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 JJM85^T (=KCTC 22372^T =DSM 21424^T).

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 Lányí (1985). Cellulose hydrolysis and flexirubin pigment

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JJM85^T is AM983542.

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′-AGAGTTTGATCMTGGCTCAG-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.

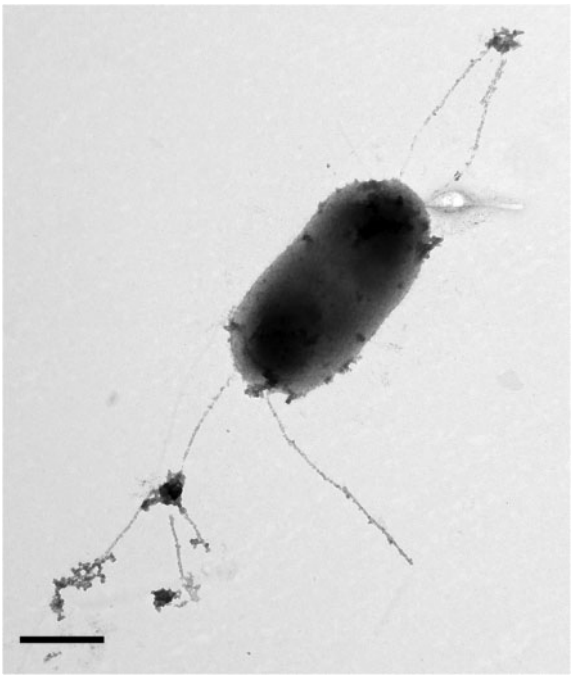

Fig. 1. Transmission electron micrograph of a cell of strain JJM85^T grown on MA at 25 °C for 2 days. Bar, 0.5 μm.

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 $C_{18:1}\omega7c$, but they differed from each other by the presence/absence of the minor components 11-methyl $C_{18:1}\omega7c$ and $C_{16:1}\omega7c$. 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

Table 1. Differential characteristics of strain JJM85^T and species of the genus *Loktanella*

Taxa: 1, strain JJM85^T (this study); 2, *L. salsilacus* (Van Trappen *et al.*, 2004); 3, *L. fryxellensis* (Van Trappen *et al.*, 2004); 4, *L. vestfoldensis* (Van Trappen *et al.*, 2004); 5, *L. hongkongensis* (Lau *et al.*, 2004); 6, *L. agnita* (Ivanova *et al.*, 2005); 7, *L. rosea* (Ivanova *et al.*, 2005); 8, *L. koreensis* (Weon *et al.*, 2006); 9, *L. maricola* (Yoon *et al.*, 2007); 10, *L. atrilutea* (Hosoya & Yokota, 2007). +, Positive; −, negative; W, weak; V, variable reaction depending on strain; ND, no data available. All taxa are Gram-stain-negative, aerobic rods and grow at 10–30 °C. They are positive for catalase and oxidase, but negative for hydrolysis of agar and starch, arginine dihydrolase, hydrogen sulfide and indole production.

Characteristic	1	2	3	4	5	6	7	8	9	10
Motility	+	−	−	−	−	−	−	−	−	+
Colony colour	Pink	Beige	Pink–beige	Pink	Pink–white	Whitish	Pink	Beige	Light orange	Beige
Diffusible pigment	−	−	−	−	Brown	−	−	−	−	−
Growth on TSA and NA	−	−	−	−	W	−	−	−	−	−
Temperature range for growth (°C)	4–30	5–30	5–25	5–37	8–44	8–35	4–35	5–30	4–34	8–30
NaCl range for growth (%, w/v)	1–12	0–10	0–5	0–10	2–14	3–6	1–12	1–5	1–7	0–8
Nitrate reduction	+	−	−	−	−	W	−	+	−	−
Hydrolysis of:										
Urea	−	−	−	+	−	−	−	−	−	−
Gelatin	−	−	−	−	−	−	−	W	−	ND
Aesculin	+	+	+	+	ND	ND	ND	+	−	ND
Tween 80	−	+	+	+	−	−	W	−	W	+
Enzyme activity										
α-Galactosidase	−	+	−	−	ND	+	−	−	−	+
β-Galactosidase	W	−	+	W	ND	ND	ND	W	−	−
β-Glucosidase	−	W	+	W	ND	ND	ND	W	−	−
Trypsin	+	−	−	+	ND	−	−	−	−	−
Carbohydrate metabolism	+	−	−	−	V	−	−	−	+	V
DNA G+C content (mol%)	67.5	59.6–60.4	65.7–66.4	62.1–63.1	65.9–66.2	59.1	60.5–61.8	60.0	56.8	66.3

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 $C_{18:1}\omega7c$. Q-10 is the major ubiquinone. The polar lipids are diphosphatidylglycerol, phosphatidylcholine 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 and indole production (API 20NE). Does not grow

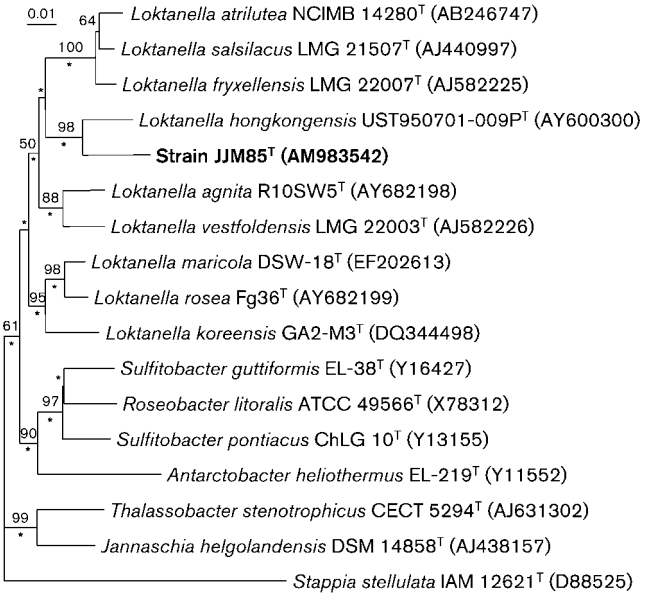

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship between strain JJM85^T, *Loktanella* species and other related taxa. The tree was constructed based on an evolutionary distance matrix by using the neighbour-joining method (Saitou & Nei, 1987) and the model of Jukes & Cantor (1969). The sequence of *Stappia stellulata* IAM 12621^T was used the outgroup. Bootstrap support values (⩾50 %) are shown at branch points. Branches found in both maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) trees are indicated by asterisks. Bar, 0.01 substitutions per nucleotide position.

on TSA. Hydrolyses aesculin, but not agar, DNA, starch, Tween 80, tyrosine 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), α-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 $C_{18:1}\omega7c$. The DNA G+C content of the type strain is 67.5 mol%.

The type strain is JJM85^T (=KCTC 22372^T =DSM 21424^T), isolated from sea sand taken from Pyoseon Beach, Jeju, Republic of Korea.

Table 2. Cellular fatty acid content of strain JJM85^T and *L. hongkongensis* NRRL B-41039^T

Strains: 1, JJM85^T; 2, *L. hongkongensis* NRRL B-41039^T. Data were obtained in this study; both strains were grown on MA for 3 days at 25 °C. Values are percentages of total fatty acids; values less than 1 % of the total fatty acids were omitted. −, Not detected.

Fatty acid	1	2
Saturated		
$C_{16:0}$	6.5	4.0
$C_{18:0}$	1.3	3.0
Unsaturated		
$C_{16:1}\omega7c$	1.9	−
$C_{18:1}\omega7c$	87.4	85.6
11-Methyl $C_{18:1}\omega7c$	−	1.1
Hydroxy		
$C_{10:0}$ 3-OH	1.4	1.8
$C_{12:0}$ 3-OH	1.2	1.8

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*.

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