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

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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, H₂ and CO₂ 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, vibrions 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-3T, 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-3T
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 strictly 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 *Ornatilinea 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, pH 7.52, 46 μ. In August 2009, a microbial mat sample was collected from Parabel' district, Tomsk region, Russia (58° 50′ 05.3″ N 81° 30′ 07.5″ E). Enrichment culture was obtained using strictly 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 *Ornatilinea 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, the presence of elemental sulfur (5 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), mannnitol 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 C14:0 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
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 jejune HY-35-12T, 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 11016T (Fig. 2). Together with a clade consisting of C. jejune HY-35-12T and Clostridium xylanovorans HESP1T, 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-3T, 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-3T: it is halotolerant and does not grow on fructose, galactose or sucrose. The differences between strain P3M-3T and its closest relative, C. aminovalericum ATCC 13725T, 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-3T 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 C16:0, C14:0 and C18:0 3-OH; dimethylacetals are also found (C14:0, C16:0, C18:1). 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-3T 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,
Table 1. Characteristics of strain P3M-3T and type strains of the closest relatives of the genus *Clostridium*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Microbial mat, Parabel', Russia</td>
<td>Sewage sludge</td>
<td>Soil sample, Jeju, Korea</td>
<td>Methanogenic digester</td>
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<tr>
<td>Flagellation</td>
<td>From subpolar to laterally-inserted flagellum</td>
<td>ND</td>
<td>Peritrichous</td>
<td>From subpolar to laterally-inserted flagella</td>
</tr>
<tr>
<td>Min./opt./max. growth temperature (°C)</td>
<td>25/37/47</td>
<td>ND/31–37/ND</td>
<td>10/30/40</td>
<td>25/37/42</td>
</tr>
<tr>
<td>Min./opt./max. pH for growth</td>
<td>6.6/7.0–7.5/9.4</td>
<td>7.0/7.4–7.7/ND</td>
<td>5.5/7.0/9.5</td>
<td>6.0/7.0/8.0</td>
</tr>
<tr>
<td>Min./opt./max. NaCl (% w/v)</td>
<td>0/1.5/8.5</td>
<td>ND</td>
<td>0/0–0.5/1.5</td>
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<tr>
<td>Doubling time (h)</td>
<td>1</td>
<td>ND</td>
<td>10.5</td>
<td>ND</td>
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<tr>
<td>Growth on substrates</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Yeast extract</td>
<td>+</td>
<td>+/- (+)*</td>
<td>–</td>
<td>–</td>
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<td>Lactose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>Starch</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Galactose</td>
<td>–</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>–</td>
<td>+*</td>
<td>+</td>
<td></td>
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<tr>
<td>Sucrose</td>
<td>–</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Arabinose</td>
<td>–</td>
<td>+/ (+)*</td>
<td>+</td>
<td>–</td>
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<td>Xylose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Avicel</td>
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<td>ND</td>
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<tr>
<td>Cellulose</td>
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<td>+*</td>
<td>(+)</td>
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<td>Xylan</td>
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<td>Growth products†</td>
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<td>F (Pr, V)</td>
<td>L, P, F</td>
<td>B, Et, F</td>
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<td>Gas products†</td>
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<td>H₂ (NH₃)</td>
<td>H₂</td>
<td>H₂, CO₂</td>
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<td>DNA G+C content (mol%)</td>
<td>33</td>
<td>33</td>
<td>41</td>
<td>40</td>
</tr>
</tbody>
</table>

*Data from Jeong et al. (2004).
†Growth products and gas products during glucose or PYG broth fermentation; growth products and gas products during aminovalerate fermentation are given in parentheses. L, lactate; Pr, propionate; P, pyruvate; V, valerate; B, butyrate; Et, ethanol; F, formate.

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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