Desertibacter xinjiangensis sp. nov., isolated from the soil of a Euphrates poplar forest, and emended description of the genus Desertibacter

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A pale pink and strictly aerobic bacterium, designated strain M71T, was isolated from the soil of a Euphrates poplar forest in Xingjiang, PR China. Cells of the strain were Gram-reaction-negative, rod-shaped and motile by means of a single polar flagellum. Growth occurred at 10–37 °C (optimum 30 °C), at pH 6.0–9.0 (optimum pH 7.0–8.0) and with 0–2.0 % NaCl (w/v, optimum 0 %). Phylogenetic analysis, based on 16S rRNA gene sequences, indicated that strain M71T belongs to the genus Desertibacter in the family Rhodospirillaceae. The 16S rRNA gene sequence of this strain showed 96.2 % sequence similarity with the type strain of Desertibacter roseus 2262T. The respiratory quinone was Q-10 and the predominant cellular fatty acids were C₁₈ : ₁₋₇c (53.2 %), C₁₆ : ₁₋₆c (11.0 %), summed feature 3 (C₁₆ : ₁₋₇c and/or C₁₆ : ₁₋₆c, 10.2 %) and C₁₆ : ₀ (8.5 %). The DNA G+C content was 71.2 mol% (HPLC). The strain contained phosphatidylcholine and phosphatidylethanolamine as the predominant polar lipids. On the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain M71T is considered to represent a novel species of the genus Desertibacter, for which the name Desertibacter xinjiangensis sp. nov. is proposed. The type strain is M71T (=CCTCC AB 209291T= =CIP 110127T).

The genus Desertibacter, first proposed by Liu et al. (2011), belongs to the family Rhodospirillaceae of the order Rhodospirillales, class Alphaproteobacteria, phylum Proteobacteria. At the time of writing, the genus comprises only one species with a validly published name, Desertibacter roseus, which was isolated from a desert soil (Liu et al., 2011). During the course of an investigation of the culturable aerobic bacterial community in soil from a Euphrates poplar forest in Xinjiang province (41° N 88° E), a Gram-reaction-negative, rod-shaped bacterial strain (M71T) was isolated. On the basis of 16S rRNA gene sequence analysis, this isolate was considered to be a Desertibacter-like strain. To determine its exact taxonomic position, M71T was subjected to a detailed investigation using a polyphasic taxonomic approach, including phylogenetic, chemotaxonomic and classical phenotypic analyses. These results indicate that M71T should be placed in the genus Desertibacter as a representative of a novel species.

For strain isolation, the soil sample was diluted serially with a sterile 0.85 % (w/v) NaCl solution, and these dilutions were plated onto R2A agar (BD) plates. Single colonies on these plates were purified by transferring them onto new plates and subjecting them to additional incubation for 5 days at 30 °C. Strain M71T was routinely cultivated in the same medium at 30 °C and stored after lyophilization.

Growth was evaluated at 30 °C on several standard bacteriological media: R2A agar (BD), 0.3 × R2A agar (BD), marine broth 2216 (MB; BD) agar, tryptic soy broth (TSB; BD) agar, 0.1 × TSB (BD) agar, nutrient agar (NA; BD), 0.1 × NA (BD) and MacConkey agar (BD). Cell morphology was examined by phase-contrast (Olympus

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain M71T is KC625488.

Four supplementary figures and one supplementary table are available with the online version of this paper.
Strain M71T was strictly aerobic, Gram-reaction-negative, 20NE and API ZYM kits (bioMe`rieux), according to the characteristics were determined by using the API 20E, API observed. Additional physiological and biochemical character-
sensitivities to that particular antibiotic. Resistance to an

Temperature (4, 10, 18, 20, 25, 28, 30, 32, 37, 42 and

Sudan black (Smibert & Krieg, 1994). Growth at different

was tested on R2A agar supplemented with 0, 0.5, 1, 2,

pH range for growth was 6.0–9.0 (optimum pH 7.0–8.0). The
growth was investigated on R2A agar for up to 2 weeks. The

the colony (Barrow & Feltham, 1993). Antibiotic sensitivity

impregnated with the following antibiotics (concentration

per disc): chloramphenicol (30 μg), erthyromycin (15 μg),
gentamicin (10 μg), kanamycin (30 μg), nitrofurantoin

(30 μg), penicillin G (10 μg), bacitracin (10 μg), poly-

myxin B (30 μg), tetracycline (30 μg), vancomycin (30 μg),
amikacin (30 μg), ceftriaxone (30 μg) and streptomycin

(10 μg). Any sign of growth inhibition was scored as

sensitivity to that particular antibiotic. Resistance to an

antimicrobial drug was indicated if no inhibition zone was

observed. Additional physiological and biochemical char-

acteristics were determined by using the API 20E, API

20NE and API ZYM kits (bioMérieux), according to the

manufacturer's instructions.

Strain M71T was strictly aerobic, Gram-reaction-negative, rod-shaped and motile by means of a single polar flagellum (Fig. S1; available in the online Supplementary Material). The strain tested positive for catalase and oxidase activities. Colonies were pale-pink, circular, convex, smooth and 1–2 mm in diameter after growth for 4 days at 30 °C on R2A agar plates. Growth occurred at 10–37 °C (optimum 30 °C) and at pH 6.0–9.0 (optimum pH 7.0–8.0). The

range of NaCl concentrations for growth was 0–2.0 %

(w/v); optimum growth occurred without NaCl. Other

physiological characteristics of strain M71T are sum-

marized in the species description and Table S1. Selected characteristics that differentiate strain M71T from related species of the genus Desertibacter are shown in Table 1.

Genomic DNA extraction, PCR-mediated amplification of the 16S RNA gene and sequencing of PCR products were carried out as described by Lin et al. (2004). Sequence similarity was searched by NCBI BLAST and calculated by pairwise alignments obtained from the EzTaxon database (Kim et al., 2012). Phylogenetic analysis was performed by using the software package MEGA version 6.0 (Tamura et al., 2013) after multiple alignments of the data with CLUSTAL W (Thompson et al., 1994). Phylogenetic trees were recon-structed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-like-lihood (Felsenstein, 1981) methods. Evolutionary distances for the neighbour-joining algorithm were calculated with Kimura’s two-parameter method (Kimura, 1980). Bootstrap values were determined based on 1000 replications (Felsenstein, 1985).

### Table 1. Differential characteristics of strain M71T and related species of the genus Desertibacter

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Pigmentation</td>
<td>Pale pink</td>
<td>Pink</td>
</tr>
<tr>
<td>Temperature range for growth (°C)</td>
<td>10–37</td>
<td>12–42</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>6.0–9.0</td>
<td>7.0–10.0</td>
</tr>
<tr>
<td>Growth on 2% (w/v) NaCl</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of Tween 20</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Antibiotic resistance (concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>per disc)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymyxin B (30 μg)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Bacitracin (10 μg)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>71.2 %</td>
<td>71.4 %</td>
</tr>
</tbody>
</table>

*Data from Liu et al., 2011.
The 16S rRNA gene sequence of strain M71<sup>T</sup> was a continuous stretch of 1410 bp. Sequence comparisons with 16S rRNA gene sequences from the EzTaxon database revealed that strain M71<sup>T</sup> had highest similarity with <i>D. roseus</i> 2262<sup>T</sup> (96.2%). No other recognized bacterial species showed more than 92% 16S rRNA gene sequence similarity to the novel isolate. These values are well below the threshold for demarcating bacterial species (Stackebrandt & Goebel, 1994). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain M71<sup>T</sup> was affiliated to the genus <i>Desertibacter</i> in the family Rhodospirillaceae, and formed a robust clade with <i>D. roseus</i> 2262<sup>T</sup> in the neighbour-joining tree (Fig. 1). The same relationship was also found in trees reconstructed using the maximum-parsimony (Fig. S2) and maximum-likelihood (Fig. S3) algorithms. These results suggest that strain M71<sup>T</sup> represents a novel species within the genus <i>Desertibacter</i>.

Respiratory quinones were extracted and identified by HPLC (Dionex UltiMate 3000) as described by Xie & Yokota (2003). For cellular fatty acid analysis, strain M71<sup>T</sup> and the related type strain, <i>D. roseus</i> CCTCC AB.

![Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship of strain M71<sup>T</sup> and related taxa. Percentage bootstrap values (1000 replications) greater than 70% are shown at nodes. The sequence of Escherichia coli ATCC 11775<sup>T</sup> was used as an outgroup.](image-url)
208152T, were grown on R2A agar plates at 30 °C and harvested at the late-exponential phase. Methods for harvesting, saponification, methylation and extraction of cellular fatty acids were used according to the protocols of the Sherlock Microbial Identification System (MIDI) version 6.0. Separation and identification of fatty acid methyl esters were performed using a Hewlett Packard 6890N gas chromatograph with MIDI Sherlock TSBA6 (version of the database) (Sasser, 1990). The DNA G+C content of strain M71T was determined by HPLC according to the method of Mesbah et al. (1989). For polar lipid analysis, strain M71T and the reference strain, D. roseus CCTCC AB 208152T, were grown on R2A broth at 30 °C. The polar lipids were extracted and analysed by two-dimensional TLC (Merck silica gel plates, layer thickness 0.2 mm) according to the protocol of Tindall (1990).

Strain M71T contained ubiquinone 10 (Q-10) as the sole respiratory quinone. The predominant cellular fatty acids of strain M71T were C18:1ω7c (53.2 %), C16:1ω5c (11.0 %), summed feature 3 (C16:1ω7c and/or C16:1ω6c, 10.2 %) and C16:0 (8.5 %), which were all consistent with the data recorded for D. roseus CCTCC AB 208152T. However, differences in the fatty acid content of strain M71T and the type species of the genus Desertibacter demonstrate that strain M71T is not a strain of a known species of the genus Desertibacter (Table 2). The DNA G+C content of strain M71T was 71.2 mol%, a value slightly lower than that reported for other species of the genus Desertibacter (Table 1). The major polar lipids of strain M71T and the reference strain were phosphatidylcholine and phosphatidylethanolamine. Phosphatidyglycerol, diphasatidylglycerol, two unknown aminolipids and two unknown polar lipids were also detected in the two strains (Fig. S4). However, the larger proportions of an unknown aminolipid (AL1) distinguished the novel isolate from the reference strain.

On the basis of the data presented, strain M71T is considered to represent a novel species of the genus Desertibacter, for which the name Desertibacter xinjiangensis sp. nov. is proposed.

### Emended description of the genus Desertibacter Liu et al. 2011

The main characteristics are the same as those previously described by Liu et al. (2011), with the following modifications. The predominant cellular fatty acids are C18:1ω7c, C16:1ω5c, summed feature 3 (C16:1ω7c and/or C16:1ω6c) and C16:0. The major polar lipids are phosphatidylcholine and phosphatidylethanolamine.

### Description of Desertibacter xinjiangensis sp. nov.

Desertibacter xinjiangensis (xin,jiang.en’sis. N.L. masc. adj. xinjiangensis pertaining to Xinjiang, an autonomous region in north-western China, where the type strain was isolated).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>iso-C14:0 3-OH</td>
<td>1.4</td>
<td>–</td>
</tr>
<tr>
<td>C16:1ω5c</td>
<td>11.0</td>
<td>6.7</td>
</tr>
<tr>
<td>C16:0</td>
<td>8.5</td>
<td>5.1</td>
</tr>
<tr>
<td>C17:1ω6c</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>C16:0 3-OH</td>
<td>3.2</td>
<td>1.1</td>
</tr>
<tr>
<td>C18:1ω9c</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>C18:1ω7c</td>
<td>53.2</td>
<td>65.4</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.9</td>
<td>2.3</td>
</tr>
<tr>
<td>C18:1 2-OH</td>
<td>2.9</td>
<td>tr</td>
</tr>
<tr>
<td>C18:0 3-OH</td>
<td>tr</td>
<td>2.0</td>
</tr>
<tr>
<td>Summed feature 2</td>
<td>2.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Summed feature 3</td>
<td>10.2</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Aerobic, Gram-reaction-negative, rod-shaped and motile by means of a single polar flagellum. Colonies are pale-pink, circular, convex, smooth and 1–2 mm in diameter after growth for 4 days at 30 °C on R2A agar plates. Growth occurs at 30 °C on R2A agar, 0.3 × R2A agar, MB agar, 0.1 × TSB agar, NA and 0.1 × NA, but not on TSB agar or MacConkey agar. Growth occurs at 10–37 °C (optimum 30 °C) and at pH 6.0–9.0 (optimum pH 7.0–8.0). The NaCl concentration range for growth is 0–2.0 % (w/v); optimum growth occurs without NaCl. Tests for catalase and oxidase are positive. Poly-β-hydroxybutyrate granules are accumulated. Bacteriochlorophyll a is not detected. Nitrate is reduced to nitrite. Hydrolyses aesculin and Tween 20, but not chitin, starch, DNA, tyrosine, gelatin, agar, CM-cellulose, casein or Tween 80. In the API ZYM test, positive reactions for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and leucine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucosidase, and negative reactions for lipase (C14), valine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galacturonidase, α-galactosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. In API 20E and 20NE strips, positive reactions for the utilization of citrate, production of acetoin, nitrate reduction, aesculin hydrolysis and β-galactosidase activity. Negative reactions for l-tyrosine decarboxylase, ornithine decarboxylase, gelatinase, urease, trypothan deaminase and arginine dihydrolase activities, production of H₂S and indole, oxidation of mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose, assimilation of glucose, arabinose, mannose, mannitol, N-acetylgalactosamine, maltose, potassium gluconate, capric acid, adipic acid, malate and phenylacetic acid and fermentation of glucose. Sensitive
to chloramphenicol, erythromycin, gentamicin, kanamycin, nitrofurantoin, penicillin G, tetracycline, vancomycin, amikacin, ceftriaxone and streptomycin, but resistant to bacitracin and polymyxin B. The isoprenoid quinone is Q-10. The major cellular fatty acids are C18:1ω7c, C16:1ω5c, summed feature 3 (C16:1ω7c and/or C16:1ω6c) and C16:0. The major polar lipids are phosphatidylcholine and phosphatidylethanolamine; phosphatidylylycerol; two unknown aminolipids and two unknown polar lipids are also detected.

The type strain, M71\textsuperscript{T} (=CCTCC AB 209291\textsuperscript{T}=CIP 110127\textsuperscript{T}), was isolated from the soil of a Euphrates poplar forest in Xingjiang, PR China. The DNA G+C content of the type strain is 71.2 mol%.

Acknowledgements

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References


