

# *Loktanella aquimaris* sp. nov., Isolated from Seawater

Kunho Kim<sup>1</sup> · Sathiyaraj Srinivasan<sup>2</sup> · Sang-Seob Lee<sup>2</sup>

Received: 15 June 2015 / Accepted: 3 October 2015 / Published online: 25 November 2015  
© Springer Science+Business Media New York 2015

**Abstract** Two novel bacterial strains, designated C5<sup>T</sup> and C9 were isolated from a fish cage at Tongyeong, South Korea and were characterized to determine their taxonomic position. The strains were Gram-negative, non-motile, strictly aerobic and short rod shaped. Growth occurred between 20 and 32 °C (optimum 30 °C) and at pH 6.0–9.0 (optimum pH 8.0) and at 0–10 % NaCl (optimum at 2 % NaCl). Based on the 16S rRNA gene sequence analysis, they were identified as a member of the genus *Loktanella* that belongs to the phylum *Proteobacteria*. Strains C5<sup>T</sup> and C9 were analyzed by a polyphasic approach, revealing variations in their phenotypic characters but reciprocal DNA–DNA hybridization values confirmed that they belong to the same species. Phylogenetic analysis of the 16S rRNA gene sequences of closely related species indicated their similarities were below 97 %. The predominant isoprenoid quinone is ubiquinone-10 (Q-10). The major cellular fatty acids were C<sub>16:0</sub>, and C<sub>18:1 ω7c</sub>. Major polar lipid contained phosphatidylglycerol (PG), phosphatidylcholine (PC), diphosphatidylglycerol (DPG), phosphatidylethanolamine

(PE), unidentified amino lipid (AL) and unidentified lipids (L<sub>1–3</sub>). Based on phylogenetic and phenotypic data, the isolated strains represent a novel species of the genus *Loktanella*, for which the name *Loktanella aquimaris* sp. nov. is proposed. The type strains were C5<sup>T</sup> (=KEMB 3-892<sup>T</sup> = JCM 30382<sup>T</sup>), and a second strain is C9 (=KEMB 3-893 = JCM 30383).

**Keywords** Bacteria · *Loktanella* · Polyphasic · Taxonomy

## Introduction

The genus *Loktanella* was first described by Van Trappen et al. [16] with three species, *Loktanella salsilacus* LMG 21507<sup>T</sup> (type strain), *Loktanella fryxellensis* LMG 22007<sup>T</sup> (type strain) and *Loktanella vestfoldensis* DSM 16212<sup>T</sup>. Many other bacterial species have been reported as belonging to this genus and at the time of writing, the genus *Loktanella* comprises more than 18 species (<http://www.bacterio.net/deinococcus.html>). Many member of the genus *Loktanella* have been found within different ecological niches, but mostly seawater and sea sand. A taxonomic study was performed on 2 isolated strains from a fish cage in Tongyeong, South Korea. Strains C5<sup>T</sup> and C9 identified as a member of a novel species of the genus *Loktanella*.

## Materials and Methods

### Isolation of Bacteria Strains and Culture Conditions

Two strains (designated as C5<sup>T</sup> and C9) were collected in South of Korea and isolated from a fish cage, Tongyeong (GPS: 34°59′38.19″N, 128°40′26.38″E). Two gram-negative, non-motile, strictly aerobic, short-rod shaped bacterial

**Electronic supplementary material** The online version of this article (doi:10.1007/s00284-015-0945-0) contains supplementary material, which is available to authorized users.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA sequence of strain C5<sup>T</sup> (=KEMB 3-892<sup>T</sup> = JCM 30382<sup>T</sup>) is KP716799 and C9 (=KEMB 3-893<sup>T</sup> = JCM 30383<sup>T</sup>) is KP883300.

✉ Sang-Seob Lee  
sslee@kyonggi.ac.kr

<sup>1</sup> Department of Biological Engineering, Graduate school, Kyonggi University, 94-6 Iui-dong Yeongtong-gu, Suwon 443-760, Republic of Korea

<sup>2</sup> Department of Life Science, College of Natural Science, Kyonggi University, 94-6 Iui-dong Yeongtong-gu, Suwon 443-760, Republic of Korea

strains designated C5<sup>T</sup> and C9 was identified as the novel member of the genus *Loktanella*. To isolate the novel strains, the filtered sample were placed in 100 ml flask, inoculated with 10 ml of 10<sup>-1</sup> diluted marine broth (Difco) and incubated aerobically in the shaking incubator (150 rpm, 28 °C) for 72 h. After incubation, using 10<sup>-5</sup>, 10<sup>-6</sup> diluted sample, 100 µl of the aliquot was spread onto marine agar (10<sup>-1</sup> dilution) and incubated at 28 °C for 3 days. Single colonies were isolated and aerobically sub-cultured into new marine agar and incubated again for 3 additional days. All experiments regarding strains C5<sup>T</sup> and C9 and its reference strains were stored and sub-cultured on marine agar media at pH 7.2 ± 0.2 aerobically. Type strains used as references were *Loktanella salsilacus* JCM 21636<sup>T</sup> [16], *Loktanella atrilutea* JCM 23210<sup>T</sup> [18], *Loktanella fryxellensis* JCM 21635<sup>T</sup> [16], *Loktanella litorea* KCTC 23883<sup>T</sup> [7] and *Loktanella cinnabarina* JCM 18161<sup>T</sup> [22] for comparative purposes.

### Phylogenetic Analysis

Strains C5<sup>T</sup> and C9 were sent for 16S rRNA sequencing on an Applied Biosystems model 3730XL automated DNA-sequencing system using 9F and 1492R primers (Applied Biosystems, USA) at Macrogen Inc. Seoul, South of Korea. The complete 16S rRNA gene sequences were compiled with SeqMan software (DNASTAR Inc.) [5, 21]. The selected colonies were tentatively identified using partial 16S rRNA gene sequences, using the EzTaxon-e server (<http://eztaxon-e.ezbiocloud.net>) [8]. Evolutionary differences were calculated using the Kimura two-parameter model. Phylogenetic trees were constructed using the neighbour-joining [14], maximum-parsimony [7] and maximum-likelihood methods in MEGA5 program [20] with bootstrap values based on 1000 replications [3, 4].

### Phenotypic and Biochemical Characteristics

Cells were grown on marine agar for 2–3 days before their descriptive examination. Gram reaction was carried out the classic Gram staining described by Doetsch [1]. API ZYM, 20NE, and ID32GN were used according to the manufacturer recommendations (bioMérieux) in order to examine their utilization of carbon source and enzymatic activities. *p*-dimethyl aminobenzaldehyde with HCl and amyl alcohol (Kovac's reagent) in SIM agar was used for indole test. For methyl red test and Voges-Proskauer test, incubated MR-VP broth for 2 days with methyl red indicator and Barritt's reagents. Citrate utilization test was checked Simmons citrate agar. Phenylalanine deaminase test was assessed using 10 % (w/v) ferric chloride solution. Nitrate reduction test was basically a nutrient broth supplemented with 0.1 % potassium nitrate as the nitrate substrate. C5<sup>T</sup> and C9

ability to reduce nitrates to nitrites was determined by the addition of sulfanilic acid and  $\alpha$ -naphthylamine. Casein test was conducted on marine agar with 2 % casein acid hydrolysate. Anaerobic growth was examined in serum bottles with marine broth and the upper air layer was replaced with nitrogen. Range and optimal temperature growth on marine broth was examined for 3 days at different combinations of temperatures (4, 10, 15, 20, 25, 30, 32, 37, 40 and 45 °C), and (4.0, 5.0, 6.0, 7.0, 8.0 and pH 9.0) at 30 °C. Optimal salt requirement on marine agar was examined for 3 days at levels 0, 1, 2, 3, 5, 7, 10, 15, 20 and 25 % NaCl (w/v). Growth media was observed on only marine agar.

### Chemotaxonomic Characteristics

Respiratory quinones were extracted from freeze-dried bacterial cells of strains C5<sup>T</sup> and C9 with chloroform/methanol (2:1, v/v), evaporated under vacuum and re-extracted in hexane. The quinone solution was purified using silica Sep-Pak Vac 6 cc cartridges (Waters Solid Phase Extraction) and subsequently analyzed by high performance liquid chromatography (HPLC), as described previously [6].

The analysis of fatty acid methyl ester was performed after growth of bacteria on marine agar for 3 days at 30 °C and exponential phase bacterial cells were harvested in order to prepare, separate, and identify the fatty acid methyl esters with the Sherlock Microbial Identification System, produced by MIDI, Inc., Newark, DE, USA [15].

For detection of polar lipids, cells of strains C5<sup>T</sup> and C9 were grown in marine broth at 30 °C, examined by two-dimensional TLC [9, 12, and 13] and identified by two-dimensional TLC followed by spraying with appropriate detection reagents. Total lipid profile was detected by spraying with molybdophosphoric acid solution (Sigma-Aldrich; St. Louis, MO) followed by heating at 150 °C; free-aminolipids by spraying with 0.2 % (w/v) ninhydrin solution followed by heating at 100 °C for 5 min; phospholipids by spraying with Zinzadze reagent (Sigma-Aldrich; St. Louis, Mo).

### Genomic Characteristics

In order to determine the DNA G + C content of strains C5<sup>T</sup> and C9, genomic DNA was extracted using the Marmur method [10]. DNA was separated by nuclease P1 (100 U/ml, pH 5.3) at 37 °C for 1 h. The separated nucleosides were then analyzed using reverse-phase HPLC [11, 19]. DNA–DNA hybridizations were carried out with photobiotin-labelled probes in microplate wells as described by Ezaki et al. [2] using a Multilabel Reader (Perkin Elmer) for fluorescence measurements.

**Table 1** Comparison of phenotypic characters of C5<sup>T</sup>, C9 and the type strains of related *Loktanella* species

Characteristic	1	2	3	4	5	6	7
Morphology	short rods	short rods	short rods	rod	short rods	rod	rod
Colony color	Beige	Beige	Beige	Beige	Beige	Light yellow	cinnabar
Motility	–	–	–	+	–	–	–
NaCl (%)	0–10	0–10	0–10	0–8	0–5	0–6	1–8
Ph	7–9	7–9	6–9	6–8	5–8	6–8	6–8
Temperature (°C)	25–32	25–32	5–30	8–30	5–25	15–37	15–35
Enzyme activity (API ZYM)							
Lipase (C14)	–	–	–	+	–	–	+
Cystine arylamidase	–	+	+	+	–	+	–
α-Galactosidase	–	–	+	–	+	–	+
β-Galactosidase (ONPG)	–	–	–	+	–	–	–
α-Glucosidase	–	–	+	+	–	–	+
(starch hydrolysis)							
Assimilation (API ID32GN&20 NE)							
Nitrate reduction to NO <sub>2</sub>	+	+	–	+	+	+	+
Urease	–	+	+	+	+	+	–
D-Glucose	w	w	+	+	w	+	w
L-Arabinose	–	–	+	+	+	–	+
D-Mannose	–	–	+	–	w	+	w
D-Maltose	+	–	+	+	–	+	–
Gluconate	–	–	–	–	–	–	w
Citrate	–	–	+	+	+	+	+
N-Acetyl-D-glucosamine	+	+	+	+	–	–	+
Suberate	+	+	+	+	–	+	–
L-Alanine	+	+	+	–	+	–	+
Glycogen	+	+	+	+	w	–	+
L-Histidine	+	+	–	–	–	–	–
GC mol%	61.0 %	61.4 %	62.1 %	66.3 %	67.4 %	57.6 %	69.3 %

All the data obtained in the study and experiments were conducted under same condition. +, positive; –, negative; w, week reaction. Taxa: 1, Strain C5<sup>T</sup>; 2, Strain C9; 3, *L. salsilacus* JCM 21636<sup>T</sup>; 4, *L. atrilutea* JCM 23210<sup>T</sup>; 5, *L. fryxellensis* JCM 21635<sup>T</sup>; 6, *L. litorea* KCTC 23883<sup>T</sup>; 7, *L. cinnabarina* JCM 18161<sup>T</sup>

## Results and Discussion

### Morphological and Physiological Characteristics

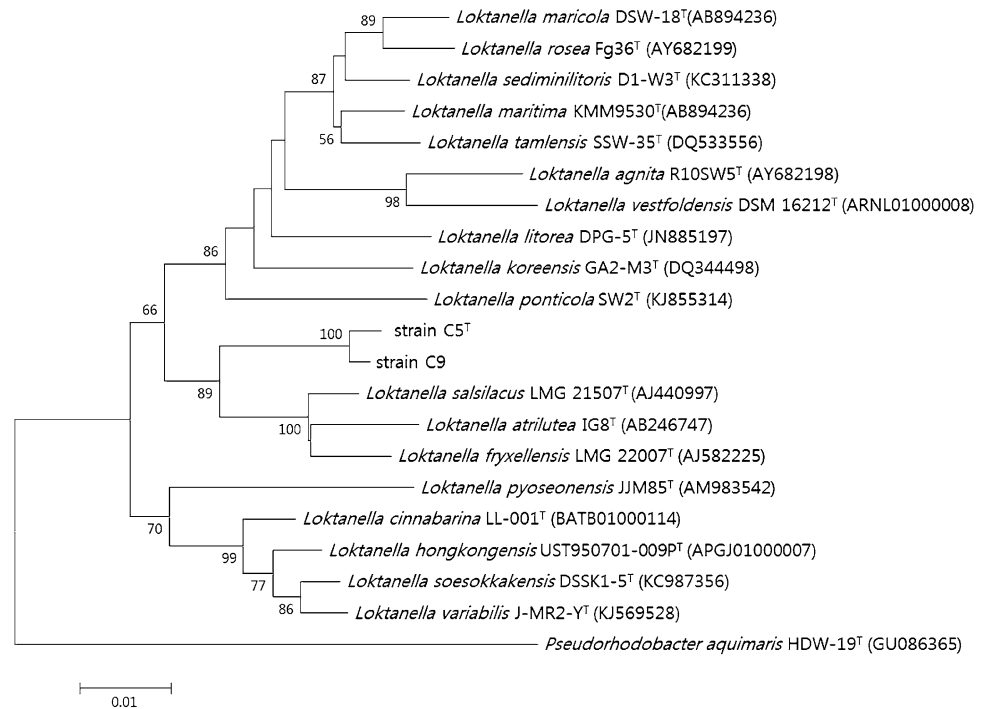
Strains C5<sup>T</sup> and C9 were found to be Gram negative, strictly aerobic, short rod shaped, 1.0–3.0 μm long and 0.5–1.0 μm wide. When cultured on marine agar at 30 °C, colonies were beige color, circular, and convex. Strains C5<sup>T</sup> and C9 grew between 25–32 °C and pH 7.0–9.0. Optimum growth of this novel strains were observed at 30 °C and pH 8.0. It also grew in the presence of 0–10 % NaCl (w/v). Optimum NaCl concentration was 2 % (w/v). Results for the physiological characteristics of Strains C5<sup>T</sup> and C9 are summarized in the species description and

comparison of discriminating characteristics of reference strains are shown in Table 1.

### Phylogenetic Analysis

Almost complete sequences of the 16S rRNA genes were compiled with SeqMan software (DNASTAR Inc.). The 16S rRNA gene sequence of strains C5<sup>T</sup> and C9 (GenBank/EMBL/DDBJ accession numbers KP716799<sup>T</sup> and KP883300, respectively) show 99.2 % similarity. A phylogenetic analysis of the 16S rRNA gene sequences showed that strains (C5<sup>T</sup> and C9) belongs to the phylum *Proteobacteria*, class *Alphaproteobacteria*, order *Rhodobacteriales* and family *Rhodobacteraceae*. *Loktanella*

**Fig. 1** Phylogenetic tree of strains C5<sup>T</sup> and C9 with closely related *Loktanella* type strains based on 16S rRNA gene sequence comparisons. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationship between strain C5<sup>T</sup> and the type strains of closely related members of the genus *Loktanella*. Bootstrap values of >50 % (percentages of 1000 replications) are shown at branching points. The sequences used for the comparative study are included in parentheses



**Table 2** Fatty acid composition of strain C5<sup>T</sup>, C9 and the type strains of closely related species in the genus *Loktanella*. Taxa: 1, Strain C5<sup>T</sup>; 2, Strain C9; 3, *L. salsilacus* JCM 21636<sup>T</sup>; 4, *L. atrilutea* JCM 23210<sup>T</sup>; 5, *L. fryxellensis* JCM 21635<sup>T</sup>; 6, *L. litorea* KCTC 23883<sup>T</sup>; 7, *L. cinnabarina* JCM 18161<sup>T</sup>

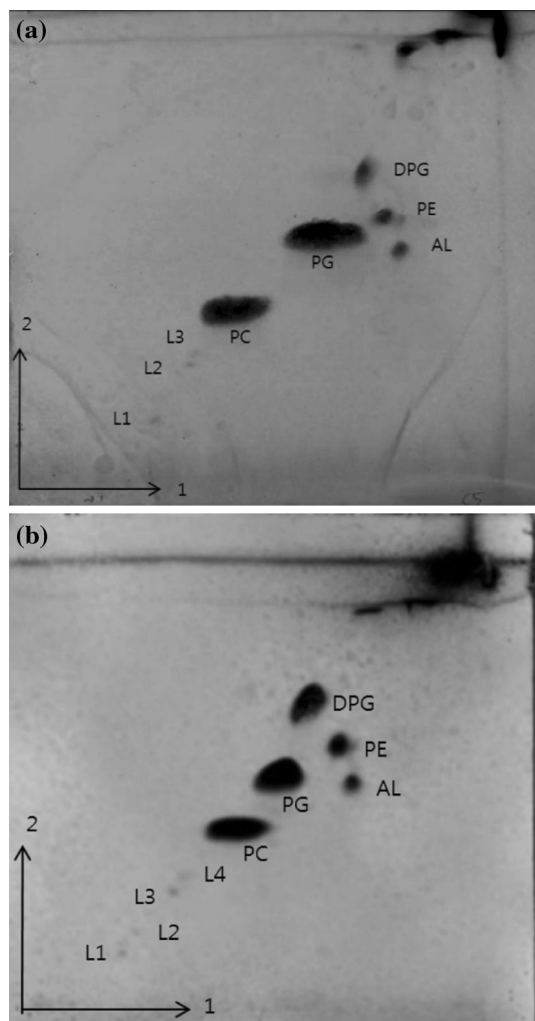
Fatty acid	1	2	3	4	5	6	7
C <sub>10:0</sub> 3OH	3.0	1.2	1.6		1.0	2.8	1.2
C <sub>14:0</sub>	0.2	0.3					0.4
C <sub>14:0</sub> 3OH/C <sub>16:1</sub> iso I	0.7	0.1	0.5	3.1	0.3		
C <sub>16:1</sub> ω7c/16:1 ω6c summed feature 3	1.1	0.9	3.0	1.0	2.6	1.1	4.7
C <sub>16:0</sub>	11.8	12.0	4.0	23.1	3.9	5.3	19.5
C <sub>17:1</sub> ω8c	0.2	0.2	0.6	2.5	0.7	0.4	
C <sub>17:0</sub>	0.8	0.3	1.1		1.7	0.8	
C <sub>18:1</sub> ω7c	78.0	77.2	82.1	52.9	81.9	67.5	68.1
C <sub>18:0</sub>	2.0	1.8	2.2	4.4	2.0	2.4	4.1
C <sub>18:1</sub> ω7c 11-methyl	1.2	3.5	2.3	5.6	2.9	17.2	0.4
C <sub>19:0</sub> 10-methyl	0.5	0.4					
Un18.846/C <sub>19:1</sub> ω6c summed feature 7	0.4	1.4	2.5		2.4	1.4	

All data were obtained in this study. Cells were grown on marine agar for 72 h at 30 °C. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. The *cis* isomers were indicated by the suffixes

*salsilacus* LMG 21507<sup>T</sup> (96.8 %), *Loktanella atrilutea* IG8<sup>T</sup> (96.2 %), *Loktanella fryxellensis* LMG 22007<sup>T</sup> (96.0 %), *Loktanella litorea* DPG-5<sup>T</sup> (95.4 %), *Loktanella cinnabarina* LL-001<sup>T</sup> (95.1 %) were determined to be the most closely related neighbours. The strains C5<sup>T</sup> and C9 joined the cluser comprised of the member of the genus *Loktanella* by neighbor-joining tree (Fig. 1) with the high bootstrap (89 %) value. The phylogenic position of the trains are also cofnrimed by maximum likelihood and maximum parsimony phylogenetic trees (See supplementary figures S1 and S2).

### Chemotaxonomic Characteristics

The cellular fatty acids of strains C5<sup>T</sup> and C9 were C<sub>18:1</sub> ω7c (>77 %) and C<sub>16:0</sub>(>10 %). Minor amounts (<10 %) were found for C<sub>10:0</sub> 3OH, C<sub>14:0</sub>, C<sub>14:0</sub> 3OH/C<sub>16:1</sub> iso I, C<sub>16:1</sub> ω7c/C<sub>16:1</sub> ω6c (Summed Feature 3), C<sub>17:1</sub> ω8c, C<sub>17:0</sub>, C<sub>18:0</sub>, C<sub>18:1</sub> ω7c 11-methyl, Un18.846/C<sub>19:1</sub> ω6c (Summed Feature 7), C<sub>19:0</sub> 10 methyl. The fatty acid profile of strains C5<sup>T</sup> and C9 are shown in Table 2 and compared with other *Loktanella* species.



**Fig. 2** Result of polarlipid picture  $C5^T$  (a) and C9 (b)

**Table 3** DNA–DNA hybridization of strain  $C5^T$  and C9 with *L. salsilacus* JCM 21636<sup>T</sup>

Strains	DNA relatedness (%) with:		
	$C5^T$	C9	JCM 21636 <sup>T</sup>
$C5^T$	100	71.94	29.60
C9	88.61	100	24.45
JCM 21636 <sup>T</sup>	28.96	24.72	100

Polar lipid profile contained phosphatidylglycerol (PG), phosphatidylcholine (PC), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), unidentified amino lipid (AL) and unidentified lipids ( $L_{1-3}$ ). Strains  $C5^T$  and C9 are compared polar lipid in Fig. 2 but strain C9 have unidentified lipid more than  $C5^T$ . *Loktanella* species are characterized as possessed Q-10 as the sole respiratory quinone. Strain  $C5^T$  and C9 also possessed Q-10 as the respiratory quinone.

## Genomic characteristics

The genomic DNA G + C content of strains  $C5^T$  and C9 was 61.0 and 61.6 mol%. Both the strains exhibited high levels of DNA–DNA relatedness values (>72 %), reciprocally, indicating them to belong to the same species (Table 3) [17].

## Taxonomic Conclusion

To conclude, the morphological, physiological, chemotaxonomic and phylogenetic data obtained during this study clearly showed that strains  $C5^T$  and C9 belong to the genus *Loktanella*. Assimilation of L-arabinose, citrate, gluconate, D-mannitol, indole can easily distinguish these strains from phylogenetically close species. We propose strain  $C5^T$  as the novel species of the genus *Loktanella*, with the name *Loktanella aquimaris* sp. nov.

## Description of *Loktanella aquimaris* sp. nov

*Loktanella aquimaris* (a.qui.máris. L. n. *aqua*, water; L. gen. n. *maris*, of the sea; N.L. gen. n. *aquimaris*, of the water of the sea).

Gram negative, strictly aerobic, non-motile, short rod shaped, 1.0–3.0  $\mu\text{m}$  long and 0.5–1.0  $\mu\text{m}$  wide. Colonies were circular, convex and beige on marine agar. Growth of strain  $C5^T$  and C9 occurred grew between temperatures 25–32  $^{\circ}\text{C}$  (optimum 30  $^{\circ}\text{C}$ ). The pH range is 7.0–9.0 (optimum pH 8.0). Growth on salt level 0–10 % NaCl (w/v) (optimum 2–3 %). Casein, citrate,  $\text{H}_2\text{S}$ , nitrate and nitrite reduction, carbonate fermentation, gelatin hydrolysis tests was negative but indole, methyl red, phenylalanine deaminase tests was positive. Positive for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl- $\beta$ -glucosaminidase, Esterase (C4), esterase (C8),  $\beta$ -glucosidase (esculin hydrolysis),  $\beta$ -galactosidase (PNPG), D-glucose, glycogen, L-histidine, indole, leucine arylamidase, suberate, L-alanine and valine arylamidase as a sole carbon and energy source. Variable results are obtained for adipate, *N*-acetyl-D-glucosamine, cystine arylamidase, L-fucose, gluconate, D-glucose, 3-hydroxybenzoate, 4-hydroxybenzoate, 2-ketogluconate ( $\alpha$ ), D,L-lactate, lipase (C14), D-mannose, D-mannitol, D-maltose, D-melibiose, urease and *n*-valerate. Acetate, L-arabinose, arginine dihydrolase, caprate,  $\alpha$ -chymotrypsin, citrate,  $\alpha$ -fucosidase,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase,  $\beta$ -galactosidase (ONPG),  $\alpha$ -glucosidase (starch hydrolysis), D,L-3-hydroxybutyrate, myo-Inositol, 5-ketogluconate,  $\alpha$ -mannosidase, phenyl acetate, protease, L-proline, propionate, D-ribose, salicin, D-sorbitol and trypsin was not utilized as a sole carbon source



and enzyme. All data were obtained in this study. +, positive; −, negative; w, weak (Table 1).

Major polar lipids were phosphatidylglycerol (PG), phosphatidylcholine (PC), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), unidentified amino lipid (AL) and minor polar lipids were unidentified lipid (Fig. 2). The predominant isoprenoid quinone detected in strain was ubiquinone-10 (Q-10). Major fatty acids and isoprenoid quinone of the type strain were C<sub>18:1</sub> ω7c and C<sub>16:0</sub> and ubiquinone-10 respectively. The DNA G + C content was 61.0–61.4 mol%. The type strains C5<sup>T</sup> (=KEMB3-892<sup>T</sup> = JCM 30382<sup>T</sup>) and C9 (=KEMB 3-893 = JCM 30383<sup>T</sup>) was isolated from seawater in Tongyeong, South of Korea.

**Acknowledgments** This research is supported by National Research foundation of Korea (NRF-2013M3A2A1067498) and (NRF-2010-0007473).

## References

- Doetsch RN (1981) Determinative methods of light microscopy. In: Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, Krieg NR, Phillips GH (eds) Manual of methods for general bacteriology. American Society for Microbiology, Washington, pp 21–33
- Ezaki T, Hashimoto Y, Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Evol Microbiol* 39:224–229
- Felsenstein J (1985) Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791
- Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 20:406–416
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98
- Hiraishi A, Ueda Y, Ishihara J, Mori T (1996) Comparative lipoquinone analysis of influent sewage and activated sludge by high performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* 42:457–469
- Yoon JH, Jung YT, Lee JS (2013) *Loktanella litorea* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* 63:175–180
- Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH et al (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 62:716–721
- Komagata K, Suzuki K (1987) Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 19:1–207
- Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* 3:208–218
- Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 39:159–167
- Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microb Methods* 2:233–241
- Minnikin DE, Patel PV, Alshamaony L, Goodfellow M (1977) Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* 27:104–117
- Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. MIDI Inc, Newark
- Stefanie VT, Joris M, Jean S (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
- Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* 44:846–849
- Shoichi H, Akira Y (2007) *Loktanella atrilutea* sp. nov., isolated from seawater in Japan. *Int J Syst Evol Microbiol* 57:1966–1969
- Tamaoka J, Komagata K (1984) Determination of DNA base composition by reversed phase high-performance liquid chromatography. *FEMS Microbiol Lett* 25:125–128
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 24:4876–4882
- Taishi T, Yasuhiro S, Mori K, Masayuki M et al (2013) *Loktanella cinnabarina* sp. nov., isolated from a deep seafloor sediment, and emended description of the genus *Loktanella*. *Int J Syst Evol Microbiol* 63:1390–1395