**Mobilitalea sibirica gen. nov., sp. nov.,** a halotolerant polysaccharide-degrading bacterium

O. A. Podosokorskaya,1 E. A. Bonch-Osmolovskaya,1 A. V. Beskorovaynyy,2 S. V. Toshchakov,3 T. V. Kolganova4 and I. V. Kublanov1

Correspondence  
O. A. Podosokorskaya  
podosokorskaya@gmail.com

1Winogradsky Institute of Microbiology, Russian Academy of Sciences, Propekt 60-Letiya Oktjabrya 7/2, 117312 Moscow, Russia  
2Gubkin Russian State University of Oil and Gas, Leninskiy Prospect 65, 119991 Moscow, Russia  
3Department of Genomic and Proteomic Research, Baltic Federal University, Botkina str. 3, 236016, Kaliningrad, Russia  
4Bioengineering Center, Russian Academy of Sciences, Propekt 60-Letiya Oktjabrya 7/1, 117312 Moscow, Russia

A novel strictly anaerobic, halotolerant, organotrophic bacterium, strain P3M-3T, was isolated from a microbial mat formed under the flow of hot water emerging from a 2775 m-deep well in Tomsk region (western Siberia, Russia). Cells of strain P3M-3T were straight and curved rods, 0.2–0.4 μm in width and 1.5–20 μm in length. Strain P3M-3T grew optimally at 37 °C, pH 7.0–7.5 and in a NaCl concentration of 15 g l\(^{-1}\). Under optimum growth conditions, the doubling time was 1 h. The isolate was able to ferment a variety of mono-, di- and polysaccharides, including microcrystalline cellulose. Acetate, ethanol, H\(_2\) and CO\(_2\) were the main products of glucose fermentation. The DNA G+C content was 33.4 mol%. 16S rRNA gene-based phylogenetic analysis showed that strain P3M-3T was a member of family Lachnospiraceae, whose representatives are also found in Clostridium cluster XIVa. 16S rRNA gene sequence similarity with Clostridium jejuense HY-35-12T, the closest relative, was 93.9%. A novel genus and species, Mobilitalea sibirica gen. nov., sp. nov., are proposed based on phylogenetic analysis and physiological properties of the novel isolate. The type strain of the type species is P3M-3T (=DSM 26468T=VKM B-2804T).

The order Clostridiales, characterized by its phenotypical, chemotaxonomical, physiological and ecological diversity (Rainey, 2009a), includes 13 families and more than 600 species with validly published names at the time of writing. A majority of these species, including members of the most numerous genus Clostridium, are characterized as obligately anaerobic, chemo-organotrophic, fermentative, spore-forming rods. Since Collins et al. (1994) demonstrated the extensive phylogenetic diversity of the species assigned to the genus Clostridium, among XIX clusters, members of cluster I, widely regarded as the Clostridium sensu stricto, and a number of species of other clusters have been reclassified to existing genera or to novel ones. Nevertheless, currently there is a significant number of species of the genus Clostridium which have not, as yet, been reassigned to new genera, and remain distributed throughout families of the order Clostridiales. Moreover, many species continue to be added to the genus Clostridium even though they are not members of cluster I, leading to taxonomic confusion associated with this taxon (Rainey et al., 2009).

The family Lachnospiraceae of the order Clostridiales was described based on phylogenetic analyses of 16S rRNA gene sequences (Rainey, 2009b). At the time of writing, this family contains 41 species with validly published names in 24 genera. Among these are several species formally affiliated with Clostridium cluster XIVa (Collins et al., 1994), whose taxonomy needs to be verified. Members of the family Lachnospiraceae are morphologically diverse and include Gram-stain-variable rods, vibrios and cocci. Nevertheless, all members are Gram-positive by cell wall structure and share similar physiology. They are mesophilic, strictly anaerobic, catalase-negative, chemo-organotrophic bacteria, able to hydrolyse polymeric substrates.

Here we describe strain P3M-3\(^{T}\), a halotolerant bacterium able to degrade different types of polysaccharides, including microcrystalline cellulose. Based on results of phylogenetic and phenotypic analyses we propose strain P3M-3\(^{T}\)
represents a novel species in a new genus of the family Lachnospiraceae.

In August 2009, a microbial mat sample was collected from the wooden surface of a chute, flooded with hot water (pH 7.52, 46 °C) emerging from a 2775-m-deep well in Parabel’ district, Tomsk region, Russia (58° 50’ 05.3” N 81° 30’ 07.5” E). Enrichment culture was obtained using strict anaerobic modified freshwater Widdel medium with microcrystalline cellulose as a substrate (Podosokorskaya et al., 2013a). After incubation at 47 °C the culture contained three different types of cells, which were further isolated using different cultivation conditions. Strain P3M-1T has been described as Ornatilinae apprima gen. nov., sp. nov. (Podosokorskaya et al., 2013a) and strain P3M-2T as Melioribacter roseus gen. nov., sp. nov. of Ignavibacteriae phyl. nov. (Podosokorskaya et al., 2013b). Strain P3M-3T was purified by serial dilution on the same medium with CM-cellulose as a substrate at 37 °C according to the method of Podosokorskaya et al. (2011). All growth experiments, described below, were done using the same mineral medium, using threefold culture transfers in two replicates (1 %, v/v, inoculum). Temperature, pH and NaCl ranges and optima of growth were determined with Avicel (2 g l⁻¹) as a substrate. Utilization of organic substrates as energy and carbon sources and use of potential electron acceptors were studied as described previously (Podosokorskaya et al., 2013b). In all cases, growth was assessed by direct cell counting under an Olympus CX41 microscope. In the case of ferrihydrite-grown cells, iron-containing precipitates were initially dissolved with a mixture of ammonium oxalate and oxalic acid (Lovley & Phillips, 1988).

Cells of strain P3M-3T were straight or bow-shaped rods, 0.2–0.4 μm in width and 1.5–20 μm in length (Fig. 1), motile and occurred singly, in pairs and in short chains. Under unfavourable conditions they markedly stretched and twisted into intricate swirls and formed terminal single spores. Electron microscopy of negatively stained cells (Bonch-Osmolovskaya et al., 1990) revealed the presence of subpolar or laterally-inserted flagellum (Fig. 1).

Strain P3M-3T was strictly anaerobic and did not grow on the medium without sodium sulfide (0.1 g l⁻¹). The catalase reaction was negative (Smibert & Krieg, 1994). Strain P3M-3T grew at a temperature range from 25 to 47 °C with optimum growth at 37 °C, and at pH 6.6–9.4 with optimum growth at pH 7.0–7.5. No growth was observed at or below 19 °C or pH 6.4, and at or above 52 °C or pH 9.7. The presence of NaCl in the medium was not mandatory; however optimal growth occurred in the presence of a NaCl concentration of 15 g l⁻¹. The maximal concentration of NaCl at which growth of strain P3M-3T was observed was 85 g l⁻¹. In the medium containing 90 g l⁻¹ of NaCl growth completely ceased. Therefore, P3M-3T was deemed to be slightly halotolerant (Larsen, 1986). Under optimal growth conditions and with xylose (2 g l⁻¹) as the growth substrate, the doubling time of strain P3M-3T was 1 h.

Utilization of growth substrates was studied using threefold transfers on the same medium in the presence of 0.1 g l⁻¹ yeast extract under anaerobic conditions. Strain P3M-3T was able to grow on the following substrates (2 g l⁻¹): yeast extract, peptone, glucose, xylose, trehalose, maltose, cellobiose, dextrin, xanthan gum, lichenan, xylan, filter paper, CM-cellulose and microcrystalline cellulose. Galactose, rhamnose, lactose, fructose, arabinose, sucrose, dextran, starch, gelatin, beta-keratin (feathers), mannitol and sorbitol (2 g l⁻¹) were not utilized. The products of glucose fermentation, determined according to Miroshnichenko et al. (2008), were acetate, ethanol, H₂ and CO₂, with traces of formate also being detected.

The presence of elemental sulfur (5 g l⁻¹), sulfate, thiosulfate, sulfite and nitrate at 10 mM of sodium salts or crystalline Fe (III) oxide in the cellobiose- or Avicel-containing medium did not influence the growth of the novel isolate. Sulfide formation (Trüper & Schlegel, 1964) was not detected when sulfur compounds were present in the culture medium.

Determination of cellular fatty acids was performed as described previously (Slobodkina et al., 2013). The major cellular fatty acid of strain P3M-3T grown on yeast extract was C₁₆:0 (30.5 %), other components were C₁₈ saturated and unsaturated fatty acids, C₁₄:₀, C₁₅:₀ and C₁₆:₁ fatty acids and several dimethylacetals (Table S1, available in the online Supplementary Material). For polar lipids analysis, the technique described previously (Podosokorskaya et al., 2013a) was followed with additional usage of polar lipids of ‘Anabaena variabilis’ CCAP 1403/4B as a reference sample (biomass of strain P3M-3T and ‘Anabaena variabilis’ CCAP 1403/4B was processed identically). The polar lipids of strain P3M-3T were represented by two unidentified lipids which were detected by phosphomolybdic acid only and nine unidentified phospholipids (Fig. S1).

DNA of strain P3M-3T was isolated according to Park (2007). The DNA G+C content of strain P3M-3T measured by the thermal denaturation method (Marmur & Doty, 1962) was calculated to be 33.4 mol%. The

Fig. 1. Electron micrograph of negatively stained cells (Bonch-Osmolovskaya et al., 1990) of strain P3M-3T showing curved single cell with laterally-inserted flagellum. Bar, 1 μm.
almost-complete 16S rRNA gene sequence of strain P3M-3T (1444 nt) was determined as described previously (Perevalova et al., 2013). Comparison of this sequence with the 16S rRNA gene sequences of species with validly published names using the EzTaxon-e server (Kim et al., 2012) revealed that the most similar sequences were those of the members of family Lachnospiraceae of the phylum Firmicutes. The 16S rRNA gene sequence similarity of strain P3M-3T and the closest relative, Clostridium jejuense HY-35-12^T, was 93.9%. In the 16S rRNA gene sequence-based phylogenetic tree, reconstructed using MEGA 5.03 software (Tamura et al., 2011), the novel isolate was a neighbour of Clostridium aminovalericum JCM 11016^T (Fig. 2). Together with a clade consisting of C. jejuense HY-35-12^T and Clostridium xylanovorans HESP1^T, this cluster formed a monophyletic genus-level group within the family Lachnospiraceae (Fig. S2). However, the absence of fatty acid profile data and phospholipid contents for the majority of the closest relatives of strain P3M-3T does not allow us to judge that these taxa all belong to the same genus.

Thus, strain P3M-3^T, being a fourth cultivated member of this group, shares several common features with other species: it is a rod-shaped, spore-forming, motile bacterium with a Gram-positive type of cell wall, is strictly anaerobic and neutrophilic, able to grow chemo-organotrophically producing acetate and does not use electron acceptors (Table 1). However, there are several genus-level discriminative features of strain P3M-3^T: it is halotolerant and does not grow on fructose, galactose or sucrose. The differences between strain P3M-3^T and its closest relative, C. aminovalericum ATCC 13725^T, include the inability to grow on starch, galactose, sucrose, arabinose and mannitol. In addition, the novel isolate forms ethanol during fermentation. Thus, the results of phylogenetic analysis along with morphological and physiological properties of the novel isolate allow us to conclude that strain P3M-3^T represents a novel species in a new genus of the family Lachnospiraceae for which the name Mobilitalea sibirica gen. nov., sp. nov. is proposed.

Description of Mobilitalea gen. nov.

Mobilitalea (Mo.bi.li.ta’le.a. L. adj. mobilis mobile; L. fem. n. talea a slender staff, a rod; N.L. fem. n. Mobilitalea a motile rod).

Cells are motile rods with a Gram-stain-positive cell wall. Terminal spores are formed. Obligately anaerobic, catalase-negative. Mesophilic, neutrophilic and halotolerant. Chemo-organoheterotrophic. Electron acceptors are not used. The main fatty acids are C_{16:0}, C_{14:0} and C_{18:0} 3-OH; dimethylacetals are also found (C_{14:0}, C_{16:0}, C_{18:1}). The genus lies in the family Lachnospiraceae. The type species is Mobilitalea sibirica.

Description of Mobilitalea sibirica sp. nov.

Mobilitalea sibirica (si.bi’ri.ca. N.L. fem. adj. sibirica originating from Siberia, referring to the site of isolation).

Straight and bow-shaped rods, 0.2–0.4 μm in width and 1.5–20 μm in length. Growth occurs between 25 and 47 °C (optimum 37 °C) and at pH 6.6–9.4 (optimum pH 7.0–7.5). Optimal and maximal concentrations of NaCl are 1.5% and 8.0%, respectively. Doubling time under optimal conditions is 1 h. Yeast extract is required for growth. In the presence of 0.1 g l^{-1} yeast extract strain P3M-3^T grows on the following substrates (2 g l^{-1}):

- Yeast extract, peptone, glucose, xylose, trehalose, maltose, cellobiose, dextrin, xanthan gum, lichenan, xylan, filter paper, CM-cellulose and microcrystalline cellulose.
- Galactose, rhamnose, lactose, fructose, arabinose, sucrose,

![Fig. 2. 16S rRNA gene-based phylogenetic tree indicating the position of strain P3M-3^T and nearest relatives within the family Lachnospiraceae. The evolutionary history was reconstructed using the neighbour-joining algorithm (Saitou & Nei 1987) integrated in the MEGA5 software package (Tamura et al., 2011). Evolutionary distances were computed using the maximum composite likelihood method (Tamura et al., 2004). Bootstrap values (Felsenstein, 1985), the percentage of 1000 replicates, are shown next to branches. Bar, 1 nt substitutions per 100 nt. The same branching was achieved using the maximum-likelihood method (Tamura & Nei, 1993).](http://ijs.sgmjournals.org/pdf/2659.jpg)

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dextran, starch, gelatin, beta-keratin (feathers), mannitol and sorbitol do not support growth. Sulfate, sulfite, thiosulfate, elemental sulfur, nitrate and crystalline Fe (III) oxide are not used as electron acceptors.

The type strain is P3M-3T (DSM 26468T, VKM B-2804T), isolated from a microbial mat formed in a wooden bath filled with hot water from a 2775 m-deep well in Tomsk region (Siberia, Russia). The DNA G+C content of the type strain is 33.4 mol%.

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