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TAXONOMIC DESCRIPTION
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Croceicoccus bisphenolivorans sp. nov., a bisphenol A-degrading bacterium isolated from seawater

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Abstract

A bisphenol A-degrading bacterium, designated as strain H4^T, was isolated from surface seawater, which was sampled from the Jiulong River estuary in southeast PR China. Strain H4^T is Gram-stain-negative, aerobic, short rod-shaped, lacking bacteriochlorophyll a, motile with multifibrillar stalklike fascicle structures and capable of degrading bisphenol A. Growth of strain H4^T was observed at 24–45 °C (optimum, 32 °C), at pH 5.5–9 (optimum, pH 7.0) and in 0–7% NaCl (optimum, 2%; w/v) . The 16S rRNA gene sequence of strain H4^T showed highest similarity to *Croceicoccus pelagius* Ery9^T (98.7%), *Croceicoccus sediminis* (98.3%), *Croceicoccus naphthovorans* PQ-2^T (98.1%) and *Croceicoccus ponticola* GM-16^T (97.6%), followed by *Croceicoccus marinus* E4A9^T (96.7%) and *Croceicoccus mobilis* Ery22^T (96.0%). Phylogenetic analysis revealed that strain H4^T fell within a clade comprising the type strains of *Croceicoccus* species and formed a phyletic line with them that was distinct from other members of the family *Erythrobacteraceae*. The sole respiratory quinone was quinone 10 (Q-10). The predominant fatty acids (>5% of the total fatty acids) of strain H4^T were summed feature 8 ($C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$), summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), $C_{17:1}\omega6c$ and $C_{14:0}$2-OH. The genomic DNA G+C content was 62.8 mol%. In the polar lipid profile, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, two unidentified phospholipids, two sphingoglycolipids and three unknown lipids were the major compounds. Based on the genotypic and phenotypic data, strain H4^T represents a novel species of the genus *Croceicoccus*, for which the name *Croceicoccus bisphenolivorans* sp. nov. is proposed. The type strain is H4^T (=DSM 102182^T=MCCC1 K02301^T).

Bisphenol A (BPA) is widely used in the manufacture of synthetic resin, plastics, polycarbonates and other products [1], and is also a well-known pollutant that shows estrogenic and mutagenic effects and acute toxicity [2]. Due to its low cost and increased environmentally friendliness when compared to physical and chemical methods, bioremediation of BPA has proved to be a promising method in recent years [3]. Several species of BPA-degrading bacteria have been reported, including *Achromobacter xylosoxidans* [4], *Bacillus cereus* and *Bacillus megaterium* [1, 5], *Cupriavidus basilensis* [6], *Sphingobium bisphenolivorans* [7], *Sphingomonas bisphenolicum* [8], *Pseudomonas putida* [3, 9], and so on. Here we describe the taxonomic characterization of a novel BPA-degrading species belonging to the genus *Croceicoccus*.

The genus *Croceicoccus* [10–12] contains six recognized species at the time of writing according to List of Prokaryotic Names with Standing in Nomenclature (https://lpsn.dsmz.de/genus/croceicoccus), and all of them were isolated from marine environments [13]. Strain H4^T was isolated from the Jiulong River estuary in southeast PR China, and BPA was detected within the region [14]. Roughly 1000 ml surface seawater (~0.5 m depth) was sampled at site JY0 [15] and filtered through 0.45 µm pore-size cellulose membranes (Millipore). Afterwards, the membrane samples were enriched in 100 ml sterilized seawater containing BPA (20 mg l^{-1}) as the major carbon and energy source in a 250 ml sterile flask and incubated in a rotary shaker at 150 r.p.m. and 30 °C. After 3 days of cultivation, we transferred 50 ml of the enriched sample to fresh sterilized seawater with BPA (40 mg l^{-1}) under the above culture conditions, and sequential

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Abbreviations: ANI, average nucleotide identity; BPA, bisphenol A.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and the GenBank accession number for the whole genome shotgun sequence of strain H4^T are KU500624 and LRSE00000000, respectively.
Four supplementary figures are available with the online version of this article.

transfers were performed eight times at intervals of 3 days. BPA-degrading bacteria were isolated by directly plating the serially diluted enrichment culture on marine agar medium 216L (CH_3COONa, 1.0 g l^{-1}; tryptone, 10.0 g l^{-1}; yeast extract, 2.0 g l^{-1}; sodium citrate, 0.5 g l^{-1}; NH_4NO_3, 0.2 g l^{-1}; seawater, 1 l; pH 7.5) according to their BPA-degrading ability. In order to carry out parallel comparisons, strain H4^T was subcultured on P5Y3 medium (peptone, 5 g l^{-1}; yeast extract, 3 g l^{-1}; sea salt, 30 g l^{-1}; pH 7.0) or R medium (NaCl, 30 g l^{-1}; $MgSO_4 \cdot 7H_2O$, 2.46 g l^{-1}; KCl, 1.5 g l^{-1}; $CaCl_2 \cdot 2H_2O$, 0.15 g l^{-1}; NaBr, 0.1 g l^{-1}; $FeCl_2 \cdot 2H_2O$, 0.016 g l^{-1}; yeast extract, 2 g l^{-1}; peptone, 5 g l^{-1}; casamino acids, 1 g l^{-1}) [11] for morphological and biochemical characterization. The medium was sterilized under 121 °C for 20 min. Characterization and classification of strain H4^T were done with polyphasic methods.

Genomic DNA was prepared using a DNeasy Blood and Tissue Kit (Qiagen), and the 16S rRNA gene was amplified by PCR using universal primers 16SF (5′-AGAGTTTGATCCTGGCTCAG-3′) and 16 SR (5′-ACGGCTACCTTGTTACGACT-3′) [16]. Sequences of related taxa were obtained from the GenBank database. Multiple sequence alignment of 16S rRNA genes was carried out using ClustalW [17] with default parameters. Phylogenetic analysis was performed using MEGA X software [18]. Distance calculations (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining [19], maximum-likelihood [20] and maximum-parsimony [21] methods were determined by using bootstrap values based on 1000 replications.

The full-length 16S rRNA gene sequence (1486 bp) of strain H4^T was obtained from its draft genome. The identification of phylogenetic neighbours and calculation of the sequence similarity of strain H4^T was carried out by using the EzBioCloud server [22]. Phylogenetic analysis indicated that strain H4^T formed a distinct evolutionary lineage within the clade enclosed by the type strains of *Croceicoccus* species (Fig. 1). Strain H4^T showed high similarity to *Croceicoccus pelagius* (99.1%), *Croceicoccus sediminis* (98.7%), *Croceicoccus naphthovorans* (98.3%), *Croceicoccus ponticola* (98.0%), *Croceicoccus marinus* (97.1%) and *Croceicoccus mobilis* (96.4%), and similarity lower than 96.1% to the type strains of other recognized species. The stable relationship between strain H4^T and

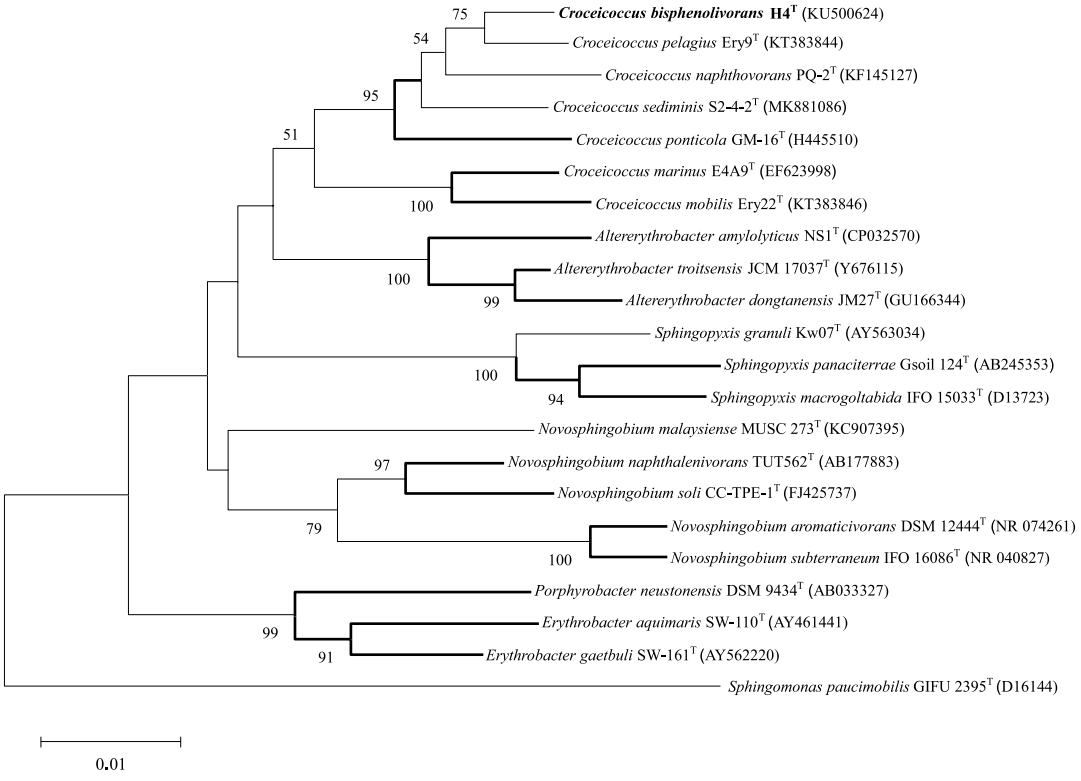

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of Croceicoccus *bisphenolivorans* H4^T, the type strains of *Croceicoccus* species and representatives of some other related taxa based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages (%) of 1000 replications) are shown at branch points. Bar, 0.01 nucleotide substitution rate (Knuc) units. Thicker branches indicate that the corresponding branches were also recovered in the trees reconstructed by the maximum-likelihood (Fig. S1) and maximum-parsimony algorithms (Fig. S2). Bootstrap percentages (above 50%) are shown. *Sphingomonas paucimobilis* GIFU 2395^T (D16144) was used as an outgroup. AQ1

the type strains of *C. pelagius* and *C. naphthovorans* was found in the trees reconstructed using the maximum-likelihood and maximum-parsimony algorithms (Figs S1 and S2, available in the online version of this article), and also confirmed by the phylogenomic tree [13], which was reconstructed based on a 92 up-to-date bacterial core gene sets by UBCG version 3.0 following the default pipeline [23]. The distinct phylogenetic and phylogenomic relationships revealed that strain H4^T should be considered to represent a novel member of the genus *Croceicoccus*.

In order to observe the genomic coherence of the strains, the average nucleotide identity (ANI) was calculated as an alternative to DNA–DNA hybridization as described before [24]. Compared with 16S rRNA gene phylogeny, the robust phylogenomic tree based on genomic similarity analyses by ANI could provide a reliable taxonomic status for the family *Erythrobacteraceae* [12]. The draft genome of strain H4^T was sequenced by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, PR China) using the Illumina Hiseq 2000 system. A library with a fragment length of 300 bp was constructed, and a total of 6983898 clean paired-end reads were generated to reach a 377-fold depth of coverage with an Illumina/Solexa Genome Analyzer II. The final genome, assembled by SOAPdenovo version 2.04 [25], contained 74 scaffolds consisting of 81 contigs (N50, 254387 bp) with a total size of 3602538 bp. The draft genome sequence of strain H4^T has been deposited at GenBank under accession number LRSE00000000. The whole genome sequences from six type strains of the genera *Croceicoccus* including *C. pelagius* Ery9^T (LYWY01000000), *C. naphthovorans* PQ-2^T (CP011770), *C. sediminis* S2-4-2^T (SUNC01000000), *C. ponticola* GM-16^T (RXOL01000000), *C. marinus* E4A9^T (CP019602) and *C. mobilis* Ery22^T (LYWZ01000000), were retrieved from the GenBank database. The ANI value was calculated by the JSpeciesWS web server (http://jspecies.ribohost.com/jspeciesws [26]). As can be seen in Table 1, ANI values of the six strains ranged from 74.7 to 78.9% using BLAST, and 84.4–86.4% using MUMmer. In all cases, the ANI results were far below the threshold interval (95–96%) that was proposed to be the species borderline [27], indicating the discrimination of strain H4^T from other *Croceicoccus* species. The ANI results in this study indicate that strain H4^T represents a putative novel species of the genus *Croceicoccus*.

Table 1. ANI analysis between strain H4^T (sequenced in this study) and six reference strains of related *Croceicoccus* species (obtained from public repositories)

ANIb, analysis using BLAST; ANIm, analysis using MUMmer

Species	H4^T	
	ANIb (%)	ANIm (%)
Croceicoccus pelagius Ery9^T	78.91	86.38
Croceicoccus naphthovorans PQ-2^T	77.61	86.01
Croceicoccus sediminis S2-4-2^T	77.89	85.68
Croceicoccus ponticola GM-16^T	77.83	84.41
Croceicoccus marinus E4A9^T	75.36	84.87
Croceicoccus mobilis Ery22^T	74.72	85.12

General cell morphology was studied under an Olympus IX73 inverted microscope using a 1-day-old culture of the strain grown on P5Y3 agar [11]. The motility of strain was tested using the semi-solid agar method [28]. For electron microscopy, exponential phase cells were harvested, suspended and absorbed on a Formvar-carbon-coated grid, and then stained with phosphotungstic acid (Fig. S3). Gram stain, catalase and oxidase activities were investigated according to Dong and Cai [29]. The optimal growth temperature was determined over the temperature range 4–60 °C using P5Y3 broth. The pH range for growth was examined at 30 °C in R broth [11] over a pH range from pH 3.0 to 12.0 (at intervals of 0.5 pH unit). The pH value was adjusted prior to sterilization using citrate/Na_2HPO_4 buffer (for pH 3.0–8.0) or $NaHCO_3$/Na_2CO_3 buffer (for pH 9.0–12.0) and pH was checked again before inoculation. Tolerance of NaCl was tested by using R broth supplemented with NaCl concentrations of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.5, 2, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0% (w/v). Other biochemical tests were carried out using API 20NE and API ZYM (bioMérieux) and Biolog GN2 kits according to the manufacturers' instructions, with the adjustment of all NaCl concentration to be 2%. *C. naphthovorans* PQ-2^T and *C. marinus* E4A9^T were tested at the same time for comparison. These results are given in the species description and Table 2.

The G+C content of the draft genome of strain H4^T was calculated by the JSpeciesWS web server (http://jspecies.ribohost.com/jspeciesws analysis). The G+C content of the draft genome of isolate H4^T was 62.8 mol% and was in the range of reported members of the genus *Croceicoccus* (Table 2).

Here we also analysed the genes possibly responsible for BPA degradation. According to the BLAST results, we found that there were five open reading frames annotating with cytochrome P450 (CYP) genes, which had been reported to be involved in BPA degradation [8, 30, 31]. However, none of them matched well with known BPA degradation CYP genes, which implies that there may be new genes involved in BPA degradation, which warrants the need to further confirm the function of CYP genes in strain H4^T.

Fatty acids in whole cells grown on P5Y3 agar at 28 °C for 72 h (exponential phase) were extracted, saponified and esterified according to the standard protocol of the MIDI Microbial Identification System, and this was followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system [32]. The fatty acid profiles of the closest species were found in parallel with strain H4^T in this study. As shown in Table 3, the major fatty acids (>5% of the total fatty acids) of strain H4^T were summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*), summed feature 3 ($C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*), $C_{17:1}$ *ω6c* and $C_{14:0}$2-OH. The overall fatty acid profile of strain H4^T (given in the species description) was similar to those reported for the type strains of *Croceicoccus* species, except for differences in the proportions of some

Table 2. Characteristics that differentiate H4^T from the type strains of its closest species

Strains: 1, H4^T (this study); 2, *Croceicoccus pelagius* Ery9^T; 3, *Croceicoccus naphthovorans* PQ-2^T (this study); 4, *Croceicoccus sediminis* S2-4-2^T; 5, *Croceicoccus ponticola* GM-16^T; 6, *Croceicoccus marinus* E4A9^T (this study); 7, *Croceicoccus mobilis* Ery22^T. All data were obtained from this study unless otherwise indicated. All strains were rod-shaped, positive for catalase, oxidase, utilization of D-glucose and activities of alkaline phosphatases, leucine arylamidase, valine aminopeptidase, valine aminopeptidase and naphthol-AS-BI-phosphohydrolase. None of the strains hydrolysed gelatin. w, Weakly positive; +, positive; –, negative; ND, not determined. DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PL, unidentified phospholipid; SGL, sphingoglycolipid; GL, unidentified glycolipid; L, unknown lipid.

Characteristic	1	2*	3	4†	5‡	6	7*
Motility	+	–	–§	–	–	+‖	+
Morphology of flagella	Multifibrillar stalk-like	No flagellum	Polar§	No flagellum	No flagellum	Multifibrillar stalk-like‖	Polar
Temperature range (optimum) for growth (°C)	25–45 (32)	15–45 (30–37)	15–50 (32)§	15–40 (30)	20–35 (30)	4–42 (25)‖	15–45 (30–37)
pH range (optimum) for growth	5.0–9.5 (8.0)	5.5–8.5 (7.0)	6.5–9.5 (7.0)§	6.0–8.0 (7.0)	5.5–ND (7.0–8.0)	6.0–9.0 (7.0)‖	5.5–8.5 (7.0–7.5)
NaCl (%) range (optimum) for growth	0–7.0 (0.5)	0–10.0 (3.0)	0.5–8.0 (2.0)§	0–10.0 (1.0)	0–4.0 (1.0–2.0)	0–10.0 (0–1.0)‖	0–7.5 (5.0)
Nitrate reduction	+	+	+	–	–	–	–
API 20NE results:							
Aesculin hydrolysis	–	+	+	+	+	+	+
L-Arabinose	w	–	w	–	–	+	–
Maltose	w	+	w	+	–	+	+
Malate	w	–	w	–	–	–	+
API ZYM results:							
Esterase (C4)	+	+	–	+	+	w	–
Esterase lipase (C8)	w	–	–	+	+	–	+
Lipase (C14)	w	–	–	w	+	–	–
Cystine aminopeptidase	w	+	–	–	+	–	+
Acid phosphatase	+	+	+	+	–	+	+
β-Glucuronidase	w	–	–	+	–	+	–
α-Glucosidase	+	+	+	w	–	+	+
DNA G+C content (mol%) (by genome)	62.8	62.8	62.6	63.0	62.1	64.5¶	62.5
Polar lipids	PC, PE, PG, PL, SGL, L	PC, PE, PG, PL, SGL, GL	PC, PE, PG, PL, SGL, L	PC, PE, PG, PL, SGL, L	PC, PE, PG,GL, SGL, L, DPG	PC, PG, PL, GL	PC, PE, PG, PL, GL, SGL

Continued

Table 2. Continued

Characteristic	1	2*	3	4†	5‡	6	7*
Isolation source	Seawater from estuary in southeast PR China	Seawater from Atlantic Ocean	Marine biofilm from a boat shell§	Coastal sediment	Seawater from the East Sea	Sediment from Pacific Ocean‖	Sediment from Indian Ocean

*Data from Wu *et al.* [34].
†Data from Huang *et al.* [13].
‡Data from Park *et al.* [35].
§Data from Huang *et al.* [11].
‖Data from Xu *et al.* [12].
¶Data from Xu *et al.* [10].

fatty acids. For instance, strain H4^T had a higher amount of 11-methyl $C_{18:1}$ ω7*c*.

Quinones were extracted and fractionated according to the method described by Qin *et al.* [7], and analysed by using reversed-phase HPLC as described previously [33]. The HPLC results showed that quinone-10 (Q-10) accounted for nearly 100% of the total quinone contents of this new isolate, which was in line with all reported members of the genus *Croceicoccus* [10, 11, 13, 34, 35].

Polar lipids were extracted and fractionated using the method described previously [7], and the analysis of polar lipids was carried out as described by Park *et al.* [36]. The major polar lipids consisted of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, two unidentified phospholipids, two sphingoglycolipids and three unknown lipids (Fig. S4). The polar lipid profile of strain H4^T was the same as *C. naphthovorans* PQ-2^T and *C. sediminis* S2-4-2^T, and phosphatidylcholine and phosphatidylglycerol were found in all *Croceicoccus* species. Compared with *C. pelagius* Ery9^T, *C. ponticola* GM-16^T, *C. marinus* E4A9^T and *C. mobilis* Ery22^T, diphosphatidylglycerol and glycolipid were absent in strain H4^T.

From the results of the physiological characterization (Table 2), we found that the motility, range and optimum growth temperature, pH and NaCl, nitrate reduction, enzyme activities in the API tests and utilization of substrates, DNA G+C content and isolation source of strain H4^T, were different from recognized *Croceicoccus* species: *C. pelagius* Ery9^T, *C. naphthovorans* PQ-2^T, *C. sediminis* S2-4-2^T, *C. ponticola* GM-16^T, *C. marinus* E4A9^T and *C. mobilis* Ery22^T. On the basis of morphological, physiological and chemotaxonomic characteristics, together with the data from 16S rRNA gene sequence and whole genome sequence comparison described above, strain H4^T represents a novel species within the genus *Croceicoccus*, for which a name *Croceicoccus bisphenolivorans* sp. nov. is proposed.

DESCRIPTION OF *CROCEICOCCUS BISPHENOLIVORANS* SP. NOV.

Croceicoccus bisphenolivorans (bis. phe. no. li. vo′rans N.L. neut. n. *bisphenol* bisphenol; L. pres. part. *vorans* eating, devouring; N.L. part. adj. *bisphenivorans* bisphenol A-eating).

Cells are 1.6–1.8 µm long and 0.8–0.9 µm wide, Gram-stain-negative, aerobic, short rod-shaped, motile with multifibrillar stalk-like fascicle structures, oxidase- and catalase-positive, and BPA-degrading. Colonies on P5Y3 agar are yellow, lack bacteriochlorophyll a, circular, smooth, slightly convex, opaque and 1.3–1.5 mm after incubation for 3 days at 30 °C. Growth of strain H4^T is observed at 24–45 °C (optimum, 32 °C), at pH 5.5–9.0 (optimum, pH 7.0) and in 0–7% (optimum, 2% w/v) NaCl. It is positive for nitrate reduction, D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, adipic acid and malic acid; but negative for indole production, D-glucose fermentation,

Table 3. Cellular fatty acid contents of strain H4^T and related species

Strains: 1, H4^T (this study); 2, *Croceicoccus pelagius* Ery9^T [34]; 3, *Croceicoccus naphthovorans* PQ-2^T (this study); 4, *Croceicoccus sediminis* S2-4-2^T [13]; 5, *Croceicoccus ponticola* GM-16^T [35]; 6, *Croceicoccus marinus* E4A9^T (this study); 7, *Croceicoccus mobilis* Ery22^T [34]. –, Not detected.

Fatty acid	1	2	3	4	5	6	7
Saturated:							
$C_{14:0}$	0.7	1.4	0.7	–	<1	0.6	1.4
$C_{16:0}$	4.1	2.1	4.4	2.7	8.0–9.6	3.0	11.8
$C_{18:0}$	1.0	–	1.1	–	–	0.8	–
Unsaturated:							
$C_{15:1}\omega6c$	0.2	–	–	–	–	0.6	–
$C_{16:1}\omega5c$	1.0	2.0	0.9	3.1	<1.0	0.4	0.9
$C_{17:1}\omega8c$	1.7	–	0.9	–	–	5.2	–
$C_{17:1}\omega6c$	7.5	1.9	5	–	2.7–4.3	33.7	6.1
$C_{18:1}\omega5c$	1.7	1.7	1.9	<1.0	1.8–2.4	6.6	1.5
Hydroxyl:							
$C_{14:0}$ 2-OH	6.2	41.9	3.5	7.3	9.2–10.6	2.5	16.9
$C_{15:0}$ 2-OH	1.4	2.7	1.0	1.5	1.0–1.7	8.1	2.1
$C_{16:1}$ 2-OH	0.3	1.6	1.4	<1.0	1.4–1.5	–	–
iso-$C_{16:0}$ 3-OH	1.0	2.5	2.7	<1.0	1.6–1.7	–	≤0.5
$C_{16:0}$ 2-OH	0.8	5.8	2.2	1.3	1.9–2.6	0.1	–
$C_{18:1}$ 2-OH	0.2	1.4	1.3	<1.0	≤1.0	–	–
11-Methyl $C_{18:1}\omega7c$	4.4	0.6	3.2	4.1	≤1.9	2.6	0.7
Cyclo $C_{19:0}\omega8c$	–	–	0.98	–	–	5.9	–
Summed features:*							
3	9.1	9.0	9.2	13.3	13.0–16.1	2.6	11.1
8	53.9	24.4	55.9	56.9	47.4–50.5	23.3	45.8

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 consisted of $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ and/or iso-$C_{15:0}$ 2-OH; summed feature 8 consisted of $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

arginine dihydrolase, urease, aesculin hydrolysis, gelatin hydrolysis, 4-nitrophenyl-β-D-galactopyranosidase, capric acid, phenylacetic acid and trisodium citrate, using the API 20NE system. Among the 95 carbon sources in the Biolog system, it is positive for α-cyclodextrin, glycogen, Tween 40, L-arabinose, D-arabitol, i-erythritol, D-galactose, m-inositol, maltose, D-mannitol, D-mannose, melibiose, D-psicose, xylitol, acetic acid, D-galactonic acid lactone, itaconic acid, α-keto butyric acid, α-keto valeric acid, DL-lactic acid, quinic acid, D-saccharic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-threonine, DL-carnitine, γ-amino butyric acid, uridine, thymidine, putrescine, 23-butanediol, glycerol and glucose-1-phosphate, weakly positive for dextrin, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosaminet, adonitol, lactose, raffinose, trehalose, β-hydroxy butyric acid, propionic acid, L-alaninamide, D-alanine, L-asparagine, L-aspartic acid, L-leucine, L-ornithine, L-proline, L-pyroglutamic acid, D-serine and inosine; negative for Tween 80, cellobiose, D-fructose, L-fucose, gentiobiose, α-D-glucose, lactulose, methyl β-D-glucoside, L-rhamnose, D-sorbitol, sucrose, turanose, methyl pyruvate, mono-methyl-succinate, *cis*-aconitic acid, citric acid, formic acid, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxy butyric acid, γ-hydroxy butyric acid, p-hydroxy phenylacetic acid, α-keto glutaric acid, malonic acid, sebacic acid, succinic acid, bromo succinic acid, succinamic acid, glucuronamide, L-alanine, L-alanyl-glycine, L-glutamic acid, glycyl-L-aspartic acid, L-phenylalanine, L-serine, urocanic acid, phenyethylamine, 2-aminoethanol, DL-α-glycerol phosphate and glucose-6-phosphate. The activity of alkaline phosphatase, esterase (C4), leucine aminopeptidase, valine aminopeptidase, acid phosphatase, naphthol-AS-BI-phosphoamidase, α-glucosidase, esterase lipase (C8), lipase (C14), cystine aminopeptidase, trypsin and β-glucuronidase

is present, but activity of *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase or *α*-fucosidase is absent in the API ZYM test. The predominant quinone is ubiquinone 10 (Q-10). The major fatty acids (>5% of the total fatty acids) of strain H4^T were summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*), summed feature 3 ($C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*), $C_{17:1}$ *ω6c* and $C_{14:0}$2-OH. The major polar lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, two unidentified phospholipids, two sphingoglycolipids and three unknown lipids. Table 2 shows characteristics used to distinguish strain H4^T from related species.

The type strain is H4^T (=DSM 102182^T=MCCC 1K02301^T) and was isolated from surface seawater sampled from the Jiulong River estuary in southeast PR China. The DNA G+C content of isolate H4^T was 62.8 mol% (by genome). The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and the GenBank accession number for the whole genome shotgun sequence of strain H4^T are KU500624 and LRSE00000000, respectively.

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

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