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Parahaliea maris sp. nov., isolated from surface seawater and emended description of the genus *Parahaliea*[§]

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A Gram-stain-negative, strictly aerobic, short-rod-shaped, and non-motile bacterial strain designated HSLHS9^T was isolated from surface seawater collected from the South China Sea. Strain HSLHS9^T could grow at 15–41°C (optimum 28°C), at pH 5.0–9.0 (optimum 6.0–7.0), and in 0–7% (w/v) NaCl (optimum 2–3%). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain HSLHS9^T shared high identities with the closely related *Parahaliea aestuarii* S2-26^T (98.6%) and *Parahaliea mediterranea* 7SM29^T (97.8%) and formed a distinct lineage within the genus *Parahaliea*. Whole-genome sequencing of strain HSLHS9^T revealed the size of 4.8 Mbp and DNA G + C content of 61.8 mol%. Strain HSLHS9^T shared the digital DNA-DNA hybridization values of 22.4% and 23.0%, and the average nucleotide identities of 79.7% and 79.9%, respectively, with the two type strains above. The predominant cellular fatty acids of the strain were summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*), summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*), $C_{17:1}$ *ω8c*, and $C_{16:0}$. The sole isoprenoid quinone was identified as Q-8. The polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, aminolipid, and two glycolipids. Based on taxonomic data obtained in this study, it is suggested that strain HSLHS9^T represents a novel species of the genus *Parahaliea*, for which the name *Parahaliea maris* sp. nov. is proposed. The type strain is HSLHS9^T (= MCCC 1A06717^T = KCTC 52307^T). An emended description of the genus *Parahaliea* is also provided.

Keywords: *Parahaliea maris*, polyphasic taxonomy, genomic analysis, novel species

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Introduction

In recent years, a large number of bacteria within the class *Gammaproteobacteria* have been isolated from saline environments, and then some isolates were identified as new species and/or new genera of this class (Garrity *et al.*, 2015). Among these new taxa, quite a few have not been allocated to much higher taxonomic ranks such as at the family and order levels due to the lack of a unified and reliable classification system within this class (Williams *et al.*, 2010). To improve the situation, a sound taxonomic framework of this class has been established through the reconstruction of evolutionary relationships of bacteria by using genome-scale data (Spring *et al.*, 2015). In the study, a novel order designated as *Cellvibrionales* was proposed, consisting of the five novel families, *Cellvibrionaceae*, *Halieaceae*, *Microbulbiferaceae*, *Porticoccaceae*, and *Spongiibacteraceae* (Oren and Garrity, 2015; Spring *et al.*, 2015). The definition of these higher taxonomic units within this class provides a solid cornerstone for the related studies, for example, the cultivation-independent diversity of *Gammaproteobacteria* from marine environments.

As of August 2019, the family *Halieaceae* comprises a total of nine genera, *Haliea* (Urios *et al.*, 2008), *Congregibacter* (Euzéby, 2009; Spring *et al.*, 2009), *Halioglobus* (Park *et al.*, 2012), *Chromatocurvus* (Csotonyi *et al.*, 2011; Euzéby, 2012), *Luminiphilus* (Oren and Garrity, 2013; Spring *et al.*, 2013), *Pseudohaliea* (Oren and Garrity, 2013; Spring *et al.*, 2013), *Parahaliea* (Lin *et al.*, 2015), *Marimicrobium* (Konkit *et al.*, 2016), and the newly established *Kineobactrum* (Chang *et al.*, 2019). Among them, the genus *Parahaliea* contained the two species, *Parahaliea mediterranea* (Lin *et al.*, 2015) and *Parahaliea aestuarii* (Jung *et al.*, 2017). To the best of our knowledge, most isolates of this genus inhabit in diverse marine environments such as seawater and sediments based on the analysis of 16S rRNA gene sequencing (Lin *et al.*, 2015; Jung *et al.*, 2017). Some strains were significantly positively correlated with total organic carbon and total nitrogen (Shi *et al.*, 2019), and could reduce nitrate to nitrite (Jung *et al.*, 2017), suggesting that the *Parahaliea* bacteria may play a crucial role in the biogeochemical cycles of carbon and nitrogen. During the study of the cultivable bacterial diversity from the surface seawater in the South China Sea, a white-color strain designated as HSLHS9^T was obtained. The preliminary analysis of 16S rRNA gene sequences indicated that strain HSLHS9^T was related to members of the genus *Parahaliea*. Here, we report the isolation, identification, and classification of strain HSLHS9^T based on phylogenetic, genomic, phenotypic, and chemotaxonomic analyses, which revealed to be a novel species of the genus *Parahaliea*.

Materials and Methods

Isolation of bacterial strain and culture conditions

A surface seawater sample was collected from the South China Sea (19°58′ N, 110° 34′ E) at Jul 28, 2010. The sample was serially diluted with aged seawater and spread onto the marine agar 2216 (MA; BD) plates, followed by incubation at 28°C for seven days. To obtain pure culture, the colonies were streaked onto the MA medium at least three times. Then a white colored bacterial strain designated HSLHS9^T was obtained. Strain HSLHS9^T was conventionally maintained on the MA medium or in marine broth 2216 (MB; BD) at 28°C. The strain was stored in 20% (v/v) glycerol suspension and maintained at -80°C for long-term preservation. Strain HSLHS9^T has been deposited in the Marine Culture Collection for China (MCCC 1A06717^T) and the Korean Collection of Type Cultures (KCTC 52307^T). To compare physiological and chemotaxonomic characteristics between HSLHS9^T and reference strains, *P. mediterranea* 7SM29^T (= DSM 21924^T, the type species of the genus) and *P. aestuarii* S2-26^T (= JCM 31547^T) were obtained from the Deutsche Sammlung von Mikroorganisem und Zellkulturen and the Japan Collection of Microorganism, respectively, and routinely cultivated on the MA medium at 28°C for three days, unless otherwise specified.

Phylogenetic and genomic analysis

The genomic DNA of strain HSLHS9^T was extracted by using the SBS extraction kit (Shanghai SBS Genetech Co., Ltd.) under the manufacturer's instructions. The 16S rRNA gene of the strain was amplified in PCR using universal bacterial primers 27F and 1492R (Weisburg *et al.*, 1991). PCR products were sequenced by using the Sanger sequencing method with the primers RP500/P300/PKCT (Lai *et al.*, 2014) in the Shanghai Majorbio Bio-Pharm Technology Co., Ltd. These sequences were assembled by using the software DNAMAN version 8 (Lynnon Biosoft, https://www.lynnon.com/). The 16S rRNA gene sequences of related taxa were obtained by using the "16S-based ID" service in the recently updated EzBioCloud database (https://www.ezbiocloud.net) (Yoon *et al.*, 2017a) and by using the standard nucleotide BLAST in the NCBI database. The multiple alignments and pairwise identities of 16S rRNA gene sequences were performed by using DNAMAN under default parameters. Phylogenetic trees based on 16S rRNA gene sequences were reconstructed by using the software MEGA version X (Kumar *et al.*, 2018) with the distance option according to Kimura's two-parameter model and clustering with the neighbor-joining (NJ) (Saitou and Nei, 1987), maximum likelihood (ML) (Felsenstein, 1981), and maximum parsimony (MP) (Czelusniak *et al.*, 1990) methods under default parameters. Bootstrap analysis based on 1,000 replicates (Felsenstein, 1985) was used to estimate the confidence level of tree topologies. The type strain *Microbulbifer mangrovi* DD-13^T (LZDE00000000) (Vashist *et al.*, 2013) was used as an outgroup in the phylogenetic analysis.

For whole-genome sequencing, the genomic DNA of strains HSLHS9^T and S2-26^T was extracted by using the SBS extraction kit. The draft genome sequences of the two strains were determined by using the Illumina HiSeq PE150 platform of Shanghai Majorbio Bio-Pharm Technology Co., Ltd. The 6,584,474 and 6,376,271 raw reads and 948,164,256 bp and 930,935,566 bp of the two strains were generated. The high-quality reads were assembled by using the software SPAdes version 3.8.1 with default parameters (Bankevich *et al.*, 2012). The genome of strain 7SM29^T was obtained from the GenBank database. Genomic qualities of the three strains were assessed by using the software CheckM version 1.0.9 (Parks *et al.*, 2015). The DNA G + C contents were determined from the respective genome sequence. Genomic relatedness including digital DNA-DNA hybridization (dDDH) values and average nucleotide identity (ANI) was estimated by using the genome-to-genome distance calculator (GGDC) version 2.1 online service with the recommended formula 2 (Auch *et al.*, 2010) and the OrthoANI algorithm in the EzBioCloud web service (Yoon *et al.*, 2017b), respectively. On the basis, all currently published genomes of type strains within the family *Halieaceae* were obtained from the GenBank database and analyzed in the study. The genome annotations were performed by using the Pathosystems Resource Integration Center (PATRIC) resource (https://www.patricbrc.org/) (Wattam *et al.*, 2014). The phylogenetic tree of the core genome was reconstructed by using the Phylogenetic Tree Building Service of the PATRIC 3.5.32 online with the FastTree method according to user guides and tutorials. The functional roles of annotated genes from genomes of the three strains were assigned and grouped in subsystem feature categories.

Phenotypic and biochemical characteristics

The morphological characteristics of colonies and cells were examined with the naked eye and by transmission electron microscope (H7650, Hitachi), respectively, by using cultures on the MA medium for 48 h according to the manual of the instrument. The motility of strain HSLHS9^T was tested in the MB supplemented with 0.5% agar. The growth of strain HSLHS9^T and the two reference strains was tested at 28°C for seven days on the MA, R2A agar, nutrient agar (NA, BD), LB agar (BD), and tryptic soy agar (TSA, BD) media. Anaerobic growth was tested in an anaerobic pouch (MGC, Mitsubishi) on MA at 28°C for 7 days. Gram-staining was performed by using a Gram-stain kit (HKM) according to the manufacturer's instructions. Catalase and oxidase activities were determined through bubble production in 10% (v/v) aqueous hydrogen peroxide solution and oxidation of 1% (w/v) *N*,*N*,*N*′,*N*′-tetramethyl-1,4-phenylenediamine, respectively. Growth was evaluated at various temperatures (4, 10, 15, 20, 28, 32, 37, 41, 43, 45, and 50°C) on MA medium. The pH range for growth was determined in the MB adjusted to pH 4.0–11.0 (at increments of one pH units) with citrate/phosphate (pH 4.0–7.0), Tris/HCl (pH 8.0–9.0), or sodium carbonate/sodium bicarbonate (pH 10.0–11.0) buffers. Tolerance to NaCl was performed in the artificially modified MB supplemented with NaCl concentrations of 0, 0.5, 1, 2, 3, 5, 7, 9, 11, 13, 15, and 18% (w/v). Hydrolysis of starch, casein, skim milk, Tweens 20, 40, 60, and 80 was tested by using the MA as the basal medium supplemented with 1% each substrate. Additional enzyme activities and biochemical properties were examined by using API ZYM, API 20NE, and API 20E strips following the manufacturer's instructions, with the single modification of adjusting the NaCl concen-

tration to 3.0% in all tests.

Chemotaxonomic characterization

For analysis of cellular fatty acids, cells of strain HSLHS9^T and reference strains were collected from the third quadrants on MA medium at 28°C. The harvested cells were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI) (Sasser, 1990). The prepared fatty acids were analyzed by gas chromatography (model 7890A; Hewlett Packard) using the Microbial Identification software package with the Sherlock MIDI 6.1 system and the Sherlock Aerobic Bacterial Database (TSBA 6.1). Polar lipids of strain HSLHS9^T were extracted by using the chloroform/methanol system (Minnikin *et al.*, 1984), and separated by two-dimensional thin-layer chromatography (silica gel 60 F254, Merck). The plate dotted with the sample was subjected to two-dimensional development, with the first solvent of chloroform-methanol-water (65:25:4, v/v) followed by the second solvent of chloroform-methanol-acetic acid-water (85:12:15:4, v/v). The extracted lipids were identified by spraying with 10% ethanolic molybdophosphoric acid, ninhydrin, molybdenum blue, α-naphthol-sulphuric acid, and Dragendorff's reagents (Supplementary data Fig. S3). Respiratory quinone of strain HSLHS9^T was extracted and determined by using the high-performance liquid chromatography (Agilent 1200; ODS 250 × 4.6 mm × 5 μm; flowing phase, methanol-isopropanol, 2: 1; 1 ml/min) according to the methods described by (Collins *et al.*, 1977).

Nucleotide sequence accession numbers

The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence of strain HSLHS9^T is MN315552; those for whole-genome sequences of strains HSLHS9^T and S2-26^T are VRZA00000000 and VRYZ00000000, respectively.

Results and Discussion

Phylogenetic and genomic analysis

The 16S rRNA gene sequence lengths of strain HSLHS9^T obtained from the Sanger's sequencing method and whole-genome sequencing were 1,458 and 1,542 bp, respectively, and the overlapping region (1,458 bp) between both sequences was identical. The sequence comparison by using the BLASTn and EzBioCloud searches revealed that strain HSLHS9^T belonged to the genus *Parahaliea* within the family *Halieaceae*, and showed the highest identity of 16S rRNA gene sequence with *P. aestuarii* S2-26^T (98.6%), followed by *P. mediterranea* 7SM29^T (97.8%), and low identities with other type strains of recognized species of the family *Halieaceae* (93.8–96.4%). The NJ phylogenetic tree showed that strain HSLHS9^T clustered with the two closely related type strains of the genus *Parahaliea* and formed a separate branch (Fig. 1A). Both ML and MP trees presented a similar topology to that of the NJ tree and thus were condensed into the NJ tree. The results clearly indicated that strain HSLHS9^T was phylogenetically affiliated to the genus *Parahaliea*.

Because 16S rRNA gene sequence identities between strain HSLHS9^T and the two closely related reference strains were higher than 97%, the genomic relatedness among the three strains was determined by using whole-genome sequences. The dDDH values between strain HSLHS9^T and the two reference strains (*P. aestuarii* S2-26^T and *P. mediterranea* 7SM29^T) were 22.4% and 23.0%, respectively; the ANI values were 79.7% and 79.9%, respectively. These values were far below

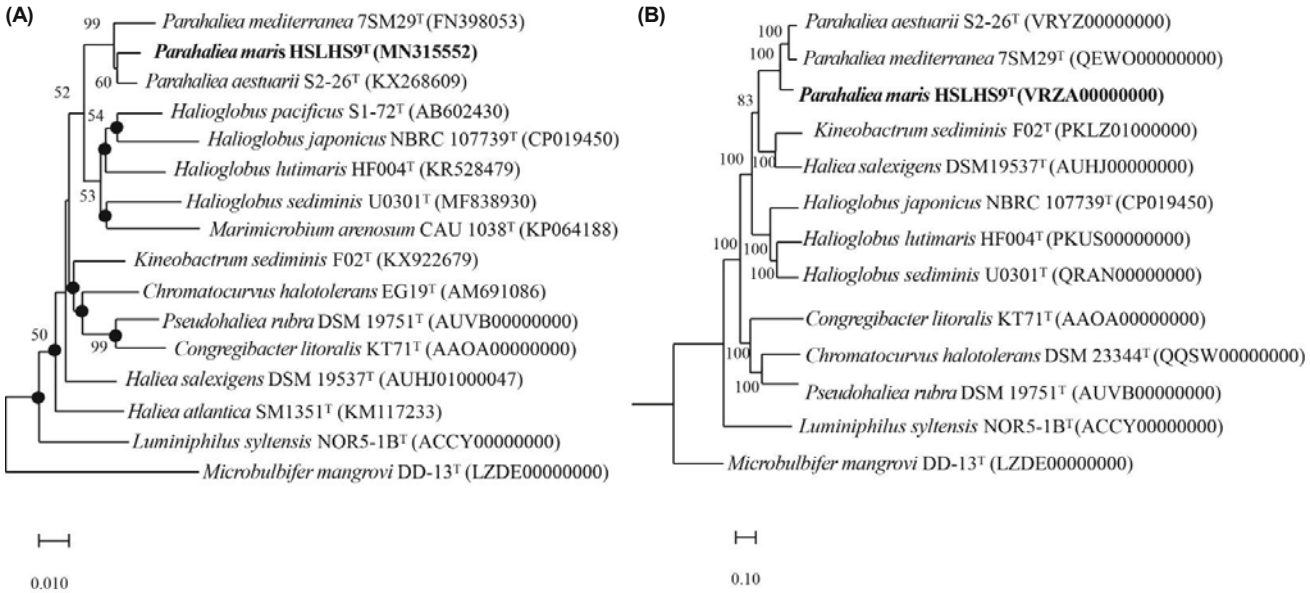

Fig. 1. The trees of strain HSLHS9^T and the related reference strains of the family *Halieaceae* based on 16S rRNA gene sequences (A) and genomic sequences (B). Filled circles indicate that nodes were also recovered in maximum likelihood and minimum-evolution trees based on the same sequences. Bootstrap values of great than 50% in 16S rRNA gene tree and great than 80% in the genomic tree were shown at branch points. Scale bar = 0.010/0.10 nucleotide substitution rate (Knuc) units.

the widely accepted thresholds of 70% dDDH (Wayne *et al.*, 1987) and 95–96.0% ANI (Richter and Rosselló-Móra, 2009) for bacterial species delineation. In the phylogenomic tree, strain HSLHS9^T formed a robust clade with *P. aestuarii* S2-26^T and *P. mediterranea* 7SM29^T, which was supported by high bootstrap values at nodes (Fig. 1B). Additionally, as shown in Fig. 1, the phylogenetic relationships of type strains of some other species within the family *Halieaceae* based on genomes were inconsistent with those based on 16S rRNA gene sequences, and thus need to be re-confirmed in the following research. As a result, the analyses of genome-based relatedness indicated that strain HSLHS9^T represented a novel species of the genus *Parahaliea*.

Genomic characteristics

The two high-quality genomes of strains HSLHS9^T and S2-26^T were obtained based on the criteria (≥ 90% of completeness and ≤ 5% of contamination) by the estimation from the CheckM (Supplementary data Table S1). The draft genome sequence of strain HSLHS9^T was 4,845,847 bp in length with about 200-fold coverage and the DNA G + C content of 61.8 mol%, consisting of 23 contigs with a N50 value of 795,689 bp.

Table 1. Differential characteristics between strain HSLHS9^T and the two reference type strains

Strains: 1, HSLHS9^T; 2, *P. aestuarii* S2-26^T; 3, *P. mediterranea* 7SM29^T. All data were from this study unless otherwise indicated. +, positive; -, negative; w, weakly positive. The data of colony colors, growth for temperatures, pH, and NaCl, and polar lipids of the two reference strains was obtained from the previously published results. All strains were positive for growth on MA medium, oxidase, catalase, hydrolysis of Tweens 20 and 40, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, N-acetyl-β-glucosaminidase, and β-glucosidase (aesculin hydrolysis). All strains were negative for hydrolysis of starch, casein, and skim milk, growth on R2A, LB, NA, and TSA media, α-galactosidase, β-galactosidase, β-glucuronidase, α-fucosidase, denitrification, indole production, D-glucose fermentation, arginine dihydrolase, urease, β-galactosidase, utilization of D-glucose, L-arabinose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, and adipic acid, β-galactosidase, lysine decarboxylase, ornithine decarboxylase, H_2S production, urease, tryptophan deaminase, and indole production. Phospholipid, PL; L, lipid.

Characteristics	1	2	3
Colony color	white	white	pale-yellow
Motility	-	+	+
Growth at :			
Temperature range (°C)	15–41	15–45	15–40
pH range	5.0–9.0	5.5–10.0	5.5–10.0
NaCl range	0.0–7.0	0.0–8.0	0.35–15.0
LB medium	-	w	-
Hydrolysis of			
Tween 40	+	+	-
Tween 80	w	+	-
API ZYM			
Lipase (C14)	+	-	+[#]
Trypsin	w	-	-
α-Chymotrypsin	w	-	-
α-Glucosidase	-	+[#]	+
α-Mannosidase	-	-	+
API 20NE			
Reduction of nitrate to nitrite	-	+	+
Gelatin hydrolysis	-	+	-[#]
Utilization of			
D-mannose, trisodium citrate	-	-	+
Capric acid, malic acid	-	+	-
Phenylacetic acid	-	+	+
API 20E			
Arginine dihydrolase			
Citrate utilization	+	-	-
Acetoin production	+	+	-
Gelatinase	-	+	+
Fermentation of	-	+	-
Glucose, rhamnose, melibiose	+	+	-
Mannitol, inositol, sorbitol	+	-	-
Saccharose	+	-	-
Amygdalin	+	-	-
Polar lipids	PG, DPG, PE, AL, 2GLs	PG, DPG, PE, PL, AL, GL, L	PG, DPG, PE, 2ALs, GL
DNA G + C content (mol%)*	61.8	62.7	63.3

[#] the results of these tests in the study were different from the previous studies.
* DNA G + C content (mol%) of the three strains was obtained from each genome.

The genomic sequence contained 4,201 genes, 4,117 protein-coding genes, 4 rRNA genes (one 5S rRNA gene, one 16S rRNA gene, and two 23S rRNA genes), and 41 tRNA genes. Likewise, the draft genome sequence of strain S2-26^T was 4,469,285 bp in length with about 200-fold coverage and the DNA G + C content of 62.7 mol%, consisting of 23 contigs with a N50 value of 505,008 bp. The genomic sequence included 3,902 genes, 3,819 protein-coding genes, 9 rRNA genes, and 43 tRNA genes. The genomic DNA G + C content of strain 7SM29^T was 63.3 mol%, which slightly higher than 62.1 mol% from the original identification by the HPLC method.

The subsystem category distributions for genomes of the three bacteria with the close phylogenetic relationship were analyzed in this study. As illustrated in Supplementary data Fig. S1, these strains showed a generally similar distribution pattern of subsystem categories. Genomes of the three strains comprised a large number of genes related to amino acids and derivatives, protein metabolism, carbohydrates, metabolism of aromatic compounds, some genes related to phosphorus metabolism, sulfur metabolism, and nitrogen metabolism. These results indicated that the bacteria within the genus *Parahaliea* may play crucial roles in the biogeochemical cycles of carbon, nitrogen, sulfur, and phosphorus. Meanwhile, genomes of the three bacteria included many genes related to stress response, virulence, disease and defense, regulation and cell signaling. These genes may assist to improve their resilience and survival in harsh environments. The analyses of metabolic features and related genes provided the genetic basis of the genus bacteria for ecological functions and environmental adaptation.

Compared with the two reference strains of S2-26^T and 7SM29^T, the genomic sequence of strain HSLHS9^T showed two distinct characteristics that could distinguish it from others, including the absence of genes related to motility and chemotaxis, and denitrification. As shown in Supplementary data Fig. S1, strain HSLHS9^T contained more genes responsible for nucleosides and nucleotides, protein metabolism, regulation and cell signaling, DNA metabolism, respiration, stress response, metabolism of aromatic compounds, amino acids and derivatives relative to *P. mediterranea* 7SM29^T with a much larger genome. These results could highlight the metabolic diversity and plasticity of strain HSLHS9^T. Besides, the genome of strain HSLHS9^T did not have the carotenoid biosynthesis-related genes and rhodopsin genes.

Phenotypic characterization

Cells of strain HSLHS9^T were Gram-stain-negative and short-rod-shaped (0.4–0.7 μm in width and 0.8–1.0 μm in length) (Supplementary data Fig. S2). Catalase and oxidase activities were positive. Anaerobic growth was not observed. Colonies were circular, convex, and white-colored with a diameter of about 2.0 mm after cultivation on MA medium at 28°C for three days. Strain HSLHS9^T grew well on MA medium but did not grow on R2A, LB, NA, or TSA media. The ranges of temperature, pH, and NaCl for growth of strain HSLHS9^T were 15–41°C, pH 6.0–9.0, and 0–7.0% (w/v), respectively. The strain grew optimally at 28°C, at pH 6.0–7.0, and in 2–3% (w/v) NaCl. Strain HSLHS9^T could hydrolyze Tweens 20, 40, 60, and 80, but not starch, casein, and skim milk. The strain was non-motile and incapable of denitrification, which was consistent with genomic analysis. The detailed physiological and biochemical characteristics of strain HSLHS9^T were shown in Table 1 and the species description. Many properties of strain HSLHS9^T such as the rod shape, catalase and oxidase activity, hydrolysis of Tweens 20 and 60 were in common with those of the two reference strains of the genus *Parahaliea*, whereas some phenotypic properties such as morphology of colonies and cells, motility, nitrate reduction to nitrite, hydrolysis of Tweens 40, 80, and gelatin, and acetoin production (Voges Proskauer) allowed the differentiation of strain HSLHS9^T from the other two type strains.

Chemotaxonomic characteristics

The major fatty acids (> 10% of total fatty acids) of strain HSLHS9^T were summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*, 22.8%), summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*, 19.5%), $C_{17:1}$ *ω8c* (12.3%), and $C_{16:0}$ (11.2%), which were consistent with those in the two reference strains of the genus *Parahaliea* (Table 2). Although the overall fatty acid profile of strain HSLHS9^T was similar to those of closely related *Parahaliea* type strains, there were some differences in proportions of some components. For example, 4.8% of the $C_{17:0}$ in strain HSLHS9^T was lower than those of strains S2-26^T (8.9%) and 7SM29^T (6.3%). The $C_{11:0}$ 2OH was detected as a trace amount (< 0.5%) in strains HSLHS9^T and 7SM29^T, but it was 4.5%

Table 2. Cellular fatty acid profiles of strain HSLHS9^T and the two reference strains

Strains: 1, HSLHS9^T; 2, *P. aestuarii* S2-26^T; 3, *P. mediterranea* 7SM29^T. Data of the fatty acids for the three strains were obtained from this study. Less than 0.5% and/or the absence of fatty acids for all strains were not shown. Values were percentages of total fatty acids. The predominant cellular fatty acids for all strains (> 10%) were in bold. tr, trace amount (< 0.5%); nd, not detected.

Fatty acids	1	2	3
$C_{9:0}$	0.6	nd	nd
$C_{10:0}$	1.2	0.9	1.0
$C_{11:0}$	1.4	2.1	1.5
$C_{12:0}$	1.0	0.9	1.2
$C_{13:0}$	1.0	tr	tr
$C_{14:0}$	2.0	1.0	1.5
$C_{15:0}$	5.8	6.3	4.5
$C_{16:0}$	**11.2**	**11.0**	**15.6**
$C_{17:0}$	4.8	8.9	6.3
$C_{18:0}$	tr	0.7	0.5
$C_{10:0}$ 3OH	2.3	1.4	1.5
$C_{11:0}$ 2OH	tr	4.5	tr
$C_{11:0}$ 3OH	4.2	nd	2.7
$C_{12:0}$ 3OH	2.2	1.9	2.4
$C_{15:1}$ *ω6c*	0.9	1.5	0.5
$C_{15:1}$ *ω8c*	1.6	0.6	0.6
$C_{17:1}$ *ω6c*	2.1	3.5	1.8
$C_{17:1}$ *ω8c*	**12.3**	**17.5**	**12.0**
iso-$C_{16:0}$	tr	0.7	tr
summed feature 3*	**19.5**	**15.7**	**22.1**
summed feature 8*	**22.8**	**17.9**	**21.7**

* Summed features represent groups of two fatty acids which could not be separated by GLC with the MIDI system. The summed feature 3: $C_{16:1}$*ω7c* and/or $C_{16:1}$*ω6c*; summed feature 8: $C_{18:1}$*ω7c* and/or $C_{18:1}$*ω6c*.

in strain S2-26^T. The polar lipids of strain HSLHS9^T were phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), an unknown aminolipid (AL), and two unidentified glycolipids (GL1 and GL2), as shown in Supplementary data Fig. S3. The PG, DPG, and PE as major polar lipids in strain HSLHS9^T was consistent with that of strains S2-26^T and 7SM29^T (Lin *et al.*, 2015; Jung *et al.*, 2017). The respiratory quinone of strain HSLHS9^T was identified as Q-8, which was in line with the two type strains of the genus *Parahaliea*.

Taxonomic conclusion

Strain HSLHS9^T shared high identities of 16S rRNA gene sequences with the two type strains of the genus *Parahaliea*. But in 16S rRNA gene and genome sequences phylogenetic trees, strain HSLHS9^T was found to form a stable lineage independent of the two taxa (Fig. 1; Supplementary data Figs. S1 and S2). Strain HSLHS9^T could be distinguished from reference strains based on differences in the phenotypic characteristics, including cellular morphology, growth temperatures, tolerance to NaCl, activities of some enzymes, and utilization and fermentation of some substrates (Table 1), thereby indicating that strain HSLHS9^T is not affiliated with any recognized species of the genus *Parahaliea*. In conclusion, the phylogenetic, genomic, phenotypic, and chemotaxonomic features support that strain HSLHS9^T represents a novel species in the genus *Parahaliea*, for which the name *Parahaliea maris* sp. nov. is proposed.

Description of *Parahaliea maris* sp. nov.

Parahaliea maris (ma′ris. L. gen. n. *maris* of the sea, isolated from surface seawater of the South China Sea). Cells are Gram-stain-negative, strictly aerobic, short-shaped, nonmotile, 0.4–0.7 μm wide, and 0.8–1.0 μm long. Colonies are white-colored, smooth, opaque, convex, and circular with entire margins, and approximately 1–2 mm in diameter on the MA medium at 28°C for three days. Growth occurs at 15–41°C (optimum 28°C), at pH 5.0–9.0 (optimum 6.0–7.0), and in 0–7% (w/v) NaCl (optimum 2–3%). Positive for catalase and oxidase, hydrolysis of Tweens 20, 40, and 60; weakly positive for hydrolysis of Tween 80; negative for hydrolysis of starch, casein, and skim milk. Growth could observe on the MA medium, but not on the R2A, LB, NA, and TSA media. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, and N-acetyl-β-Glucosaminidase; weakly positive for trypsin and α-chymotrypsin; negative for α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, α-mannosidase, and α-fucosidase. In API 20NE tests, positive for β-glucosidase (aesculin hydrolysis); negative for the remaining results. In API 20E tests, positive for arginine dihydrolase, citrate utilization, and fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, saccharose, melibiose, amygdalin, and arabinose; negative for β-galactosidase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, gelatinase, H_2S production, indole production, and acetoin production (Voges Proskauer). The major fatty acids are summed feature 8, summed feature 3, $C_{17:1}$ $\omega 8c$, and $C_{16:0}$. The polar lipids comprise phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, aminolipid, and glycolipids. The sole isoprenoid quinone is Q-8.

The type strain HSLHS9^T (= MCCC 1A06717^T = KCTC 52307^T) was isolated from surface seawater collected from the South China Sea. The DNA G + C content of the type strain is 61.8 mol%.

Emended description of the genus *Parahaliea*

The emended description of the genus *Parahaliea* is based on the description of *P. mediterranea* (Lin *et al.*, 2015), *P. aestuarii* (Jung *et al.*, 2017), and on the results of this study. Some of strains have no flagellum and cannot reduce nitrate to nitrite.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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