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ORIGINAL PAPER

Thalassospira aquimaris sp. nov. and *Winogradskyella marincola* sp. nov. two marine bacteria isolated from an agar-degrading co-culture

Zi-Yue Fu · Dao-Feng Zhang · Meng-Han Huang · Hong-Chuan Wang · Xiao-Ye Chen · Yu-Fang Yao · Yang Yuan · Wen-Jun Li

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Abstract Two novel Gram-stain-negative, aerobic, and non-motile strains, designated FZY0004^T and YYF002^T, were isolated from an agar-degrading co-culture, which was obtained from seawater of the intertidal zone of Yancheng City, the Yellow Sea of China. Strain FZY0004^T optimally grew at 28 °C, pH 7.0, and 2–6% NaCl, while strain YYF002^T optimally grew at 28 °C, pH 7.5, and 2–4% NaCl. Strain FZY0004^T possessed Q-9 as the major respiratory quinone, and its major fatty acids (> 10%) were summed feature 8 ($C_{18:1}$ $\omega7c$), $C_{16:0}$, and summed feature 3 ($C_{16:1}$ $\omega7c$/$C_{16:1}$ $\omega6c$). The polar lipids identified in strain FZY0004^T were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and several unidentified phospholipids (PL) and lipids (L). On the other hand, strain YYF002^T had MK-6 as the predominant respiratory quinone and its major fatty acids consisted of iso-$C_{15:0}$, iso-$C_{15:1}$ G, and iso-$C_{15:0}$ 3-OH. The polar lipids identified in strain YYF002^T were aminolipid (AL), PE, and several unidentified lipids. Strain FZY0004^T shared 99.5% 16S rRNA gene sequence similarity and 90.1% average nucleotide identity (ANI) with *T. povalilytica* Zumi 95^T, and strain YYF002^T shared 99.2% 16S rRNA gene sequence similarity and 88.2% ANI with *W. poriferorum* JCM 12885^T. The genomic DNA G + C contents of strains FZY0004^T and YYF002^T were 54.5% and 33.5%, respectively. The phylogenetic, phenotypic, and physiological characteristics permitted the distinction of the two strains from their neighbors, and we thus propose the names *Thalassospira aquimaris* sp. nov. (type strain FZY0004^T = JCM 35895^T = MCCC 1K08380^T) and *Winogradskyella marincola* sp. nov. (type strain YYF002^T = JCM 35950^T = MCCC 1K08382^T).

Keywords *Thalassospira aquimaris* sp. nov. · *Winogradskyella marincola* sp. nov. · Intertidal zone · Seawater · New taxa

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Z.-Y. Fu · D.-F. Zhang (✉) · M.-H. Huang · H.-C. Wang · X.-Y. Chen · Y.-F. Yao · Y. Yuan (✉) · W.-J. Li
Jiangsu Province Engineering Research Center for Marine Bio-resources Sustainable Utilization and College of Oceanography, Hohai University, Nanjing, People's Republic of China
e-mail: zdf@hhu.edu.cn

Y. Yuan
e-mail: 20240925@hhu.edu.cn

Y. Yuan · W.-J. Li
State Key Laboratory of Biocontrol, Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai) and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, Guangzhou, People's Republic of China

W.-J. Li
State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Ürümqi, People's Republic of China

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Introduction

The genus *Thalassospira* was established by López-López et al. (2002) and later emended by Liu et al. (2007) and Tsubouchi et al. (2014), belonging to the family *Rhodospirillaceae* within the class *Alphaproteobacteria*. At present, the genus contains 12 species with validly published and correct names according to the List of Prokaryotic Names with Standing in Nomenclature (LPSN) (www.bacterio.net, accessed on April 30, 2024) (Parte et al. 2020), and *T. lucentensis* is the type strain of the genus *Thalassospira*. Cells are Gram-staining negative, non-motile or motile using a polar flagellum, curved-to-spiral rod-shaped, aerobic to facultatively anaerobic, halophilic, and positive for catalase and variable to oxidase. The major cellular fatty acids are $C_{18:1}\omega7c$ and $C_{16:0}$, isoprenoid quinone is Q-9 or Q-10, and the range of genomic DNA G+C content is 46.0–62.0 mol% (Dong et al. 2018; López-López et al. 2002). From the Tara Oceans consortium metagenomic data, *Thalassospira* spp. make up to 0.1% of all marine bacteria (Sunagawa et al. 2015). The main habitat of the genus is seawater (Dong et al. 2018; Liu et al. 2016; López-López et al. 2002), marine sediments (Tsubouchi et al. 2014), and oil-contaminated water (Liu et al. 2007). Studies demonstrated the biodegradation ability of *Thalassospira* and suggested that these strains play an important role in marine contaminated ecosystems because of their potential in eliminating marine oil pollution, especially in polycyclic aromatic hydrocarbons degradation and polyvinyl-alcohol degradation (Nogi et al. 2014; Santisi et al. 2022; Wang et al. 2010). In addition, some species have potential beneficial properties of long-chain polyunsaturated fatty acids production for fish feed (Romano et al. 2020).

The genus *Winogradskyella* is a member of the family *Flavobacteriaceae* in the phylum *Bacteroidota* (Oren and Garrity 2021), which was first described by Nedashkovskaya et al. (2005), later emended by Ivanova et al. (2010), Yoon et al. (2011), Nedashkovskaya et al. (2012) and Begum et al. (2013). At present, the genus comprised 50 species with validly published names according to LPSN (accessed on April 30, 2024), and *W. thalassocola* is the type strain of the genus *Winogradskyella*. Members of the genus *Winogradskyella* have been isolated from different marine environments, such as alga specimens, seawater, marine sediment, coastal sediment, tidal flat, and coral (Bo et al. 2021; Lau et al. 2005; Yoon et al. 2011). Members of the genus *Winogradskyella* are heterotrophic, yellow or orange-pigmented, and Gram-stain-negative. All of them are mesophilic and slightly halophilic. The genomic DNA G+C content is less than 40.0 mol%, the major respiratory quinone is menaquinone 6 (MK-6), and phosphatidylethanolamine (PE) is the major polar lipid.

In this study, two strains FZY0004^T and YYF002^T were isolated from an agar-degrading coculture, which was obtained from seawater, and their taxonomic positions were clarified using a polyphasic approach. Based on these results, strains FZY0004^T and YYF002^T are proposed as novel species within the genera *Thalassospira* and *Winogradskyella*, respectively.

Material and methods

Isolation and culturing conditions

The seawater sample was collected from the intertidal zone (33°6′59″N, 121°51′9″E) of Yancheng City, China. Strains FZY0004^T and YYF002^T were isolated via a similar strategy as that for *Marinilongibacter aquaticus* YYF0007^T (Zhang et al. 2022). The purified strains were preserved at − 80 °C as glycerol stocks (20%, v/v). Strains *T. xianhensis* CGMCC 1.6849^T and *T. profundimaris* MCCC 1A00207^T were obtained from the China General Microbiological Culture Collection Center (CGMCC) and the Marine Culture Collection of China (MCCC), respectively, and used as the experimental control of strain FZY0004^T. Strain *W. poriferorum* JCM 12885^T (Lau et al. 2005) was obtained from the Japan Collection of Microorganisms (JCM) and used as the experimental control of strain YYF002^T.

16S rRNA gene sequencing and phylogenetic analysis

The genomic DNA was extracted and purified with an Ezup Column Bacteria Genomic DNA Purification Kit (Sangon Biotech, China). The 16S rRNA gene amplicons of the strains were obtained by PCR amplification. The PCR reaction included 2×PCR Master (Sangon Biotech) and a universal bacterial primer pair (forward primer 27F (5′-AGAGTTTGATCC

TGGCTCAG-3′) and reverse primer 1492R (5′-GGC TACCTTGTTACGACTT-3′) (Weisburg et al. 1991)). The sequence of the 16S rRNA gene was assembled using SeqMan software (DNASTAR) and submitted to the EzBioCloud (https://www.ezbiocloud.net/) (Yoon et al. 2017a) and NCBI (https://blast.ncbi.nlm.nih.gov/) database for alignment analysis with other taxa.

Evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) for the Neighbor-Joining (NJ) algorithm (Saitou and Nei 1987) and a phylogenetic tree constructed with MEGA X software package (Kumar et al. 2018) after multiple sequence alignments. For comparison with the NJ phylogenetic tree, the Maximum Likelihood (ML) tree (Felsenstein 1981) and the Maximum-Parsimony (MP) tree (Kannan and Wheeler 2012) were constructed using the MEGA X Program. MEGA X software was used to create phylogenetic trees by bootstrap analysis with 1,000 replicates (Felsenstein 1981).The 16S rRNA gene sequences of *Azorhizobium caulinodans* ORS 571^T (D11342) and *Tamlana crocina* HST1-43^T (AM286230) were used as outgroups, respectively.

Genome sequencing and genome sequence analysis

Genome sequencing of strains FZY0004^T and YYF002^T was performed using a paired-end sequencing method with the Hiseq X platform (Illumina) at Personalbio Company, Shanghai, China. The genome of strain *W. poriferorum* JCM 12885^T was also sequenced in this study because there is no genome sequence available for it. Sequence assembly was performed with SPAdes v3.13.0 (Nurk et al. 2013). The genomic DNA G+C content was calculated based on the whole-genome sequence. Genomic annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/) to enhance the understanding of the genome. The genome sequence data of strains FZY0004^T and YYF002^T, along with their affiliated genera *Thalassospira* and *Winogradskyella*, were analysed using EasyCGTree version 4.2 (https://github.com/zdf1987/EasyCGTree4) under Windows operation system (OS) (Zhang et al. 2023) to clarify the phylogenetic relationship. This allowed the construction of a phylogenomic tree, providing insights into the evolutionary connections and taxonomic positioning of strains with its closely related species. The phylogenetic tree was constructed using the 120 ubiquitous single-copy protein-coding genes (Parks et al. 2018) from all the publicly available genomes of the genus *Thalassospira* or *Winogradskyella*, along with the outgroup. The average nucleotide identity (ANI) was estimated by using an ANI Calculator tool (https://www.ezbiocloud.net/) (Yoon et al. 2017b) and the average amino identity (AAI) was estimated by using the AAI calculator (http://enve-omics.ce.gatech.edu/aai/) (Qin et al. 2014). Digital DNA-DNA hybridization (dDDH) was assessed using the Genometo-Genome Distance Calculator (GGDC) (https://ggdc.dsmz.de) (Meier-Kolthoff et al. 2013) with the recommended formula 2. Function gene enrichment was performed on the predicted coding genes of strains FZY0004^T and YYF002^T using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (www.genome.jp/kegg/) (Kanehisa et al. 2016).

Morphology, physiology, and biochemical analysis

A routine cultivation on the R2A plates at 28 °C until the late-exponential growth phase was employed to investigate the morphological and physiological properties of strains FZY0004^T and YYF002^T. Gram-staining was tested by using a Gram Staining kit (G1060, Solarbio, China). Growth conditions were tested at 28 °C for 3 days on R2A, tryptic soy agar (TSA), marine agar 2216 (MA), and Luria–Bertani agar (LB), which were supplemented with 2.5% sea salt except for MA. NaCl tolerance (0, 0.5, 1.0, 2.0, 3.0, 5.0, 6.0, 7.0, 10.0, and 12.0%, w/v) was tested at 28 °C with initial pH 7.0 on R2A medium. The pH range for growth (adjusted from pH 4.0–10.0 in increments of 0.5 pH unit) was tested with the pH buffer system described by Xu et al. (2005) by culturing the strains in R2A broth. The optimal temperature range required for growth (4, 10, 20, 24, 28, 37 and 42 °C) was tested at pH 7.0 on R2A plates. The growth of the strains cultured at 4 °C and 42 °C was observed after two weeks. The morphology of cells and size were examined by scanning electron microscopy (Regulus 8100, HITACHI, Japan). After 3 days of incubation at 28 °C, the cell diffusion in semisolid R2A containing 0.5% agar was observed to determine the motility of the cells. Oxidase activity was tested using an oxidase reagent kit (bioMérieux) according

to the manufacturer's instructions and catalase activity was tested by observing the production of bubbles with 3% (w/v) H_2O_2. Various biochemical tests, including hydrolysis of starch, hydrolysis of cellulose, urea, Tween 20, Tween 60, Tween 80, indole production, and H_2S production. Tests for other physiological or biochemical characteristics were performed using API 20E, API ZYM (all from bioMérieux), and GEN III MicroPlates (BIOLOG, USA), according to the manufacturer's instructions, except that the salinity of the media was adjusted to 2.5%. Type strains of taxonomically related species *T. xianhensis* CGMCC 1.6849^T, *T. profundimaris* MCCC 1A00207^T, and *W. poriferorum* JCM 12885^T were also tested for biochemical and chemical taxonomic analyses to allow a more complete comparison.

Chemotaxonomic characterization

Strains FZY0004^T, YYF002^T, and their reference strains were cultured in R2A broth at 28 °C for 48 h. Cells were collected by centrifugation and washed with distilled water. For fatty acid analysis cells were streak (quaternary streaking) plated on TSA agar. Cellular fatty acids were saponified, methylated and extracted following the classical method of MIDI protocol (Sherlock Microbial Identification System, version 6.2B) (Sasser 1990). Quinones were extracted with a chloroform/methanol (2:1, v/v) mixture and analysed using high-performance lipid chromatography (HPLC) (HIiraishi et al. 1996). Polar lipids were extracted and identified by using two-dimensional thin-layer chromatography as the previously described method (Athayle et al. 1984).

Results and discussion

Phylogenetic analysis based on 16S rRNA gene sequences

The comparative analysis of 16S rRNA gene sequences from the EzBioCloud database revealed that strain FZY0004^T (accession number, OQ714501) was closely related to *T. povalilytica* Zumi 95^T (99.5%), *T. australica* NP3b2^T (99.5%), *T. profundimaris* WP0211^T (99.4%), *T. xiamenensis* M-5^T (99.4%), *T. xianhensis* P-4^T (99.4%), *T. tipidiphila* 1-1B^T (99.3%), *T. indica* PB8B^T (99.0%), *T. lucentensis* DSM 14000B^T (98.7%), *T. lohafexi* 139Z-12^T (98.7%), *T. alkalitolerans* MBE#61^T (98.0%), *T. mesophila* MBE#74^T (97.9%), and *T. marina* CSC3H3^T (97.8%). Strain YYF002^T (accession number, OQ714211) was closely related to *W. poriferorum* UST030701-295^T (99.2%) and *W. aquimaris* DPG-24^T (97.0%). The strains annotated in the EzBioCloud database with 16S rRNA gene similarity exceeding 97% are the same as those annotated in the NCBI database. Phylogenetic analysis using the NJ, ML, and MP algorithms showed that strain FZY0004^T formed a phyletic lineage with *T. australica* NP3B2^T, *T. povalilytica* Zumi 95^T, *T. profundimaris* WP0211^T, *T. indica* PB8B^T and *T. tipidiphila* 1-1B^T within the genus *Thalassospira* (Fig. S1). The results of phylogenetic analysis based on the NJ and MP method indicated that YYF002^T formed a phyletic lineage with *W. poriferorum* UST030701-295^T, and the corresponding ML trees showed similar topologies that *W. poriferorum* UST030701-295^T was its closest neighbor, which supported the proposal that strains YYF002^T belonged to the genus *Winogradskyella* (Fig. S2).

Genomic characteristic

The whole genome sequence size of FZY0004^T was 4,982,113 bp. The N50 was 375,977 bp and the DNA G+C content was 54.5%. The genome of FZY0004^T contained 4,573 genes in total and included 4,502 protein-coding genes, 3 rRNAs, 56 tRNAs, and 34 contigs. The draft genome sequence size of YYF002^T was 3,524,355 bp, with an N50 of 437,365 bp and a DNA G+C content of 33.5%. The genome of YYF002^T contained 3,166 genes in total and included 3,116 protein-coding genes, 3 rRNAs, 38 tRNAs, and 29 contigs. The draft genome sequence size of *W. poriferorum* JCM 12885^T was 3,621,352 bp, with an N50 of 739,931 bp and a DNA G+C content of 33.5%. The genome of JCM 12885^T contained 3,284 genes in total and included 3,228 protein-coding genes, 3 rRNAs, 42 tRNAs, and 17 contigs (Table S1). The number of genes associated with the KEGG functional pathways in strains FZY0004^T and YYF002^T and the reference type strains were shown in Tables S2 and S3.

In the phylogenomic bac120 tree, FZY0004^T and *T. povalilytica* Zumi 95^T made a separate clade (Fig. 1), as did YYF002^T and *W. poriferorum* JCM

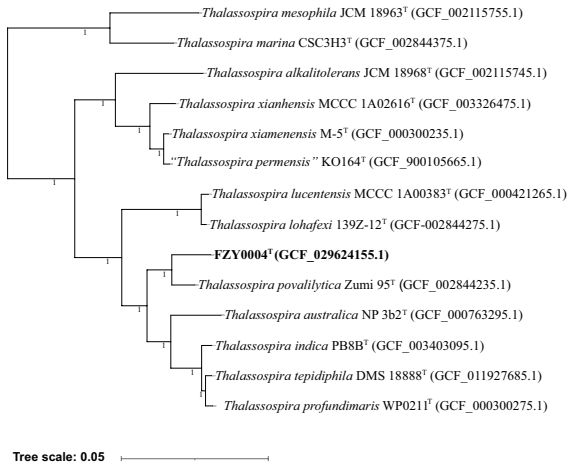

Fig. 1 A maximum-likelihood tree based on the 120 ubiquitous single-copy protein-coding genes showing the phylogenetic relationship of FZY0004^{T} in the genus *Thalassospira*. Bootstrap values based on 1000 replicates are shown at the branch points nodes. The RefSeq assembly accession number is indicated in the bracket. *Azorhizobium caulinodans* ORS 571^{T} (GCF_0000105525.1) is used as an outgroup and hidden in the tree. Bar, 0.05 substitutions per nucleotide position

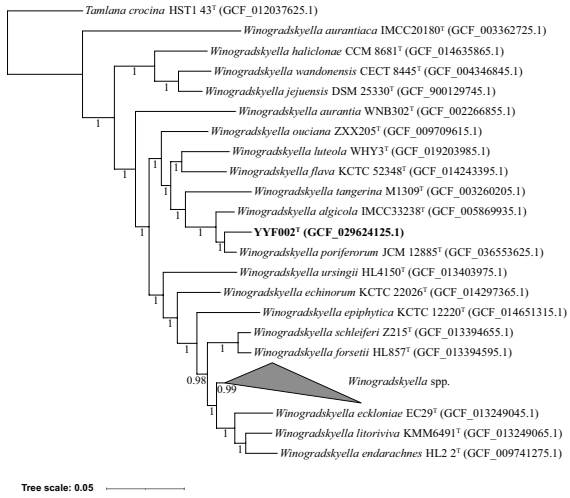

Fig. 2 A maximum-likelihood tree based on the 120 ubiquitous single-copy protein-coding genes showing the phylogenetic relationship of YYF002^{T} in the genus *Winogradskyella.* Bootstrap values based on 1000 replicates are shown at the branch points nodes. The RefSeq assembly accession number is indicated in the bracket. The type strain of the genus *Winogradskyella*, *W. thalassocola* DSM 15363^{T} (GCF_900099995.1) was included in the clade. *Tamlana crocina* HST1-43^{T} (GCF_012037625.1) is used as an outgroup and hidden in the tree. Bar, 0.05 substitutions per nucleotide position

12885 ^{T} (Fig. 2), which is consistent with the results for the phylogenetic tree based on 16S rRNA gene sequences. The ANI values for FZY0004^{T} and the reference strains *T. profundimaris* WP0211^{T} and *T. xianhensis* MCCC 1A02616^{T} were 85.0% and 79.3%, respectively, and those for YYF002^{T} and the reference strain *W. poriferorum* JCM 12885 ^{T} was 88.2%. The AAI values for FZY0004^{T} and the reference strains *T. profundimaris* WP0211^{T} and *T. xianhensis* MCCC 1A02616^{T} were 81.2% and 78.1%, respectively, and those for YYF002^{T} and the reference strain *W. poriferorum* JCM 12885 ^{T} was 89.9%. The dDDH values for FZY0004^{T} and the reference strains *T. profundimaris* WP0211^{T} and *T. xianhensis* MCCC 1A02616^{T} were 23.6% and 21.5%, respectively, and those for YYF002^{T} and the reference strain *W. poriferorum* JCM 12885 ^{T} was 34.7%. All values were below the threshold for species delineation, which are 95%, 95%, and 70% for ANI, AAI, and dDDH, respectively (Meier-Kolthoff et al. 2013; Richter and Rossello-Mora 2009; Wayne et al. 1987). Detailed values of the two novel strains and their close species are given in Tables 1 and 2. As a result, a phylogenetic tree was conducted based on coding sequences of 120 protein clusters and revealed that FZY0004^{T} and YYF002^{T} represent members of the genera *Thalassospira* and *Winogradskyella*, respectively.

Physiology and biochemical analysis

Cells of strains FZY0004^{T} and YYF002^{T} were observed to be Gram-stain-negative. SEM images of strain FZY0004^{T} cell showed curved rods with 0.3–0.5 µm width and 1.0–1.8 µm length (Fig. 3a), YYF002^{T} showed rod-shaped profile (0.3–0.4 µm wide and 1.5–1.8 µm long (Fig. 3b). Srains FZY0004^{T} and YYF002^{T} could grow on R2A, TSA and MA plates, with the best growth on R2A plates. Both strains FZY0004^{T} and YYF002^{T} were positive for oxidase and catalase activity. Results of strain FZY0004^{T} and the reference type strains *T. profundimaris* MCCC 1A00207^{T} and *T. xianhensis* CGMCC 1.6849 ^{T} in BIOLOG GEN III microtest were shown in Table S4. Results of strain YYF002^{T} in BIOLOG GEN III microtest weren't shown, as the positive control failed. The comparisons of phenotypic and biochemical characteristics of strains FZY0004^{T}

Table 1 Comparative genomic characteristics of strain FZY0004^T and phylogenetically related species of the genus *Thalassospira*

Organism	ANI (%)	AAI (%)	DDH (%)	16S rRNA gene Similarity (%)
T. profundimaris WP0211^T	85.0	81.2	23.6	99.4
T. xianhensis MCCC 1A02616^T	79.3	78.1	21.5	99.4
T. xiamenensis M-5 ^{T}	79.5	78.3	21.7	99.4
T. povalilytica Zumi 95^T	90.1	85.5	29.2	99.5
T. australica NP 3b2^T	84.5	80.7	23.3	99.5
T. indica PB8B^T	84.6	81.3	23.6	99.0
T. tepidiphila 1-1B^T	84.8	81.1	23.6	99.3
T. lucentensis DSM 14000B^T	81.1	83.0	22.2	98.7
T. lohafexi 139Z-12^T	81.2	83.1	22.3	98.7
T. alkalitolerans MBE#61^T	79.8	79.1	21.0	98.0
T. mesophila MBE#74^T	77.9	71.0	19.6	97.9
T. marina CSC3H3^T	79.1	71.0	20.9	97.8

The 16S similarity results originate from EzbioCloud. Strains (Genome accession number): FZY0004^T (ASM2962415v1); *T. profundimaris* WP0211^T (ASM30027v1); *T. xianhensis* MCCC 1A02616^T (ASM332647v1); *T. xiamenensis* M-5^T (ASM30023v1); *T. povalilytica* Zumi 95^T (ASM284423v1); *T. australica* NP 3b2^T (ASM76329v1); *T. indica* PB8B^T (ASM166283v1); T. tepidiphila 1-1B^T (ASM166287v1 1); *T. lucentensis* DSM 14000B^T (ASM42126v1), *T. lohafexi* 139Z-12^T (ASM284427v1), *T. alkalitolerans* MBE#61^T (ASM211574v1), *T. mesophila* MBE#74^T (ASM211575v1), and *T. marina* CSC3H3^T (ASM284437v1)

Table 2 Comparative genomic characteristics of strain YYF002^T and phylogenetically related species of the genus *Winogradskyella*

Organism	ANI (%)	AAI (%)	DDH (%)	16S rRNA gene Similarity (%)
W. poriferorum JCM12885^T	88.2	89.9	34.7	99.2
W. algicola IMCC33238^T	84.1	86.0	27.7	96.7
W. tangerina M1309^T	75.7	77.1	19.4	95.2
W. flava KCTC 52348^T	76.3	76.8	19.4	95.9
W. luteola WHY3^T	76.9	77.5	20.1	95.4
W. ouciana ZXX205^T	77.1	78.5	20.2	95.0

The 16S similarity results originate from EzbioCloud. Strains (Genome accession number): YYF002^T (ASM2962412v1); *W. poriferorum* JCM12885^T (ASM3655362v1); *W. algicola* IMCC33238^T (ASM586993v1); *W. tangerina* M1309^T (ASM326020v1); *W. flava* KCTC 52348^T (ASM1424339v1); *W. luteola* WHY3^T(ASM1920398v1); *W. ouciana* ZXX205^T (ASM970961v1)

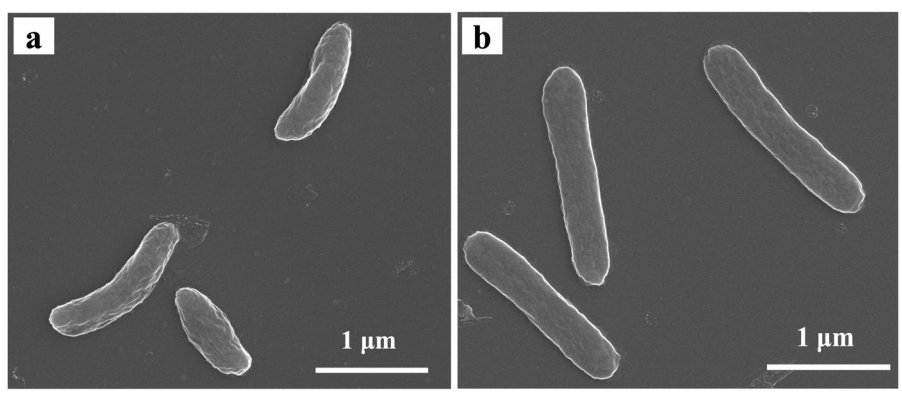

Fig. 3 Scanning electron micrograph of strains FZY0004^T (**a**) and YYF002^T (**b**). Cells were grown on R2A media at 28 °C for 2 days. Bar, 1.0 µm

Table 3 Differential characteristics between strain FZY0004^T and the reference strains

Characteristics	1	2	3
Colony colour	White	Opalescent	Cream–yellow
Cell size (μm)	0.3 – 0.5 × 1.0 – 1.8	0.3 – 0.8 × 0.8 – 2.3[a]	0.3 – 0.6 × 1.0 – 1.6[b]
Optimum temperature	28 °C	22 °C	30 °C
Optimum NaCl (%, w/v)	2–6%	3 – 4%	3–6%
Optimum pH	7.0	7.5	7.5
DNA G + C content (%)	54.5%	47.0%	61.2%
Major respiratory quinone(s)	Q – 9	Q – 10	Q – 9
Polar lipids	PE, PG, PL, L	PE, AL, GL, L[a]	PE, PG, PL, L
Main fatty acids (> 10%)	$C_{16:0}$, summed feature 3, summed feature 8	$C_{16:0}$, cyclo-$C_{17:0}$, cyclo-$C_{19:0}$ *ω8c*, summed feature 8	$C_{16:0}$, cyclo-$C_{17:0}$, summed feature 8
Assimilation of (API 20NE)			
L-arabinose	+	+	−
D-mannose	+	−	+
D-maltose	−	−	+
Potassium gluconate	−	+	+
Malic acid	+	−	−
Reduction of nitrate	−	+	+
Enzyme activities (API ZYM)			
Valine arylamidase	−	+	−
Acid phosphatase	w	+	+
Naphthol-AS-BI-Phosphohydrolase	w	+	+
B-galactosidase	+	−	−
α-glucosidase	−	−	+
β-glucosidase	+	−	−
α-fucosidase	+	−	−

Strains: 1, FZY0004^T; 2, *T. profundimaris* MCCC 1A00207^T; 3, *T. xianhensis* CGMCC 1.6849^T

All data are from this study unless otherwise indicated. A requirement for sea salts indicates that Na^+ alone does not support growth; instead, the strain requires sea salts for growth. Both strains are catalase- and oxidase- positive, aerobic and non-motile. According to the API 20NE and API ZYM kits, both strains were positive for D-glucose, D-mannitol, hydrolysis of esculin, *N*-acetylglucosamine, alkaline phosphatase, esterase(C_4), leucine arylamidase and lipase(C_{14}). All other results were negative. AL, aminolipid; GL, glycolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unidentified phospholipid; L, unidentified polar lipid. +, positive; −, negative; w, weakly positive

Data from: [a]Ivanova et al. (2016); [b]Zhao et al. (2010)

and YYF002^T with those of the reference strains are described in detail in Tables 3 and 4.

Chemotaxonomic characterization

The major fatty acids (> 10%) of strain FZY0004^T were summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) (14.5%), $C_{16:0}$ (22.2%) and summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*) (24.2%). $C_{16:0}$ is the most plentiful fatty acid in the closely related species of the genus *Thalassospira* investigated in this study. The detailed fatty acid compositions are presented in Table S5. However, some quantitative differences in fatty acid composition were observed between FZY0004^T and the other closely associated species of the genus *Thalassospira*. For example, FZY0004^T contained 24.2% summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*), but *T. profundimaris* MCCC 1A00207^T and *T. xianhensis* CGMCC 1.6849^T contained 12.1% and 29.7%, respectively. The major fatty acids (> 10%) of YYF002^T were iso-$C_{15:0}$ 3-OH (12.0%) and iso-$C_{15:1}$ G (29.3%), with iso-$C_{15:0}$ (33.1%) being the most plentiful fatty acid in the closely related species of the

Table 4 Differential characteristics between strain YYF002^T and the reference strain

Characteristics	1	2
Colony colour	Yellowish	Yellow
Cell size (μm)	0.3 − 0.4 × 1.5 − 1.8	ND
Motility	−	+
Optimum temperature	28 °C	28 °C
Optimum NaCl (%, w/v)	2–4%	2–3%
Optimum pH	7.5	7.0
Requirement for sea salts	+	−
DNA G + C content (%)	33.5%	33.5%
Polar lipids	AL, PE, L	AL, PE, L
Main fatty acids (> 10%)	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{15:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:0}$ 3-OH
Hydrolysis of gelatin	+	−
Assimilation of (API 20NE)		
L-arabinose	−	+
Potassium gluconate	−	+
Adipic acid	+	−
Enzyme activities (API ZYM)		
Esterase lipase (C_8)	w	+
Lipase (C_{14})	−	+
β-glucuronidase	+	−
α-glucosidase	+	−
α-mannosidase	+	−

Strains: 1, YYF002^T; 2, *W. poriferorum* JCM 12885^T

All data are from this study unless otherwise indicated. A requirement for sea salts indicates that Na^+ alone does not support growth; instead, the strain requires sea salts for growth. Both strains are catalase- and oxidase- positive, aerobic, have MK-6 as the major respiratory quinone. According to the API 20NE and API ZYM kits, both strains were positive for D-glucose, D-maltose, hydrolysis of esculin, acid phosphatase, alkaline phosphatase, esterase (C_4), leucine arylamidase, naphthol-AS-BI, phosphohydrolase, valine arylamidase, α-chymotrypsin, α-fucosidase, β-galactosidase and β-glucuronidase. All other results were negative. AL, aminolipid; PE, phosphatidylethanolamine; L, unidentified polar lipid. +, positive; −, negative; w, weakly positive; ND, not determined

genus *Winogradskyella* investigated in this study. The detailed fatty acid compositions are presented in Table S6. The polar lipids of strain FZY0004^T were found to be phosphatidylethanolamine (PE), phosphatidylglycerol (PG), unidentified phospholipids (PL), and unidentified lipids (L), with the major polar lipids being PE and PG. Polar lipids of YYF002^T included unidentified amino lipid (AL), phosphatidylethanolamine (PE), and unidentified lipids (L). The major polar lipids of YYF002^T were AL and PE, which are also the major polar lipids found in members of the genus *Winogradskyella*. The detailed polar lipid profiles of the two novel strains are provided in Fig. S3. The predominant respiratory quinones of strain FZY0004^T were identified as Q-9, whereas that detected in strain YYF002^T was MK-6.

Taxonomic conclusion

In summary, the phylogenetic topologies and phenotypic and chemotaxonomic characteristics supported that strains FZY0004^T and YYF002^T represent novel species of the genera *Thalassospira* and *Winogradskyella*, respectively, for which the names *Thalassospira aquimaris* sp. nov. and *Winogradskyella marincola* sp. nov. are proposed, respectively.

Description of *Thalassospira aquimaris* sp. nov.

Thalassospira aquimaris (a.qui.ma'ris. L. fem. n. aqua water; L. neut. n. mare the sea; N.L. gen. n. aquimaris of the water of the sea).

Cells are Gram-stain-negative, aerobic, non-motile, and curved rods with a size of 1.0–1.8 μm long and 0.3–0.5 μm wide. Colonies are white coloured, circular, convex, and 0.3–0.5 mm in diameter after 2 days of cultivation at 28 °C on R2A agar. The optimum temperature and pH for growth are 28 °C (range 10–37 °C) and 7.0 (6.0–9.0), respectively. NaCl is not essential for growth (tolerate up to 10% w/v; optimum, 2–6% w/v). Positive for H_2S production and Tween 80 hydrolysis, but negative for hydrolysis of starch, Tween 20, and Tween 60, and indole production. Catalase and oxidase are positive. The major fatty acids are summed feature 8 ($C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6c*), $C_{16:0}$, and summed feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*). The major respiratory quinone is Q-9 and the major polar lipids consist of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), unidentified phospholipids (PL), and unidentified lipids (L).

The type strain, FZY0004^T (=JCM 35895 ^{T}=MCCC 1K08380^T), was isolated indirectly from seawater in the Yellow Sea, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain FZY0004^T is OQ714501 and its draft genome was deposited in GenBank/EMBL/DDBJ under the accession number JARSBO000000000. The DNA G+C content of the type strain is 54.5% (by genome).

Description of *Winogradskyella marincola* sp. nov.

Winogradskyella marincola (mar.in'co.la. L. neut. n. *mare*, the sea; L. masc./fem. n. *incola* inhabitant; N.L. fem. n. *marincola,* inhabitant of the sea).

Cells are Gram-stain-negative, aerobic, non-motile, and rod-shaped and are 1.5–1.8 μm long and 0.3–0.4 μm wide. When grown on R2A agar at 28 °C for 2 days, colonies are circular, convex, glistening, viscid, translucent, yellowish, and 0.3–0.4 mm in diameter. Growth does not occur without sea salts. Cells grow at 10–37 °C (optimum, 28 °C), in the presence of 0.5–5% (w/v) NaCl (optimum, 2–4%) and at pH 5.0–9.0 (optimum, pH 7.5). Catalase and oxidase are positive. Cells hydrolyse Tween 20, Tween 60, and Tween 80, but do not hydrolyse starch, cellulose, and urease, and are negative for H_2S and indole production. The major fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G, and iso-$C_{15:0}$ 3-OH. The major respiratory quinone is MK-6 and the major polar lipids consist of unidentified aminolipid (AL), phosphatidylethanolamine (PE), and several unidentified lipids (L).

The type strain, YYF002^T (=JCM 35950 ^{T}=MCCC 1K08382^T), was isolated indirectly from seawater in the Yellow Sea, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YYF002^T is OQ714211 and its draft genome was deposited in GenBank/EMBL/DDBJ under the accession number JARSBN000000000. The DNA G+C content of the type strain is 33.5% (by genome).

Emended description of *Winogradskyella poriferorum* (Lau et al. 2005)

The description is as given previously (Lau et al. 2005) with the following amendment. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JCM 12885 ^{T} is AY848823 and its draft genome was deposited in GenBank/EMBL/DDBJ under the accession number JAZHOU010000000. The DNA G+C content of the type strain is 33.5% (by genome).

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Author contributions ZYF and DFZ designed the research and project outline. ZYF, MHH, HCW, XYC, and YFY performed isolation, deposition, and polyphasic taxonomy. ZYF performed genome analysis and drafted the manuscript. ZYF, DFZ, YY, and WJL, revised the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Competing interests The authors declare that they have no conflict of interest.

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