


Marinobacter profundus sp. nov., a slightly halophilic bacterium isolated from a deep-sea sediment sample of the New Britain Trench

Junwei Cao  · Ping Liu · Renju Liu · Hainan Su · Yuli Wei · Rulong Liu · Jiasong Fang

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Abstract A piezotolerant, cold-adapted, slightly halophilic bacterium, designated strain PWS21^T, was isolated from a deep-sea sediment sample collected from the New Britain Trench. Cells were observed to be Gram-stain negative, rod-shaped, oxidase- and catalase-positive. Growth of the strain was observed at 4–45 °C (optimum 37 °C), at pH 5.0–9.0 (optimum 7.0) and in 0.5–20% (w/v) NaCl (optimum 3–4%). The optimum pressure for growth was 0.1 MPa (megapascal) with tolerance up to 70 MPa. 16S rRNA gene

sequence analysis showed that strain PWS21^T is closely related to *Marinobacter guineae* M3B^T (98.4%) and *Marinobacter lipolyticus* SM19^T (98.2%). Multilocus sequence analysis (MLSA) based on sequences of housekeeping genes *gyrB*, *recA*, *atpD*, *rpoB* and *rpoD* indicates that strain PWS21^T represents a distinct evolutionary lineage within the genus *Marinobacter*. Furthermore, strain PWS21^T showed low ANI and diDDH values to the closely related species. The principal fatty acids were identified as C_{12:0}, C_{12:0} 3-OH, C_{16:1}ω9c, C_{16:0} and C_{18:1}ω9c. Ubiquinone-9 was identified as the major respiratory quinone. The polar lipids were identified as phosphatidylethanolamine (PE), phosphatidylglycerol

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J. Cao (✉) · P. Liu · Y. Wei · R. Liu · J. Fang (✉)
Shanghai Engineering Research Center of Hadal Science and Technology, College of Marine Sciences, Shanghai Ocean University, Shanghai 201306, People's Republic of China
e-mail: jwcao@shou.edu.cn

J. Fang
e-mail: jsfang@shou.edu.cn

J. Cao · P. Liu · Y. Wei
National Engineering Research Center for Oceanic Fisheries, Shanghai Ocean University, Shanghai 201306, People's Republic of China

R. Liu
Key Laboratory of Marine Genetic Resources, The Third Institute of State Oceanic Administration, Xiamen 361005, People's Republic of China

H. Su
State Key Laboratory of Microbial Technology, Marine Biotechnology Research Center, Shandong University, Jinan 250100, People's Republic of China

J. Fang
Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, People's Republic of China

J. Fang
Department of Natural Sciences, Hawaii Pacific University, Honolulu, HI 96813, USA

(PG), diphosphatidylglycerol (DPG), aminophospholipid (APL), two unidentified lipids and an unidentified phospholipid (PL). The G + C content of the genomic DNA was determined to be 60.3 mol%. On the basis of phenotypic, chemotaxonomic and molecular data, we conclude that strain PWS21^T represents a novel species of the genus *Marinobacter*, for which the name *Marinobacter profundus* sp. nov. is proposed (type strain PWS21^T = KCTC 52990^T = MCCC 1K03345^T).

Keywords Halophilic · *Marinobacter profundus* · New Britain Trench · Piezotolerant · Polyphasic taxonomy

Abbreviations

MCCC	Marine Culture Collection of China
KCTC	Korean Collection for Type Cultures
NCBI	National Center for Biotechnology Information
diDDH	The digital DNA–DNA hybridization
ANI	The average nucleotide identity

Introduction

The genus *Marinobacter*, belonging to the family *Alteromonadaceae*, was first proposed by Gauthier et al. (1992) and is comprised of 43 species isolated from diverse environments (Cui et al. 2016; Han et al. 2017). Species of this genus are Gram-stain negative, aerobic, and rod-shaped bacteria. The DNA G + C content of the genus ranges from 53.7 to 63.5 mol%, and the major fatty acids are C_{12:0}, C_{16:1}ω9c, C_{16:0} and C_{18:1}ω9c, and the principal respiratory quinone is Q-9 (ubiquinone 9) (Gauthier et al. 1992; Cui et al. 2016; Han et al. 2017; Kim et al. 2017). The genus *Marinobacter* includes halophilic bacteria from marine-related environments and the type species of the genus, *Marinobacter hydrocarbonoclasticus* ATCC 49840^T, is extremely halotolerant (Gauthier et al. 1992; Guo et al. 2007; Huo et al. 2008; Montes et al. 2008; Xu et al. 2008; Rani et al. 2017). During our recent screening of halophilic bacteria from samples collected from the New Britain Trench, we isolated bacterial strain PWS21^T, which was able to grow in 20% NaCl. Strain PWS21^T, isolated at a depth of 3908 m, was also found to be able to tolerate high

hydrostatic pressure. The strain is phylogenetically related to members of the genus *Marinobacter*. In this study, characterization and classification of strain PWS21^T were achieved using a polyphasic approach.

Materials and methods

Isolation and cultivation

Sediment samples were collected at a depth of 3908 m from the New Britain Trench (149.8°E, 6.6°S) in July 2016. Marine agar 2216 (MA; BD Difco) supplemented with NaCl (10%) was used to enrich the microbial consortium. Strain PWS21^T was isolated by the standard dilution plating technique at 30 °C on MA. The routine cultivation of the strain and phenotypic tests were carried out on MA, unless noted otherwise. Strain PWS21^T was maintained in marine broth 2216 (MB; BD Difco) with 20% (v/v) glycerol at – 80 °C. The type strains of *Marinobacter guineae* MCCC 1A00540^T (= M3B^T) and *Marinobacter lipolyticus* MCCC 1A03253^T (= DSM 15157^T = SM19^T), provided by Marine Culture Collection of China (MCCC; Xiamen, China), were used as reference strains.

High pressure cultivation

High pressure growth experiments were performed in pressure vessels under a range of hydrostatic pressures (0.1–80 MPa) at 30 °C, supplemented with oxygen-saturated Fluorinert (FC-40, Sigma. 25% of total volume) to supply oxygen as previously described (Kato et al. 1995; Fang et al. 2006).

Phenotypic and chemotaxonomic analyses

Cellular morphology and flagellum formation were observed using atomic force microscopy (Multimode Nanoscope VIII; Bruker AXS) with cells grown on MA at 30 °C for 24 h (Su et al. 2012). Gram-staining, oxidase and catalase activity, Voges–Proskauer reaction, methyl red test, H₂S production, indole production, and hydrolysis of starch, gelatin, casein, Tween 20, Tween 60 and Tween 80 were performed according to methods described by Dong and Cai (2001). The optimal growth temperature (4, 10, 20, 28, 37, 40, 45 and 50 °C) and pH (pH 4.0–11.0 in 0.5 unit

increments, by using HOMOPIPES, MES, PIPES, HEPES and CAPS buffers, respectively), growth at different NaCl concentrations (0, 0.5, 1, 2, 3, 4, 5, 10, 15 and 20%, w/v) were determined as previously described (Lai et al. 2014). Anaerobic growth was evaluated in MB and in MB in the presence of NaNO₃ (10 mM), prepared with a N₂ gas phase (200 kPa) in sealed sterile vials and incubated at 30 °C for 7 days. Other biochemical tests were carried out using API 20NE, API ZYM and API 50CH strips (bioMérieux) according to the manufacturer's instructions, with the NaCl concentration adjusted to 3.0% (w/v). Some tests in API strips, such as nitrate reduction, fermentation of D-glucose, aesculin hydrolysis and citrate utilization, were also re-examined by using conventional methods described by Dong and Cai (2001). To test carbon utilization, SO4PNSalts medium described by Cao et al. (2016), with a final concentration of 0.2% (w/v) carbon source was used.

Fatty acids in whole cells grown on MB at 30 °C for 48 h were saponified, extracted and methylated using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analysed by gas chromatography (Agilent Technologies 6850) and identified using the TSBA6.0 database of the Microbial Identification System (Sasser 1990). The fatty acid profile of strain *M. guineae* MCCC 1A00540^T (= M3B^T) was performed in parallel with strain PWS21^T under the same conditions (at the end of exponential growth phase). The respiratory quinone was extracted from cells of strain PWS21^T and analysed by high pressure liquid chromatography (Agilent 1200 and Thermo Finnigan LCQ DECA XP MAX mass spectrometer) (Wu et al. 2015). Polar lipids were extracted from 100 mg of freeze-dried cellular material, separated by two-dimensional silica gel TLC (Merck) and then identified according to a previously described method (Tindall et al. 2007). The polar lipid profiles of strains *M. guineae* MCCC 1A00540^T (= M3B^T) and *M. lipolyticus* MCCC 1A03253^T (= DSM 15157^T = SM19^T) were performed in parallel with strain PWS21^T under the same conditions.

Phylogenetic analyses

Genomic DNA was prepared according to the method of Ausubel et al. (2002) and the 16S rRNA gene was amplified by PCR using primers Bac8F (5'-AGA GTT

TGA TCA TGG CTC AG-3') and U1492R (5'-GGT TAC CTT GTT ACG ACT T-3') (Cao et al. 2016). Sequence similarity was determined using the EzBioCloud server (<https://www.ezbiocloud.net/>; Yoon et al. 2017). The phylogenetic analysis was performed using MEGA version 5.0 (Tamura et al. 2011). Distances were calculated using the Kimura two-parameter model and clustering was performed with the neighbor-joining (NJ) (Saitou and Nei 1987), maximum likelihood (ML) (Felsenstein 1981) and minimum evolution (ME) (Rzhetsky and Nei 1992, 1993) methods supported with bootstrap values based on 1000 replications. Multilocus Sequence Analysis (MLSA) based on sequences of housekeeping genes, *gyrB* (DNA gyrase β subunit), *recA* (recombinase A), *atpD* (ATP synthase β subunit), *rpoB* (RNA polymerase β subunit) and *rpoD* (RNA polymerase, sigma 70), was performed with the NJ, ML and ME methods supported with bootstrap values based on 1000 replications. Sequences of the housekeeping genes were downloaded from the NCBI database.

DNA–DNA relatedness

The draft genome sequences of strain PWS21^T and *M. guineae* MCCC 1A00540^T (= M3B^T) were determined at Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China), using Solexa paired-end (500 bp library) sequencing technology. The de novo assembly of the reads was performed using SOAPdenovo 2.04 and GapCloser1.12 (Luo et al. 2012). The genome sequence of *M. lipolyticus* SM19^T (ASAD000000000, Papke et al. 2013), *M. gudaonensis* CGMCC 1.6294^T (GCA_900115175), *M. salinus* Hb8^T (GCA_001854125), *M. mobilis* CN46^T (GCA_900106945), *M. adhaerens* HP15^T (GCA_000166295), *M. signi-crescens* SS011B1–4^T (GCA_900111555), *M. pelagius* HS225^T (GCA_900114925), *M. hydrocarbonoclasticus* ATCC 49840^T (GCA_000284615) and *M. salarii* R9SW1^T (GCA_000831005) were downloaded from the NCBI database. The average nucleotide identity (ANI) was calculated with the algorithm of Goris et al. (2007) using the EZGenome web service. The digital DNA–DNA hybridization (diDDH) estimate values were analysed using the genome-to-genome distance calculator (GGDC2.0) (Auch et al. 2010a, b; Meier-Kolthoff et al. 2013). The G + C content of the genomic DNA was determined from the draft genome sequence.

Results and discussion

High pressure growth

The optimum pressure for growth was determined to be 0.1 MPa, with tolerance up to 70 MPa (Fig. 1), which is much higher than that at the depth of origin (3908 m). This result shows that strain PWS21^T is piezotolerant.

Phylogenetic analysis

A nearly full-length 16S rRNA gene sequence (1409 nt, GenBank/EMBL/DDJB accession MF800963) of strain PWS21^T was determined. Comparative 16S rRNA gene sequence analysis showed that strain PWS21^T formed a cluster within the genus *Marinobacter*, within the family *Alteromonadaceae* (Fig. 2). In all three trees (NJ, ME and ML trees), strain PWS21^T formed a clade with *M. guineae* M3B^T (Montes et al. 2008). Strain PWS21^T shares high sequence similarity of 98.4% with *M. guineae* M3B^T, followed by *M. lipolyticus* SM19^T (98.2%) (Martin et al. 2003), *M. goseongensis* En6^T (98.0%) (Roh et al. 2008), *M. gudaonensis* CGMCC 1.6294^T (97.9%) (Gu et al. 2007), *M. salinus* Hb8^T (97.5%) (Rani et al. 2017), *M. mobilis* CN46^T (97.4%) (Huo et al. 2008), *M. adhaerens* HP15^T (97.2%) (Kaepfel et al. 2012), *M. segnicrescens* SS011B1–4^T (97.2%) (Guo et al. 2007), *M. pelagius* HS225^T (97.2%) (Xu et al. 2008), *M. hydrocarbonoclasticus* ATCC 49840^T (97.1%) (Gauthier et al. 1992), *M. salarius* R9SW1^T (97.1%) (Ng et al. 2014) and *M. maritimus* CK47^T (97.0%) (Shivaji et al. 2005). The level of 16S rRNA gene sequence similarities with closely

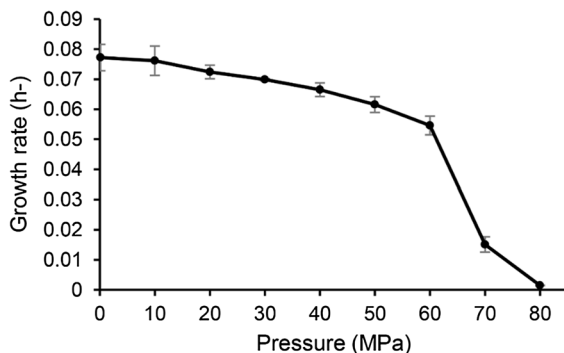


Fig. 1 Growth rate of strain PWS21^T at 30 °C and different pressures

related *Marinobacter* species showed that strain PWS21^T displayed sufficient molecular differences for delineation at the species level, because the values fall well below the threshold value (98.65–98.70%) currently recommended for demarcation of distinct species (Stackebrandt and Ebers 2006; Kim et al. 2014; Chun et al. 2018). Further MLSA showed that strain PWS21^T formed a cluster with the genus *Marinobacter* (Fig. 3) but represents a distinct evolutionary lineage within the genus *Marinobacter*. These results further affirmed that this strain represents a novel species.

The genome sequencing generated 1.04 and 1.17 G bytes clean data for strain PWS21^T and *M. guineae* MCCC 1A00540^T (= M3B^T), respectively. The de novo assembly resulted in 39 contigs (strain PWS21^T) and 37 contigs [*M. guineae* MCCC 1A00540^T (= M3B^T)]. The average genome coverage is 257 (strain PWS21^T) and 261 (*M. guineae* MCCC 1A00540^T). The draft genome accession numbers for strain PWS21^T and *M. guineae* MCCC 1A00540^T (= M3B^T) are NTFH000000000 (4,034,600 bp with 39 contigs, N50 = 354,806 bp) and NTFI000000000 (4,458,854 bp with 37 contigs, N50 = 847,285 bp), respectively. The ANI values of strain PWS21^T to *M. guineae* MCCC 1A00540^T (= M3B^T), *M. lipolyticus* SM19^T, *M. gudaonensis* CGMCC 1.6294^T, *M. salinus* Hb8^T, *M. mobilis* CN46^T, *M. adhaerens* HP15^T, *M. segnicrescens* SS011B1–4^T, *M. pelagius* HS225^T, *M. hydrocarbonoclasticus* ATCC 49840^T and *M. salarius* R9SW1^T are 76.2%, 75.9%, 76.4%, 75.1%, 74.7%, 76.2%, 75.3%, 77.8%, 76.3% and 76.2%, respectively, which are below the standard ANI criterion for species identity (95–96%) (Richter and Rosselló-Móra 2009). The diDDH estimate values between strain PWS21^T and *M. guineae* MCCC 1A00540^T (= M3B^T), *M. lipolyticus* SM19^T, *M. gudaonensis* CGMCC 1.6294^T, *M. salinus* Hb8^T, *M. mobilis* CN46^T, *M. adhaerens* HP15^T, *M. segnicrescens* SS011B1–4^T, *M. pelagius* HS225^T, *M. hydrocarbonoclasticus* ATCC 49840^T and *M. salarius* R9SW1^T were 20.6 ± 2.5%, 20.3 ± 2.4%, 20.6 ± 2.5%, 20.3 ± 2.4%, 19.5 ± 2.4%, 20.7 ± 2.4%, 21.9 ± 2.4%, 21.7 ± 2.5%, 21.2 ± 2.5% and 20.7 ± 2.4%, respectively, which are far below the standard criterion (70%) for delineation of prokaryotic species (Wayne et al. 1987). These results confirm that strain PWS21^T represents a novel genomic species of the genus *Marinobacter*.

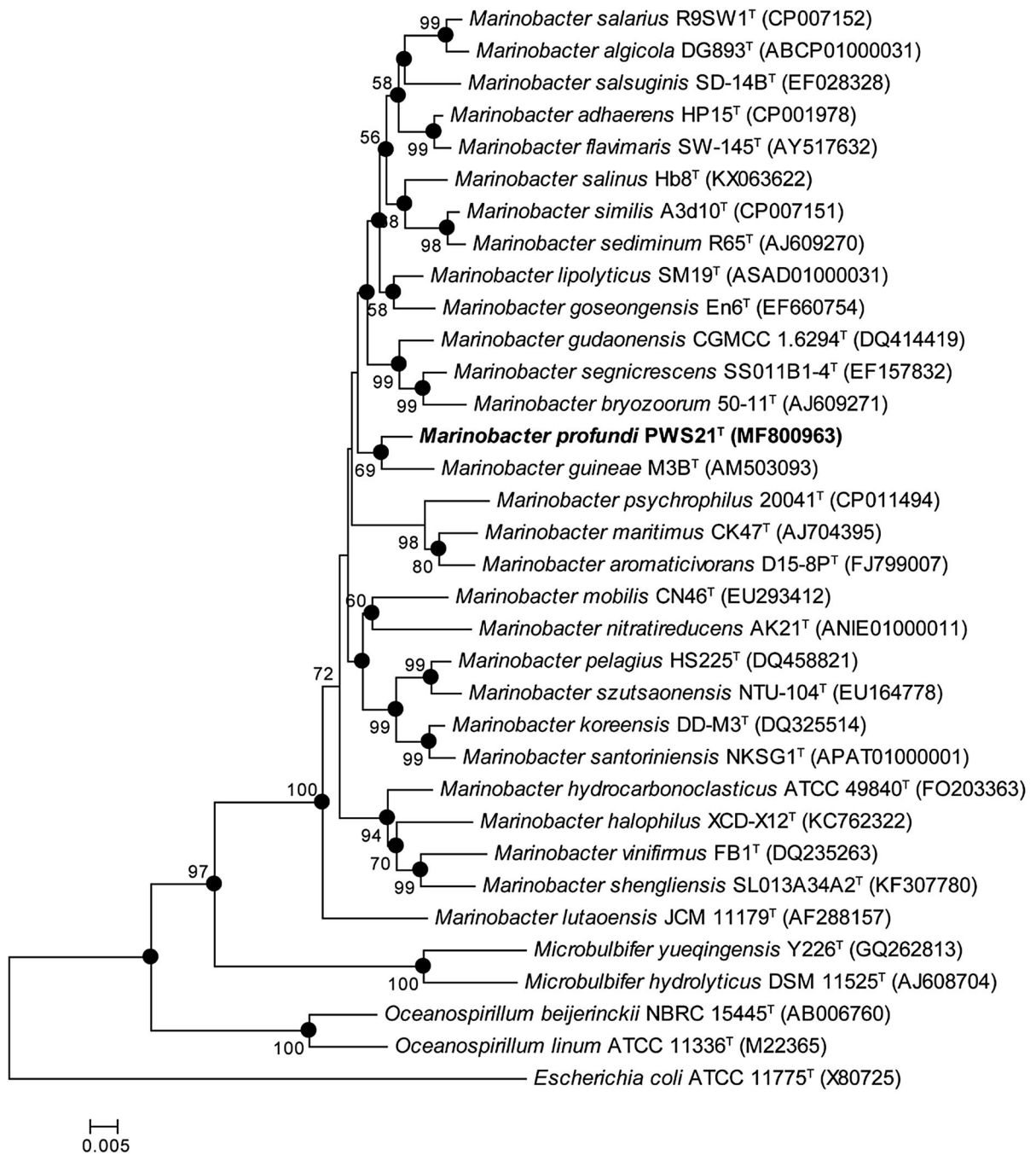


Fig. 2 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain PWS21^T and related species of the genus *Marinobacter*. *Escherichia coli* ATCC 11775^T (X80725) was used as an outgroup. Filled circles indicate nodes that were also recovered in the maximum-

likelihood and minimum evolution trees for the same sequences. Bootstrap values with neighbor-joining method (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.005 substitutions per nucleotide

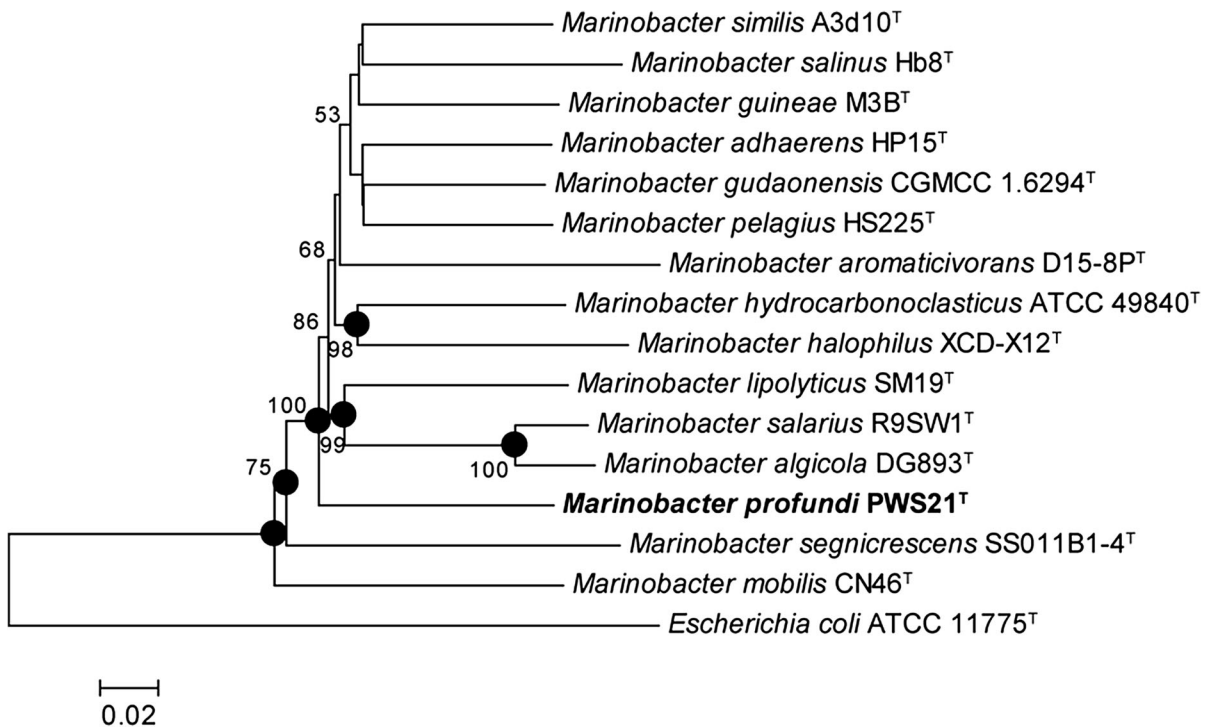


Fig. 3 MLSA of strain PWS21^T and closely related species. *Escherichia coli* ATCC 11775^T (JMST01000065) was used as an outgroup. The neighbor-joining tree was reconstructed based on five concatenated gene sequences (*gyrB*, *recA*, *atpD*, *rpoB* and *rpoD*). Filled circles indicate nodes that were also recovered

in the maximum-likelihood and minimum evolution trees for the same sequences. Bootstrap values with neighbor-joining method (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.02 substitutions per nucleotide

Phenotypic characteristics

Cells of strain PWS21^T were observed to be rod-shaped, approximately 1.2–2.3 μm long and 0.5–0.7 μm wide (Supplementary Fig. S1), catalase and oxidase positive, and Gram-stain negative. Colonies were observed to be smooth beige with regular edges on MA medium after 2 days of incubation at 30 °C. The strain was found to grow in 0.5–20% of NaCl (optimum 3–4%), from pH 5 to 9 (optimum 7), and at 4–45 °C (optimum 37 °C), but not at 50 °C within 2 weeks. Growth was observed under anaerobic conditions in the presence of NaNO₃. Aesculin, Tween 80, Tween 60 and Tween 20 were observed to be hydrolysed, but gelatin, casin and starch were not. Voges–Proskauer reaction, methyl red test, glucose fermentation, indole production and H₂S production was found to be negative. Nitrate reduction was observed to be positive. The strain was found to utilize D-glucose, lactose, adipic acid, pyruvate, malic acid, trisodium citrate and phenylacetic acid, but not

formate, D-mannitol, N-acetyl-glucosamine, fumarate, D-mannose, D-maltose, D-fructose, potassium gluconate, L-arabinose, lactate, or dextrin. In the API ZYM tests, strain PWS21^T was found to be positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and N-acetyl-β-glucosaminidase; negative for trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, or α-fucosidase. In the API 20NE tests, strain PWS21^T was found to utilize adipic acid, malic acid, trisodium citrate and phenylacetic acid, but not D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, or capric acid. Positive for reduction of nitrate, denitrification, beta-glucosidase (aesculin hydrolysis), but negative for indole production, D-glucose fermentation, gelatin hydrolysis or β-galactosidase. In the API 50CH tests, strain PWS21^T was found to produce

acid from glycerol, D-fructose, D-mannitol and D-glucose, but not others. The morphological, physiological and biochemical characteristics that differentiate strain PWS21^T from closely related species are listed in Table 1.

Chemotaxonomic characteristics

The predominant fatty acids of strain PWS21^T were identified as C_{12:0} (14.9%), C_{12:0} 3-OH (19.4%), C_{16:1}ω9c (18.6%), C_{16:0} (13.3%) and C_{18:1}ω9c (15.5%) (Table S1). C_{12:0}, C_{16:1}ω9c, C_{16:0} and C_{18:1}ω9c are characteristic of the genus *Marinobacter* (Cui et al. 2016), as well as strain PWS21^T and *M. guineae* MCCC 1A00540^T (= M3B^T). These results indicate that strain PWS21^T belongs to the genus *Marinobacter*, although several fatty acid features distinguished the novel strain

from the closely related species, *M. guineae* MCCC 1A00540^T (= M3B^T). For example, a distinct feature was that *M. guineae* MCCC 1A00540^T (= M3B^T) contained considerable amounts of summed feature 3 (C_{16:1} ω7cl C_{16:1}ω6c, 17.4%), of which there was only 2.5% in strain PWS21^T.

The polar lipids of strain PWS21^T were identified as phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), aminophospholipid (APL), two unidentified lipids and an unidentified phospholipid (PL), as shown in Supplementary Fig. S2A. The polar lipid profile of strain PWS21^T was similar to those of *M. guineae* MCCC 1A00540^T (Fig. S2B) and *M. lipolyticus* MCCC 1A03253^T (Fig. S2C), except some minor differences in unidentified lipids. The major respiratory quinone of strain PWS21^T was identified as ubiquinone 9 (Q-9). This

Table 1 Characteristics that differentiate strain PWS21^T from the closely related species of the genus *Marinobacter*

Characteristics	1	2	3
Cells size (μm)	0.5–0.7 × 1.2–2.3	0.4 × 1.4–4.0	0.3–0.5 × 2.5–3.5
Growth at 45 °C	+	–	–
Growth at 4 °C	+	+	–
Growth in 20% NaCl	+	–	–
Reduction of nitrate	+	+	–
Anaerobic growth (NO ₃ [–])	+	+	–
β-Glucosidase	+	+	–
α-Glucosidase	–	–	+
Urease activity	+	–	+
DNA G + C content (mol%)	60.3	57.0	56.8
<i>Growth on</i>			
N-acetyl-glucosamine	–	+	+
Dextrin	–	–	+
Lactose	+	+	–
D-Maltose	–	–	+
D-Fructose	–	+	–
Potassium gluconate	–	w	+
Adipic acid	+	–	–
Trisodium citrate	+	–	+
Phenylacetic acid	w	+	–
<i>Acid production from</i>			
D-Fructose	+	+	–
D-Mannitol	+	–	–
D-Maltose	–	–	+
Potassium gluconate	–	–	+
Amidon (Starch)	–	–	+
Glycogen	–	–	+

Strain: 1, PWS21^T; 2, *M. guineae* MCCC 1A00540^T (= M3B^T); 3, *M. lipolyticus* MCCC 1A03253^T (= SM19^T). All data were experimentally determined in this study under the same conditions, except for the data on cell size and DNA G + C content. Characteristics are scored as: w, weakly positive; +, positive; –, negative

trait is in accordance with the properties of the genus *Marinobacter*. These results suggested that strain PWS21^T is a member of the genus *Marinobacter*.

The DNA G + C content of strain PWS21^T was determined to be 60.3 mol%, which is in the range previously reported for *Marinobacter* species (54.0–63.5 mol%) (Cui et al. 2016).

Strain PWS21^T exhibits the typical characteristics of the genus *Marinobacter*. It has C_{12:0}, C_{16:1}ω9c, C_{16:0} and C_{18:1}ω9c as the major fatty acids, Q-9 as the major respiratory quinone, and PE, PG, DPG, APL and PL as the major polar lipids. The differences in physiological, biochemical and chemotaxonomic characteristics of strain PWS21^T and the closely species are given in Table 1. The data clearly shows differences between strain PWS21^T and the reference strains. On the basis of these characteristics, together with their low ANI and diDDH values, we conclude that strain PWS21^T represents a novel species of the genus *Marinobacter*, for which the name *Marinobacter profundus* sp. nov. is proposed.

The Digital Protologue database (Rosselló-Móra et al. 2017) TaxoNumber for strain PWS21^T is TA00453.

Description of *Marinobacter profundus* sp. nov.

Marinobacter profundus (pro.fun'di. L. gen. n. *profundus* of the depth of the ocean).

Cells are rods, about 1.2–2.3 μm long and 0.5–0.7 μm wide, catalase and oxidase positive, and Gram-stain-negative. On MA medium, produces smooth beige colonies with regular edges after 2 days of incubation at 30 °C. Growth occurs in 0.5–20% of NaCl (optimum 3–4%), from pH 5–9 (optimum 7), and at 4–45 °C (optimum 37 °C), but not at 50 °C within 2 weeks. The type strain is piezotolerant. The optimum pressure for growth is 0.1 MPa, with tolerance up to 70 MPa. Growth is observed under anaerobic conditions in the presence of NaNO₃. Aesculin, Tween 80, Tween 60 and Tween 20 are hydrolysed, but gelatin, casin and starch are not. Nitrate reduction is positive, but Voges–Proskauer reaction, methyl red test, glucose fermentation, indole production and H₂S production are negative. Principal fatty acids are C_{12:0}, C_{12:0} 3-OH, C_{16:1}ω9c, C_{16:0} and C_{18:1}ω9c. The major respiratory quinone is Q-9. The polar lipids comprise phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol

(DPG), aminophospholipid (APL), two unidentified lipids and an unidentified phospholipid (PL). The G + C content of the type strain is 60.3 mol%.

The type strain, PWS21^T (= KCTC 52990^T = MCCC 1K03345^T), was isolated from a deep-sea sediment sample collected at a depth of 3908 m from the New Britain Trench, Solomon Sea (149.8°E, 6.6°S). The GenBank accession number for the 16S rRNA gene sequence of strain PWS21^T is MF800963. The GenBank accession number for the genome sequence of strain PWS21^T is NTFH00000000.

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Conflict of interest The authors declare that they have no conflict of interest.

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