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SHORT COMMUNICATION

# *Maribacter hydrothermalis* sp. nov., Isolated from Shallow-Sea Hydrothermal Systems Off Kueishantao Island

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## Abstract

A Gram-stain-negative, strictly aerobic, yellow-pigmented bacterial strain, designated as T28$^T$, was isolated from seawater of the shallow-sea hydrothermal system, Kueishantao Islet, Taiwan, China. Cells were oxidase-negative and catalase-positive rods without gliding motility. Growth was observed at 10–40 °C (optimum, 30 °C), at pH 5.0–8.0 (optimum, pH 6.0) and in the presence of 0–5% (w/v) NaCl (optimum, 2.5%). Strain T28$^T$ contained menaquinone 6 as the only isoprenoid quinone. The main cellular fatty acids were iso-$C_{15:0}$, iso-$C_{15:1}$ G, and iso-$C_{17:0}$3-OH, summed feature 3 ($C_{16:1}$ω7c/ω6c). Polar lipids contain diphosphatidylglycerol, phosphatidylethanolamine, two unidentified phospholipids, six unidentified lipids, an unidentified aminolipid, and one unidentified aminophospholipids. The genomic DNA G + C content was 34.4 mol%. The 16S rRNA gene sequence of strain T28$^T$ shared highest similarity with *Maribacter arcticus* (97.7%). Phylogenetic analysis based on 16S rRNA gene sequences revealed that the strain T28$^T$ belongs to the genus *Maribacter.* On the basis of phylogenetic data and several distinct phenotypic characteristics, strain T28$^T$ represents a novel species, for which the name *Maribacter hydrothermalis* sp. nov. is proposed. The type strain is T28$^T$ (=CGMCC 1.15788$^T$ = JCM 31510$^T$).

## Introduction

The genus *Maribacter*, a member of the family *Flavobacteriaceae* of the phylum Bacteroidetes, was initially proposed by Nedashkovskaya et al. in 2004 [1] and was subsequently emended by Barbeyron et al. [2], Nedashkovskaya et al. [3], Lo et al. [4], Weerawongwiwat et al. [5], and Hu et al. [6]. At the time of writing, this genus comprises 29 species with validly published names (https://lpsn.dsmz.de/genus/Maribacter), which were all isolated from marine environment such as marine sediment [4] and sea water [7]. It has been reported that the bacteria of genus *Maribacter* isolated from *Ascophyllum nodosum* are able to degrade algal-polysaccharides [8]. Zhan et al. reported the complete genome sequence of strain T28$^T$, which revealed the potential ability to degrade polysaccharide [9]. The purpose of this study is to establish the taxonomic position of stain T28$^T$ by genotypic, chemotaxonomic, and phenotypic approaches.

## Materials and Methods

### Bacterial Isolation and Culture Conditions

The seawater sample for this study was collected from surface seawater of the shallow-sea hydrothermal system, Kueishantao Islet, Taiwan, China. The sample was preserved at 4 °C before laboratory experiment. The seawater sample was sucked by pipette gun with 100 µL pipette tips and spread uniformly on marine agar 2216 (MA; Difco). Several days after aerobic inoculation at 28 °C, a yellow colony was picked out and cultured on new plates with MA. After three rounds of plating and picking from plates, cultures were considered pure and the following study was carried out. The isolate was preserved at −80 °C with 15% (v/v) glycerol. Two strains, *Maribacter chungangensis* KCTC 23735$^T$ and *Maribacter aquivivus* KCTC 12968$^T$, were used as reference stains. These strains were grown under comparable conditions for parallel testing where appropriate.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the complete genome sequence of strain T28T are KX022625 and CP018760.

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### Phylogenetic and Genotypic Analysis

Genomic DNA was extracted using a TIANamp bacterial genomic DNA kit (China) according to the manufacturer's instruction and the 16S rRNA gene sequence was amplified by PCR using two primers, 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-AAGGAGGTGATCCAGCC-3′). The amplified 16S rRNA gene sequence was determined and compared to validly named bacterial type strains in the EzBioCloud database (https://www.ezbiocloud.net/) [10]. The complete 16S rRNA gene sequence was extracted from the genome data with the help of ContEst16S [11]. Based on the EzBioCloud results, phylogenetic analysis was performed using the neighbor-joining [12] and maximum-likelihood [13] algorithms within the MEGA version 7 [14] and minimum-evolution [15] algorithms within the MEGA version X [16]. To evaluate the stability of the phylogenetic tree, bootstrap analysis was performed (1000 replications) [17].

The whole-genome sequencing of strain T28$^T$ was performed on Pacbio RS II sequencing platform (Pacific Biosciences, Menlo Park, CA, USA). The low-quality reads were filtered by the SMRT 2.3.0, and the filtered reads were assembled to generate one contig without gaps [18]. The final assembled genomes were automatically annotated and analyzed via the RAST platform (http://rast.nmpdr.org).

Average nucleotide identity (ANI) values between strain T28$^T$ and the closely related strains were calculated in ANI calculator (https://www.ezbiocloud.net/tools/ani) [19] DNA–DNA hybridization (DDH) between strain T28$^T$ and the nearest phylogenetic neighbor was not attempted, as strains differing by <98.7% 16S rRNA gene sequence similarity are unlikely to exhibit >70% relatedness at the whole-genome level [20], the threshold value recommended for the assignment of genomic species [21]. For genome-based comparison, whole-genome sequences of closest species (*M. arcticus*) in phylogenetic tree and reference strain (*M. aquivivus*) were obtained from GenBank (GenBank accession numbers NZ_FUYL00000000.1 and NZ_FQZX01000003.1 respectively).

### Phenotypic, Physiological, and Biochemical Characteristics

To determine the phenotypic, physiological, and biochemical characteristics, marine broth (MB; Difco) or rich organic (RO) media was used for growth test for 3 days at 28 °C. NaCl requirement and tolerance were tested in RO medium at 28 °C with NaCl concentrations ranging from 0–10% (in 1% increment, w/v). The range of temperature (4, 10, 15, 23, 28, 30, 35, 37, 40, 45 °C) for growth was assessed on MB. The pH range for growth was determined on RO medium at 28 °C at pH 3–12 (with intervals of 1.0 units) using the following buffers: $Na_2HPO_4$/Citric Acid for pH 3.0–8.0 and Glycine/NaOH for pH 9.0–10.0, $Na_2HPO_4$/NaOH for pH 11.0–12.0). Gram staining was performed using Gram stain kit (Haibo, China). Motility was determined by the semi-solid puncture method [22]. Cell size and shape of strain T28$^T$ were studied by transmission electron microscopy (Tecnai Spirit G2, FEI) using cells from the exponential growth phase. Activities of catalase and oxidase were determined according to the methods of Smibert and Krieg [23]. To determine the utilization of different organic substrates as carbon and energy sources, Biolog GEN III microplates were used with cells suspending in IF-A. Other biochemical and enzymatic characterizations of strain T28$^T$ were performed by the API ZYM and API20NE systems, according to the manufacturer's instructions. Susceptibility to antibiotics was detected on Mueller-Hintonincubated agar by using the disk diffusion plate method [24] with disks containing penicillin G (10 U), ampicillin (10 μg), rifampicin (5 μg), kanamycin (30 μg), gentamicin (10 ± 2.5 μg), novobiocin (30 μg), tetracycline (30 μg), chloramphenicol (30 μg), cephalothin (30 μg), vancomycin (30 μg), and erythromycin (15 μg).

### Chemotaxonomic Characteristics

With 3 days cultivation, three plates were covered with homogeneous colonies and harvested. The cellular fatty acids of microbial cells were saponified, methylated, and analyzed by the method described by Komagata and Suzuki [25]. The Microbial Identification System (MIDI) is Sherlock version 6.0 and the library TSBA6 6.00 was used for comparison of fatty acid profiles. Respiratory isoprenoid quinones were extracted from 400 mg of freeze-dried cell material, then separated into their different classes by thin-layer chromatography on silica gel [26, 27], and analyzed by high-performance liquid chromatography (HPLC). Polar lipids were extracted as described by Kates [28] using a chloroform/methanol system and analyzed using two-dimensional thin-layer chromatography (TLC).

## Results and Discussion

### Molecular Phylogenetic and Genotypic Analysis

A nearly full-length 16S rRNA gene sequence (1428 bp) of strain T28$^T$ was determined. One complete 16S rRNA gene sequence (1450 bp) of strain T28$^T$ was read from the whole-genome sequence by ContEst16S. The EzBioCloud

analysis of the 16S rRNA gene sequence of strain T28$^T$ showed highest sequence similarities to *Maribacter arcticus* (97.7%) and other members of the genus *Maribacter* (<97%). Neighbor-joining tree based on 16S rRNA gene sequences show that strain T28$^T$ is the member of genus *Maribacter* (Fig. 1). Maximum likelihood phylogenetic tree (Fig. S1) and minimum-evolution phylogenetic tree (Fig. S2) also support its taxonomic position. These results strongly support the taxonomic position of strain T28$^T$ as a member of the genus *Maribacter*.

As reported before, the complete genome shows that strain T28$^T$ contains a circular chromosome of 4,271,158 bp. A total of 3695 genes were predicted in the chromosome, including 42 tRNA, 9 rRNA, and 3 sRNA genes [9]. The G+C content was 34.4 mol% based on entire genomes. Zhan et al. revealed the potential ability of strain T28$^T$ to degrade polysaccharide according to genomic analysis, with 38 genes coding for glycoside hydrolases and 6 genes coding for polysaccharides lyase on genome. Growth experiments showed that strain T28$^T$ could utilize xylan, alginate, and pectin [9]. The ANI values between strain T28$^T$ and phylogenetically related type strains, *M. arcticus* and *M. aquivivus*, were 79.5% and 72.4%, respectively. The values were below 95%, which was generally accepted for species delineation [29].

## Phenotypic, Physiological, and Biochemical Characteristics

By electron microscopy, rod-shaped cell with approximately 1.4–1.6 μm in width and 2.8–3.1 μm in length was clearly visible (Fig. S3). Cells of strain T28$^T$ were Gram-stain-negative and aerobic. The cultural, physiological and biochemical properties of strain T28$^T$ are summarized in the species description and Table 1. As for susceptibility to antibiotics, Strain T28$^T$ was susceptible to cephalothin, novobiocin, kanamycin, and gentamicin. Strain T28$^T$ shared many characters with the members of the genus *Maribacter*, such as rod-shaped, non-motile, Gram-negative, and catalase-positive. However, some other phenotypic characteristics are quietly

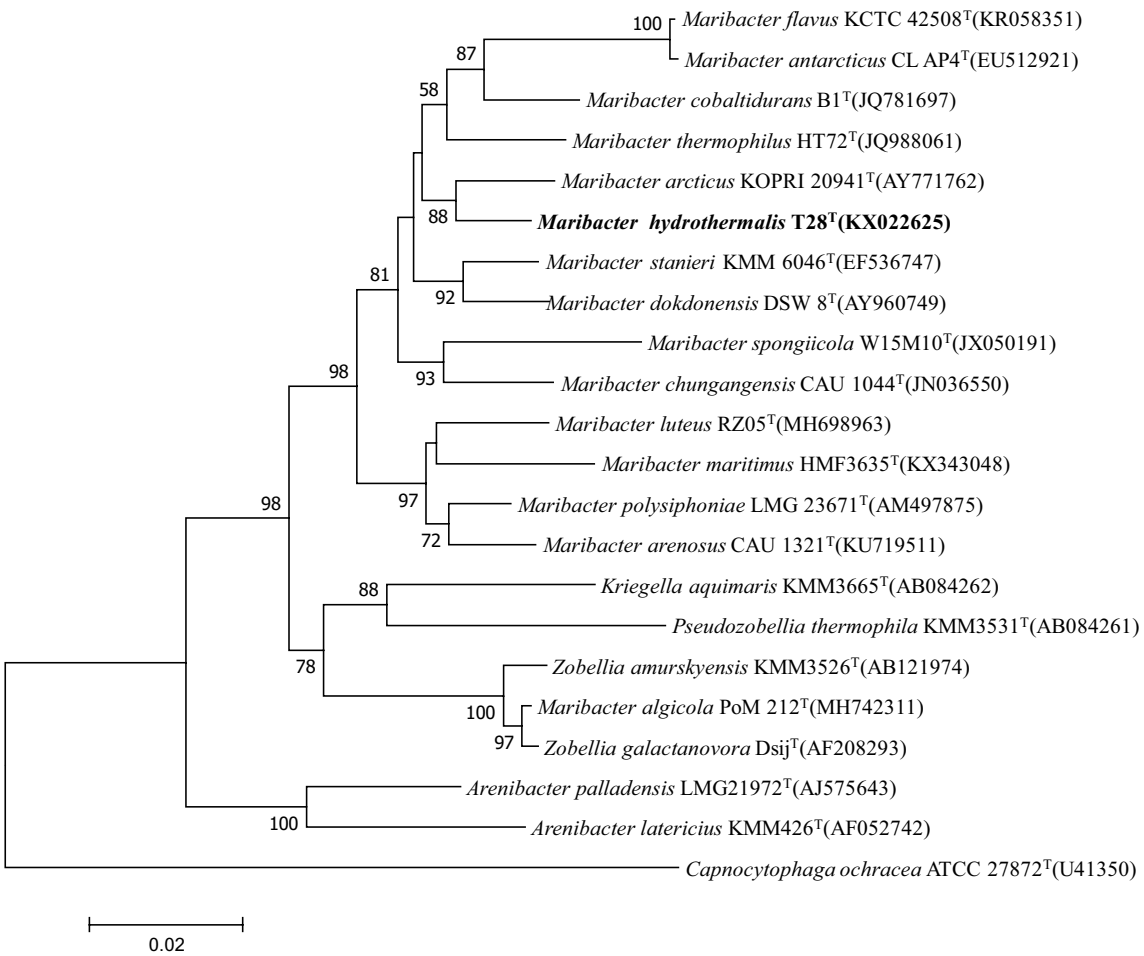


**Fig. 1** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain T28$^T$ within the genus *Maribacter*. Bootstrap values are shown at nodes as percentages of 1000 replicates. Bar, 0.02 changes per nucleotide position

**Table 1** Differential comparison of phenotypic properties of strain T28$^T$, *M. aquivivus* KCTC 12968$^T$, and *M. chungangensis* KCTC 23735$^T$

| Characteristic | 1 | 2 | 3 |
|---|---|---|---|
| Source of isolation | Sea water | Green alga[a] | Green seaweed[b] |
| Cell size (μm) | 1.4–1.6×2.8–3.1 | 1.5–3.0×0.3–0.5[a] | 1.4–2.8×0.3–0.4[b] |
| Growth range | | | |
| Temperature (°C) | 10–40 (30) | 4–15 (10)[a] | 4–30 (30)[b] |
| NaCl (%, w/v) | 0–5 (2.5) | 2–6 (3–4)[a] | 0–7 (–)[b] |
| pH | 5–8 (6) | 7–9 (8)[a] | 6.0–10.0 (8.5)[b] |
| Oxidase activity | – | + | + |
| Catalase activity | + | + | + |
| Enzymic activity (API ZYM) | | | |
| Alkaline phosphatase | + | + | + |
| Esterase (C4) | + | + | + |
| Esterase Lipase (C8) | + | + | + |
| Lipase (C14) | w | – | w |
| Valine arylamidase | + | w | + |
| Cystine arylamidase | w | – | w |
| Trypsin | + | w | + |
| α-chymotrypsin | + | – | – |
| α-galactosidase | + | + | – |
| Hydrolysis (API 20NE) | | | |
| Nitrate reduction | + | – | – |
| TRP | – | – | – |
| *β*-glucoside (esculin) | + | + | + |
| *β*-galactosidase (para-nitrophenyl-*β*-D-galactopyranoside) | + | + | + |
| Utilization of | | | |
| Glucuronamid | + | –[a] | –[b] |
| L-malic acid | + | –[a] | –[b] |
| Citrate | – | –[a] | +[b] |
| D-Glucose | – | +[a] | –[b] |
| Lactose | – | +[a] | –[b] |
| D-Mannose | – | +[a] | –[b] |
| Sucrose | – | +[a] | –[b] |
| Gelatin | – | –[a] | +[b] |
| Starch | – | –[a] | +[b] |
| Tween 20 | – | +[a] | –[b] |
| Tween 80 | – | –[a] | –[b] |
| DNA G+C content (mol%) | 34.4 | 37.11[a] | 40.2[b,c] |

[a]Date taken from Nedashkovskaya et al.[1]

[b]Date taken from Weerawongwiwat et al.[5]

[c]The DNA G+C content of *M. chungangensis* KCTC 22053$^T$ was determined using the thermal melting method, while two other strains were determined using HPLC

different from the related *Maribacter* type strains, including growth ranges of temperature, NaCl and pH, oxidase activity, and utilization of glucuronamid and L-malic acid.

## Chemotaxonomic Characteristics

With API test, strain T28$^T$ is positive in alkaline phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, trypsin, α-chymotrypsin, and α-galactosidase. The only respiratory isoprenoid quinone system identified is MK-6, which is in accordance with those identified from all *Maribacter* members [1]. Fatty acid analysis showed that branched chained fatty acids dominated the composition of all the three strains. Strain T28$^T$ has iso-$C_{15:0}$ (12.17%), iso-$C_{15:1}$ G (20.62%), iso-$C_{17:0}$ 3-OH (14.76%), and summed feature 3 (21.44%, comprising $C_{16:1}$ω7c/ω6c) as major fatty

acids (>10%) (Table S1). The major polar lipids contain diphosphatidylglycerol (DPG), phosphatidylethanolamine, two unidentified phospholipids (PL1-2), six unidentified lipids (L1-6), an unidentified aminolipid (AL), and one unidentified aminophospholipids (APL) (Fig. S4).

## Taxonomic Conclusion

Based on the above analysis, strain T28$^T$ represents a novel species within the genus *Maribacter*, for which the name *Maribacter hydrothermalis* sp. nov. is proposed.

### Description of *Maribacter hydrothermalis* sp. nov.

*Maribacter hydrothermalis* (hy.dro.ther.ma'lis. N.L. masc. adj. *hydrothermalis* from a hydrothermal area).

Cells are Gram-negative, strictly aerobic, non-motile, oxidase-negative, and catalase-positive. Cells are rods, approximately 1.4–1.6 μm in width and 2.8–3.1 μm in length. Colonies are circular and convex with yellow color. It grows at 10–40 °C (optimum of 30 °C) and pH 5–8 (optimum of pH 6) and occurs in sea salt concentrations of 0–5% (w/v) (optimum 3.5%). In Biolog GEN III Microplate system, glucuronamidand L-malic acid are oxidized. In API ZYM test, strainT28$^T$ is positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, trypsin, α-chymotrypsin, and $\alpha$-glucosidase. With API 20NE, $\beta$-glucoside (esculin) and $\beta$-galactosidase are positive. The major fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH, and summed feature 3 (comprising $C_{16:1}$ω7c/ω6c). Diphosphatidylglycerol (DPG), phosphatidylethanolamine, two unidentified phospholipids (PL1-2), six unidentified lipids (L1-6), an unidentified aminolipid (AL), and one unidentified aminophospholipids (APL) are major polar lipids. MK-6 is the only isoprenoid quinone. The DNA G+C content is 34.4 mol% (from the whole-genome sequence).

The type strain is T28$^T$ (=CGMCC 1.15788$^T$ = JCM 31510$^T$), isolated from seawater in shallow-sea hydrothermal systems off Kueishantao Island, Taiwan, China.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and the complete genome sequence of strain T28$^T$ are KX022625 and CP018760.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00284-021-02519-4.

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**Author Contributions** HL and KT designed the experiments. HL, MZ, JY, and JS performed the experiments. HL, DL, and KT analyzed the data and writing the manuscript. All authors read and approved the final manuscript.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

## References

1. Nedashkovskaya OI, Kim SB, Han SK et al (2004) *Maribacter* gen. nov. a new member of the family Flavobacteriaceae, isolated from marine habitats, containing the species *Maribacter sedimenticola* sp. nov. *Maribacter aquivivus* sp. nov. *Maribacter orientalis* sp. Nov. and *Maribacter ulvicola* sp. nov. Int J Syst Evol Microbiol 54(4):1017
2. Barbeyron T, Carpentier F, L'haridon S, Schüler M, Michel G, Amann R (2008) Description of *Maribacter forsetii* sp. nov. a marine Flavobacteriaceae isolated from north sea water, and emended description of the genus *Maribacter*. Int J Syst Evol Microbiol 58(4):790–797
3. Nedashkovskaya OI, Kim SB, Mikhailov VV (2010) *Maribacter stanieri* sp. nov. a marine bacterium of the family *Flavobacteriaceae*. Int J Syst Evol Microbiol 60(1):214
4. Lo N, Jin HM, Jeon CO (2013) *Maribacter aestuarii* sp. nov. isolated from tidal flat sediment, and an emended description of the genus *Maribacter*. Int J Syst Evol Microbiol 63(Pt9):3409–3414
5. Weerawongwiwat V, Kang H, Jung MY, Kim W (2013) *Maribacter chungangensis* sp. nov. isolated from a green seaweed, and emended descriptions of the genus *Maribacter* and *Maribacter arcticus*. Int J Syst Evol Microbiol 63(Pt7):2553–2558
6. Hu J, Yang QQ, Ren Y, Zhang WW, Zheng G, Sun C, Pan J, Zhu XF, Zhang XQ, Wu M (2015) *Maribacter thermophilus* sp. nov. isolated from an algal bloom in an intertidal zone, and emended description of the genus *Maribacter*. Int J Syst Evol Microbiol 65(Pt1):36–41
7. Yoon JH (2005) *Maribacter dokdonensis* sp. nov. isolated from sea water off a Korean island, Dokdo. Int J Syst Evol Microbiol 55(5):2051–2055
8. Martin M, Barbeyron T, Martin R, Portetelle D, Michel G, Vandenbol M (2015) The cultivable surface microbiota of the brown alga *Ascophyllum nodosum* is enriched in macroalgal-polysaccharide-degrading bacteria. Front Microbiol 6:1487
9. Zhan PW, Tang K, Chen XF, Yu L (2017) Complete genome sequence of *Maribacter* sp. T28, a polysaccharide-degrading marine flavobacteria. J Biotechnol 259:1–5
10. Yoon SH, Ha SM, Kwon SJ et al (2017) Introducing EzBioCloud : a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67(5):1613–1617
11. Lee I, Chalita M, Ha SM, Na SI, Chun J et al (2017) ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. Int J Syst Evol Microbiol 67(5):2053–2057
12. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406–425

13. Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376
14. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870–1874
15. Fitch WM (1971) Toward defining the course of evolution : minimum change for a specific tree topology. Syst Biol 20:406–416
16. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549
17. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4):783–791
18. Berlin K, Koren S, Chin CS, Drake JP, Landolin JM, Phillippy AM (2015) Assembling large genomes with single-molecule sequencing and locality sensitive hashing. Nat Biotechnol 33:623–630
19. Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110(10):1281–1286
20. Rosselló-Móra R, Amann R (2015) Past and future species definitions for bacteria and archaea. Syst Appl Microbiol 38(4):209–216
21. Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O et al (1987) Report of the ad hoc committee on reconciliation of aproaches to bacterial systematics. Int J Syst Bacteriol 37(4):463–464
22. Dong XZ, Cai MY (2001) Determination of biochemical properties. In manual for the systematic identification of general bacteria. Scientific Press, Beijing, pp 353–412
23. Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, pp 607–654
24. Fraser SL, Jorgensen JH (1997) Reappraisal of the antimicrobial susceptibilities of *Chryseobacterium* and *Flavobacterium* species and methods for reliable susceptibility testing. Antimicrob Agents Chemother 41(12):2738–2741
25. Komagata K, Suzuki KI (1987) Lipid and cell-wall analysis in bacterial systematics. Method Microbiol 19:161–207
26. Tindall BJ (1990) A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. Syst Appl Microbiol 13(2):128–130
27. Tindall BJ (1990) Lipid composition of *Halobacterium lacusprofundi*. FEMS Microbiol Lett 66(1–3):199–202
28. Kates M (1986) Lipid extraction procedures. Techniques of lipidology. Elsevier, Amsterdam, pp 100–111
29. Kim M, Oh HS, Park SC, Chun J (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 64:346–351