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ORIGINAL PAPER

Shumkonia mesophila gen. nov., sp. nov., a novel representative of *Shumkoniaceae* fam. nov. and its potentials for extracellular polymeric substances formation and sulfur metabolism revealed by genomic analysis

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Abstract A microaerophilic, mesophilic, chemoorganoheterotrophic bacterium, designated Y-P2^T, was isolated from oil sludge enrichment in China. Cells of the strain were Gram-stain-negative, non-motile, non-spore-forming, rod-shaped or slightly curved with 0.8–3.0 μm in length and 0.4–0.6 μm in diameter. The strain Y-P2^T grew optimally at 25 °C (range from 15 to 30 °C) and pH 7.0 (range from pH 6.0 to 7.5) without NaCl. The major cellular fatty acids were $C_{16:0}$, 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*). The main polar liquids of strain Y-P2^T comprised phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). The respiratory quinone was Q-10. Acetate and H_2 were the fermentation products of glucose. The DNA G+C content was 66.0%. Strain Y-P2^T shared the highest 16S rRNA gene sequence similarity (90.3–90.6%) with species within *Oceanibaculum* of family *Thalassobaculaceae* in *Rhodospirillales*. Phylogenetic analyses based on 16S rRNA gene sequences and genomes showed that strain Y-P2^T formed a distinct evolutionary lineage within the order *Rhodospirillales*. On the basis of phenotypic, phylogenetic and phylogenomic data, we propose that strain Y-P2^T represents a novel species in a novel genus, for which *Shumkonia mesophila* gen. nov., sp. nov., within a new family *Shumkoniaceae* fam. nov. The type strain is Y-P2^T (=CCAM 826 ^{T}=JCM 34766 ^{T}).

Keywords Microaerophilic · Chemoorganoheterotrophic · *Shumkonia mesophila* sp. nov. · *Shumkoniaceae* fam. nov

Abbreviations

AAI	Average amino acid identity
ANI	Average nucleotide identity
ANI	Average nucleotide identity
AL	Aminolipid
CHES	N-cyclohexyl-2-aminoethanesulfonic acid
GL	Glycolipids
L	Lipids
MES	2-(N-morpholino) ethanesulfonic acid
PE	Phosphatidylethanolamine

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PG Phosphatidylglycerol
PL Phospholipid
POCP Percentage of conserved proteins
R2A Reasoner's 2A

Introduction

The order *Rhodospirillales*, a member of the class *Alphaproteobacteria* belonging to the phylum *Pseudomonadota*, was first proposed by Pfennig and Trüper (Pfennig and Trüper 1971) to replace the name *Athiorhodaceae* (Molisch 1907), with *Rhodospirillum* as the type genus (Molisch 1907). It originally comprised three families, named *Chlorobiaceae*, *Chromatiaceae*, and *Rhodospirillaceae*, there are 12 families with validly published names (https://lpsn.dsmz.de/order/rhodospirillales): *Acetobacteraceae* (Gillis and De Ley 1980), *Azospirillaceae* (Hördt et al. 2020), Geminicoccaceae (Proença et al. 2018), *Kiloniellaceae* (Wiese et al. 2009), *Reyranellaceae* (Hördt et al. 2020), *Rhodospirillaceae* (Pfennig and Trüper 1971), *Rhodovibrionaceae*, *Stellaceae*, *Terasakiellaceae*, *Thalassobaculaceae*, *Thalassospiraceae* and *Zavarziniaceae* (Hördt et al. 2020). In 2023, Koziaeva et al. (2023) suggested that the order *Rhodospirillales* should be split into six more new family-level groups according to phylogenomic analyses, including "*Magnetospiraceae*" and "*Magnetovibrionaceae*" separated from the family *Thalassospiraceae*, "*Dongiaceae*" and "*Niveispirillaceae*" separated from the family *Rhodospirillaceae*, "*Fodinicurvataceae*" from the family *Rhodovibrionaceae* and "*Oceanibaculaceae*" from the family *Thalassobaculaceae*. *Rhodospirillales* members are widely distributed in freshwater, soil, seawater, plant root and artificial ecosystems. Most members of this order are Gram-stain-negative, spiral or rod-shaped, non-spore-forming, and obligately aerobic or facultatively anaerobic bacteria with ubiquinone Q-10 as the common major respiratory quinone. The order *Rhodospirillales* is metabolically diverse group, containing chemoorganoheterotrophs, and photoorganoheterotrophs under anoxic conditions in the light (Hördt et al. 2020)

In recent years, it has revealed that *Rhodospirillales* members present in the oil reservoir related environments, such as oil-contaminated soil and polluted water (Liu et al. 2015; Abbasian et al. 2016; Elumalai et al. 2021). However, just a few of members belonging to *Rhodospirillale* have the ability to use alkane as energy source (Wu et al. 2021b). The knowledge about ecological roles of *Rhodospirillales* in oil reservoir is still limited. Elumalai et al. found *Rhodospirillales* appears in the biofilm on corroded API 5LX carbon steel in produced water of oil reservoir (Elumalai et al. 2021), implying *Rhodospirillales* probably related with microbially induced corrosion (MIC). *Rhodospirillales* members including *Rhodospirillaceae* and *Acetobacteraceae* were abundant bacteria in the biofilm on corroded steel coupons and Biodiesel Storage Tanks (Procópio 2020; Stamps et al. 2020), populations of these bacteria were accompanied by a continuous corrosion process over the coupons (López et al. 2002; Moura et al. 2018). Corrosion conducted by microorganisms usually influenced through biofilm formation, sulfur metabolism, corrosive metabolites production (such as inorganic or organic acids, and hydrogen sulfide) (Lv and Du 2018; Moura et al. 2018; Elumalai et al. 2021). Several studies have speculated that *Rhodospirillales* members participate in MIC by producing acetic acid and biofilms, as well as reducing iron (Chen et al. 2019; Chen and Zhang 2019; Procópio 2020; Stamps et al. 2020). Therefore, *Rhodospirillales* probably link to the corrosion of oil pipelines and storage tanks, suggesting the importance of isolation *Rhodospirillales* for investigating their ecological functions in oil reservoir environments. Several species have been isolated from the oil production mixture, oil-contaminated soil or oil reservoir water, such as *Roseomonas oleicola* (Wu et al. 2021a) and *Siccirubricoccus phaeus* (Li et al. 2021) of the family *Acetobacteraceae*, *Azospirillum oleiclasticum* (Wu et al. 2021b) and *Azospirillum rugosum* (Young et al. 2008) of the family *Azospirillaceae*, *Oleisolibacter albus* (Ruan et al. 2019) and *Oleiliquidispirillum nitrogeniifigens* (Li et al. 2020) of the family *Rhodospirillaceae*. All these isolates are mesophilic, aerobic or facultatively anaerobic chemoorganoheterotrophs.

In the present study, one strain, designated Y-P2^T, was isolated from the oil sludge collected from Shengli oilfield, China. The polyphasic taxonomic analyses revealed strain Y-P2^T represents a novel genus in a new family within the order *Rhodospirillales*.

Materials and methods

Enrichment and isolation

Strain Y-P2^T was obtained from the oil sludge of the Shengli oilfield in PR China (37°54'N, 118°33'E). Approximately 10 g of mixture of oil contaminated soil and oily sludge was inoculated into 50 mL of fresh medium for preparation of pre-enrichment culture, which performed as 25 °C. The pre-reduced mineral medium used for enrichment and isolation was prepared with the following components (L^{-1}): 0.5 g NaCl, 0.5 g $MgCl_2{\cdot}6H_2O$, 0.1 g $CaCl_2{\cdot}2H_2O$, 0.3 g NH_4Cl, 0.2 g KH_2PO_4, 0.5 g KCl, 2 ml trace element solution 284 (JCM medium No.284, https://www.jcm.riken.jp/cgi-bin/jcm/jcm_grmd?GRMD=284&MD_NAME=), 1 mg resazurin, 0.5 g cysteine hydrochloride and 1 L distilled water. The strain Y-P2^T was isolated with mixed substrate (short-chain fatty acid, glucose, yeast extract and tryptone mixture) by using the extinction dilution method as described previously (Zhang et al. 2018). Unless otherwise stated, R2A (Reasoner's 2A) liquid medium was used for subculturing and cultivating the strain Y-P2^T. R2A medium contained (L^{-1}): 0.25 g tryptone, 0.5 g casein hydrolysate, 0.5 g yeast extract, 0.5 g soluble starch, 0.3 g K_2HPO_4, 0.1 g $MgSO_4$, 0.3 g sodium pyruvate, 0.25 g peptone, 0.5 g glucose, and 1L distilled water. All media used in this study were prepared and dispensed under 100% N_2, and sterilized at 121 °C for 15 min. Strain Y-P2^T was deposited in the China Collection of Anaerobic Microorganisms (CCAM 826 ^{T}) and Japan Collection of Microorganisms (JCM 34766 ^{T}). *Oceanibaculum nanhaiense* KCTC 52312 ^{T} obtained from Korean Collection for Type Cultures (KCTC) was used as the reference strain.

Morphological, physiological, and biochemical tests

The strain Y-P2^T incubated at 25 °C for 5 days was used for morphology tests. Gram-staining, flagellum-staining and spore-staining were determined using commercial Gram Staining Kit, Flagellum Staining Kit and Spore Staining Kit (Solarbio, China) according to the manufacturers' instructions, respectively. The cell shape and size of strain Y-P2^T were examined using a scanning electron microscope (JEM-1400 Plus, JEOL, Japan). 0.1% melted agarose gel spread flat on slide and solidified, then take a drop of strain Y-P2^T by microscope (Nikon 80i, Japan) for observing motility.

Growth at different temperatures (15, 20, 25, 30 and 37 °C), pH (5.0–8.5, at 0.5-unit intervals), and salinities (0 – 70 g L^{-1}, at 10 g L^{-1} intervals) were determined in the R2A medium supplemented 10% (v/v) oxygen in the headspace of Hungate tubes (25 ml). The pH values were adjusted using the sterile and HCl or NaOH solution and were buffered with 20 mM MES (pH 5.5, pH 6.0 and pH 6.5), 20 mM PIPES (pH 7.0 and pH 7.5) and 20 mM Tris (pH 8.0 and pH 8.5).The final pH was determined with a pH meter (HORIBA B-712, LAQUAtwin, Japan). Growth was determined by measuring the optical density (OD) at 600 nm using a spectrophotometer (DU730, Beckman, Germany).

For biochemical tests, cells in R2A medium were collected by centrifuging at 13,000 rpm for 5 min at room temperature. Substrate utilization, nitrate reduction, and H_2S production were tested with API 20NE, API ZYM and API 20E (bioMérieux, France) and incubated aerobically at 28 °C and 37 °C, respectively, the color change was checked every 18–24 h. Oxidase activity was determined using an oxidase reagent kit (bioMérieux, France) according to the manufacturer's instructions. Oxygen requirement of strain Y-P2^T was tested under 0%, 2%,10% and 20% oxygen (O_2:N_2, v/v). The fermentation products were analysed by liquid chromatography (Aglient 1200, USA) using AminexHPX-87H column (300 mm × 7.8 mm, 9 μm), H_2SO_4 (5 mM) was used

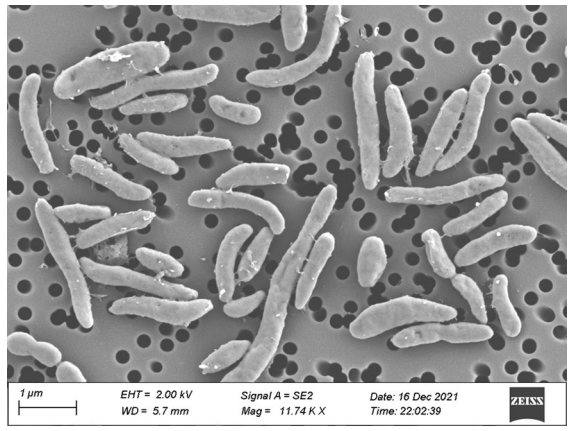


Fig. 1 Scanning electron micrograph of strain Y-P2^T. The scale bar is 1 μm

Table 1 Phenotypic traits of strain Y-P2^T and phylogenetically close genera belonging to the family *Thalassobaculaceae*, *Stellaceae* and *Geminicoccaceae*

Characteristic	1	2	3	4	5	6	7
Habitat	Oil sludge	Surface seawater	Deep seawater/ Hydrothermal field sediment	Coastal, surface waters	Deep seawater/ Coastal seawater	Soil	sludge
Oxygen requirement	Microaerophilic	ND	ND	ND	Strictly aerobic, Facultatively anaerobic	Aerobic	Aerobic
Gram reaction	–	–	–	–	–	–	–
Morphology	rods	rods	rods	rods	slightly curved rod/ straight rod	flat, six–pronged stars	coccus/coccobacillus
Motility	–	+	+	+	–/+	–	–
Spore–forming	–	–	ND	ND	ND	–	–
Cells (μm)	0.4–0.6 × 0.8–3.0	0.8–1.0 × 1.5–2.5	1.7–2.5 × 0.5–1.5	2.5 ± 0.6 × 0.9 ± 0.2	0.3–1.2 × 2.0–8.0	ND	1·5–4·0
Temperature (optimum, °C)	15–30 (25–30)	10–45 (25–37)	10–45 (25–37)	15–44 (30)	20–40 (30–35)	28–30	20–30 (25–30)
pH (optimum)	7.1	6–10 (8–9)	6–11 (7–9)	5.0–9.0 (6.0)	5.0–10.0 (6.5–8.0)	near neutral	5.0–8.5 (7·5–8·0)
NaCl (optimum, g/L)	0–100 (0)	0–90 (20)	0–90 (5–70)	0–60 (20)	0–100 (20–40)	10	ND
DNA G + C content (mol%)	66.0	65.1	64.8–67.7	60.5–60.6	65.0–69.0	69.8	66
Utilization of sugars	–	–	+	+	+	–	+
Citrate utilization	–	+	+/ ND	ND	ND	+	–
Gelatinase	–	+	+/–	ND	+	ND	ND
Reduction of nitrate	–	+	+/ variable	ND	+/–	ND	+(weak)
Denitrification	–	+	+/–	ND	ND	ND	ND
Urease	+	–	–/+	ND	+	ND	+
Gelatin hydrolysis	–	weak	weak /–	ND	+	ND	+(weak)
Oxidase	+	+	+/–	+	+	+	–
Catalase	–	+	+/+	+	+	+	+
Fatty acid	$C_{16:0}$, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$	summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), $C_{16:0}$ and $C_{18:1}$ 2–OH	$C_{16:0}$, $C_{18:1}\omega7c$, $C_{18:1}$ 2–OH and $C_{19:0}\omega8c$ cyclo	$C_{18:1}\omega7c$, $C_{16:1}\omega7c$ and $C_{16:0}$	$C_{18:1}\omega7c$, $C_{16:0}$	ND	ND
Quinone	Q–10	Q–10	Q–10	Q–10	Q–10	ND	ND

Table 1 (continued)

Characteristic	1	2	3	4	5	6	7
Polar lipid	AL, PE, PG, PL, L	DPG, PE, PME, PG, PL, L	DPG, PE, PME, PG, PL, L	PG, APL, PL	PG, APL, PL, AL, L	ND	ND

1. *Shumkonia mesophila* Y-P2^T, in this study; 2. *Oceanibaculum nanhaiense* KCTC 52312^T (Du et al. 2017); 3. *Oceanibaculum* (Du, et al. 2017, Dong et al., 2010, Lai et al., 2009a, b); 4. *Nisaea* (Urios et al. 2008); 5. *Thalassobaculum* (Su et al., 2016, Zhang et al. 2008, Urios et al., 2008); 6. *Stella* (Vasilyeva 1985); 7 *Defluviicoccus* (Maszenan et al. 2005).+, positive; −, negative; ND, no data available. A, Aerobic; MA, microaerobic; FA, facultatively anaerobic

as the mobile phase at a flow rate of 0.6 ml min^{-1}. H_2 in the overhead of the tubes were analysed by gas chromatography (Shimadzu GC2010, Japan).

Chemotaxonomic analysis

For cellular fatty acids, polar lipids and respiratory quinones analyses, cells of strains Y-P2^T and *O. nanhaiense* KCTC 52312^T incubated in R2A medium at 25 °C for 5 days were collected by centrifugation. The fatty acids were separated using gas chromatography (Aglient 8860, USA) and identified with Sherlock software (version 6.3) according to the instructions of Microbial Identification Inc. (MIDI) protocol (Sasser 1990). Respiratory quinones of strain Y-P2^T were detected using the protocol described previously (Komagata and Suzuki 1988; Tindall 1990). Polar lipids were extracted using a chloroform / methanol system and were analysed using one- and two-dimensional thin-layer chromatography (TLC) following the method described by Kates et al. (1986).

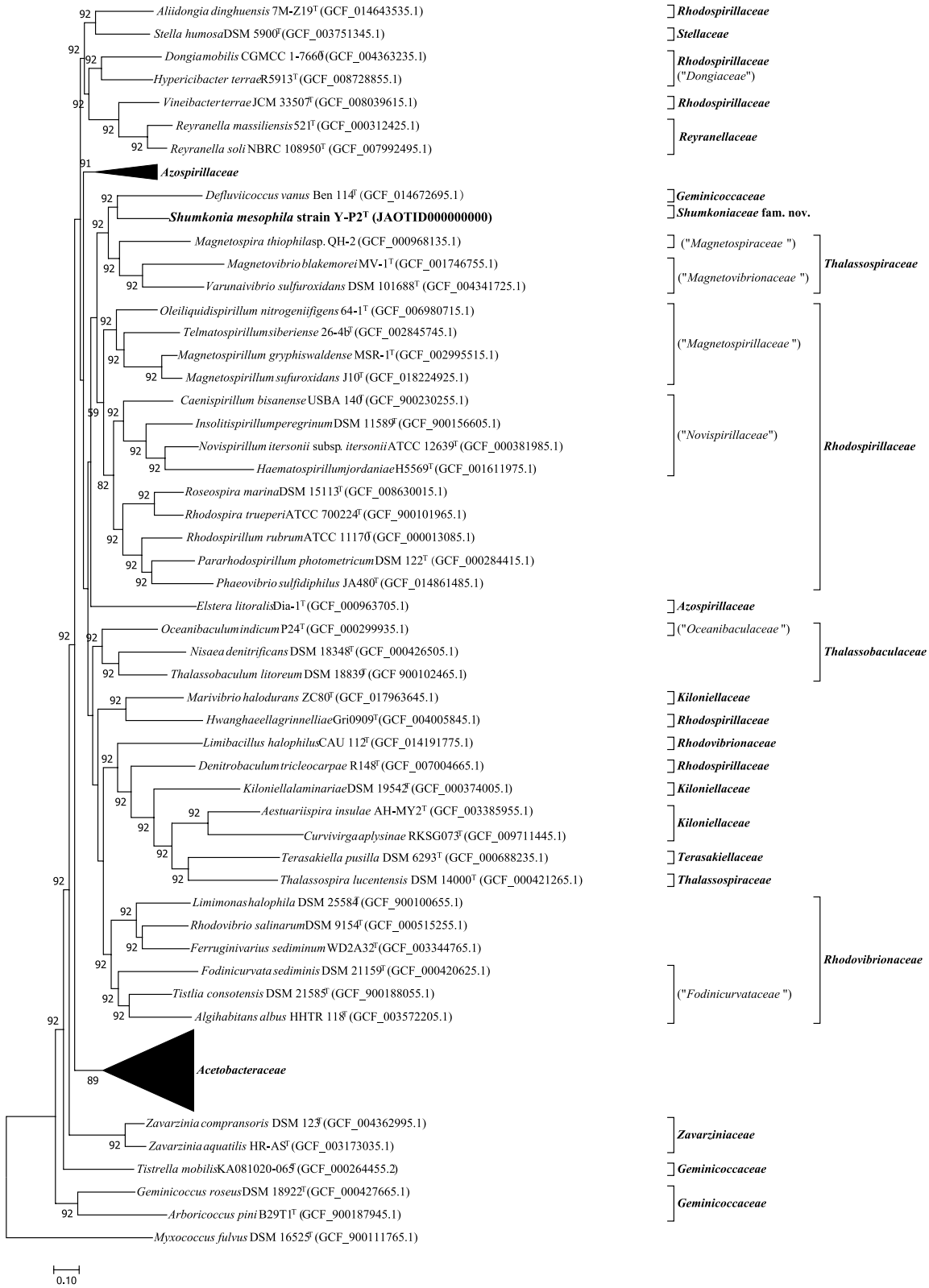
16S rRNA gene sequencing and phylogenetic analysis

Cells incubated at 25 °C for 5 d was used for extracting genomic DNA using bacteria DNA extraction kit (DP302, TIANGEN, China) following the manufacture's instruction. 16S rRNA gene fragments were amplified by PCR using the universal primers 27F (5′AGAGTTTGATCMTGGCTCAG3′) and 1492R (5′TACGGYTACCTTGTTACGACTT3′) and were sequenced by ABI 3730XL DNA anazlyzer. The sequence obtained was compared with the available 16S rRNA sequences in EzBioCloud database (https://www.ezbiocloud.net/identify) and NCBI database with nucleotide Basic Local Alignment Search Tool (blastn, https://blast.ncbi.nlm.nih.gov/Blast.cgi), respectively. 16S rRNA gene sequences of closely related species that validly published were derived from genomes from NCBI database or downloaded from EzBioCloud, and then were multiply aligned through MUSCLE v3 (https://drive5.com/muscle/downloads_v3.htm). The phylogenetic analyses were performed by fasttree and MEGA X using the neighbor-joining method and maximum-likelihood method (Felsenstein 1981), respectively. Bootstrap values were calculated based on 1000 replicates. Evolutionary distances were calculated using the Kimura two-parameter method (Kimura 1980). The phylogenetic tree was modified by Itol (https://itol.embl.de/).

Whole–genome sequencing and phylogenomic analysis

The whole genome of strain Y-P2^T was sequenced by Novogene Corporation Inc. (Beijing, China) using NovaSeq 6000 System (Illumina, USA). Phylogenomic analysis based on the GTDB taxonomy database (release r95) was performed by using the method described previously (Parks et al. 2018). Genome assembly and annotation were performed using methods described previously (Fan et al. 2023).

Up-to-date bacterial core gene (UBCG) and GTDB phylogenomic trees were reconstructed by UBCG (https://www.ezbiocloud.net/tools) and GTDB-Tk (https://gtdb.ecogenomic.org/), respectively, and were optimized in Evolview website (https://www.evolgenius.info/evolview/#/treeview). The pairwise genomic average nucleotide identity (ANI) and average amino acid identity (AAI) were calculated using OrthoANIu (https://www.ezbiocloud.net/tools/orthoaniu) and Compare M (https://github.com/dparks1134/CompareM), respectively. Percentage of conserved proteins (POCP) between two microbial genomes was calculated as the following formula: (conserved protein number of genome A + conserved protein number of genome B) / (total number of proteins being compared in genome A + total number


Aliidongia dinghuensis 7M-Z19T (GCF_014643535.1)
Stella humosa DSM 5900T (GCF_003751345.1)
Dongia mobilis CGMCC 1-7660T (GCF_004363235.1)
Hypericibacter terrae R5913T (GCF_008728855.1)
Vineibacter terrae JCM 33507T (GCF_008039615.1)
Reyranella massiliensis 521T (GCF_000312425.1)
Reyranella soli NBRC 108950T (GCF_007992495.1)
Azospirillaceae
Defluviicoccus vanus Ben 114T (GCF_014672695.1)
Shumkonia mesophila strain Y-P2T (JAOTID000000000)
Magnetospira thiophila sp. QH-2 (GCF_000968135.1)
Magnetovibrio blakemorei MV-1T (GCF_001746755.1)
Varunaivibrio sulfuroxidans DSM 101688T (GCF_004341725.1)
Oleiliquidispirillum nitrogeniifigens 64-1T (GCF_006980715.1)
Telmatospirillum siberiense 26-4bT (GCF_002845745.1)
Magnetospirillum gryphiswaldense MSR-1T (GCF_002995515.1)
Magnetospirillum sufuroxidans J10T (GCF_018224925.1)
Caenispirillum bisanense USBA 140T (GCF_900230255.1)
Insolitispirillum peregrinum DSM 11589T (GCF_900156605.1)
Novispirillum itersonii subsp. itersonii ATCC 12639T (GCF_000381985.1)
Haematospirillum jordaniae H5569T (GCF_001611975.1)
Roseospira marina DSM 15113T (GCF_008630015.1)
Rhodospira trueperi ATCC 700224T (GCF_900101965.1)
Rhodospirillum rubrum ATCC 11170T (GCF_000013085.1)
Pararhodospirillum photometricum DSM 122T (GCF_000284415.1)
Phaeovibrio sulfidiphilus JA480T (GCF_014861485.1)
Elstera litoralis Dia-1T (GCF_000963705.1)
Oceanibaculum indicum P24T (GCF_000299935.1)
Nisaea denitrificans DSM 18348T (GCF_000426505.1)
Thalassobaculum litoreum DSM 18839T (GCF 900102465.1)
Marivibrio halodurans ZC80T (GCF_017963645.1)
Hwanghaeellagrinnelliae Gri0909T (GCF_004005845.1)
Limibacillus halophilus CAU 112T (GCF_014191775.1)
Denitrobaculum tricleocarpae R148T (GCF_007004665.1)
Kiloniellalaminariae DSM 19542T (GCF_000374005.1)
Aestuariispira insulae AH-MY2T (GCF_003385955.1)
Curvivirga aplysinae RKSG073T (GCF_009711445.1)
Terasakiella pusilla DSM 6293T (GCF_000688235.1)
Thalassospira lucentensis DSM 14000T (GCF_000421265.1)
Limimonas halophila DSM 25584T (GCF_900100655.1)
Rhodovibrio salinarum DSM 9154T (GCF_000515255.1)
Ferruginivarius sediminum WD2A32T (GCF_003344765.1)
Fodinicurvata sediminis DSM 21159T (GCF_000420625.1)
Tistlia consotensis DSM 21585T (GCF_900188055.1)
Algihabitans albus HHTR 118T (GCF_003572205.1)
Acetobacteraceae
Zavarzinia compransoris DSM 123T (GCF_004362995.1)
Zavarzinia aquatilis HR-AST (GCF_003173035.1)
Tistrella mobilis KA081020-065T (GCF_000264455.2)
Geminicoccus roseus DSM 18922T (GCF_000427665.1)
Arboricoccus pini B29T1T (GCF_900187945.1)
Myxococcus fulvus DSM 16525T (GCF_900111765.1)
0.10
Rhodospirillaceae
Stellaceae
Rhodospirillaceae ("Dongiaceae")
Rhodospirillaceae
Reyranellaceae
Geminicoccaceae
Shumkoniaceae fam. nov.
("Magnetospiraceae")
("Magnetovibrionaceae")
Thalassospiraceae
("Magnetospirillaceae")
("Novispirillaceae")
Rhodospirillaceae
Azospirillaceae
("Oceanibaculaceae")
Thalassobaculaceae
Kiloniellaceae
Rhodospirillaceae
Rhodovibrionaceae
Rhodospirillaceae
Kiloniellaceae
Kiloniellaceae
Terasakiellaceae
Thalassospiraceae
("Fodinicurvataceae")
Rhodovibrionaceae
Zavarziniaceae
Geminicoccaceae
Geminicoccaceae

◂**Fig. 2** Phylogenomic tree reconstructed based on up-to-date bacterial core gene set (UBCG, concatenated alignment of 92 core genes) of representative species of genera in the order *Rhodospirillales*. Bar, 0.1 substitutions per position. Gene support indices (GSIs) are given at branching points. Latin names with quotes have not been validly published

of proteins being compared in genome B) (Qin et al. 2014).

Results and discussion

Morphological, physiological, and biochemical characteristics

Cells of strain Y-P2^T were Gram-stain-negative, non-spore-forming, straightly rod-shaped or slightly curved with 0.8 – 3.0 × 0.4 – 0.6 μm in length and in width (Fig. 1). Growth occurred at 15–30 °C (optimum at 25 °C) and pH 6.0–7.5 (optimum pH 7.0) in absence of NaCl (Fig. S1). Weak growth was observed under anaerobic conditions, but the optimum growth occurred in the presence of oxygen up to approx. 10% (v/v), no growth was observed when oxygen increase to 20% (v/v) (Fig. S2), indicating that strain Y-P2^T was microaerophilic.

According to API 20NE and API 20E tests (Table 1), strain Y-P2^T was positive for urease and gelatinase. The results of API 20NE tests revealed that strain Y-P2^T was negative for reduction of nitrate or denitrification, was positive for oxidase activity, but negative for catalase activity, Strain Y-P2^T was unable to reduce thiosulfate or ferment tested carbohydrates. In API ZYM test, activities of alkaline phosphatase, esterase (C 4), estase lipase (C 8), acid phosphatase and Naphtol-AS-BI-phosphohydrolase were positive. Acetate and H_2 were produced from glucose.

The GenBank accession numbers of the 16S rRNA gene sequence for strain Y-P2^T was MZ270534. The GenBank accession number of genome sequence for strain Y-P2^T was JAOTID000000000.

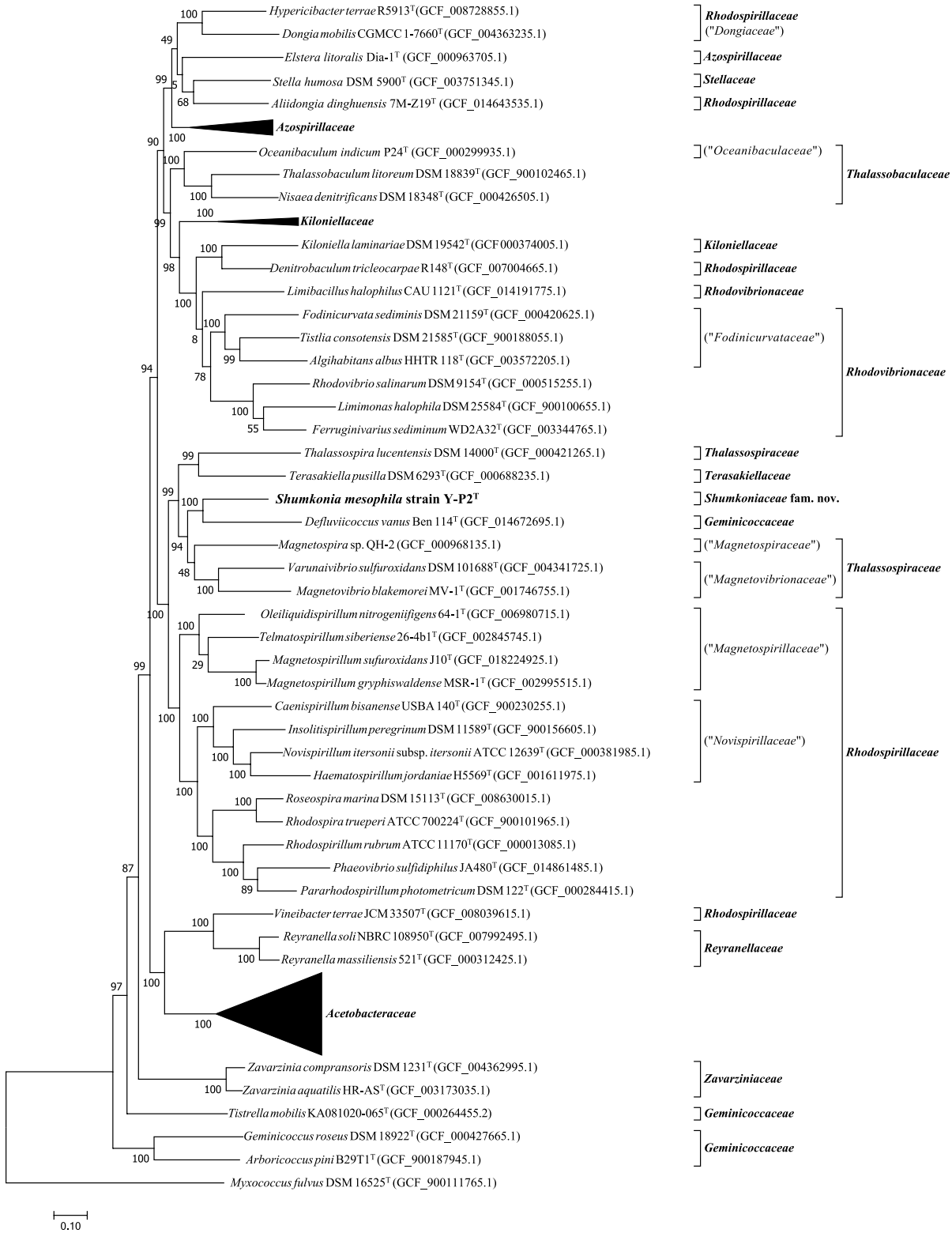
Chemotaxonomic characteristics

The predominant fatty acids (> 10%) of strain Y-P2^T were $C_{16:0}$ (34.6%), summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*, 23.1%), and summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*,17.3%), which accounted for 75.0% of the total fatty acids (Table S1). Differently, *O. nanhaiense* KCTC 52312^T contained sum in feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$, 50.0%) and $C_{16:0}$ (12.6%). The polar liquids of strain Y-P2^T comprised one unidentified aminolipid (AL), one phosphatidylethanolamine (PE), one phosphatidylglycerol (PG), three unidentified phospholipids (PLs) and four unidentified polar lipids (Ls) (Fig. S3). The respiratory quinone was Q-10.

Phylogenetic analyses

The complete 16S rRNA gene sequence analysis performed using blastn at NCBI showed that strain Y-P2^T shares the highest 16S rRNA gene sequence similarity with species in the genus *Oceanibaculum* (90.3–90.6%) which was affiliated to "*Oceanibaculaceae*" (formerly *Thalassobaculaceae*) within the order *Rhodospirillales*, followed by *Stella humosa* DSM 5900^T (90.0%) in family *Stellaceae*, *Thalassobaculum* and *Nisaea* (≤ 89.9%) in *Thalassobaculaceae*, *Varunaivibrio* and *Magnetovibrio* (≤ 89.8%) in "*Magnetovibrionaceae*" (formerly *Thalassospiraceae*), *Zavarzinia compransoris* LMG-5821^T (89.1%) in *Zavarziniaceae* (Table S2). These 16S rRNA gene sequence similarities were lower than the 94.5% threshold for the delineation of genera and the median sequence identity for the delineation of families (Yarza et al. 2014), suggesting that strain Y-P2^T belongs to a new genus or may represent a higher rank taxon.

To confirm the phylogenetic relationship between strain Y-P2^T and *Rhodospirillales* members, maximum-likelihood phylogenetic trees based on 16S rRNA gene sequences of strain Y-P2^T and the representative genera within order *Rhodospirillales* were constructed. On the phylogenetic tree, strain Y-P2^T was placed in the clade with genera *Zavarzinia* of the family *Zavarziniaceae*, but formed an independent evolutionary lineage that is distinguishable among *Rhodospirillales* families (Fig. S4). The phylogenomic trees reconstructed based on bacterial core gene set (Fig. 2) and 120 single copy genes (Fig. 3) both indicated that strain YP2^T clusters with members of "*Magnetospiraceae*" (*Magnetospira*), "*Magnetovibrionaceae*" (*Magnetovibrio*, *Varunaivibrio*), and *Geminicoccaceae* (*Defluviicoccus*),


Hypericibacter terrae R5913T (GCF_008728855.1)
Dongia mobilis CGMCC 1-7660T (GCF_004363235.1)
Elstera litoralis Dia-1T (GCF_000963705.1)
Stella humosa DSM 5900T (GCF_003751345.1)
Aliidongia dinghuensis 7M-Z19T (GCF_014643535.1)
Azospirillaceae
Oceanibaculum indicum P24T (GCF_000299935.1)
Thalassobaculum litoreum DSM 18839T (GCF_900102465.1)
Nisaea denitrificans DSM 18348T (GCF_000426505.1)
Kiloniellaceae
Kiloniella laminariae DSM 19542T (GCF 000374005.1)
Denitrobaculum tricleocarpae R148T (GCF_007004665.1)
Limibacillus halophilus CAU 1121T (GCF_014191775.1)
Fodinicurvata sediminis DSM 21159T (GCF_000420625.1)
Tistlia consotensis DSM 21585T (GCF_900188055.1)
Algihabitans albus HHTR 118T (GCF_003572205.1)
Rhodovibrio salinarum DSM 9154T (GCF_000515255.1)
Limimonas halophila DSM 25584T (GCF_900100655.1)
Ferruginivarius sediminum WD2A32T (GCF_003344765.1)
Thalassospira lucentensis DSM 14000T (GCF_000421265.1)
Terasakiella pusilla DSM 6293T (GCF_000688235.1)
Shumkonia mesophila strain Y-P2T
Defluviicoccus vanus Ben 114T (GCF_014672695.1)
Magnetospira sp. QH-2 (GCF_000968135.1)
Varunaivibrio sulfuroxidans DSM 101688T (GCF_004341725.1)
Magnetovibrio blakemorei MV-1T (GCF_001746755.1)
Oleiliquidispirillum nitrogeniifigens 64-1T (GCF_006980715.1)
Telmatospirillum siberiense 26-4b1T (GCF_002845745.1)
Magnetospirillum sufuroxidans J10T (GCF_018224925.1)
Magnetospirillum gryphiswaldense MSR-1T (GCF_002995515.1)
Caenispirillum bisanense USBA 140T (GCF_900230255.1)
Insolitispirillum peregrinum DSM 11589T (GCF_900156605.1)
Novispirillum itersonii subsp. itersonii ATCC 12639T (GCF_000381985.1)
Haematospirillum jordaniae H5569T (GCF_001611975.1)
Roseospira marina DSM 15113T (GCF_008630015.1)
Rhodospira trueperi ATCC 700224T (GCF_900101965.1)
Rhodospirillum rubrum ATCC 11170T (GCF_000013085.1)
Phaeovibrio sulfidiphilus JA480T (GCF_014861485.1)
Pararhodospirillum photometricum DSM 122T (GCF_000284415.1)
Vineibacter terrae JCM 33507T (GCF_008039615.1)
Reyranella soli NBRC 108950T (GCF_007992495.1)
Reyranella massiliensis 521T (GCF_000312425.1)
Acetobacteraceae
Zavarzinia compransoris DSM 1231T (GCF_004362995.1)
Zavarzinia aquatilis HR-AST (GCF_003173035.1)
Tistrella mobilis KA081020-065T (GCF_000264455.2)
Geminicoccus roseus DSM 18922T (GCF_000427665.1)
Arboricoccus pini B29T1T (GCF_900187945.1)
Myxococcus fulvus DSM 16525T (GCF_900111765.1)
0.10
Rhodospirillaceae ("Dongiaceae")
Azospirillaceae
Stellaceae
Rhodospirillaceae
("Oceanibaculaceae")
Thalassobaculaceae
Kiloniellaceae
Rhodospirillaceae
Rhodovibrionaceae
("Fodinicurvataceae")
Rhodovibrionaceae
Thalassospiraceae
Terasakiellaceae
Shumkoniaceae fam. nov.
Geminicoccaceae
("Magnetospiraceae")
("Magnetovibrionaceae")
Thalassospiraceae
("Magnetospirillaceae")
("Novispirillaceae")
Rhodospirillaceae
Rhodospirillaceae
Reyranellaceae
Zavarziniaceae
Geminicoccaceae
Geminicoccaceae

◂**Fig. 3** Phylogenomic tree reconstructed based on 120 single copy conservative marker genes of all species with sequenced genomes in the order *Rhodospirillales* with GTDB pipelines. Bar, 0.1 substitutions per position. Percentage bootstrap values are given at branching points. Latin names with quotes have not been validly published

and formed a clade with *Defluviicoccus vanus* Ben 114 ^{T}.

To further figure out the taxonomic rank of strain Y-P2^{T}, reanalysis of pairwise 16S rRNA gene sequence similarity and indices of pairwise genomic relatedness including ANI, AAI and POCP were performed. Strain Y-P2^{T} had 89.2 ± 0.8% of 16S rRNA sequence similarities to representative species of the family "*Oceanibaculaceae*" and *Thalassobaculaceae* (Fig. 4), which were close to the minimum sequence similarity for defining a novel family (Yarza et al. 2014). Meanwhile, the ANI and POCP values between strain Y-P2^{T} and these members were ≤ 69.8 and 43.0% (Table S2), respectively, far lower than the cutoff values for distinguishing genera (83 and 50%, respectively) (Luo et al. 2014; Qin et al. 2014; Jain et al. 2018), and were in the range of relevant inter-family values (ANI 63.7–70.7 and POCP 25.3–49.9%) (Fig. 4); the AAI values were all ≤ 57.8% (Table S2), which were lower the boundary (approximately 60%) for *Rhodospirillales* families proposed by Koziaeva et al. (2023). In addition, strain Y-P2^{T} was separated from other *Rhodospirillales* families with the average values of 16S rRNA gene sequence similarity and AAI (86.0 ± 2.0 and 55.3 ± 1.5%, respectively) below the thresholds for family delineation (Fig. 4) (Yarza et al. 2014). All these results suggested strain Y-P2^{T} could be distinguished from families in *Rhodospirillales* and represents a novel family within *Rhodospirillales*.

Genomic characteristics

The genome of strain Y-P2^{T} had a total length of 5,388,487 bp, and contained 5053 ORFs, 49 tRNA genes, one 5 s rRNA, one 16 s rRNA and one 23 s rRNA (Table S3). The G + C content was 66.0%. 2314, 3540 and 3592 genes were annotated in KEGG, Swiss and GO database, respectively (Table S3).

Glycan biosynthesis pathways

Extracellular polymeric substance (EPS) is a key component of biofilm causing processes of MIC. Strain Y-P2^{T} was able to form EPS when grown under microaerobic condition (Fig. S5). Results based on KEGG annotation showed that 66 genes associated with glycan biosynthesis and metabolism are identified in the genome of strain Y-P2^{T} (Table S4), 20 of which (more than 30%) involved in the biosynthesis of lipopolysaccharide (LPS) which may effect on the Co-Cr and Ti alloys corrosion (Yu et al. 2016). Genes *lpxA*, *lpxCD*, *lpxI*, *lpxB* and *lpxK* encoding homologs that consist of pathway synthesizing lipid IVA from UDP-*N*-acetyl-α-D-glucosamin or a (3R)-3-hydroxytetradecanoyl-[acp] (Table S5). Meanwhile, homologs encoded by genes *kdsD*, *kdsA*, *kdsC* and *kdsB* formed a pathway for synthesizing CMP-3-deoxy-β-D-manno-octulosonate from D-ribulose 5-phosphate, providing an essential substrate for generating Kdo2-lipid A via the pathway comprised of integral membrane proteins encoded by *kdtA*, *lpxL*, *IpxK* and *lpxM* (Wang et al. 2015). However, *lpxM* was not found in genome of strain Y-P2^{T}. In addition, an ADP-L-glycero-β-D-manno-heptose biosynthesis pathway containing enzymes encoded by *gmhAC*, *gmhB* and *gmhD* was employed by Y-P2^{T} to produce the precursor for the inner core region of LPS, but only one gene (*gtrB*) related to O-antigen repeat unit synthesis was identified. We also found strain Y-P2^{T} had a complete pathway (*rmlADBC*) to synthesize dTDP-L-rhamnose which is the precursor of L-rhamnose. L-rhamnose and GDP-D-glycero-D-manno-heptose that generated via enzymes encoded by *gmhA*, *hhdA* (absent), *gmhB*, and *hhdC* are sugar components of bacterial S-layer glycoproteins (Kneidinger et al. 2001; Graninger et al. 2002). It has been demonstrated that S-layer proteins are associated with cells aggregation, bacterial adherence to substrates and surfaces, as well as biofilm formation (Gerbino et al. 2015).

Sulfur metabolism

Sulfur-cycling microorganisms are commonly considered the key players of MIC, and can participate in the process of MIC through sulfate reduction and

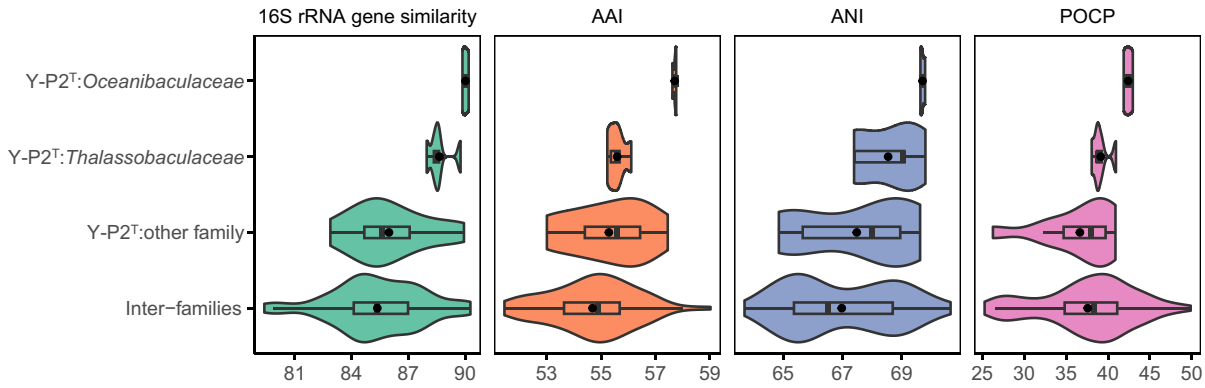


Fig. 4 Comparison of 16S rRNA gene identity, AAI, ANI and POCP between strain Y-P2^T and representatives of published families within the order *Rhodospirillales*. The violin plot lines indicate a kernel density of the identity distribution. The black dot indicates the mean and the solid horizontal line indicates the median. **Thalassobaculaceae*, members within "*Oceanibaculaceae*" and *Thalassobaculaceae* included

sulfur disproportionation (Rajala et al. 2022). The genome of strain Y-P2^T contained 24 genes for sulfur metabolism (Table S6). Gene *sta*, and the operons *aprAB* and *dsrAB* comprised a complete dissimilatory sulfate reduction and oxidation pathway for the conversion between sulfate and sulfide, which has been described in Dsr-dependent sulphate-reducing bacteria and sulphur-oxidizing bacteria (Neukirchen and Sousa 2021). SOX system oxidizing thiosulfate to sulfate via enzymes encoded by *soxDCBAZYX* operon was identified in strain Y-P2^T. In addition, a flavocytochrome c sulfide dehydrogenase was presented in genome of Y-P2^T by *fccA* and *fccB*, it has been reported that this enzyme can oxidize self-produced sulfide or exogenous sulfide to sulfite and thiosulfate under aerobic condition in *Pseudomonas aeruginosa* (Lü et al. 2017).

Conclusion

Physiological comparison revealed that strain Y-P2^T shares several common phenotypic features with *Rhodospirillales* members, such as mesophilic, Gram-stain-negative and major respiratory quinone, but is different to its phylogenetically close relatives in morphological and physiological traits (Table 2). *Thalassobaculaceae* members are motile, grow with salinity, positive for oxidase activity; "*Magnetospiraceae*" and "*Magnetovibrionaceae*" are magnetotactic; while *Geminicoccaceae* and *Stellaceae* are aerobic, having different cell shapes, *Geminicoccaceae* also has abilities to reduce nitrate and hydrolyze gelatin. Meanwhile, values of the 16S rRNA gene sequence similarity, AAI ANI and POCP between strain Y-P2^T and published members of *Rhodospirillales* families were all lower than boundaries for separating *Rhodospirillales* families. On the basis of the distinct phenotypic and chemotaxonomic characteristics, phylogenetic and genomic evidence mentioned above, strain Y-P2^T is proposed as the type strain of a novel species of a new genus, for which the name *Shumkonia mesophila* gen. nov., sp. nov. is proposed, within a new family *Shumkoniaceae* fam. nov.

Description of *Shumkoniaceae* fam. nov.

Shumkoniaceae (Shum.ko.ni.a.ce'ae. N.L. fem. n. *Shumkonia* a bacterial genus; *aceae* suffix to denote a family; N.L. fem. pl. n. *Shumkoniaceae* the *Shumkonia* family).

The description of *Shumkoniaceae* is the same as for the genus *Shumkonia*. The type genus is *Shumkonia*.

Description of *Shumkonia* gen.nov.

Shumkonia (Shum.ko'ni.a. N.L. fem. n. *Shumkonia* named in honour of ShumKo

Table 2 Differential characteristics of family *Shumkoniaceae* and related families in the order Rhodospirillales

Characteristics	1	2	3	4	5	6	7	8	9	10
Oxygen requirement	MA	A, FA	MA	MA, FA or AN	A	A, FA, AN	A to MA	A, FA	A	A
Cell shape	Rod	Diplococci, coccobacillus, rod	Variable	Vibrioid to helicoid, curved rod	rod	Rod, helical, vibrioid	Six–pronged stars, ovoid to rod	Slightly curved and straight rod	Vibrioid to spiral	Curved rod
Motility	–	(–)	+	+	+	+	–	+	+	+
Flagellum	–	Monopolar	Monopolar	Monopolar, multipolar	Monopolar	Monopolar, bipolar	ND	Monopolar	ND	Monopolar
Oxidase	–	(–)	–	+	–	(+)	–	+	+	ND
Catalase	(–)	+	–	–	+	(+)	+	+	+	+
Growth Temperature (°C):	15–30 (25–30)	15–45 (25–35)	5–37 (24–26)	4–35 (26–30)	10–42 (25–37)	15–45 (20–40)	5–30 (25–30)	10–44 (30–35)	4–40	ND (ND)
NaCl concentration (w/v)	0–10 (0)	0–3 (0.01)	ND	0.5–4.5 (1.5–2)	0–9 (0.5–7)	0–15 (0–8)	0.1–2 (0.5)	0–10 (2–4)	0–10	0–0.5 (0–2)
pH	7.1–8.1 (7.1)	5.0–11.0 (6.0–8.0)	ND (7.0)	4.5–8.5 (6.0–7.0)	6.0–11.0 (7.0–9.0)	6.0–11.0 (7.0–9.0)	6.0–8.0 (7.0)	5.0–9.0 (6.0–8.0)	ND	ND
Gelatin hydrolysis		+	ND	ND	–	–	ND	+	–	ND
Urease	+	+	ND	ND	–	–	ND	ND	ND	+
H2S production	–	–	ND	ND	ND	–	+	ND	ND	ND
Electron acceptors	Nitrate	Nitrate	ND	Nitrous oxide, nitrate, nitrite, ferric iron	Nitrate	Nitrate, sulfate	Nitrate	Nitrate, nitrite, nitrous oxide	Nitrate	Nitrate
Utilization of sugars	–	+	–	+	ND	+	+/–	+	+	–
Utilization of amino acids	–	ND	–	+	+	+		ND	ND	+
Utilization of organic acids	+	+	+	+	+	+	+	+	ND	+
Respiratory quinones	Q-10	Q-10	ND	Q-9, Q-8	ND	Q-10, Q-9, Q-8, RQ-8, Q-7, MK-7	Q-10	Q-10	ND	ND
Polar lipids	AL, PE, PG, PL, L	DPG, PG, PC, PE, PL, AL	ND	PE, PG, PL, APL	ND	PE, PG, DPG, APL, PL, GL, L	ND	PG, APL, PE, AL	ND	ND

Table 2 (continued)

Characteristics	1	2	3	4	5	6	7	8	9	10
DNA G+C content (mol%)	66.0	60.2–67.5	47.2	52.9–59.9	64.8	56.4–70.0	69.3–72.9	60.1–68	54.7	68.1

1. *Shumkoniaceae* fam.nov., in this study; 2. *Geminicoccaceae* (Foesel et al. 2007, Maszenan et al. 2005, Proença et al., 2018, Shi et al. 2002); 3. "*Magnetospiraceae*" (Williams et al. 2012); 4. "*Magnetovibrionaceae*" (Bazylinski et al. 2013, Patwardhan and Vetriani, 2016); 5. "*Oceanibaculaceae*" (Lai et al., 2009a, b); 6. *Rhodospirillaceae* (Anil Kumar et al. 2008, Chen et al. 2018, Dar Jean et al. 2016, Humrighouse et al. 2016, HYLEMON et al. 1973, Imhoff et al. 1998, Kim et al. 2019, Lai et al., 2009a, b, Lakshmi et al. 2014, Lakshmi et al. 2011, Lin et al. 2021, Pfennig et al. 1997, Ruan et al. 2019, Tang et al. 2019, Wang et al. 2020, Wang et al., 2019a, b, Yoon et al. 2007); 7. *Stellaceae* (Vasilyeva 1985, Yamada et al. 2011); 8. *Thalassobaculaceae* (Urios et al. 2008, Zhang et al. 2008); 9. *Thalassospiraceae* (Lopez-Lopez et al., 2002); 10. *Zavarziniaceae* (Lee et al. 2019). +, positive; −, negative; ND, no data available; (+), positive in most strains; (−), negative in most strains; +/−, variable. A, Aerobic; MA, microaerobic; FA, facultatively anaerobic; AN, anaerobic

(1031–1095) who found and used oil in the eleventh century in China).

Microaerophilic, mesophilic, chemoorganoheterotrophic bacterium. Gram-stain-negative, non-motile, non-spore-forming, rod-shaped or slightly curved rod. Acetate and H_2 are the fermentation products of glucose. The predominant cellular fatty acid is $C_{16:0}$, and the quinone is Q-10. The type species is *Shumkonia mesophila.*

Description of *Shumkonia mesophila* sp. nov.

Shumkonia mesophila (me.sophi. la. N.L. fem. adj. mesophila, middle temperature loving).

Microaerophilic, mesophilic, chemoorganoheterotrophic bacterium. Cells are Gram-stain-negative, non-motile, non-spore-forming, rod-shaped or slightly curved, 0.8–3.0 μm in width and 0.4–0.6 μm in length. Growth occurs optimally under the conditions of 25 °C, pH 7.0 without NaCl. Urease and oxidase positive. Acetate and H_2 are the fermentation products of glucose. Catalase, nitrate reduction and denitrification negative. Major cellular fatty acids are $C_{16:0}$, sum in feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$), sum in feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$). The polar lipids comprise phosphatidylethanolamine (PE), phosphatidylglycerol (PG), one unidentified aminolipid (AL), three unidentified phospholipid (PL) and four unidentified polar lipids (L). Respiratory quinone is Q-10. The genomic DNA G+C content was 66.0%.

The type strain is Y-P2^T (=CCAM 826 ^{T}=JCM 34766 ^{T}) isolated from oil sludge, Shengli, China.

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Author contributions Min Yang: Physiological and Biochemical experiment, data analysis and article writing ; Xue Zhang: Enrichment and isolation; Shichun Ma: Project guidance and promotion, data analysis and article revision; Qiumei Zhang: Genomic Data Analysis and phylogenetic analysis; Chenghui Peng: Physiological data collection; Hui Fan: Electron acceptor and substrate experimen; Jiang Li: Genomic Data Analysis; Lirong Dai: Strain preservation and activation; Lei Cheng: Funding support and guidance. All authors reviewed the manuscript.

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Declarations

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

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