Gracilibacillus ureilyticus sp. nov., a halotolerant bacterium from a saline–alkaline soil

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A Gram-stain-positive, halotolerant, neutrophilic, rod-shaped bacterium, strain MF38T, was isolated from a saline–alkaline soil in China and subjected to a polyphasic taxonomic characterization. The isolate grew in the presence of 0–15 % (w/v) NaCl and at pH 6.5–8.5; optimum growth was observed with 3.0 % (w/v) NaCl and at pH 7.0. Chemotaxonomic analysis showed menaquinone MK-7 as the predominant respiratory quinone and anteiso-C15 : 0, anteiso-C17 : 0, iso-C15 : 0, C17 : 0 and C16 : 0 as major fatty acids. The genomic DNA G+C content was 35.3 mol%. 16S rRNA gene sequence similarities of strain MF38T with type strains of described Gracilibacillus species ranged from 95.3 to 97.7 %. Strain MF38T exhibited the closest phylogenetic affinity to the type strain of Gracilibacillus dipsosauri, with 97.7 % 16S rRNA gene sequence similarity. The DNA–DNA reassociation between strain MF38T and G. dipsosauri DSM 11125T was 45 %. On the basis of phenotypic and genotypic data, strain MF38T represents a novel species of the genus Gracilibacillus, for which the name Gracilibacillus ureilyticus sp. nov. (type strain MF38T = CGMCC 1.7727T = JCM 15711T) is proposed.

The genus Gracilibacillus was first proposed by Waino et al. (1999) with the type species Gracilibacillus halotolerans, and Bacillus dipsosauri (Lawson et al., 1996) was reclassified in the genus as Gracilibacillus dipsosauri at the same time. Seven further species, Gracilibacillus orientalis (Carrasco et al., 2006), G. boraciitolerans (Ahmed et al., 2007), G. lacisalsi (Jeon et al., 2008), G. halophilus (Chen et al., 2008a), G. quinghaiensis (Chen et al., 2008b), G. saliphilus (Tang et al., 2009) and G. thailandensis (Chamroensaksri et al., 2010), have since been described. Most of them were isolated from saline lakes, the exceptions being G. dipsosauri (from a desert iguana; Lawson et al., 2006), G. boraciitolerans (from a soil containing high levels of boron; Ahmed et al., 2009) and G. thailandensis (from fermented fish; Chamroensaksri et al., 2010). Here, we present the results of a polyphasic study describing a novel halotolerant Gracilibacillus strain isolated from a saline–alkaline soil in China.

The saline–alkaline soil sample was collected from Minfeng country located in Xinjiang Province, China, in December 2005. The sample contained some granulated salts and was alkaline (pH 8.5). Approximately 100 mg soil sample was incubated for 30 min in modified halophilic medium (HM) containing 10 % NaCl (w/v) without carbon source. The modified HM medium contained (per l distilled water): NaCl as indicated, 2.0 g KCl, 1.0 g MgSO4, 0.36 g CaCl2.2H2O, 0.23 g NaBr, 0.06 g NaHCO3, trace FeCl3, 1.0 g yeast extract (Difco), 0.5 g peptone (Difco) and 0.1 g glucose (pH 7.5) (Ventosa et al., 1982). The liquid was plated on modified HM agar plates, using a tenfold dilution series. After 3 days of incubation at 25 °C, a cream-coloured colony, designated MF38T, was picked. The strain was purified by repeated restreaking; purity was confirmed by the uniformity of colony morphology.

The 16S rRNA gene was amplified and PCR products were cloned into pMD 19-T vector (TaKaRa) for sequencing (Xu et al., 2007b). An almost-complete 16S rRNA gene sequence (1485 nt) was obtained and compared with closely related sequences of reference organisms from the FASTA and EzTaxon services (Chun et al., 2007). Sequence data were aligned with CLUSTAL W 1.8 (Thompson et al., 1994).
Phylogenetic trees were constructed by the neighbouring-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA 4 program package (Tamura et al., 2007) and the maximum-likelihood method (Felsenstein, 1981) with the TreePuzzle 5.2 program. Evolutionary distances were calculated according to the algorithm of Kimura’s two-parameter model (Kimura, 1980) for the neighbour-joining method.

Comparisons of 16S rRNA gene sequences showed that strain MF38T should be positioned within the genus *Gracilibacillus*, related most closely to the type strain of *G. dipsosauri*, with 97.7% similarity. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain MF38T had the closest phylogenetic affinity to the type strain of *G. dipsosauri*, with high levels of bootstrap support (Fig. 1). The topologies of phylogenetic trees built using the maximum-parsimony and maximum-likelihood algorithms also supported the notion that strain MF38T formed a stable clade with *G. dipsosauri* (Supplementary Figs S1 and S2, available in IJSEM Online).

Growth at various NaCl concentrations (0, 0.5, 1.0, 2.0, 3.0, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, 22.5 and 25.0 %, w/v) was investigated in trypticase soy broth (TSB; Difco) with 1 M KCl according to Lawson et al. (1996). The pH range for growth was determined at pH 5.0–10.0 (at intervals of 0.5 pH units) in TSB with 1 M KCl using the following buffers at 40 mM: MES (pH 5.0–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) and CAPSO (pH 9.0–10.0). The temperature range for growth was determined by incubation at 4, 10, 15, 20, 25, 30, 35, 37, 42, 45 and 50 °C. Cell morphology and motility were examined by optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL). The NaCl concentration, pH and temperature for growth of strain MF38T were 0–15 % (w/v), pH 6.5–8.5 and 10–45 °C. Cells of strain MF38T were Gram-stain-positive, spore-forming rods and motile by means of peritrichous flagella (Supplementary Fig. S3).

**Table 1.** Differentiating characteristics of strain MF38T from its closest phylogenetic relative, *G. dipsosauri* DSM 11125T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain MF38T</th>
<th>G. dipsosauri DSM 11125T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis of urea</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Susceptibility to:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin (10 μg)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Ampicillin (10 μg)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Bacitracin (0.04 IU)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Penicillin G (10 IU)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>API ZYM tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-Galactosidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>W</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>W</td>
</tr>
<tr>
<td>Fermentation/oxidation of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Melibiose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Arabinoose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>35.3</td>
<td>39.4*</td>
</tr>
</tbody>
</table>

*Data from Lawson et al. (1996).*
tests. Detailed results are given in the species description and in Table 1 and Supplementary Table S1.

Fatty acid methyl esters obtained from cells grown on MA (Difco) for 2 days at 35 °C were analysed by using GC/MS (Kuykendall et al., 1988). Isoprenoid quinones were analysed using reversed-phase HPLC as described previously (Komagata & Suzuki, 1987). Phospholipids and glycolipids were separated on silica gel plates (10 × 10 cm) by TLC and were analysed according to Xu et al. (2007a). The purified DNA was hydrolysed with P1 nuclease and the nucleotides were depolymerized with calf intestine alkaline phosphatase; the G+C content of the resulting deoxyribonucleosides was determined by reversed-phase HPLC and calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) (Mesbah & Whitman, 1989). The HPLC and calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) (Mesbah & Whitman, 1989). The major fatty acids of strain MF38 T were anteiso-C15 : 0 (dG) and thymidine (dT) (Mesbah & Whitman, 1989). The purified DNA was hydrolysed with P1 nuclease and the nucleotides were depolymerized with calf intestine alkaline phosphatase; the G+C content of the resulting deoxyribonucleosides was determined by reversed-phase HPLC and calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) (Mesbah & Whitman, 1989). The major fatty acids of strain MF38 T were anteiso-C15 : 0 (dG) and thymidine (dT) (Mesbah & Whitman, 1989). The HPLC and calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) (Mesbah & Whitman, 1989).

Strain MF38 T exhibited the closest phylogenetic affinity with G. dipsosauri DSM 11125 T. Nevertheless, the absence of C12 : 0, iso-C13 : 0, iso-C18 : 0, C18 : 1

Description of Gracilibacillus ureilyticus sp. nov.

Gracilibacillus ureilyticus (u.re.i'lyi.ti.cus. N.L. n. urea urea; N.L. adj. lyticus able to dissolve; N.L. masc. adj. ureilyticus urea-dissolving).

Cells are Gram-stain-positive, spore-forming, motile rods, 0.7–1.0 μm wide and 1.5–4.5 μm long. Colonies on MA are 1–2 mm in diameter, rough, slightly elevated and cream-coloured with irregular edges after 2 days at 37 °C. Growth occurs at NaCl concentrations of 0–15% (w/v), with optimum growth at 3.0%, and at pH 6.5–8.5 and 10–45 °C (optimum growth at pH 7.0 and 35–37 °C). Oxidase- and catalase-positive. Aesculin, gelatin, starch, Tween 20 and urea are hydrolysed. Casein, DNA, Tweens 40, 60 and 80 and tyrosine are not hydrolysed. Arginine dihydrolase, indole production, lysine and ornithine carbonylases, citrate utilization, tryptophan deaminase and fermentation of amygdalin, L-arabinose, D-glucose, inositol, D-mannitol, melibiase, L-rhamnose, D-sorbitol and sucrose are negative. Voges–Proskauer and O-nitrophenyl-β-D-galactopyranoside tests are positive. Nitrate is reduced to nitrite. H2S is not produced. The following substrates are utilized for growth: L-arabinose, cellobiose, D-galactose, gluconate, glucose, lactose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-salicin, starch, sucrose, trehalose and D-xylose. The following compounds are not utilized as sole carbon and energy sources: acetate, L-alanine, L-arginine, L-asparagine, L-aspartate, citrate, L-cysteine, ethanol, formate, fumarate, L-glutamate, L-glutamine, glycine, L-histidine, isoleucine, lactate, L-lysine, malate, malonate, L-methionine, L-ornithine, propionate, pyruvate, ribitol, L-serine, D-sorbitol, L-sorbose, succinate and L-valine. Acid is produced from L-arabinose, cellobiose, D-fructose, D-galactose, glucose, lactose, maltose, D-mannitol, raffinose, L-rhamnose, D-salicin, starch, sucrose, trehalose and D-xylose, but not from ethanol, ribitol, D-sorbitol or L-sorbose. In the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), α- and β-galactosidase and α- and β-glucosidase activities are present, whereas acid phosphatase, α-chymotrypsin, N-acetyl-β-glucosaminidase, cystine arylamidase, β-fucosidase, β-glucuronidase, leucine arylamidase, lipase (C14), α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase activities are absent. Susceptible to discs containing (μg unless otherwise stated) amoxicillin (10), ampicillin (10), bacitracin (0.04 IU), carbenicillin (100), cefotaxime (30), cefoxitin (30), chloramphenicol (30), erythromycin (15), kanamycin (30), neomycin (30), nitrofurantoin (30), novobiocin (30), penicillin G (10 IU), rifampicin (5), tetracycline (10) and tobramycin (10), but not susceptible to nystatin (100) or streptomycin (10). The predominant menaquione is MK-7. The major polar lipids include diphosphatidylglycerol, phosphatidylglycerol, three unidentified phospholipids and three glycolipids. The major fatty acids (>5%) include anteiso-C15 : 0, anteiso-C17 : 0, iso-C15 : 0, C17 : 0 and C16 : 0. The DNA G+C content of the type strain is 35.3 mol%.

The type strain, MF38 T (=CGMCC 1.7727 T =JCM 15711 T), was isolated from a saline–alkaline soil sample from Minfeng county, Xinjiang, China.

Acknowledgements

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References


