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Thalassococcus profundi sp. nov., a marine bacterium isolated from deep seawater of the Okinawa Trough

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Abstract

A novel Gram-stain-negative, strictly aerobic, rod-shaped motile bacterium with a single flagellum, designated strain WRAS1^T, was isolated from deep seawater of the Okinawa Trough. Growth occurred in the presence of 0.0–9.0 % NaCl (w/v; optimum, 3.0–4.0 %), at 4–45 °C (optimum, 28–37 °C) and pH 7.0–10.0 (optimum, pH 7.0–8.0). The major fatty acid (>10 % of total fatty acids) was summed feature 8, comprising $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$. The major polar lipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and three unidentified lipids. The major respiratory quinone was ubiquinone-10. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain WRAS1^T was in the genus *Thalassococcus* and showed the highest 16S rRNA gene sequence similarity of 97.5 % to *Thalassococcus halodurans* JCM13833^T. Genome relatedness between strain WRAS1^T and *T. halodurans* JCM13833^T was computed using both average nucleotide identity and DNA–DNA hybridization with values of 74.11 % and 22.70±2.3 %, respectively. The genomic DNA G+C content calculated from the genome sequence of strain WRAS1^T was 65.6 %. On the basis of polyphasic analyses, strain WRAS1^T is considered to represent a novel species in the genus *Thalassococcus*, for which the name *Thalassococcus profundi* sp. nov. is proposed. The type strain is WRAS1^T (=CGMCC 1.16123^T=MCCC 1K03253^T =KCTC 52696^T).

The genus *Thalassococcus*, belonging to the family *Rhodobacteraceae*, was first proposed by Lee *et al.* with the description of the type species *Thalassococcus halodurans* [1]. At the time of writing, the genus *Thalassococcus* comprises only two species with validly published names, *T. halodurans* and *Thalassococcus lentus*, which were isolated from the marine sponge *Halichondria panicea* and seawater, respectively [1, 2]. The genus *Thalassococcus* is a member of *Roseobacter* clade, and species in this genus are Gram-stain-negative, rod- or ovoid-shaped, strictly aerobic, and non-motile. NaCl is required for growth and the major respiratory quinone is ubiquinone-10 (Q-10). Both species contain $C_{18:1}\omega7c$ as the predominant fatty acid and their DNA G+C content ranges between 58.0 and 58.8 mol% [1]. In this study, we described a bacterial strain, designated WRAS1^T, which was isolated from seawater at a water depth of 1304 m in the Okinawa Trough (122° 34′ E, 25° 04′ N). On the basis of its chemotaxonomic, physiological and phylogenetic characteristics, strain WRAS1^T is proposed as representing a novel species of the genus *Thalassococcus*.

The seawater sample from a depth of 1304 m from the Okinawa Trough was collected using a remote operated vehicle during the cruise 'HOBAB4' conducted by the R/V Kexue in June 2016 and was spread onto SPG medium plates, which contained the following composition: 6.5 g l^{-1} PIPES (1,4-piperazinediethanesulfonic acid); 25 g l^{-1} NaCl; 2.7 g l^{-1} $MgSO_4{\cdot}7H_2O$; 4.3 g l^{-1} $MgCl_2{\cdot}6H_2O$; 0.25 g l^{-1} NH_4Cl; 0.5 g l^{-1} KCl; 0.14 g l^{-1} $CaCl_2{\cdot}2H_2O$; 0.14 g l^{-1} $K_2HPO_4{\cdot}3H_2O$; 0.002 g l^{-1} $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$; 2.48 g l^{-1} $Na_2S_2O_3{\cdot}5H_2O$; 1 ml trace elements solution [3]; 10 ml vitamin solution [4]. Cultures were incubated at 37 °C for 7 days. Strain WRAS1^T was purified by streaking three times and maintained on marine agar 2216E (MA; Becton Dickinson) at 37 °C and stocks were preserved in sterile 0.85 % (w/v) saline supplemented with 15 % (v/v) glycerol at −80 °C. *T. halodurans* JCM13833^T, obtained from the Japan Collection of

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Abbreviations: AL, unidentified aminolipid; ANI, average nucleotide identity; DDH, DNA–DNA hybridization; L, unidentified lipid; MA, marine agar; MB, marine broth; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; Q-10, ubiquinone-10.
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The GenBank accession number for the 16S rRNA gene sequence of *Thalassococcus profundi* WRAS1^T is KY352637. The Whole Genome Shotgun project of strain WRAS1^T has been deposited at DDBJ/ENA/GenBank under the accession QPMK00000000. The version described in this paper is version QPMK01000000.
Two supplementary tables and four supplementary figures are available with the online version of this article.

Microorganisms (JCM), and *T. lentus* YCS-24^T [2] were used as control strains. The type strain was cultured in the same condition as for strain WRAS1^T [MA/marine broth 2216E (MB; BD), 37 °C], unless otherwise specified.

For 16S rRNA gene sequencing, the genomic DNA of strain WRAS1^T was extracted and purified using standard methods [5]. The 16S rRNA gene was amplified, purified, cloned and sequenced according to Zhang *et al.* [6]. The near-complete 16S rRNA gene sequence was manually checked and submitted to GenBank. Pairwise similarity values between strain WRAS1^T and closely related type strains were calculated using the EzBioCloud (www.ezbiocloud.net/, [7]). The 16S rRNA gene sequences of related strains were retrieved from the NCBI database (www.ncbi.nlm.nih.gov) and phylogenetic trees were conducted using neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms implemented in MEGA version 7.0 [8]. The genetic distance matrices of the first two trees were estimated by Kimura's two-parameter model [9]. The topologies of phylogenetic trees were evaluated based on bootstrap resampling method with 1000 replicates.

The 16S rRNA gene sequence of strain WRAS1^T was 1426 nt long and showed the highest similarity to *T. halodurans* JCM13833^T (97.54 %), followed by *T. lentus* YCS-24^T (97.47 %) and other bacteria belonging to the family *Rhodobacteraceae* with lower sequence similarities. The closer relationship between strain WRAS1^T and *T. halodurans* was confirmed by the phylogenetic trees obtained based on NJ (Fig. 1), ML and MP algorithms (Figs S1 and S2, available in the online version of this article). However, the relatively low sequence similarity of strain WRAS1^T to the type strain of recognized species in the genus *Thalassococcus* implied that strain WRAS1^T may represent a novel species.

Gram-staining and flagellum-staining were performed using standard methods [10]. The cell morphology was observed by transmission electron microscopy (JEM-1200EX, JEOL) after cells had been negatively stained with 1 % (w/v) phosphotungstic acid. Growth under anaerobic conditions was determined on MA added cysteine (0.01 %, w/v) as a reductant and resazurin (0.02 %, w/v) as an indicator of anaerobic conditions in an anaerobic jar filled with nitrogen and a packet of AneroPack-Anaero (Mitsubishi Gas Chemical Co.) at 37 °C for at least 1 month. The temperature range for growth was determined on MA plates by incubating cultures at 10–45 °C (10, 16, 20, 24, 28, 32, 37, 42 and 45 °C) for 5 days, and at 0, 4 and 47 °C for at least 30 days. Salinity and pH ranges for growth within the first 48 h were investigated in test tubes. Salt tolerance was investigated using synthetic marine ZoBell broth (5 g peptone, 1 g yeast extract and 0.01 g $FePO_4$ in 1 l distilled water) supplied with various concentrations of NaCl (0, 0.5 and 1–15 %, w/v, at intervals of 1 %). The pH range was determined in MB at pH 5.0–11.0 (at 1 pH unit intervals) using the following buffer systems: MES (pH 5.0–6.0), MOPS (pH 7.0), tricine (pH 8.0), TAPS (pH 9.0), CAPS (pH 10.0) and Na_2CO_3/$NaHCO_3$ (pH 11.0). The physiological–biochemical characteristics of strain WRAS1^T and the reference strain JCM13833^T, including catalase and oxidase activities, degradation of alginate, starch, CM-cellulose, casein, gelatin and Tweens (20, 40 and 80) were carried out according to the standard protocols [11] with the modification that filter-sterilized seawater was substituted for distilled water. DNase activity was investigated by using DNase agar (Qingdao Hope Biotechnology) according to the manufacturer's instructions. Degradation of chitin was examined on chitin agar with sterile seawater [12]. Activities of constitutive enzymes and other physiological properties were determined after growth on MA at 37 °C for one days by using API 20E, API 20NE, API 50CH, API ZYM strips (bioMérieux) and GN2 MicroPlates (Biolog) according to the manufacturers' instructions except that sterile seawater was used to prepare the inocula. The morphological, physiological and biochemical characteristics of strain WRAS1^T are given in the species description, Table 1 and Fig. S3.

For fatty acid analysis, strain WRAS1^T and *T. halodurans* JCM13833 ^{T} were cultured on MA at 37 °C. Cells were collected at the late exponential growth stage according to the four quadrant streak method [13]. Subsequently, fatty acids of the two strains were saponified, methylated and extracted according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.10) and identified by the TSBA 6.0 database of the Microbial Identification System [14]. For respiratory quinione and polar lipid analysis, cells were harvested from MB after shaking at 37 °C for 2–3 days and freeze-dried. The major respiratory quiniones were extracted with chloroform/methanol (2 : 1, v/v), separated by thin-layer chromatography (TLC) and identified by high performance liquid chromatography (HPLC) [15]. Polar lipids were extracted according to the methods described by Minnikin *et al.* [16], and separated by two-dimensional TLC on silica gel 60 F254 plates (Merck) using chloroform/methanol/water (65 : 25 : 4, by vol.) and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.) for the two dimensions, respectively [17]. The identification of extracted lipid was performed by spraying the plates with appropriated detection reagents [18]. In brief, 5 % ethanolic molybdophosphoric acid was used to detect all the lipids. Ninhydrin spray was applied to determine most of the lipids with free amino groups, while spraying the plate with the lipid phosphate reagent of Dittmer and Lester after the ninhydrin spray could reveal the presence of phospholipids. For genome sequencing, the genomic DNA of strain WRAS1^T was extracted according to the procedure described previously [19]. The genome of strain WRAS1^T was sequenced using the Illumina HiSeq platform and assembled using the assembly pipeline A5 [20]. Gene prediction and annotation was processed using RAST sever [21]. The detailed draft genome features of strain WRAS1^T are summarized in Table S2.

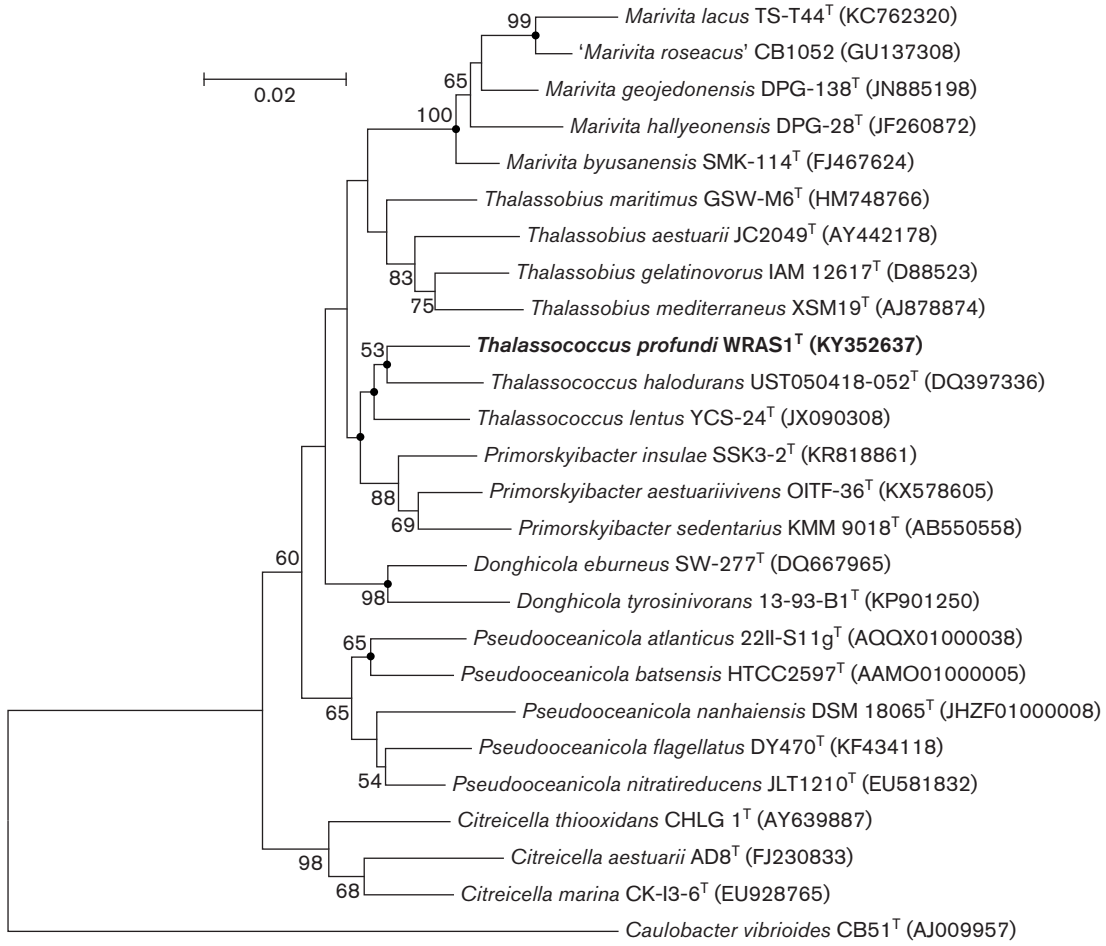

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain WRAS1^T, the type strains of recognized *Thalassococcus* species and representatives of other genera in the family *Rhodobacteraceae*. Percentage bootstrap values≥50 % (1000 replicates) are shown at branch nodes. *Caulobacter vibrioides* CB51^T (AJ009957) was used as an outgroup. Closed circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bar, 0.02 substitutions per nucleotide position.

The cellular fatty acid profiles of strain WRAS1^T and the reference strain JCM13833^T are given in Table S1. The overall fatty acids profiles of the two strains were similar, in which their significant dominant fatty acids were $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$ (summed feature 8), occupying a proportion of more than 70 %. The content of other fatty acids detected in strain WRAS1^T was no more than 10 %, while strain JCM13833^T contained a relatively higher abundance of $C_{12:1}$ 3-OH (11.6 %). The polar lipid profile of strain WRAS1^T comprised one phosphatidylcholine (PC), one phosphatidylglycerol (PG), one phosphatidylethanolamine (PE) and three unidentified lipids (L1–L3) (Fig. S4), and most polar lipids were found in *T. halodurans* JCM13833^T except for L1 [2].

For investigation of genome relatedness, the average nucleotide identity (ANI) value between strain WRAS1^T and *T. halodurans* JCM13833^T was calculated using the EzBioCloud (www.ezbiocloud.net/) with the USEARCH-based OrthoANIu algorithm [22]. The genome-to-genome distance analysis was performed using the Genome-to-Genome Distance Calculator (GGDC 2.1; http://ggdc.dsmz.de/ggdc.php) [23]. The ANI value calculated for the estimation of the degree of pairwise genome-based relatedness between strain WRAS1^T and *T. halodurans* JCM13833^T was 74.11 %, which was below the ANI cut-off value (95–96 %) proposed for delineating bacterial species [24]. Consistently, the DNA–DNA hybridization (DDH) value estimated by the GGDC was 22.70±2.3 % between strain WRAS1^T and *T. halodurans* JCM13833^T. Both the ANI and the DDH results indicated that strain WRAS1^T represented a distinctive species in genus *Thalassococcus*. The genomic DNA G+C content of strain WRAS1^T and *T. halodurans* JCM13833^T were calculated using genome sequences. The genomic DNA G+C content of strain WRAS1^T was 65.6

Table 1. Differential characteristics between strain WRAS1 and the control strains

Strains: 1. WRAS1^T; 2. *Thalassococcus halodurans* JCM13833^T, data from Lee *et al.* [1] unless indicated otherwise; 3. *Thalassococcus lentus* YCS-24^T, data from Park *et al.* [2]. +, Positive reaction; −, negative reaction. All strains are positive for activities of catalase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase; utilization of D-xylose, cellobiose, D-fructose, D-glucose, pyruvate and succinate. All strains are negative for hydrolysis of starch, gelatin and casein, utilization of sucrose and trehalose, and activities of urease, lipase (C14), β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.

Characteristic	1	2	3
Cell shape	Rod	Ovoid	Rod or ovoid
Flagellum	+	−*	−
Range (optimum) for growth:			
Temperature (°C)	4–45 (28–37)	10–45 (28–37)*	15–30 (25–28)
NaCl (%)	0.0–9.0 (3.0–4.0)	0.0–12.0 (2.0–6.0)*	1.0–4.0 (2.0)
pH	7.0–10.0 (7.0–8.0)	6.0–10.0 (7.0–8.0)*	6.0–9.5 (7.0–7.5)
Oxidase	+	+*	−
Nitrate reduction to nitrite	−	+*	−
Hydrolysis of Tween 80	−	−*	+
Production of:			
Valine arylamidase	+	+	−
Cystine arylamidase	+	−	−
Trypsin	+	−	−
α-Chymotrypsin	+	−	−
α-Galactosidase	+	+	−
β-Galactosidase	+	+	−
α-Glucosidase	+	+	−
β-Glucosidase	+	+	−
Utilization of:			
L-Arabinose	+	+	−
D-Galactose	−	+	+
Salicin	−	−	+
D-Mannose	−	+	+
Citrate	−	+	+
Maltose	+	−	−
DNA G+C content (%)	65.6	58.0	58.0

*Data of *Thalassococcus halodurans* JCM13833^T from this study.

mol%, which was higher but close to that of the reference strain JCM13833^T (58.0 mol%; Table 1).

Strain WRAS1^T shared the same characteristics with the control strains *T. halodurans* JCM13833^T and *T. lentus* YCS-24^T in terms of production of catalase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. All strains contained Q-10 as the major respiratory quinone. Strain WRAS1^T and the most closely related species *T. halodurans* JCM13833^T contained $C_{18:1}$ *ω6c* and/or $C_{18:1}$*ω7c* (summed feature 8) as the most abundant fatty acid component, while the predominant fatty acid of strain YCS-24^T was $C_{18:1}$*ω7c*. PC, PG and one L were present in all the strains except that strain WRAS1^T and *T. halodurans* JCM13833^T contained PE while *T. lentus* YCS-24^T had an AL. Moreover, the phylogenetic analyses showed that strain WRAS1^T clustered together with the type species *T. halodurans* JCM13833^T. All the above characteristics demonstrated that strain WRAS1^T belonged to the genus *Thalassococcus*. However, the low similarity of the 16S rRNA gene, together with the low level of estimated ANI and DDH values strongly implied that strain WRAS1^T represented a novel species in genus *Thalassococcus*. Moreover, some unique features also distinguished strain WRAS1^T from *T. halodurans* JCM13833^T and *T. lentus* YCS-24^T, including cell shape, flagellum, temperature and NaCl ranges (optima), the physiological characteristics and enzyme activities (such as gelatinase) listed in Table 1, the proportion of the dominant fatty acids (summed feature 8) and kinds of polar lipids (Fig. S4). Besides, the genomic DNA G+C content of strain WRAS1^T was higher than that of *T. halodurans* JCM13833^T. On the basis of phenotypic and physiological characteristics and phylogenetic inferences, strain WRAS1^T is considered to represent a novel species of the genus *Thalassococcus*, for which the name *Thalassococcus profundi* sp. nov. is proposed.

DESCRIPTION OF *THALASSOCOCCUS PROFUNDI* SP. NOV.

Thalassococcus profundi (pro.fun′di. L. gen. n. *profundi* of/from the depths of the sea).

Cells are Gram-stain-negative, strictly aerobic, rod-shaped (approximately 0.35–0.45 µm long and 0.1–0.2 µm wide) with a single flagellum (Fig. S3). Colonies on MA are beige white, round, regular, convex, smooth and with an entire margin of 1–1.5 mm in diameter after incubation for 24 h at 37 °C. Growth occurs at 4–45 °C (optimum, 28–37 °C), at pH 7.0–10.0 (optimum, pH 7.0–8.0) and with 0.0–9.0 % NaCl (w/v; optimum, 3.0–4.0 %). Oxidase and catalase are positive. Tweens 20 and 40 are hydrolysed, but alginate, starch, CM-cellulose, gelatin, casein, DNA, gelatin, chitin and Tween 80 are not. In API 20E/20NE strips, there are positive results for utilization of aesculin ferric citrate and 4-nitrophenyl-β-D-galactopyranoside, and acetoin production. Using API ZYM strips, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase activities are present, while other characteristics are absent. According to the results of API 50CHB/E strips, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, methyl β-D-xylopyranoside, D-glucose, D-fructose, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, cellobiose, maltose, lactose, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate are fermented. In the GN2 MicroPlate system, there are positive results for pyruvic acid/methyl ester, succinic acid/monomethyl ester, formic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, malonic acid, L-alanyl-glycine, L-glutamic acid, L-pyroglutamic acid, L-serine, 2,3-butanediol and glycerol, while other substrates are negative. The major fatty acid is $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$ (summed feature 8). The major respiratory quinone is Q-10. The major polar lipids include one PC, one PG, one PE and three Ls.

The type strain, WRAS1^T (=CGMCC 1.16123^T=MCCC 1K03253^T=KCTC 52696^T), was isolated from deep seawater of the Okinawa Trough (122° 34′ E, 25° 04′ N). The DNA G+C content of the type strain is 65.6 mol%.

The GenBank accession number for the 16S rRNA gene sequence of *Thalassococcus profundi* WRAS1^T is KY352637. The Whole Genome Shotgun project of strain WRAS1^T has been deposited at DDBJ/ENA/GenBank under the accession QPMK00000000. The version described in this paper is version QPMK01000000.

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

References

1. Lee OO, Tsoi MM, Li X, Wong PK, Qian PY. *Thalassococcus halodurans* gen. nov., sp. nov., a novel halotolerant member of the *Roseobacter* clade isolated from the marine sponge *Halichondria panicea* at Friday Harbor, USA. *Int J Syst Evol Microbiol* 2007;57:1919–1924.
2. Park S, Jung YT, Kim SI, Yoon JH. *Thalassococcus lentus* sp. nov., an alphaproteobacterium isolated from seawater of a seaweed farm. *Antonie van Leeuwenhoek* 2013;103:465–473.
3. Steinsbu BO, Thorseth IH, Nakagawa S, Inagaki F, Lever MA *et al.* *Archaeoglobus sulfaticallidus* sp. nov., a thermophilic and facultatively lithoautotrophic sulfate-reducer isolated from black rust exposed to hot ridge flank crustal fluids. *Int J Syst Evol Microbiol* 2010;60:2745–2752.
4. Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS. Methanogens: reevaluation of a unique biological group. *Microbiol Rev* 1979;43:260–296.
5. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG *et al.* *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, 3rd ed. New York: Wiley; 1995.
6. Zhang Z, Yu T, Xu T, Zhang XH. *Aquimarina pacifica* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* 2014;64:1991–1997.
7. 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.
8. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* 2016;33:1870–1874.
9. 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.
10. Beveridge TJ, Lawrence JR, Murray RG. Sampling and staining for light microscopy. 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. 19–33.
11. Tindall BJ, Sikorski J, Smibert RA, Krieg NR. Phenotypic Characterization and the Principles of Comparative Systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM *et al.*. (editors). *Methods for General and Molecular Microbiology*. Washington, DC: American Society of Microbiology; 2007. pp. 330–393.
12. Hsu SC, Lockwood JL. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Appl microbial* 1975;29:422–426.
13. Bruns A, Rohde M, Berthe-Corti L. *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* 2001;51:1997–2006.
14. Sasser M. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc; 1990.
15. Xie CH, Yokota A. Phylogenetic analyses of *Lampropedia hyalina* based on the 16S rRNA gene sequence. *J Gen Appl Microbiol* 2003;49:345–349.
16. 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.

17. **Collins MD, Shah HN.** Fatty acid, menaquinone and polar lipid composition of *Rothia dentocariosa*. *Arch Microbiol* 1984;137:247–249.
18. **Komagata K, Suzuki K-I.** Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1988;19:161–207.
19. **Moore ERB, Arnscheidt A, Krüger A, Strömpl C, Mau M *et al.*** Simplified protocols for the preparation of genomic DNA from bacterial cultures. *Mol Microbial Ecol Manual* 1999;1:1–15.
20. **Tritt A, Eisen JA, Facciotti MT, Darling AE.** An integrated pipeline for de novo assembly of microbial genomes. *PLoS One* 2012;7: e42304.
21. **Aziz RK, Bartels D, Best AA, Dejongh M, Disz T *et al.*** The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 2008;9:75.
22. **Yoon SH, Ha SM, Lim J, Kwon S, Chun J.** A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
23. **Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M.** Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14: 60.
24. **Richter M, Rosselló-Móra R.** Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.