Loktanella tamlensis sp. nov., isolated from seawater

Soon Dong Lee

Correspondence

Soon Dong Lee
sdlee@jejunu.ac.kr

Department of Science Education, Jeju National University, Jeju 690-756, Republic of Korea

An aerobic, Gram-reaction-negative, chemo-organotrophic bacterium, designated strain SSW-35^T, was isolated from seawater in Jeju, Republic of Korea. Cells were motile, short rods; colonies were circular, smooth, convex, translucent and beige in colour. No diffusible pigment formed on any of the media tested. The bacterium grew at 4–30 °C and pH 7.1–10.1. Phylogenetic analysis based on 16S rRNA gene sequences showed that the organism was related to members of the genus *Loktanella*, its closest recognized relatives being *Loktanella rosea* Fg36^T (98.1 % sequence similarity) and *Loktanella maricola* DSW-18^T (97.8 %). Levels of 16S rRNA gene similarity between strain SSW-35^T and other recognized species of the genus *Loktanella* were all <97 %. Polar lipid analysis revealed the presence of diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and an unknown lipid as major components, as well as small amounts of two unknown phospholipids. The predominant ubiquinone was Q-10. The major cellular fatty acid was C_{18:1} (summed feature 7), and the 3-hydroxy fatty acids detected were C_{12:1} 3-OH and C_{10:0} 3-OH. The genomic DNA G + C content was 55.0 mol%. In DNA–DNA hybridization experiments, the relatedness values between strain SSW-35^T and the type strains of the phylogenetically closest recognized species were all <11 %. On the basis of the phenotypic and genotypic characteristics, phylogenetic analysis and DNA–DNA relatedness, a novel species, *Loktanella tamlensis* sp. nov., is proposed. The type strain is SSW-35^T (=KCTC 12722^T=JCM 14020^T).

The genus *Loktanella* (Van Trappen *et al.*, 2004), which was recently emended by Moon *et al.* (2010), encompasses strictly aerobic, chemo-organotrophic, Gram-reaction-negative, moderately halotolerant, rod-shaped bacteria that show interspecific variability in their cell motility. At the time of writing, the genus contains ten recognized species: *Loktanella salsilacus* as the type species, *L. fryxellensis*, *L. vestfoldensis* (Van Trappen *et al.*, 2004), *L. hongkongensis* (Lau *et al.*, 2004), *L. agnita*, *L. rosea* (Ivanova *et al.*, 2005), *L. koreensis* (Weon *et al.*, 2006), *L. maricola* (Yoon *et al.*, 2007), *L. atrilutea* (Hosoya & Yokota, 2007) and *L. pyoseonensis* (Moon *et al.*, 2010). Members of the genus *Loktanella*, which belongs to the *Rhodobacter* group of the class *Alpha-proteobacteria*, have been isolated from various sources, such as microbial mats in Antarctic lakes, marine biofilms, seawater, sediments and sea sand. This study describes the classification and identification, by a polyphasic taxonomic approach, of a novel *Loktanella* strain isolated from seawater.

During a study of polysaccharide-producing marine bacteria, strain SSW-35^T was isolated from surface seawater

at Samyang Beach in Jeju, Republic of Korea. The water sample was directly transferred onto yeast extract-malt extract-seawater (YE-SW) agar (4 g yeast extract, 10 g malt extract, 4 g glucose and 18 g agar in a mixture of 600 ml seawater and 400 ml distilled water, pH 7.2) before the plate was incubated for 5 days at 30 °C. The pure culture was stored as glycerol suspensions supplemented with 60 % (v/v) seawater and 20 % (v/v) distilled water at both –20 °C and –80 °C. For phenotypic comparison and DNA–DNA hybridization experiments, *L. rosea* LMG 22534^T and *L. maricola* KCTC 12863^T were grown on marine agar (MA, Difco).

Cell morphology and motility, in a 3-day culture grown on YE-SW agar at 30 °C, were investigated using phase-contrast light microscopy and transmission electron microscopy. Colonial morphology was determined using a stereoscopic dissecting microscope. Growth on yeast extract-malt extract (YE) agar, nutrient agar (NA; Difco), Trypticase soy agar (TSA; Difco) and MA was investigated. Growth was also determined at 4, 10, 20, 30, 35, 40 and 42 °C on YE-SW agar at pH 7.2 and then at pH 4.0–12.0 (at intervals of 1.0 pH unit) on the same medium at 30 °C. The pH of the agar was adjusted, with 6 M HCl and 10 M NaOH, after autoclaving. Hydrolysis tests were performed on MA supplemented with 1 % (w/v) agar, 1 % (w/v)

The GenBank accession number of the 16S rRNA gene sequence of SSW-35^T is DQ533556.

Two supplementary figures are available with the online version of this paper.

skimmed milk, 0.4 % (w/v) carboxymethylcellulose, 1 % (w/v) soluble starch, 1 % (w/v) Tween 80, 0.5 % (w/v) DL-tyrosine, 0.4 % (w/v) hypoxanthine or 0.4 % (w/v) xanthine. Catalase activity was determined with a 3 % (v/v) hydrogen peroxide solution. Hydrolysis of DNA was determined by monitoring the colour change on DNase test agar (Difco) supplemented with 2 % (w/v) sea salts (Sigma) and methyl green. The methods of MacFaddin (1980) were used to investigate Gram reaction and oxidase activity. Tolerance to NaCl was explored using NA supplemented with 0–9 % (w/v) NaCl (at intervals of 1 %). Other physiological and biochemical properties were investigated using API 20 NE, API ZYM and API 50 CH test strips (bioMérieux) according to the manufacturer's instructions.

Cells were short rods (1.1–1.7 µm in length and 0.7 µm wide) that occurred singly or in pairs and were motile, each bearing a polar flagellum (see Supplementary Fig. S1, available in IJSEM Online). Colonies on YE-SW agar were beige in colour, circular, smooth, convex, translucent and 1–3 mm in diameter. The other physiological and biochemical properties of strain SSW-35^T are given in Table 1 and in the species description.

For the analyses of polar lipids and isoprenoid quinones, cell biomass was obtained from a culture grown on YE-SW broth at 30 °C for 3 days. Polar lipids were extracted by the small-scale method of Minnikin *et al.* (1984) and separated by two-dimensional TLC with aluminium-backed silica gel plates (5514, Merck). The phospholipid composition was determined by using various stains and co-developed authentic samples (Minnikin *et al.*, 1977). Isoprenoid quinones were determined by HPLC, as described by Kroppenstedt (1985). For cellular fatty acid analysis, cells were grown on MA for 3 days at 30 °C. The fatty acid profile was then determined by GC, following the standard protocol of version 2.11 of the Sherlock Microbial Identification System (MIDI) and using the TSBA (version 3.9), CLIN (version 3.9) and MI7H10 (version 3.8) databases in the AEROBE package for the identifications.

In the polar lipid analysis (see Supplementary Fig. S2), diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and an unknown lipid were detected as major components. Two unknown phospholipids were also detected in small amounts. The predominant ubiquinone was Q-10. The cellular fatty acid

Table 1. Characteristics that differentiate strain SSW-35^T from recognized members of the genus *Loktanella*

Taxa; 1, strain SSW-35^T (data from this study); 2, *L. agnita* (Ivanova *et al.*, 2005); 3, *L. atrilutea* (Hosoya & Yokota, 2007); 4, *L. fryxellensis* (Van Trappen *et al.*, 2004); 5, *L. hongkongensis* (Lau *et al.*, 2004); 6, *L. koreensis* (Weon *et al.*, 2006); 7, *L. maricola* (Yoon *et al.*, 2007 and this study); 8, *L. pyoseonensis* (Moon *et al.*, 2010); 9, *L. rosea* (Ivanova *et al.*, 2005 and this study); 10, *L. salsilacus* (Van Trappen *et al.*, 2004); 11, *L. vestfoldensis* (Van Trappen *et al.*, 2004). +, Positive; –, negative; w, weakly positive; ND, no data available; v, variable according to the test used.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Motility	+	–	+	–	–	–	–	+	–	–	–
Colony colour	Beige	Whitish	Beige	Pink–beige	Pink–white	Beige	Light orange	Pink	Pink	Beige	Pink
Diffusible pigment	–	–	–	–	Brown	–	–	–	–	–	–
Growth on TSA and NA	–	–	–	–	w	–	–	–	–	–	–
Temperature range for growth (°C)	4–30	8–35	8–30	5–25	8–44	5–30	4–34	4–30	4–35	5–30	5–37
NaCl range for growth (% w/v)	1–6	3–6	0–8	0–5	2–14	1–5	1–7	1–12	1–12	0–10	0–10
Nitrate reduction	–	w	–	–	–	+	–	+	–	–	–
Hydrolysis of:											
Urea	–	–	–	–	–	–	–	–	–	–	+
Gelatin	–	–	ND	–	–	w	+	–	–	–	–
Starch	+	–	–	ND	ND	–	–	–	–	–	–
Tween 80	–	–	+	+	–	–	w	–	w	+	+
Enzyme activity											
α-Galactosidase	–	+	+	–	ND	–	–	–	–	+	–
α-Glucosidase	+	ND	+	w	ND	–	–	–	w	w	w
β-Galactosidase	+	ND	–	+	ND	w	+	w	+	–	w
β-Glucosidase	–	ND	–	+	ND	w	–	–	w	w	w
Leucine arylamidase	+	ND	+	+	ND	+	w	+	–	+	w
N-Acetyl-β-glucosaminidase	+	ND	–	–	ND	–	–	w	–	–	–
Trypsin	–	–	–	–	ND	–	–	+	–	–	+
Carbohydrate metabolism	–	–	v	–	v	–	+	+	–	–	–
DNA G + C content (mol%)	55.0	59.1	66.3	65.7–66.4	65.9–66.2	60.0	56.8	67.5	60.5–61.8	59.6–60.4	62.1–63.1

*Result differs from that reported by Yoon *et al.* (2007).

profile of strain SSW-35^T was characterized by the predominance of straight, saturated and unsaturated acids, with small amounts of hydroxyl fatty acids. This pattern is typical of the genus *Loktanella* (Van Trappen *et al.*, 2004; Lau *et al.*, 2004; Ivanova *et al.*, 2005; Weon *et al.*, 2006; Yoon *et al.*, 2007; Moon *et al.*, 2010). The predominant cellular fatty acid was C_{18:1} (summed feature 7; 60.6%), with C_{12:1} 3-OH and C_{10:0} 3-OH present as 3-hydroxy fatty acids. The cellular fatty acid profiles of strain SSW-35^T and other members of the genus *Loktanella* are summarized in Table 2. Strain SSW-35^T could be differentiated from its phylogenetically closest established relatives, *L. rosea* LMG 22534^T and *L. maricola* KCTC 12863^T, by the presence/absence of 3-hydroxy fatty acids and some saturated and 10-methyl fatty acids.

For genotypic and phylogenetic study, cells were grown in YE-SW broth at 30 °C for 3 days. Chromosomal DNA was extracted and purified by using the Wizard genomic DNA

purification kit (Promega) according to the manufacturer's instructions. Amplification and sequencing of the 16S rRNA gene were performed as described by Lee (2006). Multiple alignments of sequences were made in CLUSTAL_X (Thompson *et al.*, 1997) and then manually optimized according to the secondary structure of the 16S rRNA of *Escherichia coli* (Brosius *et al.*, 1978). Phylogenetic analyses were carried out by using neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. The evolutionary distances used for constructing a neighbour-joining tree, with *Hyphomonas polymorpha* DSM 2665^T (GenBank accession no. AJ227813) as the outgroup taxon, were calculated according to the model of Jukes & Cantor (1969). Bootstrap analysis (Felsenstein, 1985) was performed, with 1000 data resamplings. The G+C content of the genomic DNA of strain SSW-35^T was determined by HPLC (Mesbah *et al.*, 1989).

Table 2. Cellular fatty acid profiles of strain SSW-35^T and recognized members of the genus *Loktanella*

Taxa; 1, strain SSW-35^T (this study); 2, *L. rosea* LMG 22534^T (this study); 3, *L. maricola* KCTC 12863^T (this study); 4, *L. hongkongensis* NRRL B-41039^T (this study); 5, *L. pyoseonensis* JJM85^T (this study); 6, *L. agnita* R10SW5^T (Ivanova *et al.*, 2005); 7, *L. atrilutea* IG8^T (Hosoya & Yokota, 2007); 8, *L. koreensis* GA2-M3^T (Weon *et al.*, 2006); 9, *L. fryxellensis* (12 strains; Van Trappen *et al.*, 2004); 10, *L. salsilacus* (10 strains; Van Trappen *et al.*, 2004); 11, *L. vestfoldensis* (four strains; Van Trappen *et al.*, 2004). All strains were grown on MA for 2 days at 30 °C. Values shown are percentages (or mean percentages $\pm$ standard deviation) of total fatty acids. —, <1 % of total fatty acids or not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
C _{10:0} 3-OH	3.0	—	2.3	2.5	1.7	1.0	1.5	2.4	3.7 $\pm$ 1.1	2.4 $\pm$ 0.7	6.1 $\pm$ 1.5
C _{11:0} 2-OH	—	—	1.8	—	—	—	—	—	—	—	—
C _{12:1} 3-OH	2.2	3.0	2.8	—	—	—	—	3.4	—	—	5.6 $\pm$ 1.4
C _{12:0}	—	5.8	—	—	—	—	—	—	—	—	—
C _{12:0} 3-OH	—	—	—	1.4	1.0	—	—	—	—	—	—
C _{14:0}	1.6	—	1.1	1.2	1.1	4.6	—	—	—	—	—
C _{14:1}	—	—	—	—	—	4.6	—	—	—	—	—
C _{15:0}	—	—	—	—	—	4.6	—	—	—	—	—
C _{16:0}	14.8	11.5	13.3	14.2	14.7	4.6	9.5	6.9	2.7 $\pm$ 1.1	2.9 $\pm$ 0.9	2.9 $\pm$ 0.7
C _{16:1} ω 7c	—	—	—	—	—	1.9	—	—	—	—	—
C _{17:0}	—	—	—	—	—	1.7	—	—	—	—	—
C _{18:1} (summed feature 7)*	65.0	63.4	63.5	64.9	74.0	83.4	74.4	66.6	84.9 $\pm$ 3.7	87.7 $\pm$ 1.9	74.1 $\pm$ 3.1
C _{18:1} ω 9c	—	—	—	—	—	—	—	—	—	—	—
C _{18:0}	5.2	6.6	5.9	5.2	3.9	—	2.2	9.3	1.6 $\pm$ 0.9	1.4 $\pm$ 0.8	1.8 $\pm$ 0.3
C _{18:1} ω 7c 11-methyl	—	—	—	—	—	—	6.3	8.7	—	—	1.9 $\pm$ 0.8
C _{18:2}	—	—	—	—	—	1.8	—	—	—	—	—
C _{19:1} cyclo	—	—	—	—	—	1.7	—	—	—	—	—
C _{18:0} 10-methyl	—	—	1.0	1.0	—	—	—	—	—	—	—
Other summed features											
2	—	—	—	—	—	—	3.8	—	1.7 $\pm$ 0.7	—	—
3	—	—	—	—	—	—	—	—	—	2.8 $\pm$ 0.9	—
4	1.9	—	—	—	—	—	—	—	—	—	—
Unknown ECL 9.521†	—	5.2	—	—	—	—	—	—	—	—	—
Unknown ECL 11.799	—	—	—	—	—	—	—	—	—	—	2.3 $\pm$ 1.2

*Summed features are groups of two or more fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 2 comprises C_{12:0} aldehyde, C_{16:1} iso I, C_{14:0} 3-OH and/or unknown 10.928; summed feature 4 comprises C_{16:1} ω 7c and/or C₁₅ iso 2-OH; summed feature 7 comprises C_{18:1} ω 7c, ω 9c and/or ω 12t.

†ECL, equivalent chain length.

The almost complete 16S rRNA gene sequence (1352 nt) of strain SSW-35^T determined in this study was compared with representative sequences of members of the genus *Loktanella* and related taxa. A phylogenetic tree (Fig. 1) indicated that strain SSW-35^T belonged to the genus *Loktanella* and was closely related to *L. rosea* FG36^T (98.1 % sequence similarity) and *L. maricola* DSW-18^T (97.8 %). The 16S rRNA gene sequence similarities between strain SSW-35^T and the other recognized species of the genus *Loktanella* were <97.0 %. At 55.0 mol%, the genomic DNA G+C content of strain SSW-35^T was lower than the values (59.1–67.5 %) reported previously for the genus *Loktanella* (Moon *et al.*, 2010).

The photobiotin-label method (Ezaki *et al.*, 1989) was used to study DNA–DNA hybridizations, with DNA–DNA relatedness determined fluorometrically (Yoon *et al.*, 2007) using five replications for each sample. Strain SSW-35^T showed levels of DNA–DNA relatedness of 11 % and 8 % to the type strains of its closest neighbours, *L. rosea* LMG 22534^T and *L. maricola* KCTC 12863^T, respectively. The level of DNA–DNA relatedness for a reciprocal DNA–DNA hybridization, of *L. rosea* LMG 22534^T to strain SSW-35^T, was 15.5 %.

The characteristics of strain SSW-35^T that distinguish it from the recognized species of the genus *Loktanella* are given

in Tables 1 and 2. Although cells of strain SSW-35^T were motile, each having a polar flagellum, those of *L. rosea* LMG 22534^T and *L. maricola* KCTC 12863^T are non-motile. Colony pigmentation, temperature, pH and NaCl ranges for growth, hydrolysis properties, some enzyme activities and genomic DNA G+C content could also be used to separate the novel strain from its recognized, phylogenetically close neighbours (Table 1). Phenotypic and genetic characteristics and phylogenetic analysis demonstrate that strain SSW-35^T represents a novel species within the genus *Loktanella*, for which the name *Loktanella tamlensis* sp. nov. is proposed.

Emended description of the genus *Loktanella* Van Trappen *et al.* 2004 emend. Moon *et al.* 2010

The description is as given by Moon *et al.* (2010) with the following amendment. The range of genomic DNA G+C contents is 55.0–67.5 mol%.

Description of *Loktanella tamlensis* sp. nov.

Loktanella tamlensis (tam.len'sis. N.L. fem. adj. *tamlensis* of or belonging to Tamla, the old name of Jeju, Republic of Korea, referring to the site where the type strain was isolated).

Fig. 1. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between strain SSW-35^T and other members of the genus *Loktanella*. *Hyphomonas polymorpha* DSM 2665^T was used as the outgroup. Asterisks indicate branches of the tree that were also recovered using maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) treeing algorithms. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branch points. Bar, 0.02 substitutions per nucleotide position.

Aerobic, Gram-reaction-negative, oxidase- and catalase-positive. Cells are short rods (1.1–1.7 µm in length and 0.7 µm wide) that occur singly or in pairs and are motile by means of a polar flagellum. After incubation for 3 days on YE-SW agar, colonies are beige-coloured, circular, smooth, convex, translucent and 1–3 mm in diameter. No diffusible pigment has been detected on any of the media tested. Endospores are not formed. Does not grow on YE agar, TSA or standard NA. Mesophilic. Growth occurs on YE-SW agar at 4–30 °C (optimum between 25 and 30 °C), but not at ≥35 °C, on the same medium at pH 7.0–10.0 (optimum between pH 7.0 and 8.0) and on NA in the presence of 1–6 % (w/v) NaCl. Produces H₂S. Hydrolyses agar, casein and carboxymethylcellulose but does not degrade hypoxanthine, DL-tyrosine or xanthine. No growth is observed with any carbohydrate as a single carbon source (API 20 NE) and acid is not produced from any substrate (API 50 CH). In API ZYM tests, positive reactions for alkaline phosphatase, esterase (C4), esterase lipase (C8) and acid phosphatase, but negative reactions for lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, α-mannosidase and α-fucosidase. In API 20 NE tests, positive result for aesculin degradation and β-galactosidase but negative result for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. The other physiological and biochemical properties are given in Table 1. Polar lipid analysis reveals the presence of diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and an unknown lipid as major components, plus two unknown phospholipids in small amounts. Q-10 is the predominant ubiquinone. The predominant cellular fatty acid is C_{18:1} (summed feature 7). The 3-hydroxy fatty acids are C_{12:1} 3-OH and C_{10:0} 3-OH.

The type strain SSW-35^T (=KCTC 12722^T=JCM 14020^T) was isolated from seawater collected from Samyang Beach in Jeju, Republic of Korea. The genomic DNA G+C content of the type strain is 55.0 mol%.

Acknowledgements

This work was supported by a research grant from the Chuongbong Academic Research Fund of the Jeju National University that was awarded in 2009. The author thanks H. L. Yang for the analyses of cellular fatty acids and the DNA–DNA hybridizations.

References

Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W. M. (1971). Towards defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Hosoya, S. & Yokota, A. (2007). *Loktanella atrilutea* sp. nov., isolated from seawater in Japan. *Int J Syst Evol Microbiol* **57**, 1966–1969.

Ivanova, E. P., Zhukova, N. V., Lysenko, A. M., Gorshkova, N. M., Sergeev, A. F., Mikhailov, V. V. & Bowman, J. P. (2005). *Loktanella agnita* sp. nov. and *Loktanella rosea* sp. nov., from the north-west Pacific Ocean. *Int J Syst Evol Microbiol* **55**, 2203–2207.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

Lau, S. C. K., Tsoi, M. M. Y., Li, X., Plakhotnikova, I., Wu, M., Wong, P.-K. & Qian, P.-Y. (2004). *Loktanella hongkongensis* sp. nov., a novel member of the α-Proteobacteria originating from marine biofilms in Hong Kong waters. *Int J Syst Evol Microbiol* **54**, 2281–2284.

Lee, S. D. (2006). *Kineococcus marinus* sp. nov., isolated from marine sediment of the coast of Jeju, Korea. *Int J Syst Evol Microbiol* **56**, 1279–1283.

MacFaddin, J. F. (1980). *Biochemical Tests for Identification of Medical Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Moon, Y. G., Seo, S. H., Lee, S. D. & Heo, M. S. (2010). *Loktanella pyoseonensis* sp. nov., isolated from beach sand, and emended description of the genus *Loktanella*. *Int J Syst Evol Microbiol* **60**, 785–789.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Van Trappen, S., Mergaert, J. & Swings, J. (2004). *Loktanella salsilacus* gen. nov., sp. nov., *Loktanella fryxellensis* sp. nov. and *Loktanella vestfoldensis* sp. nov., new members of the *Rhodobacter* group, isolated from microbial mats in Antarctic lakes. *Int J Syst Evol Microbiol* **54**, 1263–1269.

Weon, H.-Y., Kim, B.-Y., Yoo, S.-H., Kim, J.-S., Kwon, S.-W., Go, S.-J. & Stackebrandt, E. (2006). *Loktanella koreensis* sp. nov., isolated from sea sand in Korea. *Int J Syst Evol Microbiol* **56**, 2199–2202.

Yoon, J.-H., Kang, S.-J., Lee, S.-Y. & Oh, T.-K. (2007). *Loktanella maricola* sp. nov., isolated from seawater of the East Sea in Korea. *Int J Syst Evol Microbiol* **57**, 1799–1802.