CGGIGACTGGGACTTCTGG and *pmoR*: 5'-GCGAATTC-GAASGCIGAGAAGAASGC. The content of the PCR mixture was the same as described above. The following program was applied: the initial cycle at 94 °C for 3 min, 55 °C for 30 s and 72 °C for 30 s was followed by 35 cycles (94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s) and a final

extension step at 72 °C for 7 min. The amplification of *mmoC* and *mmoX*, genes coding for soluble methane monooxygenase subunits, was performed with the primer set *mmoC* f542–*mmoC* r986 developed for *mmoC* genes by McDonald *et al.* (1995); and with primer set *mmoXA*–*mmoXB* developed for *mmoX* genes by Auman *et al.* (2000) according to the previously described protocols. The PCR products were gel-purified using the Wizard PCR-Prep kit (Promega). Sequencing was performed using a Big Dye Terminator v.3.1 sequencing reaction kit and a DNA automatic sequencer (ABI 3730; Applied Biosystems). The sequences obtained in this study were first compared with those from GenBank using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/BLAST>; Altschul *et al.*, 1997). The nucleotide and inferred amino acid sequences were aligned with similar sequences retrieved from the GenBank using CLUSTAL W [online software at the EBI (<http://www.ebi.ac.uk>; Larkin *et al.*, 2007)]. The phylogenetic tree was constructed using the neighbour-joining algorithm using TREECONW (Van de Peer & De Wachter, 1994).

Two types of colonies developed from the methanotrophic enrichments of *Sphagnum* peat tundra wetland samples collected from the Vorkuta region on the 2A agar medium with methane as the sole electron donor and carbon source after 4 weeks of incubation at 10 °C. One of the colony types was circular, non-mucoid, opaque/creamy-coloured and 1–3 mm in diameter. The second type of colony was more convex, mucoid, dense, pale and 0.5–0.7 mm in diameter. Observations of the cells from the first colony type by phase-contrast microscopy showed that they were morphologically similar to those previously isolated from Vorkuta tundra soil and belonging to the genus *Methylocella* (Dedysh *et al.*, 2004). The second type of colony contained big, rod-shaped cells with bipolar flagella. The colonies with the latter morphotype were obtained on 2A agar medium with methanotrophic enrichments from the Chukotka and Yugorsky Peninsulas. The colonies formed by the bipolar-flagellated cells were transferred to solid and liquid 2A media with methane and methanol as substrates. Three strains (V-022^T, Ch-022 and Yu-022) were selected, each representing one of the three methanotrophic enrichments used for isolation. In the media with methane, the new isolates could only grow for three consecutive inoculation/cultivation rounds, in contrast to those grown with media containing methanol, which exhibited stable growth. The purity of these cultures was confirmed by microscopy. Cells of these three strains were Gram-negative (as confirmed by microscopy), long, bipolar rods (Fig. 1a). The cells were 0.5–0.6 µm wide and 1.3–4.5 µm long. They reproduced through an irregular division which resulted in a short phase with motile cellular forms. Young cultures thus contained a large number of motile short rods with a subpolar flagellum (Fig. 1b). Forked cells were observed occasionally. An accumulation of mucous material was observed at one of the cellular poles, which may possibly mediate the cell-to-surface attachment and formation of forks (Fig. 1a, c). Old cultures contained larger numbers of forked cells.

Fig. 1. Morphology of *Methylosula polaris* sp. nov. (a) Mucous material at one of the cellular poles; (b) young cell with subpolar flagellum; (c), phase-contrast photomicrograph illustrating the formation of rosettes. Bars, 0.5 µm (a and b), 1 µm (c). Parts (a) and (b) were negatively stained with uranyl acetate.

Cultures grown in liquid media tended to flocculate upon achievement of later growth phases.

Microscopic analysis of the thin sections of cells grown on methanol revealed a Gram-negative cell wall type (Fig. 2). A larger periplasmic space was typically observed in young cells.

A variety of carbon compounds were tested for their ability to support the growth of the isolates (Table 1). The isolates were able to use a broad spectrum of organic compounds, including C₁-compounds, mono-, di- and trisaccharides, polysaccharides, sugar, alcohols, amino acids and Casamino acids. Certain differences were observed in the utilization pattern of various compounds by the three isolates. In contrast to strain V-022^T, strain Ch-022 did not utilize raffinose, glycerol or L-phenylalanine, but was able to utilize

Fig. 2. Ultrastructure of *Methylosula polaris* sp. nov. Bar, 0.5 μ m.

cysteine. Strain Yu-022, unlike the other isolates, did not utilize trehalose, inosine, ethanol or isoleucine. None of the strains utilized butanol, pectin, amino acids (histidine and methionine) or salts of organic acids (pyruvate, lactate, succinate, malate, formate, acetate, propionate and butyrate) and were not autotrophic with hydrogen as the electron donor. Thus the isolates were heterotrophic bacteria that, in addition to C_1 -compounds, were able to utilize a broad variety of organic compounds including methylated ones. The strains were able to use ammonium, nitrate and yeast extract as nitrogen sources. The three novel strains were unable to fix atmospheric nitrogen under the conditions tested in nitrogen-free media.

Table 1. Comparative characteristics of strains V-022^T, Ch-022 and Ju-022

All strains were able to grow with methanol, methylamines (mono-, di-, tri-), monosaccharides, disaccharides maltose, lactose and sucrose, polysaccharides starch and dextrin, sugar alcohols, dulcitol, sorbitol and mannitol, amino acids alanine, glutamine, arginine and proline; yeast extract and Casamino acids. None of the strains were able to grow with butanol, with amino acids histidine or methionine, or with salts of organic acids.

Characteristic	V-022 ^T	Ch-022	Ju-022
Growth on:			
Trehalose	+	+	–
Raffinose	+	–	+
Glycerol	+	–	+
Inosine	+	+	–
Ethanol	+	+	–
Isoleucine	+	+	–
Phenylalanine	+	–	+
Cysteine	–	+	–
Serine	–	–	+
DNA G + C content (mol%)	65.2	64.0	65.0

Table 2. Cellular fatty acid compositions of strain V-022^T, *Methylocella tundrae* and *Methylocapsa acidiphila* of the family Beijerinckiaceae

Taxa: 1, V-022^T; 2, *Methylocella tundrae* T4^T (Data from Dedysh *et al.*, 2004); 3, *Methylocapsa acidiphila* B2^T (Dedysh *et al.*, 2002). ND, not detected.

Fatty acid	1	2	3
C _{14:0}	ND	2.6–4.1	ND
C _{15:0}	ND	ND	ND
C _{16:0} iso	0.7	ND	ND
C _{16:1} ω 7c	3.6	7.2–11.3	4.7
C _{16:1} ω 5c	6.8	ND	0.1
C _{16:0}	6.5	7.2–7.7	7.3
C _{17:0} iso	0.5	0.4–0.6	0.6
C _{17:1} ω 8c	ND	0–0.3	ND
C _{17:1} ω 7c	ND	ND	1.0
C _{17:1} ω 6c	ND	0–0.1	ND
C _{17:0} cyclo	1.7	5.0–6.5	ND
C _{18:0} iso	0.3	ND	ND
C _{18:1} ω 7c	66.8	59.2–61.7	78.3
C _{18:0}	1.7	0.4–0.6	7.6
C _{19:0} ω 8c cyclo	11.1	7.9–13.6	ND
C _{19:0}	0.4	ND	ND

The isolates were moderately acidophilic organisms showing growth at pH 4.0–7.8 (optimum at pH 5.5–6.0) (Table 1). The strains were psychrotolerant with a temperature range for growth of 4–30 °C (optimum, 20–25 °C). No growth occurred at 37 °C. The culture generation time with methanol (0.1%, v/v) calculated from increases in OD₆₀₀ at the exponential phase (over a total of 7 days) was 25–36 h. No growth inhibition was observed in the presence of NaCl in the medium at concentrations of 0.1–0.5% (w/v). Growth rates were inhibited by 50% at concentrations of 1–1.5% NaCl (w/v), whereas concentrations above 2.0% completely inhibited growth.

Table 2 shows the fatty acid composition of strain V-22^T. The fatty acids comprised mainly straight-chain saturated

Table 3. Enzyme activities in cell extracts of strain V-022^T grown with methylamine

The activities are expressed as nmol min^{–1} mg^{–1} of protein.

Enzyme	Cofactor	Enzyme activity
Methylamine dehydrogenase	PMS	90
Formaldehyde dehydrogenase	NAD ⁺	2
	NAD ⁺ , GSH	520
Formate dehydrogenase	NAD ⁺	140
Hydroxypyruvate reductase	NADH	–
	NADPH	–
3-Hexulose phosphate synthase		–
Ribulose-1,5-bisphosphate carboxylase		10

and unsaturated fatty acids together with a diagnostic amount of cyclopropane fatty acids showing 17 and 19 carbon atoms. The major component of the phospholipid fatty acid (PLFA) profiles of the novel isolates was 11-*cis*-octadecenoic acid ($C_{18:1\omega7c}$), which comprised 66.78% of the total PLFAs. This composition was similar to that found previously for *Methylocella tundrae* and *Methylocapsa acidiphila*. The fatty acid pattern of the novel organisms was most similar to that found in the type strain of *Methylocella tundrae* (Dedysh *et al.*, 2004). The distinctive feature of the

PLFA profiles of the novel methylotrophs was the presence of $C_{16:1\omega5c}$ fatty acids and small amounts of iso-branched fatty acids ($C_{16:0}$ iso and $C_{18:0}$ iso). The DNA G + C content of strain V-022^T was 65.2 mol%.

Measurements of the activities of key enzymes of C_1 -metabolism of the methylamine-grown cells indicated the presence of methylamine dehydrogenase activity (Table 3). Formaldehyde was further oxidized by GSH-dependent formaldehyde dehydrogenase to formate. The latter was

Fig. 3. 16S rRNA gene sequence-based dendrogram showing the phylogenetic position of strains V-022^T, Ch-022 and Yu-022 in relation to representative members of the class *Alphaproteobacteria*. Evolutionary distances were calculated by the method of Jukes & Cantor (1969). The root was determined by using the 16S rRNA gene sequence of *Methylococcus capsulatus* ATCC 19069^T (GenBank accession no. X72770) as the outgroup. The numbers at the nodes are bootstrap values as derived from 1000 data resamplings; values >75 were considered as significant. Bar, 0.05 substitutions per nucleotide position.

Fig. 4. PCR analysis for determination of functional genes of the novel methylotrophic strains. (a) *mxrF* gene. Lanes: 1, 0.1 kb marker (Sibenzyme, Russia); 2, V-022^T; 3, Ch-022; 4, Yu-022; 5, *Methylocella tundrae* T4^T (positive control); 6, *Methylosinus sporium* NCIMB 11126^T (positive control); 7, *Escherichia coli* (negative control); 8, control without DNA; 9, 0.1 kb marker (Sibenzyme, Russia). (b) *MMO* genes. Lanes: 1, 6, 11 and 17, strain V-022^T; 2, 7, 12 and 18, strain Yu-022; 3, 8, 13 and 19, *Methylocella tundrae* T4^T (positive control); 4, 9, 14 and 20, *Escherichia coli* (negative control); 5, 10, 15 and 21, control without DNA; 16 and 22, 0.1 kb marker (Sibenzyme, Russia).

finally oxidized to CO₂ by NAD-linked formate dehydrogenase. Carbon dioxide was assimilated via the RuBP pathway (Benson–Calvin cycle), as confirmed by the presence of ribulose-1,5-bisphosphate carboxylase. Neither the serine nor the ribulose monophosphate pathway of C1 assimilation was operative, due to the absence of the appropriate specific enzymes: hydroxypyruvate reductase and hexulose phosphate synthase.

Comparative sequence analysis of the 16S rRNA gene fragments (almost 1400 nt) revealed that strains V-022^T, Ch-022 and Yu-022 possessed almost identical 16S rRNA gene sequences (99.9% sequence similarity). The 16S rRNA gene-based phylogenetic analyses demonstrated that the isolates belonged to the family *Beijerinckiaceae* in the class *Alphaproteobacteria* and represented a novel independent lineage with 97.0% sequence similarity to the nearest relative (Fig. 3). Since the new isolates formed a separate lineage with uncertain branching among other members of the family *Beijerinckiaceae*, key functional genes, such as *mxrF* (methanol dehydrogenase) and pMMO and sMMO (methane monooxygenase) were also analysed. The *mxrF* gene was easily detectable in the novel isolates (Fig. 4a), but all attempts to amplify pMMO or sMMO genes, using different primer sets, failed (Fig. 4b). Phylogenetic analysis of translated amino acid sequences (162 aa) of the *mxrF* gene fragments (486 nt) from strains V-022^T and Yu-022 confirmed the 16S rRNA gene-based data. This placed the

novel isolates amongst the methylotrophic alphaproteobacteria as a separate branch, which confirmed their unusual position among known methylotrophs (Fig. 5).

In this study, three strains of a new facultatively methylotrophic bacterium were isolated that belong to the class *Alphaproteobacteria* and form a separate monophyletic lineage within the family *Beijerinckiaceae*. These new organisms are phenotypically distinct from the other known representatives of the family (Table 4). These novel methylotrophic organisms reproduce by irregular division, are motile by means of a subpolar flagellum and do not produce dormant forms. The isolates do not fix atmospheric nitrogen as found for some other members of the family *Beijerinckiaceae*. In comparison with the methanotrophic representatives of the family *Beijerinckiaceae* from the genera *Methylocella* and *Methylocapsa*, cells of the novel strains do not utilize methane as a source of carbon and energy and use the RuBP pathway for the assimilation of one-carbon compounds. In contrast to the members of the genera *Beijerinckia* and *Methylocella*, the new isolates do not utilize organic acids, but are able to use methylated amines and amino acids. The G + C content in the DNA of the novel organisms was higher than that previously found for the other representatives of the family *Beijerinckiaceae*.

On the basis of a polyphasic analysis of their phenotypic and genotypic properties, the novel strains of acid-tolerant, facultative methylotrophic alphaproteobacteria from three

Fig. 5. Unrooted phylogenetic tree of methylotrophic members of the class *Proteobacteria* based on comparative analysis of conceptual translations of *mxaF* genes. Bar, 5% sequence variation. Bootstrap values >75% (based on 1000 data resamplings) are indicated at the nodes.

different tundra wetland sites are proposed as representing a new genus and novel species, for which the name *Methylorosula polaris* gen. nov., sp. nov. is proposed. The type strain is strain V-022^T (=DSM 22001^T=VKM V-2485^T).

Description of *Methylorosula* gen. nov.

Methylorosula [Me.thyl.o.ro'su.la. N.Gr. n. *methyl* (from Gr. n. *methu* wine and Gr. n. *hulê* wood); L. fem. n. *rosula* a little rose; N.L. fem. n. *Methylorosula* methyl-using forming rosette cells].

Cells are Gram-negative rods that are not capable of nitrogen fixation. Aerobic, facultative methylotrophic bacteria. Chemoorganoheterotrophic. Use the RuBP pathway (Benson–Calvin cycle) for carbon assimilation. The dominant cellular fatty acid is C_{18:1ω7c}. A member of the family *Beijerinckiaceae* in the class *Alphaproteobacteria*. *Methylosula polaris* is the type species of the genus.

Description of *Methylosorusula polaris* sp. nov.

Methylorosula polaris (po.la'ris. N.L. fem. adj. *polaris* pertaining to the geographical zone in Northern Russia, where the type strain was isolated).

Displays the following properties in addition to those described for the genus. Cells are long, bipolar rods ($0.5\text{--}0.6 \times 1.3\text{--}4.5\text{ }\mu\text{m}$) that are single or form rosettes. Reproduces by irregular fission. Young cells are motile by means of a subpolar flagellum. Forms pale, non-translucent slimy (mucoid) colonies with an uneven edge. After 2 weeks of growth on methanol at $20\text{ }^{\circ}\text{C}$, colonies are $1.5\text{--}2.0\text{ mm}$ in size. Utilizes C_1 -compounds, methanol and methylamines, sugars, polysaccharides, sugar alcohols and amino acids as a carbon and energy source. The temperature and pH ranges for growth are $4\text{--}30\text{ }^{\circ}\text{C}$ (optimum, $20\text{--}25\text{ }^{\circ}\text{C}$) and pH $4.0\text{--}7.8$ (optimum, pH $5.5\text{--}6.0$), respectively. NaCl is not required for growth and is tolerated up to a concentration of 2.0% (w/v). The major fatty acid is $\text{C}_{18:1}\omega 7c$.

Table 4. Major characteristics that distinguish *Methylosula* gen. nov. from other genera of the family *Beijerinckiaceae*

Taxa: 1, *Methylosula* gen. nov.; 2, *Methylocella* (data from Kennedy, 2005; Dedysh *et al.*, 2005a); 3, *Beijerinckia* (Kennedy, 2005); 4, *Methylocapsa* (data from Dedysh *et al.*, 2002).

Characteristic	1	2	3	4
Cell morphology	Rods	Rods	Rods	Coccoid
Size (µm)	0.5–0.6 × 1.3–4.5	0.6–1.0 × 1.0–2.5	0.5–1.5 × 7–4.5	0.7–1.0 × 0.8–1.2
Motility	Motile, monotrichous	Non-motile, peritrichous	Motile/non-motile	Non-motile
Division	Irregular	Regular	Regular	Regular
Rosette formation	+	–	–	–
Type of resting cells	–	Exospores	Cysts, capsules	<i>Azotobacter</i> -type cysts
Substrates:				
CH ₄	–	+	–	+
Methanol	+	+	+ / –	≤ 0.05 %
Methylamines	+	–	–	–
Sugars	+	–	+	–
Ethanol	+	+	–	–
Amino acids	+	–	–	–
Organic acids	–	+	+	–
One-carbon compound assimilation	RuBP	Serine	RuBP	Serine
N ₂ fixation	–	+	+	+
DNA G + C content (mol%)	65.2	61.2	54.7–60.7	63

The type strain, V-022^T (=DSM 22001^T=VKM V-2485^T), was isolated from tundra wetland soil. The DNA G + C content of the type strain is 65.2 mol%.

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