**BMSAB GENUS CHAPTER TEMPLATE WITH INSTRUCTIONS ON FORMAT AND INFORMATION TO INCLUDE**

**Use this template as an example for the format and content of your chapter. Feel free to include additional information that is available for the genus you are covering. Your text should be entered in place of the example information provided below in black text. Continuous line numbers should be maintained throughout the manuscript including any pages containing Tables or Figures. Heading in maroon text should not be removed or edited.**

**1. PHYLUM/CLASS/ORDER/FAMILY:**

**ENTER THE TAXONOMIC PATH IN ITALICS eg.**

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*Proteobacteria/Alphaproteobacteria/Rhizobiales/Beijerinckiaceae/*

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**3. CHAPTER TITLE:**

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*Methylocella*

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**4. DEFINING PUBLICATION:**

**ENTER THE REFERENCE TO THE DEFINING PUBLICATION, EFFECTIVE PUBLICATION AND EMENDMENTS AS APPROPRIATE. SEE THE CONTRIBUTOR GUIDELINES FOR DETAILS ON THE NEW FORMAT. THE PAGE NUMBER IS NO LONGER REQUIRED.**

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Dedysh et al. 2000VP emend. Dedysh et al. 2004

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**5. AUTHORS NAMES AND INSTITUTIONS:**

**ENTER THE NAMES, INSTITUTIONS, CITY AND COUNTRY OF AUTHORS. THE ADDRESS SHOULD BE IN ITALICS eg.**

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Svetlana N. Dedysh,*Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia*

Peter F. Dunfield,*Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada*

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**6. ETYMOLOGY:**

**ENTER THE ETYMOLOGY AS PRESENTED IN THE ORIGINAL TAXONOMIC DESCRIPTION OR THE LPSN WEBSITE, eg.**

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Me.thy.lo.cel’la. N.L. pref. *methyl,* pertaining to the methyl radical; L. fem. n. *cella,* a room, and in biology, a cell; N.L. fem. n. *Methylocella,* a methane-using cell.

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**7. ABSTRACT:**

**ENTER THE ABSTRACT OF THE CHAPTER. THE ABSTRACT SHOULD BE A SUMMARY OF THE INFORMATION CONTAINED IN THE MANUSCRIPT THAT GIVES THE READER AN OVERVIEW OF THE TAXON BEING COVERED. INFORMATION ON THE MORPHOLOGY, PHYSIOLOGY, PHYLOGENY AND ECOLOGY SHOULD BE INCLUDED eg.**

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The genus *Methylocella* accommodates mildly acidophilic, facultatively methanotrophic bacteria that perform the first step of methane oxidation using only a soluble methane monooxygenase, rather than the more common particulate methane monooxygenase. Cells of these methanotrophs are Gram-negative, aerobic, non-motile, polymorphic, slightly curved rods or ovoids, which reproduce by normal cell division and occur singly or in in shapeless aggregates. Cells possess bipolar appearance due to large, highly refractile, intracellular poly-ß-hydroxybutyrate granules,which form at each cell pole one. An extensive intracytoplasmic membrane system common to most known methanotrophic bacteria is absent from cells. Like other methanotrophs, *Methylocella* species are capable of growth on methane, methanol, methylamine and formate; C1 compounds are assimilated via the serine pathway. However, they are also capable of growth on ethanol, some organic acids, ethane, propane and some other multicarbon compounds, but sugars are not utilized. Acetate is preferred over methane, and leads to a downregulation of methane oxidation. These methanotrophs are capable of atmospheric nitrogen fixation under reduced oxygen tension. They are mesophilic and psychrotolerant bacteria, which prefer dilute media of low salt content. The major fatty acid is 18:1*ω*7*c*. DNA G + C content is 61.2 – 63.3 (Tm). Known habitats are acidic and neutral peatlands, soils, and sediments.

*Type species*:  **Methylocella palustris** Dedysh et al. 2000VP.

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**8. KEYWORDS:**

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aerobe, methanotroph, methylotroph, acidic peatlands, soil, serine pathway

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**9. DESCRIPTION:**

**ENTER THE INFORMATION THAT DESCRIBES THE GENUS BEING CONSIDERED. IMPORTANT AND DEFINING CHARACTERISTICS SHOULD BE IN BOLD FONT. THE TYPE SPECIES AND DEFINING PUBLICATION, THE NUMBER OF VALIDLY PUBLISHED SPECIES AND THE FAMILY CLASSIFICATION SHOULD BE INCLUDED eg.**

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Gram-negative, aerobic, polymorphic, slightly curved rods with rounded ends or ovoids. Produce large, **highly refractile, intracellular poly-ß-hydroxybutyrate granules,** one at each pole. Reproduce by normal cell division. Cells occur singly or in shapeless aggregates, but do not form rosettes. Non-motile. Encapsulated. **Cells lack an extensive intracytoplasmic membrane system** typical of most described methanotrophic bacteria, but **contain a vesicular membrane system** composed of singular flattened or ovoid vesicles connected to the cytoplasmic membrane. **Facultatively methanotrophic.** Methane is oxidized by a **soluble methane monooxygenase;** particulate form of this enzyme is absent. C1 compounds are assimilated via the serine pathway.Tricarboxylic acid cycle is complete. **Capable of growth on ethanol, some organic acids,** **ethane, propane** and some other multicarbon compounds, but sugars are not utilized. **Acetate is preferred over methane**, and leads to a downregulation of methane oxidation. **Fix atmospheric nitrogen** via an oxygen-sensitive nitrogenase. **Moderately acidophilic,** mesophilic and psychrotolerant. Prefer dilute media of low salt content. The major fatty acid is 18:1*ω*7*c*. The major quinone is Q-10. Members of the class *Alphaproteobacteria*, family *Beijerinckiaceae*. Known habitats are acidic peatlands and soils.

*DNA G + C content (mol %)***:** 61.2 – 63.3 (Tm).

*Type species:***Methylocella palustris** *Dedysh* et al. 2000VP

Number of species with validly published names: 3.

Family classification: The genus *Methylocella* is classified within the family *Beijerinckiaceae* (fbm00164).

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**10. NUMBER OF SPECIES WITH VALIDLY PUBLISHED NAMES:**

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**11. FURTHER DESCRIPTIVE INFORMATION:**

**A. CELL MORPHOLOGY AND ULTRASTRUCTURE**

**ENTER INFORMATION ON THE MORPHOLOGY AND ULTRASTRUCTURE OF THE GENUS BEING CONSIDERED. THIS SHOULD INCLUDE CELL SHAPES AND SIZES, UNIQUE MORPHOLOGICAL CHARACTERISTICS AND THE DESCRIPTION OF ULTRASTRUCTURE SUCH AS CELL WALL STRUCTIURE ETC. REFERENCE SHOULD BE MADE TO PHOTOMICROGRAPHS AND OTHER INCLUDED FIGURES eg.**

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Three currently described species of the genus *Methylocella*, i.e. *M. palustris*, *M. silvestris* and *M. tundrae*, were isolated from acidic peat bogs, acidic forest soil and acidic tundra wetland, respectively (Dedysh et al., 2000; Dunfield et al, 2003; Dedysh et al., 2004). Cells of all species are Gram-negative, non-motile, polymorphic rods. *M. palustris* and *M. silvestris* form slightly curved rods with rounded ends, 0.6-1.0 µm wide and 1.0-2.5 µm long (Fig. 1a), while cells of *M. tundrae* are only 1.0-1.5 µm long, and form short rods or ovoids (Table 1). Old cultures of *M. tundrae* contain a large number of cells that under phase-contrast microscopy appear phase-light in the middle and phase-dark on both edges. Cells of *Methylocella* species reproduce by normal cell division and occur singly or in shapeless aggregates, but do not form rosettes. The major distinctive feature of the cells is their bipolar appearance, which is easily observable under phase-contrast microscopy (Fig. 1a). This appearance is due to large, highly refractile, intracellular granules of poly-ß-hydroxybuturate, which form at each cell pole (Fig. 1b). When grown on solid media, cells of *M. palustris* and *M. silvestris* produce large polysaccharide capsules up to 1 µm thick, which can be stained with ruthenium red. Each cell, therefore, is separated from the others by the capsular material. In contrast to *M. palustris* and *M. silvestris*, cells of *M. tundrae* do not produce a macrocapsule. Although the rare appearance of exospore-like cell forms in several-month-old cultures was reported in the original description of *M. palustris* (Dedysh et al., 2000), this has not been confirmed by further studies. Cysts or cyst-like cells are also not formed by these bacteria.

The cell ultrastructure in *Methylocella* species is unusual compared to most characterized proteobacterial methanotrophs. The extensive, stacked intracytoplasmic membrane structures found in most other methanotrophs are absent from *Methylocella* cells. Instead, the cells contain a vesicular membrane system composed of small (40–100 nm in diameter) spherical, ovoid, or tube-shaped vesicles formed by cytoplasmic membrane invaginations (Fig. 1c). These vesicles are bounded by three-layered membranes, and each contains a homogenous matrix of lower electron density than the cytoplasm. To date this unique cell ultrastructure has been described only for *Methylocella* species and for the closely related methanotroph *Methyloferula stellata* (Vorobev et al., 2011).

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**B. COLONIAL AND CULTURAL CHARACTERISTICS**

**ENTER INFORMATION ON THE SHAPES, SIZES AND CHARACTERISTICS OF COLONIES ON SOLID MEDIA AS WELL AS GROWTH CHARACTERISTICS IN LIQUID CULTURE eg.**

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On agar media, visible colonies of *Methylocella* species appear after 2-3 weeks of incubation. After growth on plates for 6 weeks, the colonies of *M. palustris* are highly raised, have a tough slimy consistency, are circular with an entire margin and a smooth surface, and are 1-2 mm in diameter. Colonies 2-3 months-old may have a folded brain-like surface texture. Initially, the colonies are semi-transparent or uniformly turbid, but become opaque white over time. Six-week-old colonies of *M. silvestris* are also raised and circular but slightly larger in size, up to 2–4 mm in diameter. With continued growth, the closely located colonies (1-5 mm distance) may merge together to form an amorphous slimy cover on the agar surface. Colonies of *M. tundrae* are less raised and not slimy. They are circular, opaque/cream-colored and 1–3 mm in diameter. Liquid cultures of *Methylocella* species display white turbidity; a surface pellicle is not formed. Flocks of biomass are commonly formed in liquid cultures of *M. palustris* and *M. silvestris*, while *M. tundrae* shows homogenous growth (Table 1).

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**C. NUTRITION AND GROWTH CONDITIONS**

**ENTER INFORMATION PHYSIOLOGY OF THE ORGANISMS AS WELL AS THE CONDITIONS FOR GROWTH. THESE SHOULD INCLUDE PH, TEMPERATURE RANGES AND OPTIMA FOR GROWTHAS WELL AS NUTRIENT REQUIREMENTS LIKE NaCl ETC, eg.**

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Like all other methanotrophs, members of the genus *Methylocella* are able to grow on methane as the sole carbon and energy source. However, the key enzyme of most currently known methanotrophic bacteria, i.e. particulate methane monooxygenase (pMMO), is lacking in *Methylocella* species. Instead, these methanotrophs possess only a soluble methane monooxygenase (sMMO). Among the known aerobic methanotrophs, the absence of pMMO-encoding genes and the presence of only an operon encoding sMMO (*mmoXYBZDC*) are features shared only by *Methylocella* species and *Methyloferula* *stellata* (Chen et al., 2010; Dedysh et al., 2015; see gbm01403). Comparative sequence analysis of MmoX (the alpha subunit of the hydroxylase component of sMMO) shows that a distinct lineage of this gene groups *Methylocella* spp. and *Methyloferula stellata* apart from type I methanotrophs and the *Methylocystis/Methylosinus* (see fbm00169) group of type II methanotrophs (Figure 2).

Originally, *Methylocella* species were described as aerobic bacteria capable of growth on only the C1 compounds methane, methanol, methylamine and formate. Later, however, it was shown that they also grow on acetate, pyruvate, succinate, malate and ethanol (Dedysh et al., 2005), while sugars are not utilized.Acetate is preferred over methane.The growth rate and carbon conversion efficiency are higher on acetate than on methane, and when both substrates are provided in excess acetate is preferentially used and methane oxidation is shut down. Methanol is utilized in a wide range of concentrations, but the three different species possess different optima (Table 1). The range of growth substrates was further extended for *M. silvestris*, which was demonstrated to utilize propane, ethane, propanol, propanediol, acetone, methyl acetate, acetol, glycerol, propionate, tetrahydrofuran, and gluconate (Crombie, Murrell, 2014). Whether or not these substrates are are also suitable for *M. palustris* and *M.tundrae* has not been verified yet. Since the description of *Methylocella silvestris* as the first facultative methanotroph, a few other methanotroph species have also been shown to consume either acetate or ethanol (Dedysh, Dunfield, 2011; Dunfield, Dedysh, 2014). However, the diversity of substrates utilized by *Methylocella silvestris* by far exceeds that of any other known methanotrophic bacterium (Dunfield, Dedysh, 2014).

All members of the genus *Methylocella* utilize ammonium salts, nitrates, yeast extract and some amino acids as nitrogen sources. They use the glutamate cycle for NH4+ assimilation. When grown in nitrogen-free medium, they are able to fix N2 via an oxygen-sensitive nitrogenase.

The temperature range for growth of *Methylocella* spp. is 4–30°C. Different species possess slightly different optima (Table 1). They grow between pH 4.2 and 7.5, with an optimum at pH 5.0–6.0. They are highly sensitive to salt stress and, therefore, prefer diluted media with a low salt content (0.2–0.5 g L-1). Thus, members of the genus *Methylocella* can be characterized as psychrotolerant mesophiles and moderate acidophiles with a low salt tolerance.

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**D. CHEMOTAXONOMIC CHARACTERISTICS**

**ENTER INFORMATION ON THE CHEMOTAXONOMIC CHARACTERISTICS OF THE GENUS INCLUDING FATTY ACIDS, POLAR LIPIDS, QUINONES AS APPROPRIATE eg.**

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Similarly, toother methanotrophic and methylotrophic representatives of the family *Beijerinckiaceae*, the major fatty acid in *Methylocella* species is 11-cis-octadecenoic acid (18:1*ω*7*c*). It comprises 78-82% of the total fatty acids in *M. palustris* and *M. silvestris*, and 59-62% in *M. tundrae*. Besides 18:1*ω*7*c*, cells of *M. tundrae* also contain significant amounts (7-13%) of 16:1*ω*7*c* and 19:0 *ω*8*c* cyclofatty acids. Notably, 10-cisoctadecenoic acid (18:1 *ω*8*c*), which is highly characteristic of alphaproteobacterial methanotrophs belonging to the family *Methylocystaceae* (see fbm00169), is not present in cells of *Methylocella* species.

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**E. GENOME FEATURES**

**ENTER INFORMATION ON THE AVAILABLE GENOME SEQUENCES FOR SPECIES OF THE GENUS. PROVIDE INFORMATION ON GENOME SIZE AND COMPOSITION AS WELL AS THE PRESENCE OF GENES RELATED TO THE CHARACTERISTIC AND PHYSIOLOGY OF THE ORGANISM. IF INFORMATION IS AVAIALABLE INCLUDE A COMPARATIVE TABLE OF THE GENOME CHARACTERISTICS FOR EACH SPECIES eg.**

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Among the three described species of *Methylocella*, a genome sequence has to date been determined only for *M. silvestris* BL2T (Chen et al., 2010). The genome size is 4.3 Mb. Two identical rRNA operons and 3917 predicted protein-coding genes were identified. The closure of the complete circular genome has conclusively verified the absence of any *pmoCAB* genes encoding pMMO. Instead, a complete operon encoding sMMO (*mmoXYBZDC*) is present. In addition to the *mmo* operon, a gene cluster encoding an additional soluble di-iron center monooxygenase (SDIMO) was detected in the genome of *M. silvestris* BL2T. This second SDIMO was shown to be a propane monooxygenase, which acts together with sMMO to facilitate propane oxidation by this unusual methanotroph (Crombie, Murrell, 2014).

A complete operon encoding methanol dehydrogenase (*mxaFJGIRSACKLDEH*) and all genes necessary for fixation of methane-derived carbon via the serine cycle are present in the genome. However unlike in many other alphaproteobacterial methylotrophs the ethylmalonyl CoA pathway for regenerating glyoxylate for the serine cycle is missing (Tamas et al., 2013). Instead genes encoding glyoxylate bypass enzymes (i.e., isocitrate lyase and malate synthase) are present and probably assist in the assimilation of carbon for both 1-C and 2-C substrates (Crombie, Murrell, 2011). The ability to grow on the C2 compound acetate as well as on some C3 and C4 compounds is explained by the presence of a full gene set encoding enzymes of the tricarboxylic acid (TCA) cycle, including genes encoding α-ketoglutarate dehydrogenase, which are lacking in some other methanotrophs. Acetate kinase- and phosphotransacetylase-encoding genes are also present, allowing acetate to be fed into the TCA cycle.

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**F. ECOLOGY**

**ENTER INFORMATION ON THE ECOLOGY OF THE GENUS INCLUDING HABITATS FROM WHICH STRAINS HAVE BEEN ISOLATED AS WELL AS INFORMATION ON THE DETECTION OF MEMBERS OF THE GENUS IN THE ENVIRONMENT BASED ON ENVIRONMENTAL DNA STUDIES eg.**

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Members of the three described species of the genus *Methylocella* were isolated from various mildly acidic environments, i.e. *Sphagnum*-dominated peat bogs, temperate forest soil, and tundra wetland (Dedysh et al., 2000, 2004; Dunfield et al., 2003). Further use of cultivation-independent 16S rRNA gene-based methods demonstrated that *Methylocella* species are widely distributed in acidic and neutral terrestrial environments. *Methylocella*-like 16S rRNA gene sequences have been retrieved from neutral (pH 6.8) and acidic (pH 4.2-4.8) peatlands (Morris et al., 2002; Chen et al., 2008), acidic (pH 3.5-4.0) forest soils (Radajewski et al., 2002; Lau et al., 2007), and a neutral (pH 6.2) landfill cover soil (Chen et al., 2007). Using fluorescence *in situ* hybridization with species-specific, 16S rRNA-targeted oligonucleotide probes, *Methylocella palustris* was enumerated at greater than 106 cells per g of wet peat in a *Sphagnum* peat bog (Dedysh et al., 2001).

Due to the absence of pMMO in *Methylocella* species, these bacteria cannot be detected using a *pmoA*-based PCR assay considered universal and specific for all other known methanotrophs. To overcome this limitation, a *Methylocella*-specific *mmoX*-targeted assay was developed to detect and enumerate these methanotrophs in environmental samples (Rahman et al., 2011). It was revealed that *Methylocella* species are not restricted to acidic environments and can also be detected in neutral and alkaline ecosystems, although their population size is generally higher in acidic habitats. The abundance of *Methylocella* in selected samples of sediments, soils and peats determined with this assay was in the range 0.9-3.3 × 106 cells g-1.

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**12. ENRICHMENTS AND ISOLATION PROCEDURES**

**ENTER INFORMATION ON THE METHODS AND PRECEDURES USED FOR THE ISOLATION OF STRAINS OF THIS GENUS eg.**

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A key to successful isolation of *Methylocella* spp. to date has been the use of moderately acidic (pH 5.0-5.8) mineral media with a low salt content. One suitable medium for these methanotrophs is liquid mineral medium M2 of the following composition (g per liter demineralized water): KNO3, 0.25; KH2PO4, 0.1; MgSO4 × 7H2O, 0.05; CaCl2 × 2H2O, 0.01; NaCl, 0.02; 0.1% (v/v) of trace element solution; pH 5.0-5.5. Trace element solution has the following composition (g per liter distilled water): Na2EDTA, 0.5; FeSO4 × 7H2O, 0.2; H3BO3, 0.03; ZnSO4 × 7H2O, 0.01; MnCl2 × 4H2O, 0.003; CoCl2 × 6H2O, 0.02; CuSO4 × 5H2O, 0.03; NiCl2 × 6H2O, 0.002, Na2MoO4 × 2H2O, 0.003. Alternatively, diluted nitrate mineral agar salts (DNMS) medium at pH 5.8 as described by Dunfield et al. (2003) can also be used. In the case of methane-rich environments, isolation efforts can start by adding serial dilutions of environmental samples directly to solid media on Petri plates and incubating these under a methane-enriched atmosphere. A more reliable approach, however, involves obtaining enrichment cultures of methanotrophs. The sample of interest is used to inoculate a respective liquid mineral medium and is incubated under a headspace enriched in methane (10-30%, v/v) in static conditions for 3-6 weeks. Isolation of methanotrophs from the resulting enrichment cultures is achieved by plating an aliquot of the respective cell suspensions on agar medium M2 or DNMS. Solid media can also be prepared with gellan gum (Gel-Gro; ICN Biomedicals). Inoculated plates are incubated for 1-1.5 months at 20-24 °C in a closed glass desiccator containing a headspace of 20% (v/v) methane and 5% CO2 (v/v) in air. The colonies appearing on the plates are randomly picked for examination by phase microscopy for the presence of slightly curved thick rods with bipolar appearance. This procedure is continued until individual colonies of target bacteria are ultimately identified and obtained in pure culture. Additional tips for isolation and purification of methanotrophic bacteria are described by Dedysh & Dunfield (2014).

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**13. MAINTENANCE PROCEDURES**

**ENTER INFORMATION ON THE PROCEDURES USED TO MAINTAIN STRAINS OF THIS GENUS BOTH SHORT AND LONG TERM eg.**

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Strains can be maintained either on the solid media M2 and DNMS media or in liquid cultures. For growth in liquid media, screw-capped serum bottles are used with a headspace/liquid space ratio of 4 : 1. After inoculation, methanol (0.25-0.5%, v/v) is added aseptically to the cultures and the bottles are capped with silicone rubber septa to prevent loss of methanol by evaporation, or methane is added aseptically through silicone rubber septa to achieve a mixing ratio in the gas headspace of approximately 10–20 %. Bottles are incubated on a rotary shaker (100-120 r.p.m.) at 20-24 °C. Strains are sub-cultured once every 1.5-2 months. Although acetate is the preferred growth substrate of *Methylocella* species, media with acetate are not recommended for maintaining these methanotrophs due to the risk of contamination by heterotrophic bacteria. Long term storage can be achieved by addition of 5-10% dimethyl sulfoxide and freezing at -80 oC. We have retrieved samples preserved for >10 years under these conditions.

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**14. PROCEDURES FOR TESTING SPECIAL CHARACTERISTICS**

**SOME GENERA MAY BE VERY SPECIALIZED AND SO HAVE UNQUE CHARACTERISTICS THAT REQUIRE SPECIAL APPROACHES. ENTER INFORMATION ON THESE SPECIAL PROCEDURES eg.**

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The ability to grow on methane clearly differentiates *Methylocella* from non-methanotrophic members of the family *Beijerinckiaceae*, i.e. the genera *Beijerinckia* (see gbm00795), *Methylovirgula* (see gbm01405) and *Methylorosula* (see gbm01404). Characteristics that distinguish *Methylocella* from other methanotrophs of the family *Beijerinckiaceae* are listed in Table 2. Cell morphology, the absence of intracytoplasmic membranes, absence of pMMO, and the preference for growth on acetate makes *Methylocella* different from members of the genus *Methylocapsa* (see gbm01402). The inability to form rosettes and to grow at pH below 4, the absence of RubisCO activity, and the ability to utilize multicarbon compounds distinguishes *Methylocella* from *Methyloferula* (see gbm01403) species.

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**15. DIFFERENTIATION OF THE GENUS XXXXX FROM OTHER GENERA**

**ENTER INFORMATION ON THE CHARACTERISTICS THAT DIFFERENTIATE THIS GENUS FROM RELATED GENERA. A TABLE SHOULD PROVIDED THAT SHOWS THE KEY DIFFERENTIATING CHARACTERISTICS eg.**

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**16. TAXONOMIC COMMENTS**

**ENTER INFORMATION ON THE TAXONOMIC POSITION OF THE GENUS AND THE SPECIES IT CONTAINS INCLUDING THE 16S rRNA GENE BASED PHYLOGENY, THE WHOLE GENOME BASED PHYLOGENY FROM GTDB AND ANY DIFFERENCES OBSERVED BETWEEN THESE PHYLOGENIES AND RESULTING CLASSIFICATIONS. PHYLOGENTIC TREES AND ANI/AAI VALUES SHOULD BE INCLUDED AS APPROPRIATE AND AVAILABLE. A 16S rRNA OR GENOME BASED PHYLOGENETIC TREE SHOULD BE PRESENTED eg.**

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The closest neighbors of *Methylocella* based on 16S rRNA gene sequence phylogeny are sMMO-possessing obligate methanotrophs of the genus *Methyloferula* (see gbm01403), heterotrophs of the genus *Beijerinckia*, pMMO-possessing methanotrophs of the genus *Methylocapsa* (see gbm01402) and facultative methylotrophs of the genera *Methylovirgula* (see gbm01405) and *Methylorosula* (see gbm01404). All these metabolically distinct bacteria display 96-97% 16S rRNA gene sequence similarity to each other (Figure 3). Taxonomic construction based on 21 concatenated methylotrophy genes verifies the same close phylogenetic relationship of *Methylocella* to *Methylocapsa* and *Beijerinckia* (Tamas et al., 2013).

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**17. LIST OF SPECIES OF THE GENUS**

**ENTER INFORMATION ON EACH OF THE SPECIES OF THE GENUS WITH NAMES THAT HAVE BEEN VALIDLY PUBLISHED. INCLUDE THE DEFINING PUBLICATION AND THE ETYMOLOGY. MAINTAIN A SIMILAR FORMAT AND INFORMATION CONTENT ORDER FOR EACH SPECIES ENTRY. INCLUDE THE DNA G+C CONTENT, TYPE STRAIN AND CULTURE COLLECTION, THE ACCESSION NUMBERS FOR THE 16S rRNA GENE AND WHOLE GENOME SEQUENCES. SPECIES DESCRIPTION SHOULD NOT BE COPIED VERBATUM FROM THE ORIGINAL PUBLICATIONS. eg.**

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***Methylocella palustris***

1. Dedysh et al. 2000VP

pa.lus´tris. M.L. adj. *palustris* bog-inhabiting

Description as for the genus plus the following traits. Cells are polymorphic, slightly curved bipolar rods, 0.6-1.0 µm wide by 1.0-2.5 µm long. Produce large polysaccharide capsules. Colonies of are highly raised, semi-transparent or uniformly turbid, have a tough slime consistency, are circular with an entire margin and a smooth surface. Optimal growth at 20°C and at pH 5.5. Carbon sources used include methane, methanol, acetate, ethanol, pyruvate, succinate, malate. Methanol is utilized in low concentrations, up to 0.3% (v/v). Nitrogen sources are ammonium salts, nitrates and yeast extract. NaCl inhibits growth at a concentration of 0.5% (w/v). The type strain was isolated from *Sphagnum* peat from the Kyrgyznoye ombrotrophic bog in West Siberia (56° N, 85° E). The species also includes strains S6 and M131.

*DNA G* + *C content (mol %)*: 61.2 (Tm).

*Type strain*: K, ATCC 700799.

*EMBL/GenBank accession (16S rRNA gene)*: Y17144.

***Methylocella silvestris***

Dunfield et al. 2003VP

sil.ves´tris. L. adj. *silvestris* of the forest

Cells are slightly curved bipolar rods, 0.6–0.8 µm in width and 1.2–1.5 µm in length. Produce large polysaccharide capsules. Colonies are raised and circular, semi-transparent or uniformly turbid. Optimal growth occurs at 15–25°C and at pH 5.5. Capable of slow growth at 4°C. Carbon sources used include methane, methanol, methylamines, acetate, ethanol, pyruvate, succinate, malate, propanol, propanediol, acetone, methyl acetate, acetol, glycerol, propionate, tetrahydrofuran, gluconate, ethane, propane. Methanol is utilized in a wide concentration range, from 0.01 to 5% (v/v). NaCl inhibits growth at concentrations above 0.8% (w/v). The type strain was isolated from an acidic cambisol under a beech-dominated forest near Marburg, Germany. The species also includes strain A1.

*DNA G* + *C content (mol %)*: 63.0% (genome analysis).

*Type strain*: BL2, DSM 15510, NCIMB 13906.

*EMBL/GenBank accession (16S rRNA gene)*: AJ491847.

*EMBL/GenBank accession (genome)*: CP001280.

***Methylocella tundrae***

Dedysh et al. 2004VP

tun´drae. N.L. gen. fem. n. *tundra* from the tundra, the northern zone of Eurasia and North America

Cells grown on methane are curved ovoids. Old cultures contain many cells that appear phase light in the middle and phase dark on both edges. Cells do not possess a macrocapsule and colonies are not slimy like those of *M. palustris* or *M. silvestris*. Liquid cultures display homogeneous turbidity. Optimal growth occurs at 15°C and at pH 5.5–6.0. Capable of slow growth at 5°C and pH 4.2. Carbon sources include methane, methanol, methylamine, formate, acetate, ethanol, pyruvate, succinate, malate. Methanol is utilized in a wide range of concentrations from 0.01 to 2.0% (v/v). NaCl inhibits growth at concentrations above 0.8% (w/v). The distinctive feature of the PLFA profile is the presence of 19:0*ω*8*c* cyclo fatty acids. The type strain was isolated from an acidic *Sphagnum* peatland in Vorkuta region, northern Russia. The species also includes strains TCh1 and TY1.

*DNA G* + *C content (mol %)*: 63.3 (Tm).

*Type strain*: T4, DSM 15673, NCIMB 13949.

*EMBL/GenBank accession (16S rRNA gene)*: AJ555244.

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**18. OTHER SPECIES**

**ENTER INFORMATION ON OTHER SPECIES THAT HAVE BEEN DESCRIBED IN THE LITERATURE BUT DO NOT HAVE VALIDLY PUBLISHED NAMES. MAINTAIN A SIMILAR FORMAT AND INFORMATION CONTENT ORDER FOR EACH SPECIES ENTRY. INCLUDE THE DNA G+C CONTENT, TYPE STRAIN AND CULTURE COLLECTION, THE ACCESSION NUMBERS FOR THE 16S rRNA GENE AND WHOLE GENOME SEQUENCES eg.**

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***“Methylocella imadethisoneupus”***

Whitman and Dedysh 2016

i mad e thi son’e up us N.L. gen. masc. n. *imadethisoneupus* a hypothetical name to show how to include species whose names have not been validly published in your genus chapter.

The “other species” section can be used for species whose names have not been validly published, species whose names have been validated but then transferred to other genera, and species that have been misassigned to a genus. If the species name is not validated, the name is in quotes and includes the effective publication. Notice the absence of the superscripts “VP” or “AL”. For species that have been transferred or misassigned, include a brief rationale for not including the species in the “List of species”. A more extensive discussion can be given in the genus description under the section “Taxonomic Comments”. Otherwise, the description should have similar content as used for the “List of Species”.

*DNA G* + *C content (mol %)*: 00.0 (LC).

*Type strain*: None, DSM 0, NCIMB 0.

*EMBL/GenBank accession (16S rRNA gene)*: Z0000R0.

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**19. REFERENCES**

**ENTER FULL REFERENCE FOR EACH CITATION IN THE CHAPTER INCLUDING THOSE CITED IN TABLES AND FIGURE LEGENDS. REFERENCES SHOULD BE LISTED IN ALPHABETICAL ORDER BASED ON THE NAME OF THE FIRST AUTHORS. THE FORMAT PROVIDED BELOW SHOULD BE FOLLOWED EXACTLY. DOIs SHOULD BE INCLUDED WHEN AVAILABLE eg.**

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**20. TABLES AND FIGURES**

**PROVIDE TABLES AND FIGURES IN THE FORMAT PROVIDED BELOW**

Table 1. Characteristics that differentiate described species of the genus *Methylocella.*

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristic | *M. palustris* | *M. silvestris* | *M. tundrae* |
| Cell morphology | Polymorphic, slightly curved bipolar rods | Slightly curved bipolar rods | Short, slightly curved rods or ovoids |
| Cell size, μм | 0.6-1.0 × 1.0-2.5 | 0.6-0.8 × 1.2-2.0 | 0.6-0.8 × 1.0-1.5 |
| Macrocapsule formation | + | + | - |
| Colony morphology | Highly raised, tough slime, circular and semi-transparent | Raised, white or semi-transparent, circular colonies with an entire edge and a smooth surface | Less raised, not slimy, circular, opaque/cream-colored |
| Growth in liquid media | Slimy and aggregated | Slimy and aggregated | Homogenous |
| Optimal growth temperature, ˚С  | 20 | 20-25 | 15 |
| Optimal growth pH  | 5.0 - 5.5 | 5.5 | 5.5-6.0 |
| Growth on CH3OH, % (v/v) | ≤ 0.3 | ≤ 5.0 | ≤ 2.0 |
| Major fatty acids | 18:1*ω*7*c* | 18:1*ω*7*c* | 18:1*ω*7*c,* 16:1*ω*7*c,* 19:0 *ω*8*c* cyclo |
| DNA G+C content, Tm | 61.2 | 63.0\* | 63.3 |

\*- data based on genome sequence analysis

**Table 2.** Major characteristics that distinguish *Methylocella* from other methanotrophic members of the family *Beijerinckiaceae*

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | ***Methylocella*** | ***Methyloferula*** | ***Methylocapsa*** |
| Cell morphology | Bipolar straight or curved rods | Straight or curved rods | Curved coccoids |
| Cell size, μm | 0.6 – 1.0 × 1.0 – 2.5 | 0.4 – 0.65 × 1.1 – 3.0 | 0.7 – 1.2 × 0.8 – 3.1  |
| Rosette formation | – | + | – |
| Type of metabolism | Facultative methanotrophy | Obligate methanotrophy | Obligate or limited facultative methanotrophy |
| Possession of:pMMOsMMO | -+ | -+ | +- |
| Preferred growth substrate(s) | Methanol, acetate | Methanol | Methane |
| Multicarbon compounds utilized | acetate, ethanol, pyruvate, succinate, malate, propanol, propanediol, acetone, methyl acetate, acetol, glycerol, propionate, tetrahydrofuran, gluconate, ethane, propane | None | None or acetate |
| RubisCO activity | \_ | + | \_ |
| Growth at/in: pH 3.5 0.5% NaCl | – – |  + + | – – |
| G+C content (mol%) | 60-63.3 | 59.5 | 61.4-61.9 |

**21. FIGURE CAPTIONS**

**Figure 1. (a)** Phase-contrast micrograph of cells of *Methylocella silvestris* BL2T; bar, 10 µm. **(b, c)** Electron micrographs of ultrathin sections of vegetative cells of *Methylocella palustris* KT; bars, 0.5 µm. PHB, granules of poly-ß-hydroxybutyrate; PP, granules of polyphosphate; MV, membrane vesicles.

**Figure 2**. Unrooted neighbor-joining tree constructed based on 368 deduced amino acid sites of partial *mmoX* gene sequences, showing the positions of *Methylocella* species relative to other sMMO-possessing type I and type II methanotrophs. Bootstrap values (1000 data resamplings) >80% are shown. Bar, 0.05 substitutions per amino acid position.

**Figure 3**. 16S rRNA gene-based maximum-likelihood tree showing the phylogenetic position of *Methylocella* species in relation to other representatives of the *Beijerinckiaceae*. 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.

**NOTE THAT OMISSION OF THE OUTGROUP FROM THE FIGURE ENABLES A BETTER PRESENTATION IN MOST CASES BECAUSE IT ALLOWS EXPANSION OF THE RIGHTHAND SIDE OF THE FIGURE, WHICH IS MOST INFORMATIVE.**

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Figure 1.



Figure 2



Figure 3.