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ORIGINAL PAPER

Zongyanglinia huanghaiensis gen. nov., sp. nov., a novel denitrifying bacterium isolated from the yellow sea, and transfer of *Pelagicola marinus* to *Zongyanglinia* gen. nov. as *Zongyanglinia marinus* comb. nov

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Abstract Two Gram-stain-negative, oxidase- and catalase-positive, aerobic strains (CY05^T and H18S-6) were isolated from sediment samples of the Yellow Sea, China. The strains were positive for denitrification. Optimum growth was observed at 20 °C, pH 7.5–8.0 and with 2.0%–3.0% NaCl. The predominant cellular fatty acids (> 10%) were summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$), major respiratory quinone was ubiquinone-10 and main polar lipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid and one unidentified aminolipid. The approximate genome size of strains CY05^T and H18S-6 were 4.86 and 5.04 Mbp, the genomic G + C content of them were 54.2 and 54.5%, respectively. Both of the phylogenetic analysis based on 16S rRNA gene sequences and the up-to-date bacterial core gene (UBCG) sequences revealed that strains CY05^T, H18S-6 and *Pelagicola marinus* DSW4-44^T formed a distinct monophyletic clade within the family *Rhodobacteraceae*. The ANI and *is*DDH values between strains CY05^T and H18S-6 were 94.0% and 56.5%, between CY05^T and *Pelagicola marinus* DSW4-44^T were 94.1% and 59.8%, respectively, all below the accepted threshold value for species delineation. But the ANI and *is*DDH values between strains H18S-6 and *Pelagicola marinus* DSW4-44^T were 96.8% and 76.7% respectively, indicating that strains H18S-6 and *Pelagicola marinus* DSW4-44^T belong to the same species. Based on the distinctive polyphasic evidence, CY05^T represent a novel species of a novel genus of the family *Rhodobacteraceae*, for which the name *Zongyanglinia huanghaiensis* gen. nov., sp. nov. is proposed. The type strain is CY05^T (= MCCC 1K04409^T = KCTC 62200^T). Moreover, the reclassification of *Pelagicola marinus* Choi et al. 2019 as *Zongyanglinia marinus* comb. nov. (type strain DSW4-44^T = KCTC 62762^T = KCCM 43261^T = JCM 33637^T) is proposed based on the polyphasic taxonomic data obtained in this study.

Keywords *Rhodobacteraceae* · *Zongyanglinia* gen. nov. · Polyphasic taxonomy · Denitrification

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Introduction

The family *Rhodobacteraceae* is an abundant bacteria group with diverse ecology, metabolism and phenology (Garrity et al. 2005). The number of the genera and species in this family is growing rapidly. For example, 15 novel genera (Park et al. 2018a, b; Feng et al. 2018a, b; Wang et al. 2018; Yu et al. 2018; Klotz et al. 2018; Hu et al. 2018; Zhang et al. 2018; Wirth and Whitman 2018) have been described in 2018, including 6 reclassified novel genera (*Cognatishimia*, *Cognatiyoonia*, *Flavimaricola*, *Limimaricola*, *Pseudaestuariivita* and *Yoonia*) based on phylogenomic analyses (Wirth and Whitman 2018). At the time of writing, the family *Rhodobacteraceae* contains at least 160 genera encompassing nearly 592 species (List of Prokaryotic Names with Standing in Nomenclature; www.bacterio.net).

Denitrification, an important branch of nitrogen cycle, is a microbial process in which nitrate and nitrite are stepwisely reduced to gaseous forms of nitrogen. As human activities such as the high input of nitrogen-based fertilizers have nearly doubled the nitrogen input to terrestrial and marine ecosystems and consequently lead to water eutrophication, microbial denitrification is getting more attention (Kuypers et al. 2018). Some microbes showing good denitification capacity including *Pseudomonas* sp. (Sun et al. 2015), *Alcaligenes* sp. (Ozeki et al. 2001) and *Microvirgula* sp. (Patureau et al. 2000) have been applied to remove the excess nitrogen from wastewater.

In this study, two bacteria strains showing denitrification capacity were isolated from sediment samples of the Yellow Sea, China. Based on the polyphasic taxonomy study, strain CY05^T was proposed to represent a species of a novel genus in the family *Rhodobacteraceae*. In addition, we explored the existence of genes related to denitrification by analyzing their genomes.

Materials and methods

Samples and cultivation

Two surface marine sediment samples were collected at a water depth of 36 m in the Yellow Sea during a cruise as described previously (Zhang et al. 2017) in 2014. Samples were stored in sterilized plastic bags (250 mL) and transported to the laboratory at 15 °C. Bacterial strains were isolation using the standard dilution plating technique on TYM agar at 15°C as described previously (Zhang et al. 2018). Strain CY05^T was isolated from sample collected at the site H02 (36° N, 121° E) in November, and H18S-6 was isolated from sample collected at the site H18 (35° N, 121° E) in May, respectively. The temperature and salinity of the seawater at site H02 and H18 measured by CTD system was 18.2°C and 3.1%, 10.1°C and 3.1%, respectively. In addition, the following strains: *Pelagicola marinus* DSW4-44^T (obtained from Korean Collection for Type Cultures), *Pelagicola litoralis* DSM 18290^T, *Phaeobacter porticola* DSM 103148^T, *Sulfitobacter pseudonitzschiae* MCCC 1A00686^T, *Sedimentitalea nanhaiensis* MCCC 1A04178^T and *Pelagimonas varians* DSM 23678^T (obtained from Marine Culture Collection of China) were characterized alongside for comparative purposes. Unless otherwise stated, these strains were routinely cultured in marine broth 2216 (MB; Difco) or on marine agar 2216 (MA; MB with 1.5% agar) at 20°C except for *Pelagimonas varians* DSM 23678^T cultured in corresponding medium supplemented with 0.25 mg/L nicotinic acid.

Phylogenetic analysis

The 16S rRNA gene was amplified by PCR with the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Weisburg et al. 1991). PCR products were purified using the GeneJET gel extraction kit (Thermo). The purified PCR products were ligated into pMD 19-T vector (Tsingke) and sequenced using an automated DNA sequencer (model 3730xl; Applied Biosystems) at Tsingke Biological Technology Co. Ltd (Qingdao, China). The nearly complete 16S rRNA gene sequences were compared to those of species validly published through the EzBioCloud server [http://www.ezbiocloud.net/, (Yoon et al. 2017)]. Phylogenetic trees were constructed with MEGA 6 (Tamura et al. 2013) using maximum-likelihood (Felsenstein 1981), neighbour-joining (Saitou and Nei 1987) and maximum-parsimony (Fitch 1971) methods. The topologies of the phylogenetic trees were evaluated by bootstrap analyses (1000 replications).

Genomic DNA sequencing and analyses

Genomic DNA of strains CY05^T and H18S-6 were extracted using a bacterial genomic DNA isolation kit (BioTeke) following the manufacturer's instructions except that sterile ddH_2O was used for DNA elution. Genome sequencing was performed at BGI-Shenzhen (China) using the HiSeq 4000 sequencer system (Illumina). The DNA G + C % content of the two strains were calculated directly from the draft genome sequences.

The obtained draft genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al. 2016) and RAST v2.0 (Rapid Annotation using Subsystem Technology) (Brettin et al. 2015).

Phylogenomic tree reconstruction and genome comparison

Phylogenomic tree reconstruction was conducted based on an up-to-date bacterial core gene set (UBCG) consisting of 92 single-copy core genes (Na et al. 2018). The alignment file of the UBCG was generated using a JAVA program (Na et al. 2018) from the genome sequences and was used as input for the PhyML 3.0 server (www.atgc-montpellier.fr/phyml/) with smart model selection (Guindon et al. 2010; Lefort et al. 2017). Robustness estimation of the nodes is conducted using bootstrapping with 100 replicates. Genome-based similarity indexes including in silico DNA-DNA hybridization (*is*DDH), average nucleotide identities (ANI), average amino acid identities (AAI) and the percentage of conserved proteins (POCP) were also calculated. The *is*DDH values were estimated with the Genome-to-Genome Distance Calculator using the recommended formula 2. ANI values including ANIm and ANIb were calculated in JspeciesWS. AAI values were determined by the website-based AAI calculator (Rodriguez-R and Konstantinidis 2016). POCP values were calculated according to Qin et al. (2014).

Morphological, physiological and biochemical analysis

The colony morphologies of the strains were observed after incubation on MA at 20 °C for 7 days. Cellular morphologies and flagellum were observed by transmission electron microscope (HITACHI HT7700) with cells from exponentially growing cultures. Flagellum was also checked by the flagella staining method (Qingdao Hope Bio-Technology Co. Ltd., China). Gram staining was carried out using the standard Gram procedure (Murray et al. 1994). Growth at temperatures (5, 10, 15, 20, 25, 30, 37 and 42 °C) was measured in MB. Growth with different NaCl concentrations (0, 0.5% and 1.0%–10.0%, at intervals of 1.0% units; w/v) was determined in NP broth (Zhang et al. 2017) at 20 °C. Growth tests for pH range [5.0–10.0, at intervals of 0.5 pH units, buffered with MES (pH 5.0–6.0, 50 mM), MOPS (pH 6.5–7.0, 50 mM), Tris (pH 7.5–8.5, 50 mM) and CHES (pH 9.0–10.0, 50 mM)] were assessed in NP broth with 2.0% NaCl. Oxidase activity was tested by using commercial oxidase test strips (Tianhe Microorganism Reagent Co.) according to the manufacturer's instructions. Catalase activity was determined by bubble production in 3% (v/v) H_2O_2. Hydrolysis of starch, casein, Tween 40, Tween 60 and Tween 80 were examined by using MA as the basal medium and incubation at 20 °C. Growth under anaerobic condition was determined in MB supplemented with potassium nitrate (0.1%, w/v), cysteine hydrochloride (0.05%, w/v) and sodium sulfide (0.05%, w/v) in Hungate tubes filled with oxygen-free N_2 at 20°C for two weeks. The utilization of carbohydrates (0.5%, w/v) as sole carbon and energy sources was determined in basal medium (Farmer et al. 2005) at 20 °C for 14 days. Growth was scored as negative when it was less than, or equal to, that in the negative control (lacking any carbon source). Nitrate reduction, denitrification and nitrite reduction were tested in MB medium supplemented with KNO_3 (0.1%, w/v) and $NaNO_2$ (0.01%, w/v) respectively, and contained a small inverted vial. The result were examined as (Dong and Cai 2001) described. MB medium contained a small inverted vial served as control. Susceptibility to antibiotics was examined by the disc-diffusion method on MA after spreading cell suspensions (0.5 McFarland). The discs (Oxoid) contained the following antibiotics: amoxycillin (10 μg), ampicillin (10 μg), bacitracin (10 U), carbenicillin (100 μg), cefotaxime (30 μg), cefoxitin (30 μg), chloramphenicol (30 μg), nitrofurantoin (300 μg), penicillin G (10 U) and rifampicin (5 μg). Antibiotic susceptibility was assessed by measuring diameters of the inhibition zone after 5 days at 20 °C.

An inhibition zone diameter more than 10 mm (including the 6 mm diameter of the filter paper) was considered sensitive to antimicrobial agent (Wu et al. 2015). More biochemical properties were determined by using API ZYM and API 20NE strips (bioMerieux) according to the manufacturer's instructions with sterilized artificial seawater (ASW) (Tindall et al. 2007) as the cell suspension solution and AUX medium supplemented with 2.0% (w/v) NaCl. The results were read after incubation at 20 °C for 24 h, 24 and 48 h for API ZYM and API 20 NE kits, respectively.

Chemotaxonomic analysis

For the analysis of cellular fatty acids, cells were collected from the third quadrant of the quadrant streaked plate (at exponential phase) after incubated on MA medium supplemented with 0.25 mg/L nicotinic acid at 20°C. Fatty acid methyl esters were analysed by an Agilent 6850 N gas chromatograph and identified using the Sherlock Microbial Identification System (TSBA, version 6.1, MIDI) at Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, P. R. China. For the analysis of polar lipids and quinone, cells of them were grown to early stationary phase in MB at 20 °C. Polar lipids were extracted according to (Komagata and Suzuki 1988) and analysed using two-dimensional TLC on silica gel 60 F254 plates (Merck) with appropriate spraying reagents including ethanolic molybdophosphoric acid (total lipids), ninhydrin (aminolipids) and molybdenum blue (phospholipids). The respiratory quinone of strains CY05^T and H18S-6 were extracted, separated and analysed as described by (Lin et al. 2015).

Results and discussion

Phylogenetic analysis

Nearly full-length 16S rRNA gene sequence of CY05^T (1425 bp, MN790778) and H18S-6 (1425 bp, MN808553) were obtained. Strains CY05^T and H18S-6 showed the highest 16S rRNA gene sequence similarities to *Pelagicola marinus* DSW4-44^T (100% and 99.93%) and less than 96.6% sequence similarities with the closely related type strains of the family *Rhodobacteraceae* in the class *Alphaproteobacteria*. The 16S rRNA gene sequence similarity between CY05^T and H18S-6 was 99.93%. There was also relatively similar sequence similarities of strains CY05^T and H18S-6 to closely related species *Pelagicola litoralis* DSM 18290^T (96.54% and 96.47%, respectively), *Phaeobacter porticola* DSM 103148^T (96.32% and 96.32%, respectively), *Sulfitobacter pseudonitzschiae* MCCC 1A00686^T (96.17% and 96.10%, respectively), *Sedimentitalea nanhaiensis* MCCC 1A04178^T (95.96% and 95.89%, respectively) and *Pelagimonas varians* DSM 23678^T (95.75% and 95.68%, respectively). In the maximum-likelihood tree (Fig. 1), strains CY05^T, H18S-6 and *Pelagicola marinus* DSW4-44^T formed a monophyletic clade within the family *Rhodobacteraceae* with 100% bootstrap support. This stable topology was also supported by the maximum-parsimony (Fig. S1) and neighbour-joining trees (Fig. S2). On the basis of their stable monophyletic grouping, strains CY05^T, H18S-6 and *Pelagicola marinus* DSW4-44^T should be placed in a new genus of the family *Rhodobacteraceae*.

Phylogenomic tree reconstruction and genome comparison

Previous studies had shown that 16S rRNA gene sequences lack the resolution for a proper phylogenetic reconstruction inside the *Rosebacter* group (Luo and Moran 2014; Wirth and Whitman 2018) and genome sequence data was strongly recommended to use for the taxonomy of these bacteria. Therefore, a multigene, genome-based tree using the UBCG tool was constructed to complement the 16S rRNA gene-based trees. In accordance with the 16S rRNA trees, the genome-based tree (Fig. 2) also showed that strains CY05^T, H18S-6 and *Pelagicola marinus* DSW4-44^T formed a distinct phylogenetic clade (100% bootstrap value support) within the family *Rhodobacteriaceae* conforming that strains CY05^T, H18S-6 and DSW4-44^T should be considered as representing a novel genus of the family *Rhodobacteraceae*. Further, genome-based similarity indexes including *is*DDH, ANI, AAI and POCP which were useful for the taxonomy of prokaryotes were calculated and depicted in Table 1. ANI and *is*DDH percentages were genomic nucleic acid-level comparisons widely used for species delineation. As shown in Table 1, ANIb percentages between strains CY05^T and

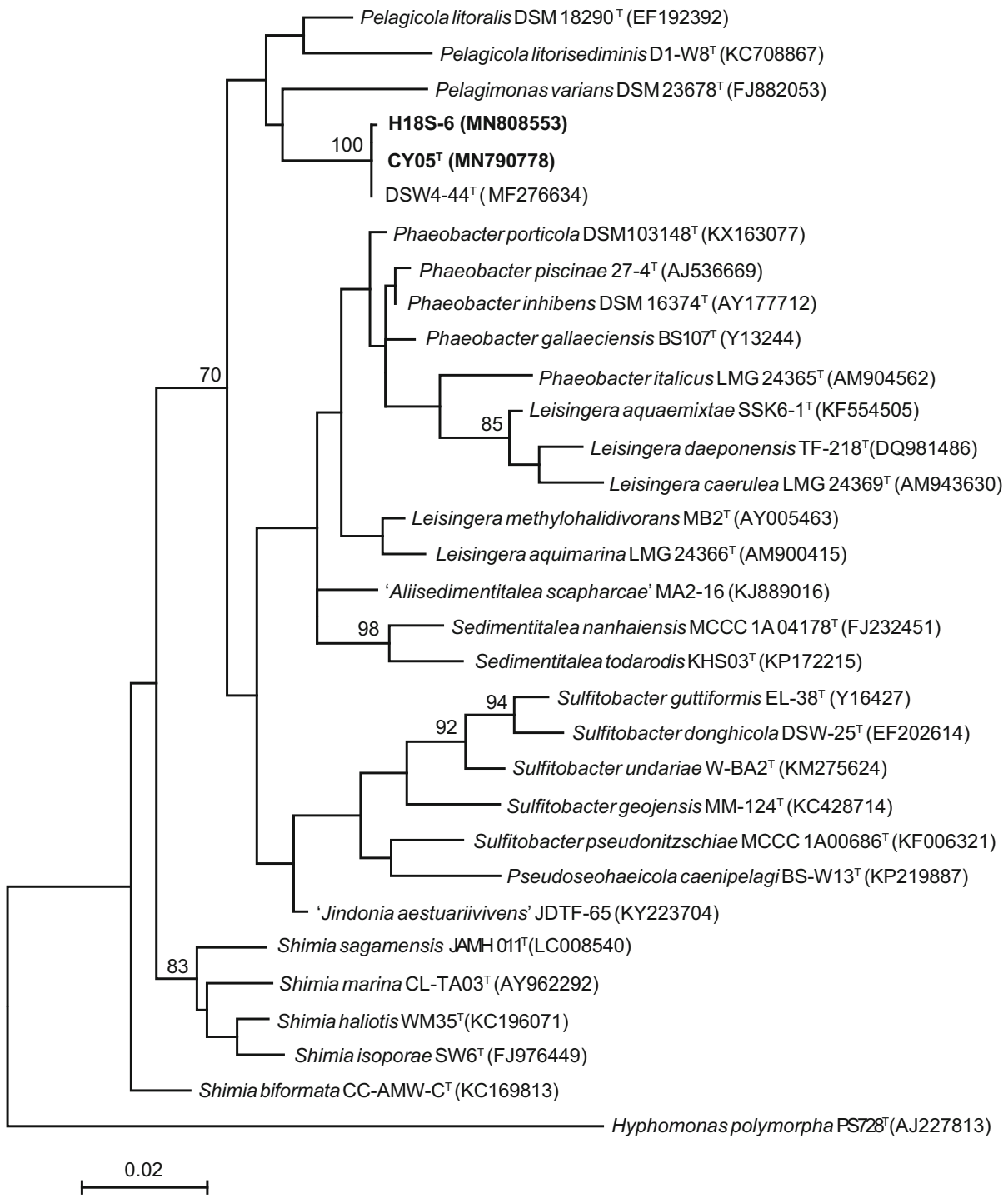

Fig. 1 Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences of strains CY05^T, H18S-6 (in bold) and members of closely related taxa. *Hyphomonas polymorpha* PS728^T was used as an outgroup. Bootstrap values (> 70%) based on 1000 replicates are shown at branch points. Bar, 0.02 substitutions per nucleotide position

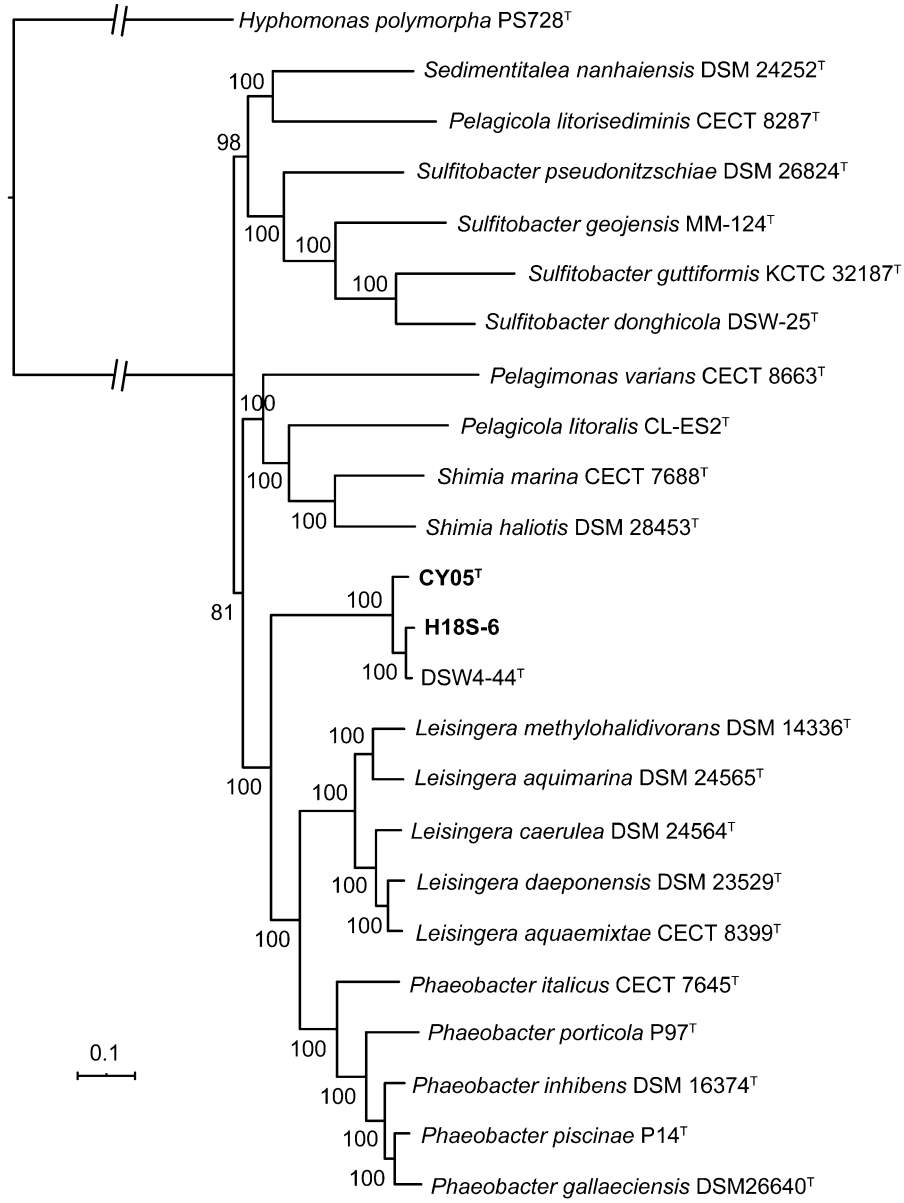

Fig. 2 UBCG maximum-likelihood tree. Tree was generated using PhyML server 3.0 with smart model selection. The numbers at the nodes indicate the bootstrap support after 100 replicates. *Hyphomonas polymorpha* PS728^T was used as an outgroup. Bar, 0.1 substitutions per position

H18S-6, *Pelagicola marinus* DSW4-44^T, *Pelagicola litoralis* DSM 18290^T, *Phaeobacter porticola* DSM 103148^T, *Sulfitobacter pseudonitzschiae* MCCC 1A00686^T, *Sedimentitalea nanhaiensis* MCCC 1A04178^T and *Pelagimonas varians* DSM 23678^T were 94.0%, 94.1%, 69.7%, 72.2%, 69.5%, 70.6% and 68.7%, respectively, and the *is*DDH values between them were 56.5%, 59.8%, 20.0%, 19.5%, 19.6%, 17.7% and 19.7%, respectively. They were all below the threshold value for ANI (95%–96%) and *is*DDH (70%) to discriminate bacterial species (Chun et al. 2018). ANI and *is*DDH percentages indicated that strains CY05^T represents a novel genomic species in the family *Rhodobacteraceae*. The *is*DDH and ANIb values were 76.7% and 96.8% after comparing strains H18S-6 and *Pelagicola marinus* DSW4-44^T.

Table 1 ANIb, ANIm, *is*DDH, AAI and POCP percentages of strains CY05^T and H18S-6 with the closely related type species. Strains: (1) CY05^T; (2) H18S-6; (3) DSW4-44^T; (4) *Pelagicola litoralis* DSM 18290^T; (5) *Phaeobacter porticola* DSM 103148^T; (6) *Sulfitobacter pseudonitzschiae* MCCC 1A00686^T; (7) *Sedimentitalea nanhaiensis* MCCC 1A04178^T; (8) *Pelagimonas varians* DSM 23678^T

	ANIb		ANIm		*is*DDH		AAI		POCP	
	1	2	1	2	1	2	1	2	1	2
1	–	94.0	–	94.4	–	56.5	–	95.8	–	84.2
2	94.0	–	94.4	–	56.5	–	95.8	–	84.2	–
3	94.1	96.8	94.5	97.0	59.8	76.7	96.2	97.9	86.5	86.8
4	69.7	69.5	83.9	83.8	20.0	19.2	66.3	66.5	59.0	57.6
5	72.2	72.2	83.2	83.1	19.5	19.2	72.5	72.4	66.7	65.4
6	69.5	69.5	83.2	83.3	19.6	19.6	64.1	64.0	53.9	53.6
7	70.6	70.5	82.6	82.5	17.7	18.0	67.9	67.5	57.1	56.1
8	68.7	68.9	83.7	83.5	19.7	20.4	62.7	63.0	56.8	56.4

Considering the recommended threshold value for species discrimination (*is*DDH < 70% and ANI < 95%–96%), it is clear that strains H18S-6 and *Pelagicola marinus* DSW4-44^T belong to the same species based on the high values of *is*DDH and ANIb values between them. AAI and POCP percentages were genomic amino acid-level comparisons mainly used for genus delineation. Previously studies showed that the AAI percentages worked perfectly for delimiting genera in the *Roseobacter* group (Wirth and Whitman 2018) by a gradient of AAI percentages defined by two values: a minimum value (80%) below which species should be separated into different genera. Otherwise, the suggested genus boundary of 50% POCP was found to be too conservative and consequently it was inappropriate for delimiting genera in the *Roseobacter* group (Wirth and Whitman 2018) and many other bacterial genera (Aliyu et al. 2016; Li et al. 2017). As shown in Table 1, AAI percentages among CY05^T, H18S-6 and *Pelagicola marinus* DSW4-44^T were 95.8–97.9%, which were significantly higher than the threshold value for AAI (> 80%) to discriminate bacterial genera (Wirth and Whitman 2018). This result indicated that they represented a genus-level taxon in agreement with the result of the 16S rRNA gene phylogeny, phylogenomic tree and the genome comparison, including ANI and *is*DDH values. For the POCP percentages, our results confirmed that 50% POCP alone could not be applied to *Roseobacter* group for genus-level circumscription because all of the calculated POCP percentages were greater than this value. All together, the genome sequence data based on ANI, *is*DDH and AAI values indicated that strains CY05^T, H18S-6 and *Pelagicola marinus* DSW4-44^T represent a novel genus of the family *Rhodobacteraceae*.

Genomic DNA sequencing and analyses

The assembled draft genome of strain CY05^T was 4,856,535 bp in length, with 40 contigs and a N50 value of 277,157 bp. According to annotation by PGAP, A total of 4660 genes were predicted, with 4557 protein-coding genes, 53 RNA genes and 50 pseudogenes. The assembled draft genome of strain H18S-6 was 5,037,133 bp in length, with 292 contigs and a N50 value of 264,929 bp. According to annotation by PGAP, A total of 4934 genes were predicted, with 4808 protein-coding genes, 69 RNA genes and 57 pseudogenes. The calculated genomic DNA G + C content of strains CY05^T and H18S-6 were 54.5 and 54.2%, similar to that of *Pelagicola marinus* DSW4-44^T (54.3%), but relatively lower than those of other type strains in related genera (Table 2).

In accordance with the phenotypic results that strains CY05^T and H18S-6 could reduce nitrate to gaseous forms of nitrogen, the genomes of strains CY05^T and H18S-6 contained a complete set of genes encoding the enzymes involved in denitrification, including nitratereductase, nitrite reductase, nitric oxide reductase and nitrous-oxide reductase (Table. S1). Although the related genes of denitrification of

Table 2 Differential characteristics of strains CY05^T, H18S-6 and the type strains of its phylogenetically closest relatives in the family *Rhodobacteraceae*. Strains: (1) CY05^T; (2) H18S-6; (3) *Pelagicola marinus* DSW4-44^T; (4) *Pelagicola litoralis* DSM 18290^T; (5) *Phaeobacter porticola* DSM 103148^T; (6) *Sulfitobacter pseudonitzschiae* MCCC 1A00686^T; (7) *Sedimentitalea nanhaiensis* MCCC 1A04178^T; (8) *Pelagimonas varians* DSM 23678^T. Symbols: + Positive; − Negative; w Weakly positive

Characteristic	1	2	3	4	5	6	7	8
Cell size (μm):								
Width	0.7–1.8	0.8–1.3	0.3–0.5[a]	0.5–1.4[b]	0.8–1.4[c]	0.6–0.7[d]	0.62–0.8[e]	0.75–3.3[f]
Length	1.6–3.9	1.9–4.5	1.5–6.0[a]	1.1–7.0[b]	1.2–2.5[c]	1.8–2.0[d]	1.6–2.96[e]	1.0–1.4[f]
Motility	−	−	−	−[b]	+[c]	−[d]	+[e]	+[f]
Reduction of nitrate	+	+	+	+	−	−	−	−
Reduction of nitrite	+	+	+	+	−	−	+	−
Denitrification	+	+	+	−	−	−	−	−
Growth at/with:								
5°C	+	+	+	−	+	+	+	+
37 °C	−	−	−	−	−	+	+	−
0.5%(w/v) NaCl	−	−	−	−	+	+	+	+
6% (w/v) NaCl	−	−	+	w	+	+	+	−
Assimilation of:								
D-glucose	+	+	−	w	+	+	w	+
Sucrose	+	+	−	−	+	+	w	w
Cellobiose	−	+	−	–	+	−	w	w
Inulin	+	w	w	w	+	+	w	+
Inositol	−	+	−	−	+	−	w	W
Enzyme activity (API ZYM):								
Cystine arylamidase	−	−	−	−	−	−	+	−
α-galactosidase	−	w	−	−	−	−	−	−
Acid phosphatase	+	+	+	+	w	+	+	+
Esterase lipase (C8)	−	−	w	−	−	+	+	+
API 20NE results								
Urease	−	−	−	−	−	+	−	−
Mannitol	−	−	−	−	+	−	−	−
Gluconate	−	−	−	−	+	−	−	−
Malate	−	−	−	−	+	−	−	−
G + C content (%)	54.2	54.5	54.3[a]	54.8[g]	58.6[c]	61.7[d]	60.5[e]	55.1[f]
Isolation source	marine sediment	marine sediment	deep-sea water[a]	coastal water[b]	harbour[c]	toxic marine diatom[d]	sandy sediment[e]	Seawater[f]

All data are from this study, except for cell size, motility and DNA G + C contents of the reference strains, which are taken from:

[a]Data form Choi et al. (2019)

[b]Data form Kim et al. (2008)

[c]Data form Breider et al. (2017)

[d]Data form Hong et al. (2015)

[e]Data form Sun et al. (2010)

[f]Data form Hahnke et al. (2013)

[g]Data form Hördt et al. (2020)

the two isolates are located in different positions far away from each other, suggesting that these genes may not function simultaneously, strains CY05^T and H18S-6 were positive for nitrate and nitrite reduction and denitrification based on the experimental results. There may be other ways to compensate and the specific reasons need to be further explored. Furthermore, based on the genome annotation and gene sequence comparison, the genome of strain H18S-6 also contained an assimilatory pathway by which this strain could reduce nitrate to ammonia for growth (Table. S2). However, whether or not these genes could function is not clear.

Morphological, physiological and biochemical analysis

Both strains were Gram negative, aerobic, rod-shaped and non-flagellated. Growth of the two strains was found to occur between 4 and 30 °C (optimum 20 °C). They were able to reduce nitrate and nitrite to gaseous forms of nitrogen. They were sensitive to amoxycillin (10 μg), ampicillin (10 μg), bacitracin (10 U), carbenicillin (100 μg), cefotaxime (30 μg), cefoxitin (30 μg), chloramphenicol (30 μg), nitrofurantoin (300 μg), penicillin G (10 U) and rifampicin (5 μg). There were many differences between the two strains. Cells of strains CY05^T and H18S-6 were (0.7–1.8) μm and (0.8–1.3) μm in width, and (1.6–3.9) μm and (1.9–4.5) μm in length, respectively (Fig. S3). Growth occurs in 1.0%–5.0% NaCl (optimum, 2.0%–3.0%) for strain CY05^T and 2.0–5.0% (optimum, 3.0%) for strain H18S-6. The pH for growth was pH 6.5–9.0 (optimum, 7.5–8.0) for CY05^T and pH 7.0–9.0 (optimum, 7.5) for H18S-6. The strain H18S-6 also distinguished from strain CY05^T by the ability to utilise D-cellobiose and inositol as single carbon resource for growth and the presence of α-galactosidase activities. The strains CY05^T differed from those of phylogenetically related taxa by the ability of nitrate reduction and denitrification, flagellum, the assimilation of carbohydrates, the tolerance to sodium chloride and temperatures. (Table 2 and S3). Further data on the morphological, physiological and biochemical characteristics of the isolates were mentioned in Table 2 and the species description.

Chemotaxonomic analysis

The chemotaxonomic results also supported the results of the phylogenetic analysis. Cellular fatty acid comparison of CY05^T, H18S-6 and the type strains of closely related species were shown in Table 3. The predominant cellular fatty acids of strains CY05^T and H18S-6 were summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$) (> 80%), which are characteristics of the family *Rhodobacteraceae*. But the proportions of some fatty acids were distinguishable. $C_{16:0}$ were the significant amounts in strains *Pelagicola litoralis* DSM 18290^T, *Phaeobacter porticola* DSM 103148^T, *Sulfitobacter pseudonitzschiae* MCCC 1A00686^T and *Pelagimonas varians* DSM 23678^T, but were detected at lower levels in strains CY05^T, H18S-6, *Pelagicola marinus* DSW4-44^T and *Sedimentitalea nanhaiensis* MCCC 1A04178^T. Strain *Sedimentitalea nanhaiensis* MCCC 1A04178^T contained a higher amount of $C_{12:0}$ 3OH, but it was not detected in CY05^T, H18S-6, *Pelagicola marinus* DSW4-44^T (Table 3). The distinctiveness of our isolates was also evident in the polar lipid profiles. The polar lipids of strain CY05^T included phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminolipid, three unidentified phospholipids, one unidentified lipid and one unidentified amino phospholipid. The polar lipids of strain H18S-6 included phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, three unidentified phospholipids, two unidentified aminolipids and one unidentified lipid. Unidentified amino phospholipid was not detected in other strains except for strains CY05^T and *Pelagimonas varians* DSM 23678^T. However, more unidentified lipids were detected in *Pelagimonas varians* DSM 23678^T than in strain CY05^T. Two unidentified aminolipids were only detected in strain H18S-6 (Fig. S4). The major respiratory quinone detected in strains CY05^T and H18S-6 was ubiquinone-10, typical in members of the family *Rhodobacteraceae* (Park and Yoon 2013).

In conclusion, strain CY05^T is not closely affiliated with any closely related genera by polyphasic characteristic differences, such as the ability of nitrate reduction, nitrite reduction and denitrification, the tolerance to sodium chloride and temperatures (Table 2). Especially significant differences between the content of polar lipids, fatty acid profiles and DNA G + C contents as described above. Furthermore, the

Table 3 Fatty acid compositions (%) of strains CY05^T, H18S-6 and the type strains of some phylogenetically related species

Fatty acid	1	2	3	4	5	6	7	8
Saturated fatty acids								
$C_{12:0}$	0.3	0.4	0.2	–	0.7	–	–	–
$C_{14:0}$	–	–	–	–	–	0.4	–	–
$C_{16:0}$	1.4	2.3	1.1	9.5	9.1	18.5	2.5	4.5
$C_{17:0}$	–	–	–	0.1	–	–	–	–
$C_{18:0}$	0.6	0.7	0.6	2.3	2.7	3.6	0.3	1.8
Unsaturated fatty acids								
C17:1ω7*c*	–	–	–	–	–	–	0.3	–
C18:1ω9*c*	–	–	0.2	–	–	–	0.2	0.8
11-methyl $C_{18:1}$ ω7*c*	–	–	–	–	5.4	3.5	0.3	–
Hydroxy fatty acids								
$C_{10:0}$ 3OH	3.9	6.8	3.0	4.9	2.2	3.8	5.2	0.5
$C_{12:0}$ 3OH	–	–	–	0.2	2.0	–	2.4	–
$C_{15:0}$ 3OH	–	–	–	–	–	0.4	–	–
$C_{16:0}$ 2OH	4.1	4.6	2.7	2.9	1.1	–	3.7	–
$C_{16:1}$ 2OH	0.3	0.5	0.4	–	–	–	–	–
$C_{12:1}$ 3OH	–	–		–	–	4.3	–	–
$C_{18:1}$ 2OH	1.1	1.4	1.1	0.5	–	–	–	–
Summed feature*								
2	0.2	0.2	0.1	–	–	–	–	–
3	0.2	0.2	0.1	0.5	–	1.3	0.4	0.3
5	–	0.2	–	0.3	–	–	–	0.4
8	88.0	82.6	90.6	78.9	76.8	64.3	84.7	91.8

Strains: (1) CY05^T; (2) H18S-6; (3) *Pelagicola marinus* DSW4-44^T; (4) *Pelagicola litoralis* DSM 18290^T; (5) *Phaeobacter porticola* DSM 103148^T; (6) *Sulfitobacter pseudonitzschiae* MCCC 1A00686^T; (7) *Sedimentitalea nanhaiensis* MCCC 1A04178^T; (8) *Pelagimonas varians* DSM 23678^T. All data are from this study. –, not detected

* Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 2: contains $C_{14:0}$ 3OH and/or iso-$C_{16:1}$ I, Summed feature 3: contains $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω7*c*, Summed feature 5: contains $C_{18:0}$ ante/$C_{18:2}$ ω6, 9*c*, Summed feature 8: contains $C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*

phylogenetic position based on the 16S rRNA gene sequences and the genome sequence also reflected this relationship. And strain CY05^T could also be distinguished from H18S-6 to be recognized as separate species, such as culture conditions, the ability to utilise D-cellobiose and inositolthe, content of polar lipids and fatty acid profiles (Table 2).

Based on the polyphasic data presented in this study, strain CY05^T should be considered to represent a novel species of a novel genus within the family *Rhodobacteraceae*, for which the name *Zongyanglinia huanghaiensis* gen. nov. sp. nov. is proposed. Moreover, the genome sequence data based on ANI, *is*DDH and AAI values indicated that strains H18S-6 and *Pelagicola marinus* DSW4-44^T belong to the same species. Combined with the above mentioned polyphasic data, such as the phylogenetic trees and the genome sequence data based on ANI, *is*DDH and AAI values, strain DSW4-44^T should be transferred to this new genus *Zongyanglinia* as *Zongyanglinia marinus* comb. nov., the type strain is DSW4-44^T (= KCTC 62762^T = KCCM 43261^T = JCM 33637^T).

Description of *Zongyanglinia* gen. nov.

Zongyanglinia (Zong.yang.li'ni.a. N.L. fem. N. *Zongyanglinia* named to honour Chinses microbiologist Zong-Yang Lin).

Cells are Gram-stain negative. The major respiratory quinone is ubiquinone-10 (Q-10). The main polar

lipids included phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, unidentified phospholipid, unidentified aminolipid and lipid. The predominant cellular fatty acids are summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$). The DNA G + C content of the genomic DNA are 54.2%–54.3%. Phylogenetically, the genus *Zongyanglinia* is affiliated to the family *Rhodobacteraceae* of the class *Alphaproteobacteria*. The type species is *Zongyanglinia huanghaiensis* CY05^T (= MCCC 1K04409^T = KCTC 62200^T).

Description of *Zongyanglinia huanghaiensis* sp. nov.

Zongyanglinia huanghaiensis (hu.ang.hai.en'sis. N.L. masc. adj. *huanghaiensis* pertaining to Huanghai, the Chinese name for the Yellow Sea, the geographical origin of the type strain).

Strain exhibits the following properties in addition to those given in the genus description. Cells are aerobic, rods or ovoid-shaped rods and approximately 0.7 μm –1.8 μm in width and 1.6 μm –3.9 μm in length. Colonies are circular with regular edges, opaque, slightly convex and approximately 0.2 mm–1.1 mm in diameter on MA agar after 7 days incubation at 20 °C. Growth occurs at 5 °C–30 °C (optimum, 20 °C) and in pH range 6.5–9 (optimum, 7.5–8.0). Grows with 1.0%–5.0% NaCl (optimum, 2.0%–3.0%), does not grow without NaCl. Positive for catalase, oxidase, nitrate and nitrite reduction and denitrification, but could not hydrolysis of casein, starch, Tween 40, Tween 60 and Tween 80. The following substances are utilised as single carbon resource for growth: D-glucose, sucrose, inulin, D-raffinose pentahydrate, D-galactose, D-mannose, D-maltose monohydrate and xylan (weakly), but not D-cellobiose, inositol, D-xylose, xylitol, D-fructose, D-mannitol, sorbitolum, L-sorbose, L-arabinose and D-trehalose. In the API ZYM strips, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-B1-phosphophydrolase, but negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. In API 20NE tests, positive for nitrate reduction, but negative for indole production, acid production from glucose, arginine dihydrolase, urease, β-glucosidase, gelatinase and beta-galactosidase, or assimilattion of glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate and phenyl-acetate.

The type strain is CY05^T (= MCCC 1K04409^T = KCTC 62200^T), isolated from sediment of the Yellow Sea. The G + C content of the genome is 54.2%, its approximate genome size is 4.86 Mbp. The GenBank accession numbers for the 16S rRNA gene sequence and the draft genome sequence of the type strain are MN790778 and WQLV00000000, respectively.

Description of *Zongyanglinia marinus* comb. nov.

Zongyanglinia marinus (ma.ri′nus. L. masc. adj. marinus of the sea, marine).

Basonym: *Pelagicola marinus* Choi et al. 2019.

The description is the same as that given by Choi et al. (2019) with the following modification. Positive for nitrite reduction and denitrification, could grow at 5°C, weakly utilize inulin as single carbon resource for growth. The type strain is DSW4-44^T (= KCTC 62762^T = KCCM 43261^T = JCM 33637^T).

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflict of interest.

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