INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Yin *et al.*, *Int J Syst Evol Microbiol* 2018;68:3316–3321

DOI 10.1099/ijsem.0.002990

MICROBIOLOGY SOCIETY

Acuticoccus kandeliae sp. nov., isolated from rhizosphere soil of the mangrove plant *Kandelia*, and emended description of *Acuticoccus yangtzensis*

Qi Yin,[1,2] Jinyou Liang,[1] Lv Zhang,[1] Ke Ma,[1] Zhang-Li Hu,[1,2] Yu Zhang[1,*] and Ying Xu[1,*]

Abstract

A novel bacterial strain, J103^T, was isolated from rhizosphere soil of the mangrove plant *Kandelia* in Mai Po Inner Deep Bay Ramsar Site, Hong Kong. The strain was aerobic, Gram-stain-negative, oval-shaped with folds in the middle, non-motile and non-spore-forming. It grew at temperatures of 20–30 °C (optimum, 25–30 °C), at pH 6.0–9.0 (optimum pH 6.0) and at NaCl concentrations of 0.5–5.0 % (w/v) (optimum 1.0–2.0 %). Strain J103^T was able to reduce nitrate to nitrite, and hydrolyse urea, Tween 40 and Tween 60. The major polar lipids were aminolipid, glycolipid, phosphatidylcholine and phosphatidylglycerol. The major fatty acids were $C_{18:1}\omega7c$ and $C_{19:0}$ cyclo $\omega8c$. The respiratory quinone was Q-10. The DNA G+C content was 68.5 mol%. Sequence analysis of the 16S rRNA gene indicated that strain J103^T belongs to the genus *Acuticoccus*, within the family *Rhodobacteraceae*. The closest phylogenetic neighbour was *Acuticoccus yangtzensis* JL1095^T, showing 96.2 % 16S rRNA gene sequence similarity. The genome size of strain J103^T was 6 478 100 bp. The average nucleotide identity and digital DNA–DNA hybridization values between strain J103^T and *Acuticoccus yangtzensis* JL1095^T were 75.44 and 16.43 %, respectively. Characterization based on phylogenetic, phenotypic, chemotaxonomic and genomic evidence demonstrated that strain J103^T represents a novel species of the genus *Acuticoccus*, for which the name *Acuticoccus kandeliae* sp. nov. is proposed. The type strain is J103^T (=DSM 104434^T=MCCC 1K03288^T).

Mangroves are coastal ecosystems dominated by woody plants and sediment-dwelling micro-organisms [1, 2]. The mangrove biome is a critical bioremediation environment against anthropogenic pollution, through the cycling of nutrients and water [3–5]. Mangrove sediments contain abundant and diverse microflora mainly composed of bacteria and fungi, which account for 91 % of the total microbial biomass in tropical mangroves [6, 7]. Numerous and diverse micro-organisms continuously convert dead mangrove vegetation and anthropogenic biowastes into sources of carbon, nitrogen and other nutrients. These nutrients and then supplied to live plants, whose root exudates serve as a food resource for micro-organisms [2, 6, 8]. Due to the richness and uniqueness of the micro-organisms in mangroves, the isolation and identification of novel species among mangrove microflora will expand our understanding of undiscovered metabolic pathways as well as reveal new genetic information and bioactive natural products.

The family *Rhodobacteraceae* is affiliated to the order *Rhodobacterales* of the class *Alphaproteobacteria*. Members of the family *Rhodobacteraceae* are mainly found across aquatic habitats, especially in coastal seawater, the open ocean and marine sediments. These organisms are phenotypically, metabolically and ecologically diverse [9, 10]. First reported in 2015, the genus *Acuticoccus* is a new member of the family *Rhodobacteraceae* and contains, at the time of writing, only one member, *Acuticoccus yangtzensis* [9].

Here, we describe a novel rhizobacteria, designated J103^T, isolated from rhizosphere soil of the mangrove plant *Kandelia* sp. Based on its phylogenetic, phenotypic, chemotaxonomic and genomic characteristics, strain J103^T is considered to represent a novel species of the genus *Acuticoccus*, within the family *Rhodobacteraceae*.

Strain J103^T was isolated from the rhizosphere soil of a mangrove plant, *Kandelia* sp., in Mai Po Inner Deep Bay

Author affiliations: [1]Shenzhen Key Laboratory of Marine Bioresource and Eco-environmental Science, Shenzhen Engineering Laboratory for Marine Algal Biotechnology, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, PR China; [2]Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, College of Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, PR China.

***Correspondence:** Yu Zhang, biozy@szu.edu.cn; Ying Xu, boxuying@szu.edu.cn

Keywords: *Acuticoccus kandeliae*; rhizosphere soil; mangrove plant.

Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization.

The GenBank/EMBL/DDBJ accession numbers for the draft genome sequence and 16S rRNA gene sequence of *Acuticoccus kandeliae* J103^T are QBBV00000000 and KY038377, respectively.

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

Ramsar Site, Hong Kong (114.05° E 22.49° N, 10 October 2015), by using a conventional dilution-plating technique on marine agar 2216 (MA; Becton Dickinson). Strain J103^T was subcultivated on MA at 25 °C and preserved in MA with 25 % glycerol suspension at –80 °C.

Genomic DNA was extracted with the FastDNA SPIN kit for soil (MP Biomedicals) and the 16S rRNA gene was amplified using universal primers 27F and 1492R [11]. The PCR product was purified using the TIAN gel Midi purification kit (TIANGEN Biotech), cloned to pM18-T (TaKaRa) and sequenced by using an automated DNA sequencer (ABI 3730; Applied BioSystems). The 16S rRNA gene sequence (1430 bp) of strain J103^T was submitted to GenBank (accession no. KY038377). Identification of the phylogenetic neighbours of strain J103^T, as well as calculation of pairwise 16S rRNA gene sequence similarities, were performed using Ezbiocloud (http://www.ezbiocloud.net/ [12]). The program CLUSTAL X1.8 [13] was used to align sequences. Phylogenetic trees were reconstructed using the neighbour-joining and maximum-likelihood methods [14, 15] implemented in the program MEGA v7.0 [16]. The topological robustness was evaluated by bootstrap analysis based on 1000 replicates [17]. Phylogenetic analysis based on the neighbour-joining (Fig. 1) and maximum-likelihood algorithms (Fig. S1, available in the online version of this article) revealed that strain J103^T was clustered in the genus *Acuticoccus* and constituted a different clade only with *Acuticoccus yangtzensis* JL1095^T. Furthermore, the isolate showed 96.2, 93.1 and 92.5 % 16S rRNA gene sequence similarity with the three phylogenetically closest type strains, namely *Acuticoccus yangtzensis* JL1095^T, *Amorphus coralli* RS.SPH.026^T and *Amorphus orientalis* YIM D10^T respectively, and less than 92.0 % similarity with those of related species of other genera in the family *Rhodobacteraceae*.

Growth conditions, including optimum growth temperature, tolerance to pH and NaCl, were determined on both MA and in marine broth 2216 (MB; Becton Dickinson). For these studies, cells were grown at 4, 8, 10, 20, 25, 30, 37, 45 and 50 °C, at initial pH values of 3–11 (at 0.5 pH unit intervals) by using the buffer system described by Liang *et al.* [18], and at NaCl concentrations of 0, 0.5 and 1–15.0 % (w/v, at intervals of 1 %). All experiments were conducted in triplicate with 7 days of incubation. After the bacterial cells were negatively stained with phosphotungstic acid, cell morphology and size were examined by transmission electron microscopy (JEM-1230; JEOL). Catalase activity was determined by the production of gas bubbles upon the addition of a drop of 3 % (v/v) H_2O_2. A Gram stain set (Difco Laboratories) was used for Gram staining according to standard procedures [19]. Tween 20, 40, 60 and 80 utilization tests were performed according to a standard protocol [20]. In addition, enzyme activities, and physiological and biochemical features were determined using API ZYM and API 20NE test kits (bioMérieux) according to the manufacturer's instructions. *Acuticoccus yangtzensis* JL1095^T, *Amorphus coralli* RS.SPH.026^T and *Amorphus orientalis* YIM D10^T were used as reference strains.

Cells of strain J103^T were Gram-stain-negative, non-motile, aerobic, non-spore-forming and oval-shaped with folds in the middle (1.0–1.5 μm in diameter, Fig. S2). The strain formed light-orange colonies with a convex surface after 3–5 days at 30 °C on MA. It grew at temperatures of 20–30 °C, with optimum growth at 25–30 °C. Growth was observed at pH 6.0–9.0, with an optimum of pH 6.0. Salt requirement ranged from 0.5 to 5.0 % (w/v) NaCl, with optimal growth in 1.0–2.0 % (w/v) NaCl. Catalase activity of strain J103^T was positive. Strain J103^T could utilize Tweens 40 and 60. The physiological and biochemical

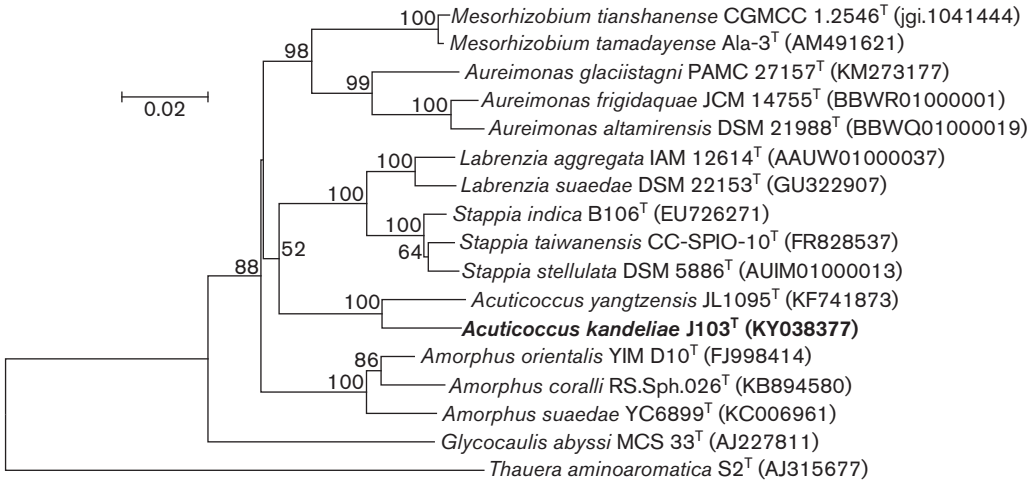

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain J103^T and other related micro-organisms. *Thauera aminoaromatica* S2^T was used as an outgroup. Percentage bootstrap values above 50 % (1000 replicates) are shown at branch nodes. Bar, 0.02 changes per nucleotide position.

characteristics of strain J103^T and the three closest related type strains are described in Table 1. According to results of API 20NE tests (Table 1), strain J103^T could reduce nitrate to nitrite, in contrast to the three phylogenetically closest related strains. This might be related to its ecological position as living in sediment associated with a mangrove plant [21–23]. Strain J103^T also showed the ability to hydrolyse urea, as with *Acuticoccus yangtzensis* JL1095^T and *Amorphus coralli* RS.SPH.026^T.

The DNA G+C content was first measured using the HPLC method by the Identification Service of the DSMZ, according to the method described by Mesbah *et al.* [24]. Polar lipids were extracted as previously described [25] and then were separated by two-dimensional TLC on silica gel 60 F254 plates (Merck) using chloroform/methanol/water (64 : 25 : 5, by vol.) for the first dimension and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.) for the second dimension [26]. Total lipid content and individual compounds were detected using molybdatophosphoric acid, and specific functional groups were detected using the following spray reagents: ninhydrin for free amino groups, and molybdenum blue reagent for phosphate esters [20]. Extraction of fatty acid methyl esters and separation by GC were performed by using the Instant FAME method of the Microbial Identification System (MIDI) version 6.1 and the RTSBA6 6.10 database [27]. Analysis of respiratory quinones were carried out by the Identification Service of the DSMZ according to procedures described previously [28, 29].

Table 1. Phenotypic properties and differential characteristics between strain J103^T and its phylogenetically closest related species

Strains: 1, J103^T; 2, *Acuticoccus yangtzensis* JL1095^T; 3, *Amorphus coralli* RS.SPH.026^T; 4, *Amorphus orientalis* YIM D10^T. Characters are scored as: +, positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4
Colony pigmentation	Light-orange	Light-orange	Cream	Cream
Cell morphology				
Shape	Oval	Oval*	Amorphous*	Rods*
Size (μm, diameter)	1.0–1.5	1.3–1.5*	0.5–3.0*	1.0–2.5*
Growth requirements				
Optimum temperature (°C)	25–30	35*	25–30*	~28*
Optimum pH	~6.0	~7.5*	7.5–8.0*	~7.5*
Optimum NaCl (%, w/v)	1.0–2.0	~3.0*	3.0–4.0*	3.0–8.0*
DNA G+C content (mol%)	68.5	68.5	67.1*	65.4*
Catalase activity	+	+	+	+
API 20NE tests				
Nitrate reduction	+	–	–	–
Urea	+	+	+	–
Gelatin hydrolysis	–	+	–	+
API ZYM tests				
Alkaline phosphatase	w	w	+	+
Esterase lipase (C8)	w	–	+	w
Valine arylamidase	–	+	–	–
Acid phosphatase	+	–	+	+

*Data from Hou *et al.* [9], Wang *et al.* [30] and Yosef *et al.* [40].

Strain J103^T showed a polar lipid pattern characteristic of the genus *Acuticoccus*, mainly consisting of aminolipid (AL), glycolipid (GL), phosphatidylcholine (PC) and phosphatidylglycerol (PG) (Fig. S3), consistent with the major polar lipids of *Acuticoccus yangtzensis* JL1095^T [9]. The major polar lipids of *Amorphus orientalis* YIM D10^T were AL, PC and phospholipid (PL) [30]. In addition, GL was predominant in strain J103^T, but not in *Acuticoccus yangtzensis* JL1095^T. Glycolipids (GLs) are well known to promote the mechanical stability and chemical resistance of cell membranes [31, 32], and therefore the high amount of GLs in strain J103^T could improve its adaptive capacity to highly contaminated environments such as mangroves. Furthermore, GLs are widely distributed in the membranes of micro-organisms, and have shown various biological functions, including as biosurfactants, antimicrobial, anti-cancer, antiviral and immunocompetence agents [33–36]. Thus, strain J103^T might be a potential source of bioactive compounds.

The fatty acid profiles of strain J103^T, *Acuticoccus yangtzensis* JL1095^T, *Amorphus coralli* RS.SPH.026^T and *Amorphus orientalis* YIM D10^T were tested under the same growth conditions (Table 2). The major fatty acids (>10 %) of strain J103^T were $C_{18:1}\omega7c$ (46.3 %) and $C_{19:0}$ cyclo $\omega8c$ (35.3 %). The four strains shared the major fatty acid $C_{19:0}$ cyclo $\omega8c$, accounting for 35.3 % in strain J103^T, 22.7 % in *Acuticoccus yangtzensis* JL1095^T, 39.8 % in *Amorphus coralli* RS.Sph.026^T and 40.4 % in *Amorphus orientalis* YIM D10^T. $C_{19:0}$ cyclo $\omega8c$ is a cyclopropane fatty acid that has an additional carbon attached to the eighth and ninth carbons from the ω end, which forms a cyclic side chain [37]. It has been recently reported to play a role in stabilizing bacterial membranes against adverse conditions while simultaneously promoting membrane fluidity [38, 39]. The high amount of $C_{19:0}$ cyclo $\omega8c$ in strain J103^T may help the bacterium to build a tenacious cell membrane with efficient substance exchange activities (as a result of good membrane fluidity), which could be an important living strategy in the hostile mangrove environment. The relatively high content of GLs and $C_{19:0}$ cyclo $\omega8c$, the ability to reduce nitrate to nitrite, as well as other minor differences mentioned above (Tables 1 and 2), indicate that strain J103^T was unique and phylogenetically distinct from other closely related strains.

The isoprenoid quinone of strain J103^T was Q-10, the same as for the phylogenetically closely related strains *Acuticoccus yangtzensis* JL1095^T and *Amorphus orientalis* YIM D10^T [9, 30]. No data in this regard were reported for *Amorphus coralli* RS.SPH.026^T [40].

Table 2. Cellular fatty acid contents (%) of strain J103^T and phylogenetically closely related species

Strains: 1, J103^T; 2, *Acuticoccus yangtzensis* JL1095^T; 3, *Amorphus coralli* RS.SPH.026^T; 4, *Amorphus orientalis* YIM D10^T. All data are from this study. TR, Trace amount (<1 %). Fatty acids of strain J103^T amounting to >10 % of the total fatty acid content are in bold type, and those comprising less than 1 % are not shown.

Fatty acid	1	2	3	4
$C_{16:0}$	3.5	3.4	1.3	3.9
$C_{17:0}$	TR	1.1	4.7	7.1
$C_{18:0}$	3.2	5.4	8.8	7.3
$C_{18:0}$ 3-OH	2.9	4.0	3.9	3.0
$C_{18:1}\omega7c$	**46.3**	35.3	7.7	10.9
11-methyl $C_{18:1}\omega7c$	TR	1.0	16.9	7.1
$C_{19:0}$ cyclo $\omega8c$	**35.3**	22.7	39.8	40.4
$C_{20:1}\omega7c$	5.1	13.4	6.2	2.6

The genomes of strain J103^T and *Acuticoccus yangtzensis* JL1095^T were sequenced on an Illumina HiSeq X-ten platform with 100× coverage. Reads were trimmed to remove low-quality, ambiguous nucleotides and adapters using Trimmomatic 0.36 [41]. Genome assembly was performed using SOAPdenovo [42]. Genomic similarity was assessed by using the average nucleotide identity (ANI) algorithm using USEARCH [43], and digital DNA–DNA hybridization (DDH) analysis using the DSMZ GGDC platform [44]. The DNA G+C content of strain J103^T was 68.5 mol% by the HPLC method, similar to values for *Amorphus coralli* RS.SPH.026^T (67.1 mol%) and *Amorphus orientalis* YIM D10^T (65.4 mol%) [30, 40] (Table 1), but was much higher than the 51.5 mol% of *Acuticoccus yangtzensis* JL1095^T described by Hou *et al.* [9]. Thus, the DNA G+C contents of strain J103^T and *Acuticoccus yangtzensis* JL1095^T were calculated from whole genome sequencing in this study. The genome size was 6 478 100 bp with a 68.51 mol% G+C content and 1 433 190 bp N_{50} value for strain J103^T (GenBank accession no. QBBV00000000), and 5 098 609 bp with a 68.52 mol% G+C content and 513 089 bp N_{50} value for *Acuticoccus yangtzensis* JL1095^T (GenBank accession no. QBBW00000000) (Table 3). Our genomic results for *Acuticoccus yangtzensis* JL1095^T are in accordance with those for the genome reported later in 2017 by the same group that originally described strain JL1095^T (5 043 263 bp genome size and 68.6 mol% G+C content, accession no. MJUX00000000) [45]. Therefore, the 51.5 mol% G+C content of *A. yangtzensis* JL1095^T described previously [9] should be emended to 68.5 mol%. For genome similarity analyses between strain J103^T and *Acuticoccus yangtzensis* JL1095^T, the ANI and DDH values were 75.44 and 16.43 % (Table 3), much lower than the species cut-off thresholds (95 and 70 %, respectively) [46]. Genome analyses thus indicated that strain J103^T should be considered as representing a separate novel species.

Based on the analysis of morphological, phylogenetic, chemotaxonomic and genomic characteristics, it is evident that strain J103^T represents a novel *Acuticoccus* species, for which the name *Acuticoccus kandeliae* sp. nov. is proposed. Based on the calculation from whole genome sequencing, the DNA G+C content of *Acuticoccus yangtzensis* JL1095^T is emended to 68.5 mol%.

Table 3. General genome statistics of strain J103^T and *Acuticoccus yangtzensis* JL1095^T

Attribute	Strain J103^T	*Acuticoccus yangtzensis* JL1095^T
Genome size (bp)	6 478 100	5 098 609
N_{50} length (bp)	1 433 190	513 089
Contig number	211	89
DNA G+C (bp)	4 438 575	3 493 657
DNA G+C content (mol %)	68.51	68.52
Total genes	6075	4547
ANI (%)	75.44	
Digital DDH (%)	16.43	

DESCRIPTION OF *ACUTICOCCUS KANDELIAE* SP. NOV.

Acuticoccus kandeliae (kan.de'li.ae. N.L. fem. gen. n. *kandeliae* of the genus *Kandelia*, referring to the rhizosphere soil from which the type strain was isolated).

Cells are Gram-stain-negative, oval-shaped with folds in the middle, 1.0–1.5 µm diameter in size, non-motile and non-spore-forming. Colonies are light-orange with a circular convex surface after 3–5 days at 30 °C. Growth occurs at 20–30 °C (optimum 25–30 °C), with 0.5–5.0 % (w/v) NaCl (optimum 1.0–2.0 %) and at pH 6.0–9.0 (optimum pH 6.0). Test for catalase is positive. Cells can reduce potassium nitrates to nitrites. Hydrolyses urea, Tween 40 and Tween 60. Negative results for L-tryptophane, D-glucose, L-arginine, aesculin, gelatin, β-galactosidase, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid in API 20NE tests. In the API ZYM system, cells are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, α-glucosidase, β-glucosidase, β-glucuronidase, α-galactosidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The major polar lipids are aminolipid, glycolipid, phosphatidylcholine and phosphatidylglycerol. The major fatty acids are $C_{18:1}\omega7c$ and $C_{19:0}$ cyclo $\omega8c$. The respiratory quinone is Q-10.

The type strain, J103^T (=DSM 104434^T=MCCC 1K03288^T), was isolated from rhizosphere soil of the mangrove plant *Kandelia*, in Mai Po Inner Deep Bay Ramsar Site, Hong Kong. The DNA G+C content is 68.5 mol%. The GenBank accession number of the genome sequence is QBBV00000000, with 6 478 100 bp genome size.

EMENDED DESCRIPTION OF *ACUTICOCCUS YANGTZENSIS* HOU *ET AL.* 2017

The description is as given by Hou *et al.* [9] with the following amendment. The DNA G+C content of the type strain is 68.5 mol%.

Funding information
This work was supported by the Open Financial Grant from Qingdao National Laboratory for Marine Science and Technology (No. QNLM2016ORP0302), the Scientific and Technical Innovation Council of Shenzhen (Nos. 827000012 and KQJSCX20170330110206042), and the 62st-class General Financial Grant from the China Postdoctoral Science Foundation (No. 2017M622754).

Acknowledgements
We are grateful to Dr Qiliang Lai from Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, State Oceanic Administration, for help in genomic analysis and editing the manuscript.

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

References

1. Duke NC, Meynecke JO, Dittmann S, Ellison AM, Anger K *et al.* A world without mangroves? *Science* 2007;317:41–42.
2. Sahoo K, Dhal N. Potential microbial diversity in mangrove ecosystems: a review. *Indian J Mar Sci* 2009;38:249–256.
3. Lovelock CE. Soil respiration and belowground carbon allocation in mangrove forests. *Ecosystems* 2008;11:342–354.
4. Kathiresan K, Bingham BL. Biology of mangroves and mangrove ecosystems. *Adv Mar Biol* 2001;40:81–251.
5. Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC *et al.* The value of estuarine and coastal ecosystem services. *Ecol Monogr* 2011;81:169–193.
6. Holguin G, Vazquez P, Bashan Y. The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. *Biol Fertil Soils* 2001;33:265–278.
7. Lang FS, Destain J, Delvigne F, Druart P, Ongena M *et al.* Characterization and evaluation of the potential of a diesel-degrading bacterial consortium isolated from fresh mangrove sediment. *Water Air Soil Pollut* 2016;227:58.
8. Krishnan KP, Fernandes SO, Chandan GS, Loka Bharathi PA. Bacterial contribution to mitigation of iron and manganese in mangrove sediments. *Mar Pollut Bull* 2007;54:1427–1433.
9. Hou L, Zhang Y, Sun J, Xie X. *Acuticoccus yangtzensis* gen. nov., sp. nov., a novel member in the family *Rhodobacteraceae*, isolated from the surface water of the Yangtze Estuary. *Curr Microbiol* 2015;70:176–182.
10. Xian S, Zhang R, Sun J, Chen Y, Deng W *et al.* *Seohaeicola westpacificensis* sp. nov., a novel member of genera *Seohaeicola* isolated from deep West Pacific Sea water. *Curr Microbiol* 2014;69:32–36.
11. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E and Goodfellow M (editors). *Nucleic Acid Techniques in Bacterial Systematics*. New York: John Wiley & Sons; 1991. pp. 115–175.
12. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M *et al.* Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
13. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997;25:4876–4882.
14. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
15. Yang Z. PAML: a program package for phylogenetic analysis by maximum likelihood. *Comput Appl Biosci* 1997;13:555–556.
16. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
17. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
18. Liang X, Lin H, Wang K, Liao Y, Lai Q *et al.* *Altererythrobacter salegens* sp. nov., a slightly halophilic bacterium isolated from surface sediment. *Int J Syst Evol Microbiol* 2017;67:909–913.
19. Gerhardt P, Murray RGE, Wood WA, Krieg NR. *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994.
20. Tindall BJ, Sikorski J, Smibert RA, Krieg NR. 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, USA: American Society of Microbiology; 2007. pp. 330–393.
21. Alongi DM. The role of bacteria in nutrient recycling in tropical mangrove and other coastal benthic ecosystems. *Hydrobiologia* 1994;285:19–32.
22. Li M, Cao H, Hong Y, Gu JD. Spatial distribution and abundances of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in mangrove sediments. *Appl Microbiol Biotechnol* 2011;89:1243–1254.
23. Reef R, Feller IC, Lovelock CE. Nutrition of mangroves. *Tree Physiol* 2010;30:1148–1160.
24. Mesbah M, Premachandran U, Whitman WB. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 1989;39:159–167.
25. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911–917.
26. Collins MD, Shah HN. Fatty acid, menaquinone and polar lipid composition of *Rothia dentocariosa*. *Arch Microbiol* 1984;137:247–249.
27. Sasser M. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc; 1990.
28. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
29. Tindall BJ. A Comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 1990;13:128–130.
30. Wang YX, Liu JH, Chen YG, Zhang XX, Wang ZG *et al.* *Amorphus orientalis* sp. nov., an exopolysaccharide-producing bacterium isolated from salt mine sediment. *Int J Syst Evol Microbiol* 2010;60:1750–1754.
31. Westerlund B, Slotte JP. How the molecular features of glycosphingolipids affect domain formation in fluid membranes. *Biochim Biophys Acta* 2009;1788:194–201.
32. Simons K, Ikonen E. Functional rafts in cell membranes. *Nature* 1997;387:569–572.
33. Manna M, Róg T, Vattulainen I. The challenges of understanding glycolipid functions: an open outlook based on molecular simulations. *Biochim Biophys Acta* 2014;1841:1130–1145.
34. Kitamoto D, Isoda H, Nakahara T. Functions and potential applications of glycolipid biosurfactants–from energy-saving materials to gene delivery carriers. *J Biosci Bioeng* 2002;94:187–201.
35. Stoffel W, Bosio A. Myelin glycolipids and their functions. *Curr Opin Neurobiol* 1997;7:654–661.
36. Feizi T, Stoll MS, Yuen CT, Chai W, Lawson AM. Neoglycolipids: probes of oligosaccharide structure, antigenicity, and function. *Methods Enzymol* 1994;230:484–519.

37. **Grogan DW, Cronan JE.** Cyclopropane ring formation in membrane lipids of bacteria. *Microbiol Mol Biol Rev* 1997;61:429–441.
38. **Poger D, Mark AE.** A ring to rule them all: the effect of cyclopropane Fatty acids on the fluidity of lipid bilayers. *J Phys Chem B* 2015;119:5487–5495.
39. **Kim BH, Kim S, Kim HG, Lee J, Lee IS *et al.*** The formation of cyclopropane fatty acids in *Salmonella enterica* serovar Typhimurium. *Microbiology* 2005;151:209–218.
40. **Zeevi Ben Yosef D, Ben-Dov E, Kushmaro A.** *Amorphus coralli* gen. nov., sp. nov., a marine bacterium isolated from coral mucus, belonging to the order *Rhizobiales*. *Int J Syst Evol Microbiol* 2008;58:2704–2709.
41. **Bolger AM, Lohse M, Usadel B.** Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–2120.
42. **Xie Y, Wu G, Tang J, Luo R, Patterson J *et al.*** SOAPdenovo-Trans: *de novo* transcriptome assembly with short RNA-Seq reads. *Bioinformatics* 2014;30:1660–1666.
43. **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.
44. **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.
45. **Hou L, Sun J, Xie X, Jiao N, Zhang Y.** Genome sequence of *Acuticoccus yangtzensis* JL1095^{T} (DSM 28604^{T}) isolated from the Yangtze Estuary. *Stand Genomic Sci* 2017;12:91.
46. **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.