INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Yan *et al.*, *Int. J. Syst. Evol. Microbiol.* 2022;72:005365

DOI 10.1099/ijsem.0.005365

MICROBIOLOGY SOCIETY

Algoriphagus algorifonticola sp. nov., a marine bacterium isolated from cold spring area of South China Sea

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Abstract

A Gram-stain-negative, aerobic, non-motile, short-rod-shaped bacterium, designated strain hg1^T, was isolated from marine sediment within the cold spring area of South China Sea and subjected to a polyphasic taxonomic investigation. Colonies were circular and 1.0–2.0 mm in diameter, coral in colour, convex and smooth after growth on marine agar at 28 °C for 3 days. Strain hg1^T was found to grow at 4–40 °C (optimum, 35–37 °C), at pH 6.5–9.0 (optimum, pH 7.5) and with 0–8% (w/v) NaCl (optimum, 1.5–2%). Chemotaxonomic analysis showed the sole respiratory quinone was MK-7, and the principal fatty acids are iso-$C_{15:0}$, summed feature 3 ($C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*), and iso-$C_{16:0}$. The major polar lipids are phosphatidylethanolamine, an unidentified phospholipid and five unidentified glycolipids. The DNA G+C content of strain hg1^T was 39.6 mol% based on the genome sequence. The comparison of 16S rRNA gene sequence similarities showed that hg1^T was closely related to *Algoriphagus ornithinivorans* DSM 15282^T (98.6% sequence similarity), *Algoriphagus zhangzhouensis* MCCC 1F01099^T (97.9%) and *Algoriphagus vanfongensis* DSM 17529^T (97.2%); it exhibited 97.0% or less sequence similarity to the type strains of other species of the genus *Algoriphagus* with validly published names. Phylogenetic trees reconstructed with the neighbour-joining, maximum-parsimony and maximum-likelihood methods based on 16S rRNA gene sequences showed that strain hg1^T constituted a separate branch with *A. ornithinivorans*, *A. zhangzhouensis*, *A. vanfongensis* in a clade of the genus *Algoriphagus*. OrthoANI values between strain hg1^T and *A. ornithinivorans*, *A. zhangzhouensis* and *A. vanfongensis* were 94.3, 74.1, 73.2%, respectively, and *in silico* DNA–DNA hybridization values were 56.2, 18.5 and 18.3%, respectively. Differential phenotypic properties, together with phylogenetic distinctiveness, demonstrated that strain hg1^T is clearly distinct from recognized species of genus *Algoriphagus*. On the basis of these features, we propose that strain hg1^T (=MCCC 1K03570^T=KCTC 72111^T) represents a novel species of the genus *Algoriphagus* with the name *Algoriphagus algorifonticola* sp. nov.

INTRODUCTION

The genus *Algoriphagus*, belonging to the family *Cyclobacteriaceae* within the phylum *Bacteroidetes*, was first proposed by Bowman in 2003 with *Algoriphagus ratkowskyi* as the type species [1]. At the time of writing, the genus has 48 species with validly published names isolated from different environments (https://www.bacterio.net/) [2], including seawater [3–5], brackish lake [6], freshwater lake [7, 8], tidal flat sediment [9–12], mangrove sediment [13], marine sediment [14, 15], estuarine environment [16–18], soil [19–21] and so on. Members of the genus share common characteristics such as having cells that are Gram-negative, non-motile, aerobic, rod-shaped and often pink-/reddish-/orange-pigmented. In this study, one novel strain, hg1^T, isolated from a cold spring area sediment of the South China Sea was studied through a polyphasic taxonomic approach and is proposed as representing a novel species within the genus *Algoriphagus*.

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Keywords: *Algoriphagus algorifonticola* sp. nov.; polyphasic taxonomy; marine bacterium.

Abbreviations: ANI, average nucleotide identity; COG, Clusters of Orthologous Groups; DDH, DNA–DNA hybridization; MA, marine agar; MB, marine broth; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain hg1^T is MN152930, and Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession VLOG00000000. The version described in this paper is version VLOG00000000.1.

Seven supplementary figures and two supplementary tables are available with the online version of this article.

ISOLATION AND CULTIVATION

Marine sediment sample collected on 8 April 2018 from a cold spring area in the South China Sea (110° 14′ 13.9″ E 16° 54′ 39.2″ N,1500 m depth), and stored at 4 °C in the lab until it was used. The sediment sample was suspended in sterile distilled water, and diluted using the standard dilution-plating method [22], spread on modified marine agar (MA; BD) medium and incubated at 28 °C for up to 7 days. The modified MA agar medium contained (per litre distilled water): 19.45 g NaCl, 12.6 g $MgCl_2{\cdot}6H_2O$, 6.64 g $MgSO_4{\cdot}7H_2O$, 1.8 g $CaCl_2$, 0.55 g KCl, 0.16 g $NaHCO_3$, 0.08 g KBr, 22 mg H_3BO_3, 4 mg $NaSiO_3$, 2.4 mg NaF, 1.6 mg NH_4NO_3, 8 mg Na_2HPO_4, 34 mg $SrCl_2$, 0.1 g ferric citrate, 5 g trypticase peptone, 1 g yeast extract and 20 g agar (pH 7.2–7.4 adjusted by NaOH). One coral colony formed after 3 days of incubation and was purified by repeated streaking method was named hg1^T. The strain was maintained for short-term storage on MA slants. For long-term preservation, cultures were preserved by lyophilization and frozen at −80 °C with 25% (v/v) glycerol [23]. Meanwhile, strain hg1^T has been deposited at the Marine Culture Collection of China (MCCC) and the Korean Collection for Type Cultures (KCTC).

PHYLOGENETIC ANALYSIS

The genomic DNA of strain hg1^T was extracted and purified using a Wizard Genomic DNA purification kit (Promega) according to the manufacturer's instructions. The 16S rRNA gene of strain hg1^T was amplified with universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). PCR products were purified by the PCR Cleanup Kit (Axygen) and cloned into pMD 19T vector (TaKaRa) for sequencing. The 16S rRNA gene sequence (1480 bp) obtained was compared with closely related sequences of reference organisms via BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the EzBioCloud (https://www.ezbiocloud.net/identify) [24]. The almost-complete sequence (1480 bp) and the complete sequence (1486 bp) obtained from the whole genome had the identical alignment results with BLASTN 2.11.0 with identities=1481/1486 (99%), gaps=0/1486(0%). The result shows that strain hg1^T has 92.8–98.6% sequence similarity to the type strains of species of the genus *Algoriphagus*. Among them, *Algoriphagus ornithinivorans* shows the highest sequence similarity (98.6%) followed by *Algoriphagus zhangzhouensis* (97.9%) and *Algoriphagus vanfongensis* (97.2%). Multiple sequence alignment based on 16S rRNA gene sequences of strain hg1^T and related taxa were performed with ClustalW 1.8 program [25]. Phylogenetic trees were reconstructed using MEGA version 7.0 [26] by the neighbour-joining (NJ; Fig. 1), maximum-parsimony (MP; Fig. S1, available in the online version of this article) and maximum-likelihood (ML; Fig. S2) methods [27]. Bootstrap analysis (1000 resample datasets) was used to evaluate the topology of the trees. Kimura's two-parameter model [28] was used for phylogeny reconstruction and evolutionary distance analysis. The topological structures of the NJ, ML and MP trees were highly similar, illustrating that strain hg1^T coherently clustered into the clade of genus *Algoriphagus* by forming a distinct lineage among the most related species including *A. ornithinivorans* DSM 15282^T, *A. zhangzhouensis* MCCC 1F01099^T and *A. vanfongensis* DSM 17529^T. As a result, these three strains were selected as reference strains for polyphasic taxonomic study. *A. ornithinivorans* DSM 15282^T and *A. vanfongensis* DSM 17529^T were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen(DSMZ), and *A. zhangzhouensis* MCCC 1F01099^T was obtained from the MCCC Unless otherwise stated, all strains were incubated on MA or in marine broth 2216 (MB; Difco) at 28 °C.

MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTIZATION

Cell morphology and motility were observed by optical microscope (BX40; Olympus) and transmission electron microscopy (JEM-1230, JEOL) for strain hg1^T that had been grown on MA for 24 h. The presence of gliding motility was checked by the hanging-drop method as described by Suzuki *et al.* [29]. Gram-staining was performed with commercial Gram-stain kit (BD) and observed by optical microscope (BX40, Olympus). The presence of flexirubin-type pigments and carotenoid was investigated as described by Bernardet *et al.* [30]. The temperature range for growth was determined in MB at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 42 and 45 °C for up to 2 weeks. The pH range for growth was determined in MB from pH 5.0 to 10.0 with 0.5 pH intervals, adjusted by appropriate buffers including MES (pH 5.0–5.5), MOPS (pH 6.0–7.5), Tricine buffer (pH 8.0–8.5) and CAPSO (pH 9.0–10.0) for up to 2 weeks. The salinity range for growth was tested by using NaCl-free MB medium with different NaCl concentrations (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4.0, 6.0, 8.0, 10.0 and 12.0%, w/v) for up to 2 weeks. The temperature, pH and NaCl ranges for growth were monitored by measuring OD_{600nm} in a UV/Visible spectrophotometer (Ultrospec 6300 pro, Amersham Biosciences). Anaerobic growth was investigated at 28 °C for 15 days in modified MA medium supplemented with 20 mM sodium thiosulfate, 5 mM sodium sulphite, 20 mM sodium sulphate, 5 mM sodium nitrite, 20 mM sodium nitrate and 5 g l^{-1} L-arginine as electron acceptor, 0.01 g l^{-1} resazurin as oxygen indicator and 0.5 g l^{-1} cysteine as deoxidizer with an AnaeroPack (Mitsubishi). Catalase activity was tested with 3% (v/v) H_2O_2 by observing the production of oxygen bubbles within 5 min. *p*-Aminodimethylaniline oxalate (1%, v/v) was used for the oxidase activity detection. The hydrolysis of starch, Tween 20, 40, 60 and 80, casein, and tyrosine were determined according to the method of Sun *et al.* [31]. Nitrate and nitrite reduction, and hydrolysis of CM-cellulose, filter paper, gelatin, xanthine and hypoxanthine were determined according to the method of Dong and Cai [32].

H_2S production was determined by observing the colour change of sterilized filter strips which were previously steeped in 10% (v/v) lead acetate and placed in the tube containing 5 ml modified MB medium with 5 g l^{-1} sodium thiosulphate. After 1% late

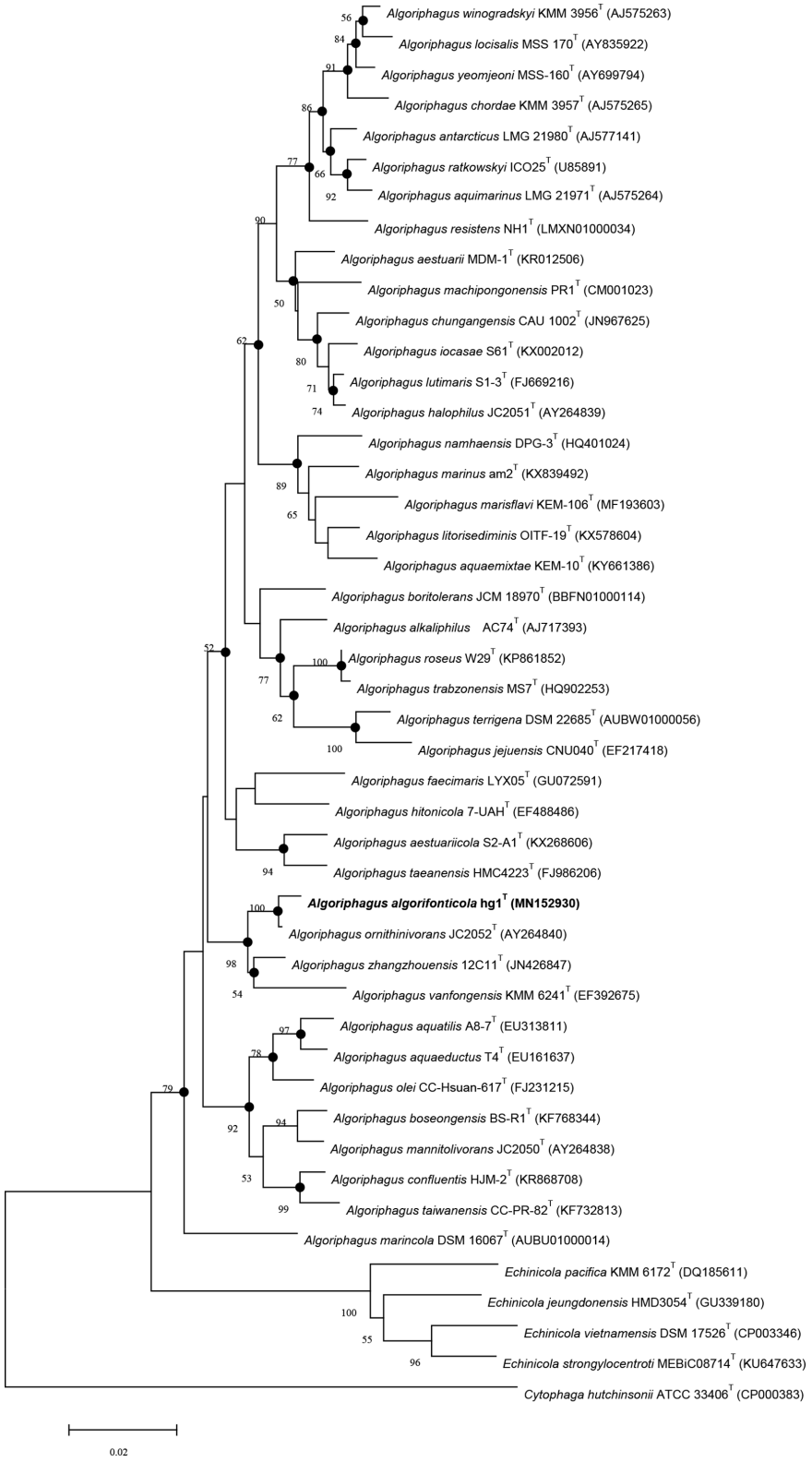

Fig. 1. Neighbour-joining phylogenetic tree using the Kimura two-parameter model based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain hg1^T and related species of the genus *Algoriphagus*. Bootstrap values are based on 1000 replicates; values >50% are shown. Filled circles indicate the same topological structures in the maximum-likelihood and maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position. *Cytophaga* and *Echinicola* were selected as outgroups.

exponential phase bacterial liquid culture was inoculated in MB, the filter strips were observed for 5–10 days at 28 °C. The methyl red and Voges–Proskauer reactions were tested according to the method of Zhang *et al.* [33]. Single carbon source utilization was observed in modified MB medium which without peptone but with 0.01% (w/v) yeast extract and corresponding filter-sterilized alcohols (0.2%, w/v), sugar (0.2%, w/v), amino acid (0.1%, w/v) or organic acid (0.1%, w/v) [34]. Acid production was determined by using API 50CH (bioMérieux) strips with MOF medium [35]. Additional physiological characteristics and enzyme activities were tested by API ZYM and API 20NE (bioMérieux) according to the manufaturer's instructions. Antibiotic sensitivity was tested on MA with antibiotic discs, each disc containing following antibiotics (μg per disc unless stated otherwise): amoxicillin (20), ampicillin (10), carbenicillin (100), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (2), neomycin (30), novobiocin (30), ofloxacin (5), penicillin (10U), polymyxin B (300 IU), rifampicin (5), streptomycin (10) and tetracycline (30). Three reference strains, *A. vanfongensis* DSM 17529^T, *A. ornithinivorans* DSM 15282^T and *A. zhangzhouensis* MCCC 1F01099^T, were used for comparison under the same condition.

Cells of strain hg1^T were Gram-strain-negative, strictly aerobic, rod-shaped, non-spore-forming, non-motile without flagella, 1.0–1.9 μm long and 0.6–0.9 μm wide (Fig. S3). No gliding motility was observed. Colonies on MA agar plates were coral in colour, convex, smooth and approximately 1–2 mm in diameter after incubation for 3 days at 28 °C. The temperature and pH range for growth were 4–40 °C (optimum at 35–37 °C) and pH 6.5–9.0 (optimum at pH 7.5). The NaCl concentration range for growth was 0–8% (optimum at 1.5–2%). No growth occurred at 10%. The result showed that NaCl was not required for growth. Carotenoid was found in strain hg1^T. No flexirubin pigments were found in strain hg1^T. Strain hg1^T was positive for catalase activity, oxidase activity, hydrolysis of starch, gelatin and casein, and susceptible to the antibiotics (μg per disc unless otherwise stated) amoxicillin (20), ampicillin (10), carbenicillin (100), chloramphenicol (30), erythromycin (15), lincomycin (2), novobiocin (30), ofloxacin (5), penicillin (10 IU) and rifampicin (5) and resistant to gentamicin (10), kanamycin (30), neomycin (30), polymyxin B (300 IU), streptomycin (10) and tetracycline (30). Details of phenotypic characteristics are shown in the Table 1, which reveal that there are many common traits between strain hg1^T and the reference strains. All strains are aerobic, non-motile, grow at NaCl concentrations ≥0%, and positive for H_2S production, oxidase, catalase and β-glucosidase. All strains are negative for reduction of nitrate to nitrite, gliding motility, and degradation of xanthine, hypoxanthine, CM-cellulose and filter paper. However, there are many phenotypic features that could distinguish strain hg1^T from the reference strains, such as strain hg1^T was positive for reduction of nitrate to nitrogen, fermentation of glucose, and acid production of L-sorbose, D-sorbitol and xylitol, while the reference strains were negative for these features. Meanwhile, the reference strains were positive for β-glucuronidase, but strain hg1^T was negative. There are other phenotypic features that enable strain hg1^T to be distinguished from the reference strains are listed in Table 1.

CHEMOTAXONOMY

Isoprenoid quinones were extracted from freeze-dried cells at exponential phase using chloroform–methanol (2:1, v/v) then evaporated to dryness at 35 °C and resuspended in chloroform–methanol (2:1, v/v). The separation was performed on GF254 silica gel plates (10×20 cm; Qingdao Haiyang Chemical) with *n*-hexane–ether (17:3, v/v). The isoprenoid quinones were analysed by HPLC-MS (Agilent 1200 chromatograph and Thermo Finnigan LCQ DECA XP MAX mass spectrometer). For the fatty acid methyl ester analysis, strain hg1^T and the reference strains were harvested during the third quadrants grown on MA at 28 °C for 3 days. Cellular fatty acid methyl esters were analysed following the instruction of the Microbial Identification System (MIDI; Microbial ID) using the RTSBA6 database. Polar lipids were extracted from late exponential phase cells then were separated by two-dimensional TLC on silica gel 60 F254 plates (10×10 cm; Merck). Different spray reagents were used to detect corresponding lipids: molybdophosphoric acid for total lipids, ninhydrin reagent for aminolipids, molybdenum blue spray reagent (Sigma) for phospholipids and sulfuric acid and α-naphthol reagent for glycolipids. The sole respiratory quinone found in hg1^T was MK-7, which is the major respiratory quinone in all *Algoriphagus* species. The predominate cellar fatty acid (>5%) of strain hg1^T was iso-$C_{15:0}$ (23.8%), which is typical of the genus *Algoriphagus*, followed by summed feature 3 ($C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*; 11.2%), iso-$C_{16:0}$ (10.8%), summed feature 9 ($C_{16:0}$ 10-methyl and/or iso-$C_{17:1}$ ω9*c*; 5.5%) and iso-$C_{17:0}$ 3-OH (5.2%), which was similar to the reference strains. There were some differences between strain hg1^T and the reference strains, the percentage of iso-$C_{15:0}$ (23.8%) was lower than that of the reference strains (30.8–42.6%), while the percentage of iso-$C_{16:0}$ (10.8%) was higher than that of the reference strains (1.7–6.1%). The fatty acid profiles also showed other minor differences between strain hg1^T and the reference strains. Strain hg1^T contained summed feature 8 ($C_{18:1}$ ω7*c*/$C_{18:1}$ ω6*c*; 0.7%), iso-$C_{14:0}$ 3-OH (0.2%) and $C_{16:0}$ 2-OH (0.1%), which were not detected in the reference strains (Table S1). Anteiso-$C_{11:0}$ and iso-$C_{15:1}$ G were detected in the reference strains while not found in strain hg1^T. Iso-$C_{16:0}$ was detected in strain hg1^T as a major fatty acid (10.8%), while it was a minor fatty acid in *A. zhangzhouensis* (1.7%) and *A. vanfongensis* (3.3%). As shown in Fig. S4, strain hg1^T possessed phosphatidylethanolamine, an unidentified phospholipid, five unidentified glycolipids and two unidentified polar lipids.

GENOME SEQUENCING AND ANALYSIS

The genome of strain hg1^T was sequenced by Solexa PE150 sequencing technology with the HiSeq platform (Novogene Technology Company) and approximately 1.164 Gb of clean data was obtained (approximate 280-fold genome coverage). The *de novo*

Table 1. Differential characteristics of strain hg1^T and the type strains of *Algoriphagus* species

Strains: 1, hg1^T; 2, *A. ornithinivorans* DSM 15282^T; 3, *A. vanfongensis* DSM 17529^T; 4, *A. zhangzhouensis* MCCC 1F01099^T; 5, *A. ratkowskyi* IC025^T. Unless stated otherwise, all data were obtained from this study under identical growth conditions. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4	5
Temperature range for growth (°C)	4–40	10–45*	12–35†	4–40	–2–25‡
Optimum temperature for growth (°C)	35–37	35–40*	28†	25§	16–19‖
NaCl for growth (%)	0–8	0–10*	0–8†	0–10¶	0.5–5.8**
Optimum NaCl for growth (%)	1.5–2.0	1*	1–4†	4§	1.0–2.0**
pH range for growth	6.5–9.0	6–13*	6–11	5.5–11.0¶	6.5–8.5**
Optimum pH for growth	7.5	7.0*	7.5	7.5§	7.5–8.0**
H_2S production	+	+	+	+	–**
Carotenoid	+	+	–	–	–‖
Degradation of:					
Starch	+	+	–	–	–‖
Casein	+	+	–	+	+‖
API ZYM assay:					
α-Galactosidase	–	–	+	–	–‖
β-Glucuronidase	–	+	+	+	–‖
Cystine arylamidase	+	+	+	+	–**
α-Fucosidase	–	–	+	+	–‖
API 20NE assay:					
Reduction of nitrate to nitrogen	+	–	–	–	–††
Fermentation of glucose	+	–	–	–	–††
Hydrolysis of gelatin	+	–	w	+	–‖
Arginine dihydrolase	–	–	–	+	+**
Assimilation of D-mannitol	–	+	–	+	–‡
Assimilation of maltose	w	–	w	+	–‡
Assimilation of malic acid	–	+	–	+	–‡
Assimilation of *N*-acetyl-glucosamine	–	–	–	w	+**
Acid production from:					
D-Xylose	+	+	+	+	+**
L-Xylose	–	–	+	w	+
L-Sorbose	w	–	–	–	-**
Raffinose	+	+	–	+	+**
L-Arabinose	+	+	+	w	+§§
L-Rhamnose	–	–	w	w	+§§
Utilization of single carbon sources:					
D-Fructose	+	+	+	+	–**
D-Mannitol	+	–	–	+	+**
Cellobiose	–	–	+	+	–‖

Continued

Table 1. Continued

Characteristic	1	2	3	4	5
D-Salicin	w	+	−	+	+**
N-Acetylglucosamine	−	w	+	+	−**
L-Arabinose	−	−	w	w	−‖
L-Ornithine monohydrochloride	−	+	−	−	−‖
Susceptibility to (μg per disc):					
Streptomycin (10)	−	−	−	−	+‡
Ampicillin (10)	+	+	−	−	−‖
Kanamycin (30)	−	w	−	−	−‖
Penicillin (10 U)	+	+	−	−	−‖

*Data from [45].
†Data from [46].
‡Data from [1].
§Data from [13].
‖Data from [47].
¶Data from [48].
**Data from [17].
††Data from [16].
‡‡Data from [49].

assembly of the reads was performed using ABySS version 2.0.2 [36]. The assembly k-value was tested from 32 to 64 to find out the optimal k-value using the abyss-pe script. The quality of genome was checked by CheckM 1.0.8 [37]. The result showed that the genomic sequence was estimated to be ≥95% complete (100%) with ≤5% contamination (0.38%) and was considered as an ideal reference genome for further analyses [37]. Rapid Annotation using Subsystem Technology (RAST) was used for the DNA G+C content and open reading frame calculations using the draft genome sequence [38]. For functional annotation, the Clusters of Orthologous Groups (COG) [39] database was used via the WebMGA server (http://weizhong-lab.ucsd.edu/webMGA/server/cog/). The antiSMASH 6.0 program was used as a tool for the identification of the secondary metabolism gene clusters [40].

The genome sequence and G+C content data for the reference strains *A. ornithinivorans* DSM 15282^T (FOVW01000000), *A. vanfongensis* DSM 17529^T (AUBX01000000) and *A. zhangzhouensis* MCCC 1F01099^T (FRXN01000000) were retrieved from the GenBank database. Average nucleotide identity (ANI) values were calculated using the Orthologous Average Nucleotide Identity Tool (OAT) from ChunLab [41]. The description and analysis methods we used matched the taxonomy of prokaryote standards proposed by Chun *et al.* [42]. *In silico* DNA–DNA hybridization (DDH) values were calculated by the Genome-to-Genome Distance Calculator [43].The phylogenomic tree based on the whole genome was reconstructed using the TYGS (https://tygs.dsmz.de/) server.

The DNA base composition (G+C content) was determined by calculating G+C from full genome sequences. The genome sequence of strain hg1^T consisted of 68 scaffolds, with 4449979 bp in total (N50 value, 126468 bp). The genome had a total of 4177 coding sequences and 33 RNA genes. The genomic DNA G+C content of strain hg1^T was 39.6 mol%, which was within the range of 35–49 mol% for members of the genus *Algoriphagus* and was distinct from *A. vanfongensis* DSM 17529^T (42.8%), *A. ornithinivorans* DSM 15282^T (39.4%) and *A. zhangzhouensis* MCCC 1F01099^T (39.3%). The reconstructed phylogenomic tree showed that strain hg1^T formed a cluster with *A. ornithinivorans* DSM 15282^T, which was located in the clade containing the genus *Algoriphagus* (Fig. S5). The *in silico* DDH value between strain hg1^T and *A. vanfongensis* DSM 17529^T (18.3%), *A. ornithinivorans* DSM 15282^T (56.2%) and *A. zhangzhouensis* MCCC 1F01099^T (18.5%) were all below the 70% threshold value for DDH proposed for the delineation of bacterial species [44]. The ANI values between strain hg1^T and *A. vanfongensis* DSM 17529^T (73.2%), *A. ornithinivorans* DSM 15282^T (94.3%) and *A. zhangzhouensis* MCCC 1F01099^T (74.1%) were all below the species circumscription threshold (95%) [28] and supported the *in silico* DDH results.

The different genomic features of the novel isolate and the reference strains based on the RAST result are listed in Table S2. On the basis of the genome annotation, a considerable number of genes associated with virulence, disease and defence were found in strain hg1^T, of which, 27 genes were related to resistance to antibiotics and toxic compounds and 10 genes were related to invasion and intracellular resistance. A number of genes associated with environmental adaptation were found in strain hg1^T, of which, there were 15 genes related to phosphorus metabolism, 15 genes for sulphur metabolism and 13 genes for nitrogen metabolism.

In addition, 21 genes responsible for stress response were found in the genome of strain hg1^T, including 10 genes associated with oxidative stress, four genes associated with periplasmic stress, three genes associated with osmotic pressure and two genes associated with detoxification. Compared with the reference strains, strain hg1^T contained 13 genes responsible for nitrogen metabolism, which was higher than most of the reference strains. The number of genes associated with potassium metabolism and phosphorus metabolism in strain hg1^T were similar to the reference strains. The result of antiSMASH 6.0 indicated that the novel isolate contained one gene cluster for an NRPS-like fragment which showed 100% similarity to carotenoid (Fig. S6).

According to function annotation by using the RPSBLAST program on the COG database (prokaryotic proteins), 3111 genes were annotated. Among them, the number of genes related to amino acid transport and catabolism was highest (270 genes). In the second place was genes associated with energy production and conversion (258 genes). There were 245 genes related to translation, ribosomal structure and biogenesis. In addition to these, there were 99 genes associated with secondary metabolites biosynthesis, transport and catabolism,

In conclusion, strain hg1^T exhibits typical characteristics of the genus *Algoriphagus*, such as having MK-7 as the sole respiratory quinone, iso-$C_{15:0}$ as the predominant fatty acid, and most of its phenotypic characteristics. However, there are still many characteristics that can distinguish strain hg1^T from the type strains of the genus *Algoriphagus*, such as range and optima of temperature and pH, hydrolysis of starch and casein, enzyme activities, carbohydrate utilization, and acid production (Table 1). On the basis of its phylogenetic, genotypic, phenotypic and chemotaxonomic characteristics, strain hg1^T was clearly distinguishable from other species within the genus *Algoriphagus*. A novel species, *Algoriphagus algorifonticola* sp. nov., with the type strain hg1^T, is proposed.

DESCRIPTION OF *ALGORIPHAGUS ALGORIFONTICOLA* SP. NOV.

Algoriphagus algorifonticola [al.go.ri.fon.ti'co.la. L. masc. n. *algor* the cold; L. masc. n. *fons, fontis* a spring; L. suff. *-cola* (from L. masc. or fem. n. *incola*) an inhabitant of a place, a resident; N.L. n. *algorifonticola* an inhabitant of a cold spring].

Cells are Gram-stain-negative, strictly aerobic, non-motile short rods (1.0–1.9 µm long and 0.6–0.9 µm wide). No gliding motility is observed. Colonies on MA agar plates are circular, coral in colour, convex, smooth and approximately 1–2 mm in diameter after incubation for 3 days at 28 °C. Growth occurs at 4–40 °C (optimum, 35–37 °C), pH 6.5–9.0 (pH 7.5) and with 0–8% (w/v) NaCl (1.5–2%). Positive for oxidase and catalase activities, methyl red reaction, H_2S production, carotenoid, reduction of nitrate to nitrogen, fermentation of glucose, hydrolysis of aesculin and gelatin, degradation of starch, casein, tyrosine and Tweens 20/40/60/80. The activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase are present. Acid production from D-xylose, D-glucose, D-fructose, D-mannose, trehalose, L-arabinose, D-ribose, methyl α-D-mannopyranoside, aesculin ferric citrate and salicin are positive. Utilization of D-xylose, D-glucose, D-fructose, D-mannose, trehalose, raffinose, lactose, sucrose, inulin and glycerol is positive, while utilization of citric acid, D-sorbitol, inositol, glycine, D-ribose, L-arginine, L-ornithine monohydrochloride and L-sorbose are negative. The sole respiratory quinone is MK-7. The principal fatty acids are iso-$C_{15:0}$, summed feature 3 ($C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*) and iso-$C_{16:0}$. The major polar lipids are phosphatidylethanolamine, an unidentified phospholipid, five unidentified glycolipids and two unidentified polar lipids.

The type strain is hg1^T (=MCCC 1K03570^T=KCTC 72111^T), isolated from marine sediment of South China Sea. The DNA G+C content of the type strain is 39.6 mol% (calculated from the genome sequence).

Funding information
This work was supported by the Key R&D Program of Zhejiang (2022C03010) and the Science and Technology Basic Resources Investigation Program of China (2017FY100300).

Acknowledgements
The authors thank Cong Sun (College of Life Sciences, Zhejiang Sci-Tech University) for phylogenomic analysis and maximum-likelihood phylogenetic tree reconstruction.

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

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