INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Jung *et al.*, *Int J Syst Evol Microbiol* 2018;68:289–293

DOI 10.1099/ijsem.0.002499

MICROBIOLOGY SOCIETY

Albirhodobacter confluentis sp. nov., isolated from an estuary

Hye Su Jung, Jaegeun Lee, Jong Woo Hyeon, Seogang Hyun* and Che Ok Jeon*

Abstract

A Gram-stain-negative and strictly aerobic, moderately halophilic bacterium, designated strain S1-47^T, was isolated from estuary sediment in South Korea. Cells were non-motile rods showing oxidase- and catalase-positive activities. Growth was observed at 10–30 °C (optimum, 25 °C), at pH 5.0–8.0 (optimum, pH 6.0–7.0) and in the presence of 0–6.0 % (w/v) NaCl (optimum, 2.0 %). Strain S1-47^T contained summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), summed feature 8 (comprising $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and $C_{16:0}$ as major cellular fatty acids and ubiquinone-10 as the sole isoprenoid quinone. Phosphatidylethanolamine, an unidentified aminolipid, an unidentified phospholipid and three unidentified lipids were detected as polar lipids. The G+C content of the genomic DNA was 69.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain S1-47^T formed a tight phylogenetic lineage with *Albirhodobacter marinus* N9^T. Strain S1-47^T was most closely related to *Albirhodobacter marinus* N9^T with a 99.4 % 16S rRNA gene sequence similarity. DNA–DNA relatedness levels between strain S1-47^T and the type strain of *Albirhodobacter marinus* were 49.8–52.2 %. Based on the phenotypic, chemotaxonomic and molecular features, strain S1-47^T clearly represents a novel species of the genus *Albirhodobacter*, for which the name *Albirhodobacter confluentis* sp. nov. is proposed. The type strain is S1-47^T (=KACC 18804^T =JCM 31536^T).

The genus *Albirhodobacter* was first proposed by Nupur [1] as a member of the family *Rhodobacteraceae* within the phylum *Proteobacteria*. At the time of writing, the genus *Albirhodobacter* comprises only one species with a validly published name, *Albirhodobacter marinus*, which was isolated from seashore water. Cells of the genus *Albirhodobacter* are Gram-stain-negative, facultatively aerobic, catalase- and oxidase-positive and non-motile rods. In addition, members of the genus *Albirhodobacter* contain ubiquinone-10 (Q-10) as the respiratory quinone and phosphatidylethanolamine as the major polar lipid [1]. In this study, a representative of a presumably novel species of the genus *Albirhodobacter*, designated strain S1-47^T, was isolated from estuary sediment and its taxonomic properties were characterized using a polyphasic approach.

Strain S1-47^T was isolated from the Asan Bay estuary of South Korea (36° 88′ 74″ N 126° 89′ 73″ E), using a previously described procedure [2] with some modifications. Briefly, an estuary sediment sample (less than 5 cm depth) was serially diluted with 0.85 % (w/v) saline, and aliquots of each serial dilution were spread on marine agar (MA; BD) and incubated at 25 °C for 5 days under aerobic conditions. The 16S rRNA genes of colonies grown on MA were PCR-amplified using the universal primers F1 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R13 (5′-TACGGYTACCTTGTTACGACTT-3′) and double-digested with *Hae*III and *Hha*I, and representative PCR amplicons showing distinct fragment patterns were partially sequenced using the primer 340F (5′-CCTACGGGAGGCAGCAG-3′). The resulting 16S rRNA gene sequences were compared with those of type strains of species with validly pulished names using the Nucleotide Similarity Search program in the EzTaxon-e server [3]. From the 16S rRNA gene sequences analysis, a representative of a putatively novel species belonging to the genus *Albirhodobacter*, designated strain S1-47^T, was selected for further phenotypic and phylogenetic analyses. Strain S1-47^T was routinely cultured aerobically on MA at 25 °C for 3 days. Strain S1-47^T was stored at −80 °C in marine broth (BD) containing 15 % (v/v) glycerol for long-term preservation. *Albirhodobacter marinus* JCM 17680^T was used as a reference strain for the comparison of phenotypic properties and fatty acid compositions and DNA–DNA hybridization.

The 16S rRNA gene of strain S1-47^T was further sequenced using the universal primers 518R (5′-ATTACCGCGGCTGCTGG-3′) and 805F (5′-GATTAGATACCCTGGTAGTC-3′). The resulting 16S rRNA gene sequence of strain S1-47^T (1418 nucleotides) and those of closely related

Author affiliation: Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea.
***Correspondence:** Seogang Hyun, sghyun@cau.ac.kr; Che Ok Jeon, cojeon@cau.ac.kr
Keywords: *Albirhodobacter confluentis*; new taxa; estuary; Proteobacteria.
Abbreviations: DDH, DNA-DNA hybridization; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; Q-10, ubiquinone-10.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S1-47^T is KX268608.
One supplementary table and two supplementary figures are available with the online version of this article.

type strains were aligned using the fast secondary-structure aware Infernal aligner available in the Ribosomal Database Project [4]. Phylogenetic relationships between strain S1-47^T and closely related type strains were inferred using the neighbour-joining (NJ) algorithm with the Kimura two-parameter model and the maximum-parsimony (MP) algorithm through a heuristic search in the PHYLIP software (version 3.695) [5], and the tree topologies were evaluated through bootstrap analyses based on a 1000-resampled dataset. A phylogenetic tree with bootstrap values based on the maximum-likelihood (ML) algorithm was also reconstructed using RAxML-HPC BlackBox (version 8.2.4) with a GTRCAT model of evolution using the Cyber-Infrastructure for Phylogenetic Research project (CIPRES, www.phylo.org; [6]). DNA–DNA hybridization (DDH) between strain S1-47^T and *Albirhodobacter marinus* JCM 17680^T was performed to evaluate their genomic DNA relatedness using a genome-probing microarray method, as described previously [7]. The DNA–DNA relatedness was confirmed using a different DDH method by the DIG High Prime DNA Labelling kit (Roche Applied Science), as described previously [8]. The DDH experiments were conducted in triplicate and confirmed by reciprocally interchanging the probe and target DNA. Signals produced by hybridization of probes to homologous target DNAs were taken to be 100 %, and signal intensities by self-hybridization of the series of dilutions were used for the calculation of the levels of DNA relatedness between strain S1-47^T and the type strain of *Albirhodobacter marinus*.

Phylogenetic analysis based on the NJ algorithm showed that strain S1-47^T formed a tight phylogenetic lineage with *Albirhodobacter marinus* N9^T with a 100 % bootstrap value (Fig. 1). Phylogenetic trees reconstructed by the ML and MP algorithms also supported that strain S1-47^T formed a tight phylogenetic lineage with *Albirhodobacter marinus* N9^T. Comparative analysis based on the 16S rRNA gene sequences revealed that strain S1-47^T was most closely related to *Albirhodobacter marinus* N9^T with a 99.4 % sequence similarity. The next closely related strains to strain S1-47^T were *Thioclava indica* DT23-4^T and *Rhodobacter ovatus* JA234^T with 95.5 and 95.3 % sequence similarities, respectively. The DNA–DNA relatedness levels between S1-47^T and the type strain of *Albirhodobacter marinus* (JCM 17680^T) using the genome-probing microarray method and the DIG High Prime Labelling kit were 52.2±9.9 and 49.8 ±1.9 %, respectively, which were clearly below the 70 % threshold generally accepted for species delineation [9]. These results clearly suggest that strain S1-47^T represents a novel species of the genus *Albirhodobacter*.

Growth of strain S1-47^T was tested at 25 °C for 3 days on MA, LB agar (BD), R2A agar (BD), nutrient agar (BD) and

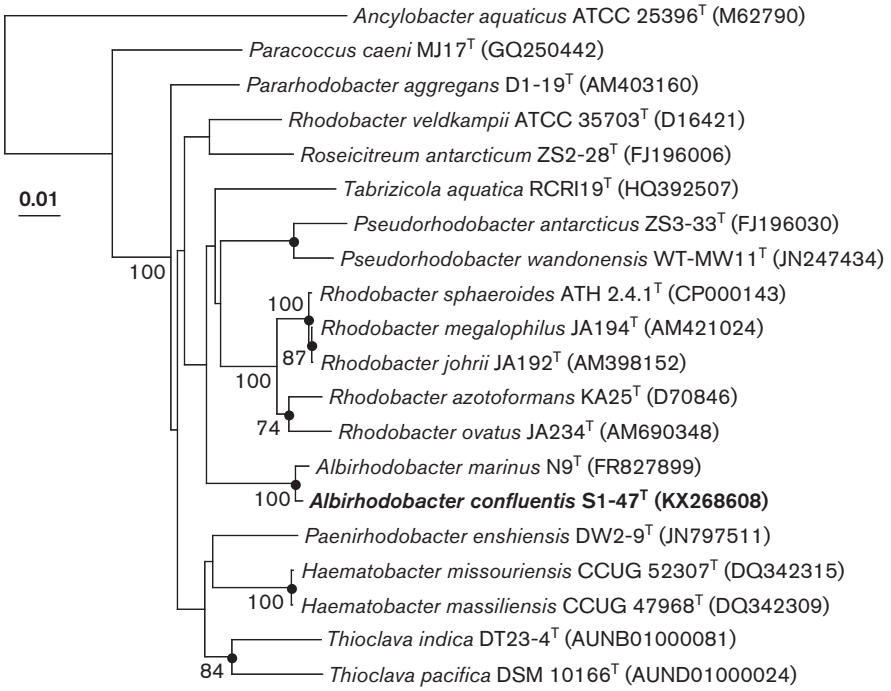

Fig. 1. NJ tree based on 16S rRNA gene sequences indicating the phylogenetic relationships of strain S1-47^T and related taxa. The phylogenetic tree was reconstructed on the basis of 16S rRNA gene sequences between sequence positions 23 and 1491 (according to *Escherichia coli* rRNA gene sequence numbering), and the bootstrap values are shown on nodes as percentages of 1000 replicates; only values over 70 % are indicated. Filled circles (•) indicate the corresponding nodes that were also recovered by the ML and MP algorithms. *Ancylobacter aquaticus* ATCC 25396^T (M62790) was used as the outgroup. Bar, 0.01 changes per nucleotide position.

tryptic soy agar (BD), which were supplemented with NaCl to be approximately 2 % (w/v) of the final NaCl concentration. Gram staining of strain S1-47^T was investigated using the bioMérieux Gram stain kit according to the manufacturer's instructions. Anaerobic growth was assessed on MA under anaerobic conditions (with 4–10 % CO_2) using the GasPak Plus system (BBL) at 25 °C for 21 days. Cell morphology and the presence of flagella were investigated using transmission electron microscopy (JEM-1010; JEOL) and phase-contract microscopy (AXIO Scope A1; ZEISS) with cells from an exponentially grown culture on MA at 25 °C. The following properties of strain S1-47^T and *Albirhodobacter marinus* JCM 17680^T were tested in parallel under the same conditions. Growth at different temperatures (4, 10, 15, 20, 25, 30, 37 and 40 °C) was checked on MA for 3 days. Growth at different pH values (pH 4.0–11.0 at 0.5 pH unit intervals) was assessed in marine broth at 25 °C for 3 days. Marine broth with pH values of below pH 5.5, pH 6.0–7.5, pH 8.0–9.5 and pH 10–11 were prepared using sodium citrate, Na_2HPO_4/NaH_2PO_4, Tris/HCl and Na_2CO_3/Na_2HCO_3 buffers, respectively [10]. After sterilization (121 °C for 15 min), the pH values were adjusted again if necessary. Growth at different NaCl concentrations (0–10 % at 1 % intervals, w/v) was evaluated using marine broth prepared in the laboratory according to the BD formula. Gliding motility was determined using the hanging drop technique with *Zeaxanthinibacter enoshimensis* DSM 18435^T as a positive control, as described by Bernardet [11]. Nitrate reduction was tested according to the method of Lányí [12]. Catalase and oxidase activities were tested by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution and the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck), respectively [13]. Hydrolysis of starch, Tween 20, Tween 80, aesculin, casein, gelatin, tyrosine and urea was investigated on MA, according to the procedures described by Lányí [12] and Smibert and Krieg [13]. Additional enzymic activities, biochemical features and oxidation of carbon sources were investigated using the API ZYM and API 20NE kits (bioMérieux) and GN2 MicroPlate (Biolog), respectively, according to the instructions of the manufacturers, except that cells were resuspended in artificial seawater (20 g NaCl, 2.9 g $MgSO_4$, 4.53 g $MgCl_2 . 6H_2O$, 0.64 g KCl and 1.75 g $CaCl_2 . 2H_2O$ per litre) were used as inocula and the test strains were incubated at their optimal growth temperatures.

Strain S1-47^T grew well on MA, nutrient agar, LB agar, tryptic soy agar and R2A agar containing 2 % NaCl (optimum, MA). Cells were Gram-stain-negative, non-motile rods (1.7–2.0 µm in length and 0.8–1.0 µm in width) (Fig. S1, available in the online version of this article). Anaerobic growth was not observed after 21 days of incubation at 25 °C. Many phenotypic properties such as nitrate reduction, catalase and oxidase activities, hydrolysis of tyrosine, enzyme activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase were in common with those considered to be characteristic for the genus *Albirhodobacter*, whereas some other phenotypic properties such as cell morphology, growth conditions, glucose fermentation, hydrolysis of aesculin, casein, gelatin and urea, and enzyme activity of leucine arylamidase, valine arylamidase, cystine arylamidase, *N*-acetyl-β-glucosaminidase and arginine dihydrolase allowed the differentiation of strain S1-47^T from *Albirhodobacter marinus* (Tables 1 and S1).

Isoprenoid quinones were extracted according to the method of Minnikin *et al.* [14] and analysed using a model LC-20A HPLC system (Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu) and a reversed-phase column (250×64.6 mm, Kromasil; Akzo Nobel), as

Table 1. Comparisons of phenotype characteristics of strain S1-47^T and the type strain of *Albirhodobacter marinus*

Strains: 1, S1-47^T (this study); 2, *Albirhodobacter marinus* JCM 17680^T. All tests except for the DNA G+C content of *Albirhodobacter marinus* were conducted under the same conditions in this study. All strains are positive for the following characteristics: nitrate reduction, oxidase, catalase, hydrolysis of tyrosine, enzyme activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase, and assimilation of D-glucose, L-arabinose, D-mannitol, maltose, malic acid and trisodium citrate. All strains are negative for the following characteristics: Gram-staining, gliding motility, indole production, hydrolysis of Tween 20, Tween 80 and starch, enzyme activity of lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase, and assimilation of capric acid, adipic acid and phenylacetic acid. +, Positive; –, negative.

Characteristic	1	2
Colony morphology		
Form	Circular	Irregular
Elevation	Convex	Flat
Growth conditions		
Temperature (optimum, °C)	10–30 (25)	15–40 (30)
NaCl (optimum, %)	0–6 (2)	1–9 (2)
pH (optimum)	5.0–8.0 (6.0–7.0)	6.0–9.0 (8.0)
Glucose fermentation	–	+
Hydrolysis of:		
Aesculin, urea	–	+
Casein, gelatin	+	–
Enzyme activity of:		
Leucine arylamidase	–	+
Valine arylamidase	–	+
Cystine arylamidase	–	+
N-Acetyl-β-glucosaminidase	–	+
Arginine dihydrolase	–	+
Assimilation of:		
Potassium gluconate	–	+
D-Mannose	+	–
N-Acetylglucosamine	+	–
DNA G+C content (mol%)	69.3	63.0*

*The DNA G+C content of *Albirhodobacter marinus* was derived from a previous study by Nupur *et al.* [1].

described by Komagata and Suzuki [15]. Strain S1-47^T and *Albirhodobacter marinus* JCM 17680^T were cultivated in marine broth at their respective optimal temperatures, and microbial cells were harvested at the same growth stage [exponential phase, optical density at 600 nm (OD_{600})=0.8] for the cellular fatty acid analysis. The cellular fatty acids of microbial cells were saponified, methylated and extracted using the standard MIDI protocol. The fatty acid methyl esters were analysed by gas chromatography (model 6890; Hewlett Packard) and identified by using the TSBA6 database of the Microbial Identification System (Sherlock version 6.0B; [16]). The polar lipids of strain S1-47^T and the type strain of *Albirhodobacter marinus* (JCM 17680^T) were analysed by TLC using cells harvested during the exponential growth phase, as described by Minnikin *et al.* [17]. The following reagents were used to detect different polar lipids: 10 % ethanolic molybdophosphoric acid reagent (for total polar lipids), Dittmer–Lester reagent (for phospholipids), ninhydrin (for aminolipids) and α-naphthol/sulfuric acid (for glycolipids). The DNA G+C content of strain S1-47^T was determined by the fluorometric method [18] using SYBR green I and a real-time PCR thermocycler (Bio-Rad).

The only respiratory quinone of strain S1-47^T detected was Q-10. The major cellular fatty acids (>5.0 % of the total fatty acids) of strain S1-47^T were summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, 29.0 %), summed feature 8 (comprising $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$, 53.1 %) and $C_{16:0}$ (6.9 %), which was in common with those of *Albirhodobacter marinus* (Table 2). However, the presence or absence of $C_{13:0}$ 2-OH, $C_{12:1}$ 3-OH and summed feature 1 (comprising iso-$C_{15:1}$ H and/or $C_{13:0}$ 3-OH) differentiated strain S1-47^T from *Albirhodobacter marinus*. Phosphatidylethanolamine was identified from strain S1-47^T as a major polar lipid (Fig. S2), which was inconsistent with that of *Albirhodobacter marinus* (Fig. S2) [1]. An unidentified aminolipid, an unidentified phospholipid and three unidentified lipids were also detected as unidentified polar lipids in strain S1-47^T, which were clearly different from those of *Albirhodobacter marinus* (Fig. S2). For example, an unidentified glycolipid was not detected from strain S1-47^T, but it was detected from *Albirhodobacter marinus*. The DNA G+C content of strain S1-47^T was 69.3 mol%, which was a little different from that of *Albirhodobacter marinus* [1]. In conclusion, the phylogenetic inference, and the physiological and biochemical properties of strain S1-47^T support its assignment as a representative of a novel species of the genus *Albirhodobacter*, for which the name *Albirhodobacter confluentis* sp. nov. is proposed.

Table 2. Cellular fatty acid contents (percentages) of strain S1-47^T and the type strain of *Albirhodobacter marinus*

Strains: 1, S1-47^T; 2, *A. marinus* JCM 17680^T. All data were obtained from this study. Data are expressed as percentages of the total fatty acids, and fatty acids amounting to less than 1.0 % in all strains are not shown. Major components (>5.0 %) are highlighted in bold type; –, not detected.

Fatty acid	1	2
Saturated		
$C_{16:0}$	**6.9**	**7.7**
Hydroxy		
$C_{10:0}$ 3-OH	5.0	3.7
$C_{13:0}$ 2-OH	–	1.0
$C_{12:1}$ 3-OH	2.1	–
Summed features*		
1	–	1.6
3	**29.0**	**25.0**
7	4.0	2.9
8	**53.1**	**58.1**

*Summed features represent groups of two fatty acids that cannot be separated by gas/liquid chromatography with the MIDI system. Summed feature 1, iso-$C_{15:1}$ H and/or $C_{13:0}$ 3-OH; summed feature 3, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 7, $C_{19:1}\omega6c$ and/or cyclo-$C_{19:0}\omega10c$; summed feature 8, $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

DESCRIPTION OF *ALBIRHODOBACTER CONFLUENTIS* SP. NOV.

Albirhodobacter confluentis (con.flu.en′tis. L. masc. gen. n. *confluentis* of a meeting place of waters).

Cells are Gram-stain-negative, strictly aerobic and circular convex rods (1.7–2.0 µm in length and 0.8–1.0 µm in width) without gliding motility. Colonies on MA are ivory-coloured, circular, convex and smooth. Growth occurs at 10–30 °C (optimum, 25 °C), at pH 5.0–8.0 (optimum, pH 6.0–7.0) and in the presence of 0–6 % (w/v) NaCl (optimum, 2 %). Oxidase- and catalase-positive. Casein, tyrosine and gelatin are hydrolysed, but Tween 80, Tween 20, aesculin, starch and urea are not. Nitrate is reduced to nitrite, but nitrogen gas is not produced. Indole production and fermentation of glucose are negative. Enzyme activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase is positive, but enzyme activity of lipase (C14), trypsin, leucine arylamidase, valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, *N*-acetyl-β-glucosaminidase, α-fucosidase and arginine dihydrolase is negative. Assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, malic acid and trisodium citrate is positive, but assimilation of potassium gluconate, capric acid, adipic acid and phenylacetic acid is negative. Phosphatidylethanolamine is a major polar lipid. An unidentified aminolipid, an unidentified phospholipid and three unidentified lipids are also detected as polar lipids. The major cellular fatty acids are summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), summed feature 8 (comprising $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and $C_{16:0}$. The only respiratory quinone is Q-10.

The type strain is S1-47^T (=KACC 18804^T=JCM 31536^T), isolated from estuary sediment in Asan, South Korea. The DNA G+C content of the type strain is 69.3 mol%.

Funding information
This work was supported by the Program for Collection of Domestic Biological Resources from the National Institute of Biological Resources (NIBR no. 2017-02-001) of the Ministry of Environment (MOE), Republic of Korea. J. L. was partially supported by a Chung-Ang University Research Scholarship Grant in 2016.

Acknowledgements
The authors would like to thank a nomenclature reviewer for support regarding the nomenclature of the micro-organism.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Nupur, Vaidya B, Tanuku NR, Pinnaka AK. *Albirhodobacter marinus* gen. nov., sp. nov., a member of the family *Rhodobacteraceae* isolated from sea shore water of Visakhapatnam, India. *Antonie van Leeuwenhoek* 2013;103:347–355.
2. Jeong SH, Jin HM, Lee HJ, Jeon CO. *Altererythrobacter gangjinensis* sp. nov., a marine bacterium isolated from a tidal flat. *Int J Syst Evol Microbiol* 2013;63:971–976.
3. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
4. Nawrocki EP, Eddy SR. Query-dependent banding (QDB) for faster RNA similarity searches. *PLoS Comput Biol* 2007;3:e56.
5. Felsenstein J. *Phylip (Phylogeny Inference Package), Version 3.6a*. Seattle, WA: Department of Genetics, University of Washington; 2002.
6. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
7. Chang HW, Nam YD, Jung MY, Kim KH, Roh SW *et al.* Statistical superiority of genome-probing microarrays as genomic DNA-DNA hybridization in revealing the bacterial phylogenetic relationship compared to conventional methods. *J Microbiol Methods* 2008;75:523–530.
8. Lee SH, Shim JK, Kim JM, Choi HK, Jeon CO. *Henriciella litoralis* sp. nov., isolated from a tidal flat, transfer of *Maribaculum marinum* Lai *et al.* 2009 to the genus *Henriciella* as *Henriciella aquimarina* nom. nov. and emended description of the genus *Henriciella*. *Int J Syst Evol Microbiol* 2011;61:722–727.
9. Stackebrandt E, Frederiksen W, Garrity GM, Grimont PA, Kämpfer P *et al.* Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* 2002;52:1043–1047.
10. Gomori G. Preparation of buffers for use in enzyme studies. In: Colowick SP and Kaplan NO (editors). *Methods in Enzymology*. New York: Academic Press; 1955. pp. 138–146.
11. Bernardet JF, Nakagawa Y, Holmes B. Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* 2002;52:1049–1070.
12. Lányí B. Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 1987;19:1–67.
13. Smibert RM, Krieg NR. Phenotypic characterization. In: Gerhardt P (editor). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994. pp. 607–654.
14. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al.* An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
15. Komagata K, Suzuki K. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–208.
16. Sasser M. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc; 1990.
17. Minnikin DE, Patel PV, Alshamaony L, Goodfellow M. Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* 1977;27:104–117.
18. Gonzalez JM, Saiz-Jimenez C. A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* 2002;4:770–773.