The genus *Loktanella*, which was described by Van Trappen *et al.* (2004), originally contained three species, *Loktanella salsilacus*, *L. fryxellensis* and *L. vestfoldensis*. Subsequently, six other species, *Loktanella 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) and *L. atrilutea* (Hosoya & Yokota, 2007), have been described. Members of the genus *Loktanella* have been isolated from microbial mats in Antarctic lakes and marine environments such as marine biofilms, sediment, sea sand and seawater. In this paper, a novel pink-coloured, obligately halophilic bacterium isolated from beach sand was studied by using a polyphasic approach.

In the course of a study on the bacterial diversity of beach sand, strain JJM85\(^T\) was isolated from sand collected from Pyoseon Beach in Jeju, Republic of Korea, in July 2007. The sand samples (1 g) were suspended in 10 ml 0.85 % (w/v) NaCl. Aliquots (100 μl) of serial dilutions were inoculated on marine agar 2216 (MA; Difco) and plates were incubated at 25 °C for 2 days. A pure culture was stored at −80 °C in marine broth 2216 (MB; Difco) supplemented with a glycerol solution containing 20 % (v/v) distilled water and 60 % (v/v) natural seawater. For phenotypic comparison, *L. hongkongensis* NRRL B-41039\(^T\) was grown on MA at 25 °C. Unless otherwise specified, all phenotypic characteristics were examined using MA as the basal medium. Growth was tested on MA, nutrient agar (NA; Difco) and trypticase soy agar (TSA; Difco). Colony morphology and pigmentation were determined using a culture grown at 25 °C for 2 days. Cell morphology was observed under an Olympus light microscope equipped with phase-contrast optics (magnification ×400). Motility was assessed on a semi-solid agar tube containing marine broth (Difco) supplemented with 0.4 % agar. Cells were inoculated by stabbing with a straight needle and the tube was incubated at 25 °C for 5 days. The presence of flagella was checked with a transmission electron microscope (JEM-1010; JEOL) using cells negatively stained with 2 % phosphotungstic acid. Gram staining was performed using a Gram stain kit (bioMérieux) according to the manufacturer’s instructions. Growth at 4–40 °C and pH 4.0–12.0 was tested on MA and MB. Sodium ion requirements for growth and tolerance of various NaCl concentrations (0–14 %) were determined on NA. Oxidase and catalase activities and degradation of agar, DNA and starch were determined according to Lányi (1985). Cellulose hydrolysis and flexirubin pigment

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### Loktanella pyoseonensis sp. nov., isolated from beach sand, and emended description of the genus *Loktanella*

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A novel Gram-stain-negative, aerobic, heterotrophic, obligately halophilic bacterium, designated strain JJM85\(^T\), was isolated from beach sand in Jeju, Republic of Korea. Cells were rod-shaped and motile by means of flagella; colonies were pink, convex and smooth with an entire edge. The organism grew at pH 5.0–10.0 and 4–30 °C. Phylogenetic analysis based on 16S rRNA gene sequences showed that the organism belonged to the genus *Loktanella* of the class *Alphaproteobacteria* and formed a tight cluster with the type strain of *Loktanella hongkongensis* (96.0 % sequence similarity). The DNA G+C content and fatty acid profile of the novel strain supported affiliation with the genus *Loktanella*. However, the novel strain could be differentiated clearly from members of this genus by cell motility, some physiological properties and low 16S rRNA gene sequence similarity (93.1–96.0 %). On the basis of the polyphasic data presented here, strain JJM85\(^T\) is considered to represent a novel species of the genus *Loktanella*, for which the name *Loktanella pyoseonensis* sp. nov. is proposed; the type strain is J JM85\(^T\) (=KCTC 22372\(^T\) = DSM 21424\(^T\)).
production were determined as described by Bowman (2000). Hydrolysis of chitin and Tweens 20, 40 and 80 was determined according to Baumann & Baumann (1981). Other physiological and biochemical properties were tested using API 20NE, API 50CH and API ZYM strips (bioMérieux) according to the manufacturer’s instructions. For these tests, cells were suspended in a solution of 2 % sea salts (Sigma). Results were recorded after 48 h incubation at 25 °C for API 20NE and API 50CH strips and after 4 h incubation at 37 °C for API ZYM strips.

Cells of strain JJM85 T were Gram-stain-negative, aerobic, motile rods (Fig. 1). Strain JJM85 T, along with L. atrilutea (Hosoya & Yokota, 2007), was motile by means of flagella, in contrast to the other species of the genus Loktanella (Van Trappen et al., 2004; Ivanova et al., 2005; Weon et al., 2006; Yoon et al., 2007). The results of the other cultural, biochemical and physiological tests are given in the species description and Table 1.

Genomic DNA was extracted and purified using a commercial genomic DNA extraction kit (Bioneer). Amplification of the 16S rRNA gene by PCR was performed using the universal bacterial primers 27F (5′-AGAGTTTGATCMTGCGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). The purified PCR product was sequenced directly using an ABI BigDye 3.1 sequencing kit (Applied Biosystems) and an automated DNA sequencer (ABI 3730XL; Applied Biosystems). 16S rRNA gene sequence fragments of strain JJM85 T were compiled using SEQMAN software (DNASTAR) and the partial 16S rRNA gene sequence (1338 bp) was determined. The result of a preliminary BLAST search against GenBank showed that the isolate was related to members of the family Rhodobacteraceae. Multiple alignments of sequences were carried out using CLUSTAL_X (Thompson et al., 1997) and phylogenetic analyses were performed by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. A phylogenetic tree was constructed using the neighbour-joining method and evolutionary distances were calculated with the model of Jukes & Cantor (1969). A bootstrap analysis (Felsenstein, 1985) was performed with 1000 resampled datasets to estimate tree topology.

A neighbour-joining tree (Fig. 2) based on 16S rRNA gene sequences showed that strain JJM85 T belonged to the genus Loktanella and formed a robust cluster with the type strain of L. hongkongensis. This branching pattern was supported by a high bootstrap value (98 %) and was also found in trees obtained by the maximum-parsimony and maximum-likelihood treeing algorithms. 16S rRNA gene sequence similarities between strain JJM85 T and members of the genus Loktanella were as follows: L. hongkongensis NRRL B-41039 T, 96.0 %; L. maricola DSW-18 T, 94.1 %; L. rosea Fg36 T, 93.9 %; L. koreensis GA2-M3 T, 93.9 %; L. agnita R10SW5 T, 93.8 %; L. salsilacus LMG 21507 T, 93.7 %; L. atrilutea NCIMB 14280 T, 93.6 %; L. fryxellensis LMG 22007 T, 93.4 %; and L. vestfoldensis LMG 22003 T, 93.1 %.

DNA–DNA hybridization experiments between strain JJM85 T and its phylogenetic neighbours were not carried out given the phenotypic distinctiveness of strain JJM85 T and 16S rRNA gene sequence similarity values, which were lower than the recommended value of 97 % used to delineate separate bacterial species (Stackebrandt & Goebel, 1994).

Cellular fatty acids of strain JJM85 T and L. hongkongensis NRRL B-41039 T were analysed according to the instructions of the Sherlock Microbial Identification System (MIDI version 6). Fatty acid methyl esters were prepared from cells grown on MA for 3 days at 25 °C. The G+C content of the DNA was determined by HPLC (Mesbah et al., 1989).

The cellular fatty acid profiles of strain JJM85 T and L. hongkongensis NRRL B-41039 T consisted of straight-chain saturated and unsaturated components with small amounts of hydroxy fatty acids; these profiles were similar to those of other members of the genus Loktanella (Van Trappen et al., 2004; Ivanova et al., 2005; Weon et al., 2006; Hosoya & Yokota, 2007; Yoon et al., 2007). The dominant fatty acid of both strains was C18:1 ω7c, but they differed from each other by the presence/absence of the minor components 11-methyl C18:1 ω7c and C16:1 ω7c. The fatty acid profiles of strain JJM85 T and L. hongkongensis NRRL B-41039 T are given in Table 2. The DNA G+C content of strain JJM85 T was determined as 67.5 mol%, whereas that of L. hongkongensis NRRL B-41039 T determined in this

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**Fig. 1.** Transmission electron micrograph of a cell of strain JJM85 T grown on MA at 25 °C for 2 days. Bar, 0.5 μm.
study was 65.7 mol%, which is within the range reported previously (Lau et al., 2004).

In our study, strain JJM85\(^T\) and \(L.\) \(hongkongensis\) NRRL B-41039\(^T\) assimilated D-glucose, L-arabinose, D-mannitol and citrate as sole carbon sources, whereas strain JJM85\(^T\) showed additional utilization of N-acetylglucosamine and maltose, in contrast to \(L.\) \(hongkongensis\) NRRL B-41039\(^T\). Both strains produced acid from D- and L-arabinose, D-fructose, lactose, D-mannitol, sucrose and L-xylose, but \(L.\) \(hongkongensis\) NRRL B-41039\(^T\) differed from strain JJM85\(^T\) in that it also produced acid from dulcitol, D-glucose, inositol, xylitol and D-xylose. Differential features of strain JJM85\(^T\) and members of the genus \(Loktanella\) are given in Table 1.

On the basis of the phenotypic features and phylogenetic evidence presented here, strain JJM85\(^T\) represents a novel species of the genus \(Loktanella\), for which the name \(Loktanella\) \(pyoseonensis\) sp. nov. is proposed. An emended description of the genus \(Loktanella\) is also presented.

**Emended description of the genus \(Loktanella\) Van Trappen et al. 2004**

Cells are Gram-stain-negative, strictly aerobic, moderately halotolerant, chemoheterotrophic, non-spore-forming and rod-shaped. Motility is variable among species; if observed, cells are motile by means of flagella. Cytochrome oxidase- and catalase-positive. Colony colours are variable (white, pink, whitish pink, beige or light orange) depending on the species. The optimal temperature for growth is 25 °C. The dominant fatty acid is \(C_{18:1}\)ω7c. Q-10 is the major ubiquinone. The polar lipids are diphasphatidylglycerol, phosphatidylycholine and phosphatidylglycerol. DNA G+C contents are 59.1–67.5 mol%. Phylogenetically, the genus belongs to the \(Rhodobacter\) group within the class \(Alphaproteobacteria\). The type species is \(Loktanella\) \(salsilacus\).

**Description of \(Loktanella\) \(pyoseonensis\) sp. nov.**

\(Loktanella\) \(pyoseonensis\) (pyo.se.o.nen’sis. N.L. fem. adj. \(pyoseonensis\) pertaining to Pyoseon Beach, Jeju, Republic of Korea, where the type strain was isolated).

Cells are Gram-stain-negative, aerobic and rod-shaped (0.6–0.8 × 1.3–3.0 μm). Motile by means of flagella. Colonies are circular, smooth, convex with an entire margin and pinkish in colour. Grows between 4 and 30 °C (optimum at 25 °C) and at pH 5.0–10.0 (optimum at pH 7.0–8.0). Grows on NA supplemented with 1–12 % (w/v) NaCl (optimum at 2–5%); growth does not occur on NA in the absence of NaCl. Positive for catalase and oxidase activities and nitrate reduction, but negative for gelatin liquefaction, glucose fermentation, arginine dihydrolase, hydrogen sulfide and indole production (API 20NE). Does not grow...
Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship between strain JMM85T, Loktanella species and other related taxa. The tree was constructed based on an evolutionary distance matrix by using the neighbour-joining method (Saitou & Nei, 1987) and the model of Felsenstein (1981) trees are indicated by asterisks. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid content of strain JMM85T and L. hongkongensis NRRL B-41039T

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>6.5</td>
<td>4.0</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.3</td>
<td>3.0</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:1ω7c</td>
<td>1.9</td>
<td>–</td>
</tr>
<tr>
<td>C18:1ω7c</td>
<td>87.4</td>
<td>85.6</td>
</tr>
<tr>
<td>11-Methyl C18:1ω7c</td>
<td>–</td>
<td>1.1</td>
</tr>
<tr>
<td>Hydroxy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C10:0 3-OH</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>C12:0 3-OH</td>
<td>1.2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

on TSA. Hydrolyses aesculin, but not agar, DNA, starch, Tween 80, tyro sine or urea. Assimilates N-acetylglucosamine, L-arabinose, citrate, D-glucose, maltose and D-mannitol as sole carbon sources. Acid is produced from D-arabinose, L-arabinose, D-fructose, lactose, D-mannitol, sucrose and L-xylose. Positive for alkaline phosphatase, esterase (C4) (weak), esterase lipase (C8) (weak), lipase (C14) (weak), leucine arylamidase, valine arylamidase (weak), cystine arylamidase (weak), trypsin, acid phosphatase (weak), x-chymotrypsin (weak), naphthol-AS-BI-phosphohydrolase (weak), β-galactosidase (weak) and N-acetyl-β-glucosaminidase (weak), but negative for α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase (API ZYM). The main cellular fatty acid is C18:1ω7c. The DNA G+C content of the type strain is 67.5 mol%.

The type strain is JMM85T (=KCTC 22372T =DSM 21424T), isolated from sea sand taken from Pyoseon Beach, Jeju, Republic of Korea.

Acknowledgements

This work was partially supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science & Technology, Republic of Korea. The authors are thankful for Dr A. P. Rooney for providing the type strain of L. hongkongensis.

References


