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Denitrobaculum tricleocarpae gen. nov., sp. nov., a marine bacterium from coralline algae *Tricleocarpa* sp

Guanghua Wang[1,2,3], Dahao Tang[1,2,3], Guangyu Li[4], Shuailiang Xu[1,2,3], Ge Dang[1,2,3], Hongfei Su[1,2,3], Zhiheng Liao[1,2,3], Biao Chen[1,2,3], Wen Huang[1,2,3], Jiayuan Liang[1,2,3] and Kefu Yu[1,2,3,*]

Abstract

A Gram-stain-negative, non-spore-forming, aerobic, motile, curved rod-shaped bacterium, designed strain R148^T was isolated from a coralline algae *Tricleocarpa* sp. collected from Weizhou island, PR China. The optimal growth of R148^T occurred at 25 °C, pH 8–9 in the presence of 0.5% (w/v) NaCl on the basis of amended marine broth 2216. The genomic DNA G+C content was 59.5 mol%. The only detected respiratory quinone was Q-10. The major polar lipids were phosphatidylmethylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, and three unidentified ninhydrin-positive lipids. The major cellular fatty acids were $C_{18:1}\omega7c$, $C_{16:1}\omega7c$, $C_{19:0}$cyclo 9, 10 DMA and $C_{18:0}$. The results of 16S rRNA gene-based global alignment indicated that the closest neighbour of strain R148^T was *Pelagibius litoralis* DSM 21314^T (93.1% similarity), the second is *Limibacillus halophilus* KCTC 42420^T (92.2%). The results of phylogenetic analysis indicated that R148^T forms a distinct branch in the robust clade of R148^T and *P. litoralis* DSM 21314^T, while the taxonomic position of this clade in the family *Rhodospirillaceae* is ambiguous among phylogenetic approaches. The low 16S rRNA gene similarity and distinct polar lipid and cellular fatty acid profile could readily distinguish R148^T from closely related type strains. So R148^T is suggested to represent a novel species in a novel genus, for which the name *Denitrobaculum tricleocarpae* gen. nov., sp. nov. is proposed. The type strain is R148^T (=MCCC 1K03781^T=KCTC 72137^T).

The family *Rhodospirillaceae* was proposed in 1971 to replace the name *Athiorhodaceae* Molisch 1907, which contained the genera *Rhodospirillum*, *Rhodopseudomonas* and *Rhodomicrobium* at that time [1]. Another family *Kiloniellaceae* was proposed with single species *Kiloniella laminariae* in 2009 [2], which usually was classified in the midst of the *Rhodospirillaceae* phylogeny, so the future of this family was highly controversial [3, 4]. Since 2005, the number of genera affiliated to the family *Rhodospirillaceae* has increased from 13 to 51 [4], the family phylogeny was so diverse that several genomic based taxonomic amendments were suggested, such as 'Diaforabacterales' ord. nov. to replace the *Rhodospirillales* [4] and 'Fodinicurvataceae' fam. nov. to accommodate *Fodinicurvata*, *Tistlia*, *Rhodovibrio* and *Limimonas* [3]. In this study, the phylogeny of a novel isolate of a member of the family *Rhodospirillaceae*, strain R148^T, is discussed. Genomic annotation indicated that this isolate is versatile in carbohydrate and peptide/amino acid metabolism, can produce vitamin B12 (VB_{12}) and degrade benzoate, which may play an important role in the algal micro-ecology.

R148^T was isolated from a coralline alga *Tricleocarpa* sp. collected from Weizhou island (21°03′42″N, 109°08′35″E), in the Beibu Gulf, PR China. Algae were stored and transported in an ice box as soon as they were collected, then washed with autoclaved natural seawater and homogenized using a mortar in the lab. The algal powder was suspended with autoclaved natural seawater and diluted by tenfold. A 100 µl sample of each dilution was spread on modified R2A plate (marine R2A plate, R2A agar was obtained from BD, which was dissolved in natural seawater, pH7.6). Bacteria were incubated at 25 °C for

Author affiliations: [1]Guangxi Key Laboratory on the Study of Coral Reefs in the South China Sea, Nanning 530004, PR China; [2]Coral Reef Research Center of China, Guangxi University, Nanning 530004, PR China; [3]School of Marine Sciences, Guangxi University, Nanning 530004, PR China; [4]Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, PR China.
***Correspondence:** Kefu Yu, kefuyu@scsio.ac.cn
Keywords: *Denitrobaculum tricleocarpae* gen. nov., sp. nov.; phylogenetic; chemotaxonomic.
Abbreviations: DPG, Diphosphatidylglycerol; NL, Unidentified ninhydrin-positive lipid; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; PME, Phosphatidylmethylethanolamine; Q, Ubiquinone.
The 16S rRNA gene sequence of strain R148^T has been deposited in GenBank under the accession number MN094886. The Whole Genome Shotgun project of strain R148^T has been deposited at DDBJ/ENA/GenBank under the accession number VHSH00000000. The Whole Genome Shotgun project of *Pelagibius litoralis* DSM 21314^T has been deposited at DDBJ/ENA/GenBank under the accession number JAAQPH000000000.
One supplementary table and five supplementary figures are available with the online version of this article.

2 weeks. A cream colony, designed strain R148^T, was isolated and purified on marine R2A plates, then stored at −70 °C with R2A broth (Haibo):glycerol (4:1, v/v). Type strains *Pelagibius litoralis* DSM 21314^T and *Limibacillus halophilus* KCTC 42420^T were used as references and were obtained from the German Collection of Microorganisms and Cell Cultures (DSM) and the Korean Collection for Type Cultures (KCTC), respectively.

The 16S rRNA gene of R148^T was obtained by using PCR amplification with the universal primers 27F and 1492R [5] and sequenced using the Sanger method. Determination of the 16S rRNA gene sequence similarities were performed using EzBioCloud [6] and the NCBI database. Alignment of 16S rRNA gene sequences was performed using the SINA software package [7] and SILVA rRNA database. Phylogenetic trees were reconstructed using the maximum likelihood [8], neighbor-joining [9] and maximum-parsimony [10] algorithms in the software package MEGA version 7.0 [11]. The phylogenetic distance matrices were estimated by the Kimura two-parameter model [12]. The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method of Felsenstein [13] with 1000 replicates. Whole-genome sequencing was performed on the HiSeq PE150 platform (Illumina). A-tailed, ligated to paired-end adaptors and PCR amplified DNA with a 350 bp insert was used for the library construction at Beijing Novogene Bioinformatics Technology (Beijing, PR China). All good quality paired reads were assembled using SOAP denovo [14, 15] (http://soap.genomics.org.cn/soapdenovo.html) into a number of scaffolds. Genome information was extracted according to the protocol of Chun *et al*. [16]. The average nucleotide identity was calculated as described by Yoon *et al*. [17]. The phylogenomic tree was reconstructed using the up-to-date bacterial core gene set (UBCG v.3) according to its manual [18].

The genome sequencing depth of R148^T was 156×, the N50 was 492275 bp. A total of 36 contigs were obtained, the obtained genome size was 6.44 Mb, and the genome DNA G+C content was 59.5 mol%. The full length 16S rRNA gene sequence was 1479 bp, which was similar to the Sanger result (1360 bp, MN094886). The genome of *P. litoralis* DSM 21314^T was also sequenced and deposit in the GenBank under the accession number JAAQPH000000000. The global alignment based on 16S rRNA gene sequence in the EzBioCloud database indicated that R148^T shares 93.1 and 92.2% sequence similarity with the most closely related type strains *P. litoralis* DSM 21314^T and *L. halophilus* KCTC 42420^T, respectively. The ANI of R148^T to *P. litoralis* DSM 21314^T and *L. halophilus* KCTC 42420^T was 71.2 and 67.9%, respectively. Phylogenetic analysis based on the maximum likelihood algorithm indicated that R148^T forms a distinct branch in a stable clade that consists of R148^T and *P. litoralis* DSM 21314^T, then forms a large loose clade with *L. halophilus* KCTC 42420^T and members of the genus *Fodinicurvata* (Fig. 1). The neighbor-joining algorithm also supported this tree topology (Fig. S1, available in the online version of this article). However, the maximum-parsimony algorithm indicated a large loose clade consisting of R148^T, *P. litoralis* DSM 21314^T, *L. halophilus* KCTC 42420^T and members of genera *Fodinicurvata*, *Kiloniella* and *Aestuariispira* (Fig. S2). The phylogenomic tree based on the up-to-date bacterial core gene set also indicated the close relationship between R148^T, *P. litoralis* DSM 21314^T and *L. halophilus* KCTC 42420^T, also forming a robust clade with members of genus *Kiloniella* (Fig. S3). These phylogenetic results indicated the close relationship of R148^T, *P. litoralis* DSM 21314^T and *L. halophilus* KCTC 42420^T and also the possibility that R148^T may represent a novel genus [16, 19], but reveal the uncertainty of the taxonomic position of the R148^T clade in current phylogenetic analysis systems.

Cell morphology was observed by using a transmission electron microscope (HT7700; Hitachi). Cell mobility was tested by using an optical microscope (BX53; Olympus) with the hanging drop technique [20]. Gram straining was determined on marine R2A plates following the protocols of Gerhardt *et al.* [21]. Catalase activity was determined by gas production upon exposure to 3% (v/v) hydrogen peroxide solution. Oxidase activity was determined by using oxidase test strains (Huankai). NaCl requirement and tolerance were tested at 25 °C for 7 days in amended marine broth 2216 with NaCl concentrations ranging from 0 to 16% (w/v), namely 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 and 16% (w/v). Growth at different pH values was tested for seven days at 25 °C in marine broth 2216 (BD) amended with different buffers (0.5 pH unit intervals, pH 5–8, 0.1 M KH_2PO_4/K_2HPO_4; pH 8.5–10, 0.1 M $NaHCO_3$/Na_2CO_3; pH 10.5–11, 0.1 M Na_2CO_3/NaOH). Optimal growth temperature was determined on marine agar 2216 (BD) plates after 7–30 days of growth at temperatures of 5, 10, 15, 20, 25, 30, 33, 37, 40 and 45 °C. The ability to form endospores and hydrolysis of starch, Tween 20, 40, 60 and 80 was tested described by Dong and Cai [22]. Carbohydrate metabolism were tested by using API 20NE and API ZYM strips according to the manufacturer's protocols except that cells were suspended in sterile aged nature seawater. Anaerobic fermentation was determined using API 50CH strips according to the manufacture's protocol, the inoculum medium was R2A liquid medium. H_2S production was tested by following standard procedures compiled by Tindall *et al.* [23].

Cells of R148^T were Gram-stain-negative, non-spore-forming, aerobic, slightly curved rods, motile with a monopolar flagellum or bipolar flagella (Fig. S4). The position of the flagellum could readily be used to differentiate strain R148^T from *L. halophilus* KCTC 42420^T, *L. halophilus* KCTC 42420^T had no flagellum [24]. In the API 20NE test, arginine dihydrolase in R148^T was negative, while *P. litoralis* DSM 21314^T and *L. halophilus* KCTC 42420^T were positive. Other characteristics of R148^T are listed in Table 1 and the species description.

Biomass of R148^T and reference strains for cellular fatty acid analysis was acquired from the third quadrant of the quadrant-streaked (exponential state) marine agar 2216 (BD) plate incubated at 25 °C for 3–5 days; *P. litoralis* DSM 21314^T required 5 days. Cellular fatty acid composition was analysed by gas chromatography (G6890N; Agilent) and identified by using the Sherlock Microbial Identification System (Version

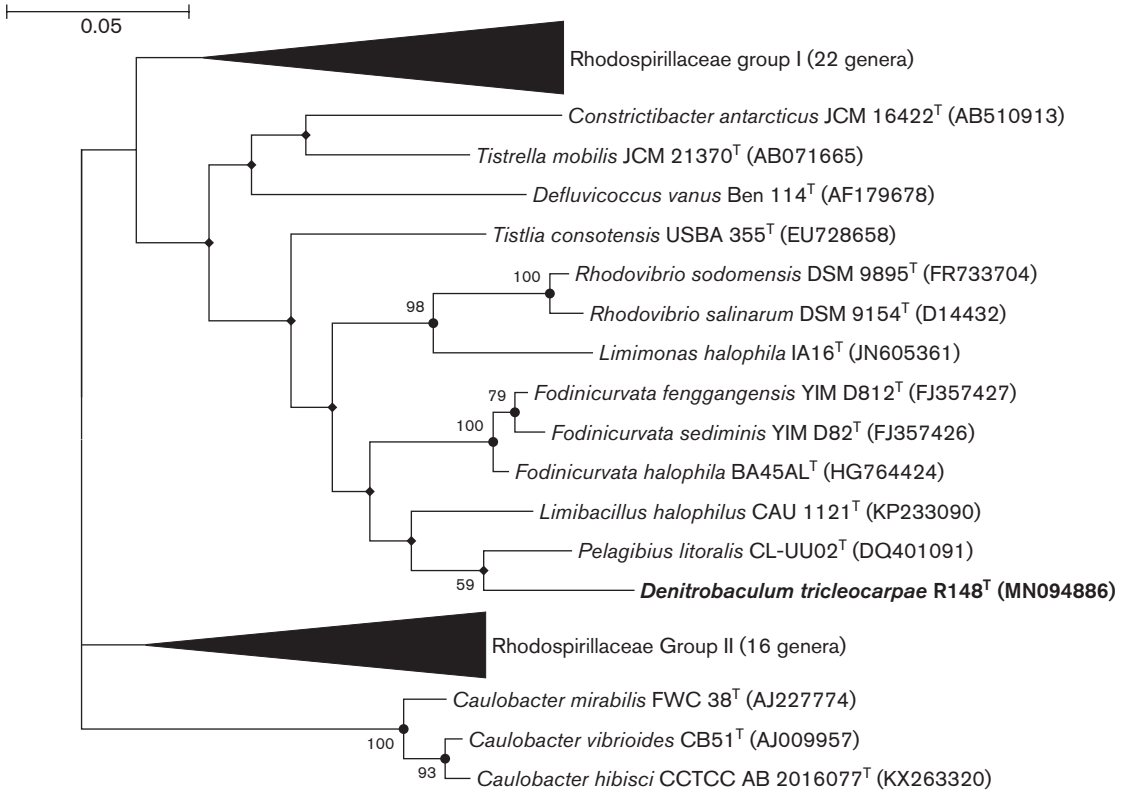

Fig. 1. Maximum likelihood phylogenetic tree based on the 16S rRNA gene sequences of R148^T and related taxa. Sequences from members of the genus *Caulobacter* were used as outgroups. Numbers at nodes indicate percentages of 1000 bootstrap resamplings; only values above 50% are shown. Bar, 0.05 substitutions per nucleotide position. Filled circles (●) at the nodes indicate that the same tree topology was obtained with the neighbour joining and maximum parsimony algorithms; filled diamonds (♦) at the nodes indicate that the same tree topology was obtained with the neighbour joining algorithm.

6.0) according to the manufacturer's instructions. Biomass for the analyses of quinones and DNA extraction was obtained from marine R2A liquid medium. Respiratory quinones were extracted as described by Collins [25] and analysed using reversed-phase HPLC [26]. The isoprenoid quinones were eluted with a mixture of methanol and 2-propanol (2:1, v/v) using a flow rate of 1 ml min^{-1} at room temperature and detected by UV absorbance at 270 nm. Biomass for polar lipid analysis was obtained from marine R2A liquid medium. Polar lipids were extracted as described by Kamekura [27], and identified by spraying with ethanolic molybdophosphoric acid, molybdenum blue, ninhydrin, α-naphthol/sulphuric acid and Dragendorff's reagent after two-dimensional TLC [28].

The major cellular fatty acids of R148^T were $C_{18:1}\omega7c$ (57.4% of total content), $C_{16:1}\omega7c$ (12.7%), $C_{19:0}$cyclo 9, 10 DMA (12.3%) and $C_{18:0}$ (7%) (Table S1). This fatty acid profile was much different from those of *L. halophilus* KCTC 42420^T and *P. litoralis* DSM 21314^T; *P. litoralis* DSM 21314^T lacked $C_{16:1}\omega7c$, *L. halophilus* KCTC 42420^T had a high $C_{18:1}\omega7c$ 10-methyl (10%) content (Table S1). The detected respiratory quinone of R148^T was ubiquinone 10 (Q-10). The major polar lipids were phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and three unidentified ninhydrin-positive lipids (Fig. S5). *L. halophilus* KCTC 42420^T and *P. litoralis* DSM 21314^T had the same major polar lipids, both of them lacked an unidentified ninhydrin-positive lipid (NL3) compared with R148^T (Fig. S5).

In summary, low 16S rRNA gene similarities to (93.1%) and different polar lipids (possession of NL3) and cellular fatty acid profiles ($C_{16:1}\omega7c$ or $C_{18:1}\omega7c$ 10-methyl) from closely related type strains indicated that R148^T should represent a novel species in a novel genus in the family *Rhodospirillaceae*, for which the name *Denitrobaculum tricleocarpae* gen. nov., sp. nov. is proposed.

DESCRIPTION OF *DENITROBACULUM* GEN. NOV.

Denitrobaculum (De.ni.tro.ba′cu.lum. L. pref. *de*, from; N.L. pref. *nitro-*, pertaining to nitrate; L. neut. n. *baculum*, a stick; N.L. neut. n. *Denitrobaculum*, nitrate-reducing rod)

Cells are Gram-stain-negative, non-spore-forming, aerobic curved rods, motile by means of a single polar flagellum. Catalase is positive while oxidase is negative. The major respiratory quinone is Q-10. The major polar lipids are

Table 1. Characteristics that differentiate strain R148^T from type strains of related species

Strains: 1, R148^T; 2, *Pelagibius litoralis* DSM 21314^T; 3, *Limibacillus halophilus* KCTC 42420^T; 4, *Fodinicurvata sediminis* YIM D82^T; 5, *Kiloniella laminariae* LD81^T. All the data were obtained from this study except where indicated. +, Positive; –, negative or not detected. All strains were catalase-positive.

Characteristics	1	2	3	4‡	5§
Source of isolate	*Tricleocarpa* sp.	Coastal seawater*	Reclaimed land†	Salt mine sediment	Marine macroalga
Cell size(μm)	0.5–0.7×0.9–3.2	0.5–1.0×1.2–2.5*	0.3–0.5×1.0–2.0†	0.3–0.5×0.7–1.5	0.5–0.6×2.5–5.0
Flagellum	Monopolar	Monopolar*	–†	+	Monopolar
Cell shape	Slightly curved rod	Slightly curved rod*	Short rod†	Vibrioid and rod	Curved spirilla
NaCl tolerance (w/v, %)	0.5–5 (0.5)	1–6 (1)	0–8 (1–4)	1.5–20	0.3–10 (3)
pH	8–9	7–10 (8–9)	6–9 (7)	6.5–8.5 (7.5)	3.5–9.5 (5.5)
Temperature (°C)	15–37	15–37	15–40	15–42	4–40
Urease	+	+	+	+	–
Arginine dihydrolase	–	+	+	+	+
Hydrolysis of gelatin	+	+	+	–	–
β-glucosidase	+	–	–	–	–
Genomic DNA G+C content (mol%)‖	59.5	62.7	58.1	60.6	51.4
ANI to R148^T (%)	100	71.2	67.9	70.0	67.9
Major polar lipids¶	PME, PE, PG, DPG, NL(1-3)	PME, PE, PG, DPG, NL(1-2)	PME, PE, PG, DPG, NL(1-2)	DPG, PME,PC,PL	PE, PG, NPLs, Ls
Major cellular fatty acids	$C_{18:1}\omega7c$, $C_{16:1}\omega7c$, $C_{19:0}$cyclo 9, 10 DMA, $C_{18:0}$	$C_{18:1}\omega7c$, $C_{19:0}$cyclo 9, 10 DMA, $C_{18:0}$	$C_{18:1}\omega7c$, $C_{16:1}\omega7c$, $C_{19:0}$cyclo 9, 10 DMA, $C_{18:1}\omega7c$ 10-methyl, $C_{18:0}$	$C_{18:1}\omega7c$, $C_{18:1}$ 2-OH, $C_{16:0}$	$C_{18:1}\omega7c$, $C_{16:1}\omega7c$, $C_{16:0}$, $C_{18:0}$ and $C_{19:0}$cyclo ω8*c*

*Data from [29]
†Data from [24].
‡Data from [30].
§Data from [31, 32].
‖Data from genome sequencing.
¶PME, phosphatidylmethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; NPL, unidentified ninhydrin-positive phospholipid; NL, unidentified ninhydrin-positive lipid; PL, unidentified phospholipid; PC, phosphatidylchlorine; L, unidentified lipid.

phosphatidylmethylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, and three unidentified ninhydrin-positive lipids. The major cellular fatty acids are $C_{18:1}\omega7c$, $C_{16:1}\omega7c$, $C_{19:0}$cyclo 9, 10 DMA and $C_{18:0}$.

The type species is *Denitrobaculum tricleocarpae*.

DESCRIPTION OF *DENITROBACULUM TRICLEOCARPAE* SP. NOV.

Denitrobaculum tricleocarpae (tri.cle.o.car′pae. N.L. gen. n. *tricleocarpae*, of/from *Tricleocarpa*, isolated from the red alga *Triclecarpa* sp.)

The description is as for the genus with the following additional properties. Cells are usually 0.5–0.7 μm wide and 0.9–3.2 μm long. Colonies are cream and circular on marine R2A plates. Cells can grow at 15–37 °C (optimum 25 °C), pH 8–9 in 0.5–5% (w/v) NaCl (optimum 0.5%) on the basis of marine broth 2216 medium. Production of H_2S does not occur. Starch and gelatin are hydrolysed. In the API 20NE test, nitrate reduction, urease, *β*-glucosidase and protease are positive. In the API ZYM test, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI phosphohydrolase, α-galactosidase, *β*-galactosidase, *β*-glucuronidase, α-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase and α-mannosidase are positive.

The type strain, R148^T (=MCCC 1K03781^T=KCTC 72137^T), was isolated from a coralline algae *Tricleocarpa* sp. collected from Weizhou island, in the Beibu Gulf, PR China. The genomic DNA G+C content is 59.5 mol%. The 16S rRNA gene sequence of R148^T has been deposited in GenBank under the accession number MN094886. The Whole Genome Shotgun project of R148^T has been deposited at DDBJ/ENA/GenBank

under the accession number VHSH00000000. The version described in this paper is version VHSH00000000.

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

References

1. Pfennig N, Trüper HG. Higher taxa of the phototrophic bacteria. *Int J Syst Bacteriol* 1971;21:17–18.
2. Wiese J, Thiel V, Gärtner A, Schmaljohann R, Imhoff JF. *Kiloniella laminariae* gen. nov., sp. nov., an alphaproteobacterium from the marine macroalga *Laminaria saccharina*. *Int J Syst Evol Microbiol* 2009;59:350–356.
3. Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A *et al*. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat Biotechnol* 2018;36:996–1004.
4. Degli Esposti M, Lozano L, Martínez-Romero E. Current phylogeny of *Rhodospirillaceae*: A multi-approach study. *Mol Phylogenet Evol* 2019;139:106546.
5. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (editors). *Nucleic Acid Techniques in Bacterial Systematics*. Chichester: Wiley; 1991. pp. 115–175.
6. Yoon S-H, Ha S-M, 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.
7. Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.
8. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
9. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
10. Swofford DL. *PAUP: Phylogenetic analysis using parsimony, version 3.1.1*. Champaign, IL: Illinois Natural History Survey; 1993.
11. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
12. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
13. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
14. Li R, Li Y, Kristiansen K, Wang J. SOAP: short oligonucleotide alignment program. *Bioinformatics* 2008;24:713–714.
15. Li R, Zhu H, Ruan J, Qian W, Fang X *et al*. De novo assembly of human genomes with massively parallel short read sequencing. *Genome Res* 2010;20:265–272.
16. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR *et al*. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466.
17. Yoon S-H, Ha S-min, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
18. SI N, Kim YO, Yoon SH, SM H, Baek I *et al*. UBCG: up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. *J Microbiol* 2018;56:280–285.
19. Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W *et al*. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 2014;12:635–645.
20. 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.
21. Gerhardt P, Murray RGE, Wood WA, Krieg NR. *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994.
22. Dong XZ, Cai MY. *Determinative Manual for Routine Bacteriology*. Beijing: Scientific Press; 2001.
23. Tindall BJ, Sikorski J, Smibert RM, Krieg NR *et al*.. Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf G, Schmidt TM *et al*. (editors). *Methods for General and Molecular Microbiology*. Washington, DC: American Society for Microbiology; 2007. pp. 330–393.
24. Kim J-H, Konkit M, Yoon J-H, Kim W. *Limibacillus halophilus* gen. nov., sp. nov., a moderately halophilic bacterium in the family *Rhodospirillaceae* isolated from reclaimed land. *Int J Syst Evol Microbiol* 2015;65:3155–3161.
25. Collins MD. Isoprenoid quinones. In: Goodfellow M, O'Donnell AG (editors). *Chemical Methods in Prokaryotic Systematics*. Chichester: John Wiley & Sons; 1994. pp. 345–401.
26. Komagata K, Suzuki K. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–207.
27. Kamekura M. Lipids of extreme halophiles. In: Vreeland RH, Hochstein LI (editors). *The Biology of Halophilic Bacteria*. Boca Raton: CRC Press; 1993. pp. 135–161.
28. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
29. Choi DH, Hwang CY, Cho BC. *Pelagibius litoralis* gen. nov., sp. nov., a marine bacterium in the family *Rhodospirillaceae* isolated from coastal seawater. *Int J Syst Evol Microbiol* 2009;59:818–823.
30. Wang Y-X, Liu J-H, Zhang X-X, Chen Y-G, Wang Z-G *et al*. *Fodinicurvata sediminis* gen. nov., sp. nov. and *Fodinicurvata fenggangensis* sp. nov., poly-β-hydroxybutyrate-producing bacteria in the family *Rhodospirillaceae*. *Int J Syst Evol Microbiol* 2009;59:2575–2581.
31. Wiese J, Thiel V, Gärtner A, Schmaljohann R, Imhoff JF *et al*. *Kiloniella laminariae* gen. nov., sp. nov., an alphaproteobacterium from the marine macroalga *Laminaria saccharina*. *Int J Syst Evol Microbiol* 2009;59:350–356.
32. Yang S-H, Seo H-S, Lee J-H, Kim S-J, Kwon KK. *Kiloniella spongiae* sp. nov., isolated from a marine sponge and emended description of the genus *Kiloniella* Wiese *et al*. 2009 and *Kiloniella laminariae*. *Int J Syst Evol Microbiol* 2015;65:230–234.