Loktanella sediminiloritis sp. nov., isolated from tidal flat sediment

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A Gram-stain-negative, non-motile, rod-shaped bacterial strain, designated D1-W3T, was isolated from tidal flat sediment of the South Sea, South Korea, and subjected to a taxonomic study using a polyphasic approach. Strain D1-W3T grew optimally at pH 7.0–8.0, at 25 °C and in the presence of 2% (w/v) NaCl. Neighbour-joining phylogenetic analyses based on 16S rRNA gene sequences revealed that strain D1-W3T fell within the clade comprising species of the genus Loktanella, clustering with the type strains of Loktanella tamlensis, Loktanella rosea and Loktanella maricola, with which it exhibited the highest 16S rRNA gene sequence similarity values (98.1–98.2%). The 16S rRNA gene sequence similarity values between strain D1-W3T and the type strains of other species of the genus Loktanella were in the range 93.5–96.5%. The DNA G+C content of strain D1-W3T was 58.1 mol% and the mean DNA–DNA hybridization values with L. tamlensis KCTC 12722T, L. rosea LMG 22534T and L. maricola DSW-18T were 13–25%. Strain D1-W3T contained Q-10 as the predominant ubiquinone and C₁₈:₁₀7c as the predominant fatty acid. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, phosphatidylethanolamine and one unidentified aminolipid. Differential phenotypic properties, together with the phylogenetic and genetic distinctiveness, showed that strain D1-W3T could be differentiated from other species of the genus Loktanella. On the basis of the data presented, strain D1-W3T represents a novel species of the genus Loktanella, for which the name Loktanella sediminiloritis sp. nov. is proposed. The type strain is D1-W3T (=KCTC 32383T =CECT 8284T).

The genus Loktanella, a member of the class Alphaproteobacteria, was created by Van Trappen et al. (2004) with the description of three novel species, including Loktanella salsilacus as the type species of the genus. At the time of writing, the genus Loktanella comprised 13 species with validly published names: Loktanella salsilacus, L. fryxellensis and L. vestfoldensis (Van Trappen et al., 2004), L. hongkongensis (Lau et al., 2004), L. agnita and L. rosea (Ivanova et al., 2005), L. koreensis (Weon et al., 2006), L. maricola (Yoon et al., 2007), L. atrilutea (Hosoya & Yokota, 2007), L. pyoseonensis (Moon et al., 2010), L. tamlensis (Lee, 2012), L. litorea (Yoon et al., 2013) and L. cinnabarina (Tsoubuchi et al., 2013). Members of the genus Loktanella have been isolated from Antarctic lakes and marine environments (Van Trappen et al., 2004; Lau et al., 2004; Ivanova et al., 2005; Weon et al., 2006; Yoon et al., 2007; Hosoya & Yokota, 2007; Moon et al., 2010; Lee, 2012). In this study, we describe a Loktanella-like bacterial strain, designated D1-W3T, which was isolated from a tidal flat in the South Sea, South Korea. The aim of the present work was to determine the exact taxonomic position of strain D1-W3T by using a polyphasic characterization that included the determination of chemotaxonomic and other phenotypic properties, a detailed phylogenetic investigation based on 16S rRNA gene sequences and DNA–DNA hybridization studies.

Strain D1-W3T was isolated by the standard dilution plating technique at 25 °C on marine agar 2216 (MA; BD) and cultivated routinely under the same conditions. Strain D1-W3T was maintained on MA at 4 °C for short-term preservation and as a glycerol suspension (20%, w/v in distilled water) at −80 °C for long-term preservation. L. maricola DSW-18T, L. rosea LMG 22534T and L. tamlensis KCTC 12722T were used as reference strains for DNA–DNA hybridization and phenotypic characterization. Cell morphology was examined by using light microscopy (BX51; Olympus) and transmission electron microscopy (JEM1010; JEOL). The latter technique was also used to assess the presence of flagella on cells from an exponentially growing MA culture. For this purpose, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was investigated by using the bioMérieux Gram stain kit according to the manufacturer’s instructions. Growth under anaerobic conditions was determined after incubation for 10 days in an anaerobic jar (MGC).
with AnaeroPack (MGC) on MA; the jar was kept overnight at 4 °C to make anoxic conditions before incubation at 25 °C. Growth at 4, 10, 20, 25, 30, 35, 37 and 40 °C was measured on MA to measure optimal temperature and temperature range for growth. The pH range for growth was determined in marine broth 2216 (MB; BD) adjusted to pH 4.5–9.5 (in increments of 0.5 pH units) by using sodium acetate/acetate acid and sodium carbonate buffers. The pH values were verified after autoclaving. Growth in the absence of NaCl and in the presence of 0.5, 1.0, 2.0 and 3.0% (w/v) NaCl was investigated by using trypticase soy broth prepared according to the formula of the BD medium except that NaCl was excluded and that 0.45% (w/v) MgCl₂.6H₂O was added. Growth at various NaCl concentrations (2.0–8.0%, w/v, in increments of 1.0%) was investigated in MB. Catalase and oxidase activities were determined as described by Lányí (1987). Hydrolysis of casein, starch, hypoxanthine, L-tyrosine and xanthine was tested on MA, overnight at 4 °C. 

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**Table 1. Differential phenotypic characteristics of strain D1-W3<sup>T</sup> and the type strains of the three phylogenetically most closely related species of the genus Loktanella**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colony colour</strong></td>
<td>Greyish-yellow</td>
<td>Brownish-orange</td>
<td>Deep orange–yellow</td>
<td>Greyish-yellow</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><strong>Hydrolysis of:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Casein</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>–</td>
<td>W</td>
<td>–</td>
</tr>
<tr>
<td><strong>Utilization of:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Citrate</td>
<td>–</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Succinate</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td><strong>Acid production from:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td><strong>Susceptibility to:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanamycin</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Polymyxin B</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td><strong>Enzyme activity (API ZYM)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Leucine arylamidase</td>
<td>–</td>
<td>W</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trypsin</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>N Acetyl-β-glucosaminidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>58.1</td>
<td>56.8†</td>
<td>61†</td>
<td>55.0†</td>
</tr>
</tbody>
</table>

*Data obtained after incubation for 7 days at 25 or 30 °C on MA.
†Data for reference strains taken from Ivanova et al. (2005), Yoon et al. (2007) and Lee (2012).

Strain D1-W3<sup>T</sup> exhibited 16S rRNA gene sequence similarity values of 98.2, 98.2 and 98.1% to the type strains of *L. tamlensis*, *L. rosea* and *L. maricola*, respectively, and of 93.5–96.5% to the type strains of other species of the genus *Loktanella*. The DNA G+C content of strain D1-W3<sup>T</sup> was 58.1 mol%, which is within the range reported for members of the genus *Loktanella* (Moon et al., 2010; Lee, 2012; Tsubouchi et al., 2013).

The predominant isoprenoid quinone detected in strain D1-W3<sup>T</sup> was ubiquinone-10 (Q-10), consistent with the data for species of the genus *Loktanella* (Moon et al., 2010; Lee, 2012). The cellular fatty acid profile of strain D1-W3<sup>T</sup> consisted of C<sub>18:1ω7c</sub> (69.3%), C<sub>16:0</sub> (13.6%), C<sub>18:0</sub> (8.2%), C<sub>12:1</sub> 3-OH (5.8%) and C<sub>10:0</sub> 3-OH (3.0%). The fatty acid profile of strain D1-W3<sup>T</sup> was similar to those of species of the genus *Loktanella* in that the predominant...
fatty acid was C₁₈:₁ω7c (Lee, 2012; Yoon et al., 2013). Major polar lipids detected in strain D1-W3ᵀ were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and one unidentified aminolipid; minor amounts of diphosphatidylglycerol, one unidentified aminophospholipid, one unidentified phospholipid and one unidentified lipid were also present (Fig. S1, available in IJSEM Online). Based on the polar lipid data of L. agnita, L. rosea and L. maricola, the genus Loktanella was described to have phosphatidylcholine, phosphatidylglycerol and diphosphatidylglycerol as the major polar lipids (Moon et al., 2010). However, diphosphatidylglycerol may not be the major polar lipid in L. rosea, due to its low proportion (Ivanova et al., 2005). Recently, L. tamlensis was reported to have phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and one unknown lipid as the major polar lipids (Lee, 2012). In the study of Yoon et al. (2013), the type strain of L. salsilacus was found to have major amounts of phosphatidylcholine and phosphatidylglycerol and a minor amount of phosphatidylethanolamine. Hence, the results obtained from chemotaxonomic analyses are sufficient to support the results of the phylogenetic analysis, i.e. showing that strain D1-W3ᵀ is a member of the genus Loktanella.

Strain D1-W3ᵀ exhibited mean DNA–DNA relatedness values of 22, 25 and 13 % to L. tamlensis KCTC 12722ᵀ, L. rosea LMG 22534ᵀ and L. maricola DSW-18ᵀ, respectively. Strain D1-W3ᵀ was distinguishable from the type strains of the three phylogenetically most closely related species of the genus Loktanella by differences in phenotypic characteristics; including motility, colony colour, utilization of some substrates, acid production from some substrates and enzyme activities (Table 1). These differences, in combination with the phylogenetic and genetic distinctiveness, are sufficient to show that the novel strain is separate from all other recognized species of the genus Loktanella (Wayne et al., 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain D1-W3ᵀ represents a novel species of the genus Loktanella, for which the name Loktanella sediminilitoris sp. nov. is proposed.
Description of Loktanella sediminilitoris sp. nov.

Loktanella sediminilitoris (se.di.mi.nlil’to.ris. L. n. sedimen -inis sediment; L. n. litus -oris the seashore, beach; N.L. gen. n. sediminilitoris of sediment, of seashore).

Cells are Gram-stain-negative, non-spore-forming, non-motile and rod-shaped, 0.4–0.8 μm in diameter and 0.9–4.0 μm in length. Colonies on MA are circular, slightly convex, smooth, glistening, greyish-yellow and 0.5–1.0 mm after incubation for 7 days at 25 °C. The optimal growth temperature is 25 °C; growth occurs at 10 and 35 °C, but not at 4 or 37 °C. The optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.5, but not at pH 5.0. Optimal growth occurs in the presence of 0–5.0 % (w/v) NaCl; growth occurs in the presence of 0–5.0 % (w/v) NaCl. Mg^{2+} ions are required for growth. Anaerobic growth does not occur on MA. Catalase- and oxidase-positive. Nitrate is not reduced to nitrite. Aesculin and Tweens 20, 40, 60 and 80 are hydrolysed, but casein, hypoxanthine, gelatin, starch, L-tyrosine and xanthine are not. L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, sucrose, D-xylate, acetate and L-malate (weak) are utilized as carbon and energy sources, but maltose, trehalose, benzoate, citrate, formate, pyruvate, succinate, L-glutamate and salicin are not. Acid is not produced from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, myo-inositol, lactose, maltose, mannitose, D-mannitol, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, D-sorbitol, sucrose, trehalose or D-xylate. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, oleandomycin, penicillin G, polymyxin B, streptomycin and tetracycline, but not to lincomycin. In assays with the API ZYM system, alkaline phosphatase, acid phosphatase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. The predominant ubiquinone is Q-10. The major fatty acids (>10 % of total fatty acids) are C_{18:1}ω7c and C_{16:0}. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, phosphatidylethanolamine and one unidentified aminolipid.

The type strain, D1-W3{\textsuperscript{T}} (=KCTC 32383{\textsuperscript{T}}=CECT 8284{\textsuperscript{T}}), was isolated from tidal flat sediment at Boseong in the South Sea, South Korea. The DNA G+C content of the type strain is 58.1 mol% (determined by HPLC).

Acknowledgements

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isolated from a deep subseafloor sediment, and emended description of


