

Pseudooceanicola lipolyticus sp. nov., a marine alphaproteobacterium, reclassification of *Oceanicola flagellatus* as *Pseudooceanicola flagellatus* comb. nov. and emended description of the genus *Pseudooceanicola*

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Abstract

A Gram-stain-negative, rod-shaped bacterium, designated 157^T, was isolated from seawater collected from the Philippine Sea. Cells of strain 157^{T} grew in medium containing 0.5–10.0 % NaCl (w/v, optimum 3 %), at pH 6.0–8.5 (optimum 7.0) and at 15-40 °C (optimum 30 °C). Tweens 20, 40 and 80 as well as urea were hydrolysed. The 16S rRNA gene sequence of strain 157^{T} had a high sequence similarity with respect to *Pseudooceanicola marinus* AZO-C^T (97.2%), and exhibited less than 97.0 % sequence similarity to other type strains of the species with validly published names. Phylogenetic analyses revealed that strain 157^T fell within a cluster comprising the *Pseudooceanicola* species and formed a coherent clade with *P. marinus* AZO-C^T and *Pseudooceanicola antarcticus* Ar-45^T. Strain 157^T exhibited average nucleotide identity values of 74.5 and 74.9 % to P. marinus LMG 23705^T and P. antarcticus Ar-45^T, respectively. In silico DNA-DNA hybridization analysis revealed that strain 157^T shared 20.2 % DNA relatedness with *P. marinus* LMG 23705^T and 20.6 % with *P. antarcticus* Ar-45^T, respectively. The sole isoprenoid quinone was ubiquinone 10. The major fatty acids were summed feature 8 ($C_{18:1}\omega^{7}c$ and/or $C_{18:1}\omega^{6}c$), $C_{19:0}$ cyclo $\omega 8c$, $C_{16:0}$ 2-OH and $C_{16:0}$. The major polar lipids were phosphatidylcholine, phosphatidylethanolamine, phosphatidylqlycerol, one unidentified aminolipid and one unidentified qlycolipid. The DNA G+C content was 64.6 mol%. According to the phylogenetic, chemotaxonomic and phenotypic data, it represents a novel species of the genus Pseudooceanicola, for which the name Pseudooceanicola lipolyticus is proposed. The type strain is 157^{T} (=KCTC 52654^T=MCCC 1K03317^T). In addition, the description of the genus *Pseudooceanicola* is emended and *Oceanicola flagellatus* is reclassified as Pseudooceanicola flagellatus comb. nov., with the type strain DY470^T (=CGMCC 1.12664^T=LMG 27871^T) proposed.

The genus *Pseudooceanicola*, belonging to the family *Rhodobacteraceae* of the class *Alphaproteobacteria*, was proposed by Lai *et al.* [1]. It was divided from the genus *Oceanicola* based on the phylogenetic analysis of 16S rRNA gene sequences [1]. Currently, the genus *Pseudooceanicola* includes six species with validly published names, *Pseudooceanicola atlanticus* (type species), *Pseudooceanicola antarcticus*, *Pseudooceanicola batsensis*, *Pseudooceanicola marinus*, *Pseudooceanicola nanhaiensis* and *Pseudooceanicola nitratireducens* [1]. The *Pseudooceanicola* species were isolated from

seawater and sediments, and are aerobic or facultatively anaerobic, Gram-negative, rod-shaped, non-motile, oxidasepositive and catalase-positive [1–6]. Members of the genus *Pseudooceanicola* possess ubiquinone 10 (Q-10) as the major respiratory quinone and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), $C_{16:0}$, 11-methyl $C_{18:1}\omega7c$ and $C_{12:0}$ 3-OH as predominant fatty acids [1]. Here, we present a phenotypic, genotypic and chemotaxonomic characterization of strain 157^T, which was isolated from seawater. In addition, based on the results from Lai *et al.* [1], *Oceanicola flagellatus* is

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 157^T is KY273603. The GenBank accession numbers for the whole genome sequences of strain 157^T, *P. marinus* LMG 23705^T and *P. antarcticus* Ar-45^T are PGTB00000000, PGTC00000000 and PGTD00000000. Two supplementary figures are available with the online version of this article.

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Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization; LTP, Living Tree Project; MA, marine agar; MB, marine broth; Q-10, ubiquinone 10; TLC, thin-layer chromatography.

therefore reclassified as *Pseudooceanicola flagellatus* comb. nov., with the type strain DY470^T (=CGMCC 1.12664^{T} =LMG 27871^T) proposed.

During research on the bacterial diversity of seawater in December 2015, samples were collected from the Philippine Sea at a depth of 100 m using a rosette sampler connected to a CTD system (SBE911 plus, Sea-Bird Electronics). Aboard the ship, the seawater sample was serially diluted and spread on natural seawater agar (natural seawater supplemented with 0.05% peptone, 0.01% yeast extract and agar 1.5 %, w/v, pH 7.2-7.4) immediately. Then, the plates were incubated at room temperature (approximately 25-28°C) for 1 month on the vessel and transported to the laboratory when the vessel returned. Colonies were picked out and purified on marine agar 2216 (MA, BD) by repeated restreaking. One cream colony, named 157^{T} , was obtained in this study and the purity was confirmed by the uniformity of cell morphology by phase-contrast microscopy (DM 5000B, Leica). The reference strain, P. marinus LMG 23705^T, was obtained from the collection of the Laboratorium voor Microbiologie en Microbiele Genetica and P. antarcticus Ar-45^T was available in our laboratory [2]. Unless otherwise stated, these strains were routinely cultured in marine broth 2216 (MB, BD) or on MA at 30 °C. For long-term storage, strain 157^T was preserved as suspensions in 25% (v/v) glycerol at -80° C.

Cellular morphology, ultrastructure and size were observed with cells from the exponential phase grown on MA. The temperature range for growth was investigated at various temperatures (4, 10, 15, 20, 25, 30, 35, 40, 45 and 50°C). The pH range for growth was measured from 5.0 to 10.0 at intervals of 0.5 at 30 °C. Biological buffers (50 mM, MES for pH 5.0-6.0, PIPES for pH 6.5-7.0, Tricine for pH 7.5-8.5 and CAPSO for pH 9.0-10.0) were supplemented in MB to maintain pH. Growth at different sea salt concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0, 4.5 and 5.0%) was tested in PY medium (5.0 g peptone, 1.0 g yeast extract and 1 l distilled water, pH 7.2). Growth at various salinities (0, 0.5, 1.0, 3.0, 5.0, 7.5, 10.0 and 15.0%) was tested in NaCl-free MB medium (prepared according to the MB formula, but without NaCl). Growth was measured at OD₆₀₀ in a UV/visible spectrophotometer (Ultrospec 6300 Pro, Amersham Biosciences). Upper and lower limits for growth were confirmed when no visible turbidity was observed after 1 month's incubation. Anaerobic growth was carried out by using the AnaeroPack (Mitsubishi). Sodium nitrate (20 mM) or sodium nitrite (20 mM) was used as a potential electron acceptor.

Unless stated otherwise, physiological and biochemical tests were performed in MB at 30 $^{\circ}$ C. Gram reaction, oxidase and catalase activities, hydrolysis of starch (1.0 %, w/v) and Tweens 20, 40 and 80 (0.5 %, w/v) were tested based on the methods given in Dong and Cai [7]. The utilization of carbohydrates as sole carbon and energy sources was determined in BM medium [8]. The corresponding filter-

sterilized complex nutrients (yeast extract, peptone or tryptone, 0.2 %, w/v), sugars (0.2 %, w/v), alcohols (0.2 %, w/v), organic acids (0.1 %, w/v) or amino acids (0.1 %, w/v)were added. Acid production tests were performed in the marine oxidation-fermentation medium [9]. Filter-sterilized substrates were added at a final concentration of 1.0 %. Other physiological and biochemical tests were performed in API 20NE and API ZYM strips (bioMérieux) according to the manufacturers' instructions. Sea salt (2.0%, w/v, Sigma) was added to the AUX medium according to the manufacturer's recommendations. Antibiotic susceptibility was tested using the plate method and the diameter of the antibacterial circles was measured. Susceptibility to antibiotics was determined following the criteria described by Wu et al. [10]. Two reference strains, P. antarcticus Ar-45^T and *P. marinus* LMG 23705^T, were used as controls in the above

Isoprenoid quinones were extracted from cells of strain 157^{T} with chloroform/methanol (2:1, v/v). Extracts were evaporated to dryness at 35 °C, resuspended in chloroform/ methanol (2:1, v/v) and purified by thin-layer chromatography (TLC) on GF254 silica gel plates (Qingdao Haiyang) with N-hexane/ether (17:3, v/v) [10]. The isoprenoid quinones were analysed by high-performance liquid chromatography-mass spectrometry (Agilent 1200 and Thermo Finnigan LCQ DECA XP MAX mass spectrometer) [10]. Total lipids were extracted and separated by two-dimensional TLC on silica gel 60 F254 plates (Merck). Four kinds of spray reagents were used to detect the corresponding lipids, including molybdophosphoric acid for total lipids, molybdenum blue for phosphorus-containing lipids, ninhydrin reagent for lipids containing free aminolipids and *p*-anisaldehyde reagent for glycolipids [11]. Cellular fatty acids methyl esters were extracted from cells collected from the quadrant three (at the late exponential phase) along the streaking axis after incubation of 48 h and analysed according to the instructions of the Microbial Identification System (MIDI).

Genomic DNA was extracted using the bacterial genomic DNA fast extraction kit (DongSheng Biotech). Bacterial domain-specific primer sets 27F and 1492R were used [12], and 16S rRNA gene sequence amplification was performed. PCR products were cloned into vector pMD 19 T (TaKaRa) and sequenced to determine the almost-complete sequence of the 16S rRNA gene. Then, the 16S rRNA gene sequence of strain 157^T was compared with closely related sequences of reference organisms via BLAST analysis (http://blast.ncbi. nlm.nih.gov/Blast.cgi) and the EzTaxon-e service [13]. Phylogenetic analysis was performed in ARB release 6.0.2 [14] in the All-Species Living Tree Project (LTP) database (LTP_s123, November 2014, [15]). The 16S rRNA gene sequence of 157^{T} was aligned with SINA (version 1.2.11) according to the SILVA seed alignment (http://www.arb-silva. de; [16]) and implemented into the LTP database. Based on the obtained BLAST, EzTaxon-e and phylogenetic resolution results, 46 species were selected for further phylogenetic

analysis and *Oceanibaculum indicum* P24^T was used as the outgroup. The multiple sequences were retrieved based on the accession numbers and aligned by using CLUSTALW [17]. Phylogenetic trees were reconstructed using the MEGA 5.0 program package [18] by neighbour-joining [19], minimum-evolution [20] and maximum-likelihood methods [21]. Bootstrap analysis (1000 resample datasets) was used to evaluate the tree topologies. Kimura's two-parameter model [22] was used for phylogeny reconstruction and evolutionary distance analyses.

The genomes of strain 157^{T} , *P. marinus* LMG 23705^{T} and *P. antarcticus* Ar- 45^{T} were sequenced by HiSeq X-Ten PE150 platform (Annoroad Gene Technology for strain 157^{T} and Novogene Technology for type strains, respectively). The sequencing generated approximate 1 Gb clean data (approximate 250-fold genome coverage). The *de novo* assembly of the reads was performed using SPAdes version 3.9.0 [23] for strain 157^{T} and ABySS version 1.5.2 [24] for *P. marinus* AZO-C^T and *P. antarcticus* Ar- 45^{T} . The quality of microbial genomes was assessed using the bioinformatics tool CheckM [25]. The average nucleotide identity (ANI) was calculated using the OrthoANIu algorithm by Chun Lab's online ANI calculator [26]. *In silico* DNA–DNA hybridization (DDH) values were calculated by using the Genome-to-Genome Distance Calculator [27].

Cells of strain 157^T were Gram-stain-negative, non-sporeforming, rod-shaped, 0.6-1.0 µm wide and 1.5-2.5 µm long (Fig. S1, available in the online version of this article). Colonies were cream, circular, convex, smooth and 1-2 mm in diameter after incubation at 30 °C on MA for 3 days. Strain 157^T grew well on MA, but not on tryptic soy agar (BD) or Luria-Betrani agar (BD). The pH and temperature range for growth were pH 6.0-8.5 (optimum at pH 7.0) and 15-40 °C (optimum at 30 °C). No growth occurred at 10 or 45 °C after 1 month's incubation. The NaCl concentration range for growth was 0.5-10.0 % (w/v, optimum at 3.0 %). Strain 157^{T} was positive for oxidase and catalase activities, hydrolysis of Tweens 20, 40 and 80 and urea, susceptible to (μ g per disc unless otherwise stated) amoxicillin (20), ampicillin (10), carbenicillin (100), cefoxitin (30), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), novobiocin (30), penicillin (10), polymyxin B (300 IU), rifamcin (5), tetracycline (30) and tobtamycin (10), weakly susceptible to nitrofurantoin (300) and streptomycin (10), and resistant to bacitracin (0.04 U), nystatin (100) and vancomycin (30). Detailed phenotypic characteristics are given in the species description and Table 1.

The almost-complete 16S rRNA gene sequence of strain 157^{T} (1452 nt) was obtained. Based on the EzTaxon service results, strain 157^{T} showed a high 16S rRNA gene sequence similarity to *P. marinus* AZO-C^T (97.2%), and exhibited less than 97.0% sequence similarity to other type strains within the family *Rhodobacteraceae*. The All-Species Living Tree indicated that the genus *Pseudooceanicola* forms a monophyletic clade and strain 157^{T} fell within the cluster comprising the *Pseudooceanicola* species (data not shown).

In the phylogenetic tree based on the neighbour-joining method, strain 157^{T} fell within the cluster of the genus *Pseu-dooceanicola* and formed a coherent clade with *P. marinus* AZO-C^T and *P. antarcticus* Ar-45^T. The clade had moderate bootstrap support and represented an independent lineage. Similar results were obtained using the maximum-likelihood and minimum-evolution algorithms (Fig. 1). Phylogenetic analysis indicated that strain 157^{T} represents a novel member of the genus *Pseudooceanicola*.

The genome completeness of strain 157^T, P. antarcticus Ar- 45^{T} and *P. marinus* LMG 23705^{T} were 96.2, 98.5 and 99.1 %, with a contamination percentage of 0.63, 0.25 and 0.9%, respectively. Genome sequences estimated to be >95 % completeness, with <5 % contamination, are considered as excellent reference genomes for deeper analyses [25]. The DNA G+C content of strain 157^{T} was 64.6 mol%, a value in the range reported for members of the genus Pseudooceanicola, i.e. 61.8-72.8 mol% [1-6]. Strain 157^{T} exhibited ANI values of 74.5 and 74.9% to P. marinus AZO-C^T and *P. antarcticus* Ar-45^T, respectively. The *in silico* DDH values (the recommended results from formula 2) indicated that strain 157^T shared 20.2 % DNA relatedness with P. marinus AZO-C^T and 20.6 % with P. antarcticus Ar-45^T, respectively. The ANI values and *in silico* DDH values were strikingly lower than the threshold values of the species boundary (ANI 94-96 % and in silico DDH 70 %) [28, 29], revealing a low taxonomic relatedness between strain 157^T and the two reference strains of the *Pseudooceanicola* species. These values suggested that strain 157^T represents a different genomic species.

The chemotaxonomic results also supported the results of the phylogenetic analysis. Fatty acid analysis revealed that summed feature 8 ($C_{18:1}\omega_7c$ and/or $C_{18:1}\omega_6c$) and $C_{19:0}$ cyclo ω_8c were the major fatty acids in strain 157^T as well as the reference strains (Table 2). The sole isoprenoid quinone was Q-10, which is a common characteristic in the genus *Pseudooceanicola*. The polar lipid profiles indicated that strain 157^T possessed phosphatidylcholine, phosphatidylglycerol and one unidentified aminolipid as the major compounds, which was similar to the reference strains (Fig. S2). In addition, strain 157^T possessed three unidentified glycolipids, one unidentified phospholipid and one unidentified lipid, which were also found in the two reference strains (Fig. S2).

The chemotaxonomic data results also showed some clear differences between strain 157^{T} and the reference strains. Differences in the presence of fatty acids and in their proportion were detected. For instance, strain 157^{T} contained $C_{16:0}$ 2-OH (6.0%) as a major fatty acid, which was not detected in the reference strains (Table 2). The percentage of $C_{10:0}$ 3-OH of strain 157^{T} (3.1%) was higher than that of the reference strains ($\leq 0.7\%$). Oppositely, the ratio of $C_{16:0}$ of strain 157^{T} (5.3%) was much lower than that of the reference strains (16.3-21.1%). In addition, phosphatidylethanolamine was detected in strain 157^{T} as one of the major polar lipids, which was not found in the reference

Table 1. Different characteristics between strain 157^{T} and its closely related *Pseudooceanicola* species

Strains: 1, strain 157^T; 2, *P. antarcticus* Ar-45^T; 3, *P. marinus* LMG 23705^T; All data was obtained from this study under identical conditions. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3
Growth in NaCl (%, w/v):			
Range	0.5-10.0	0.5-10.0*	2.0-8.0
Optimum	3.0	0.5-3.0*	3.0-5.0
Growth temperature (°C):			
Range	15-40	4-40*	4-42†
Optimum	30	35-37*	28-35
Hydrolysis of:			
Aesculin	-	+	+
Tween 20	+	+	_
Tweens 40 and 80	+	_	_
Enzyme activities:			
Acid phosphatase	+	W	+
α -Glucosidase	-	+	+
β -Glucosidase	-	_	+
N -acetyl- β -glucosaminidase	-	+	_
Trypsin	W	_	W
Assimilation of:			
Aesculin	-	_	W
Adipic acid	+	W	_
L-Alanine and sodium acetate	+	_	+
Lactose	-	_	W
D-Mannitol	+	_	W
D-Mannose	-	W	+
N-Acetyl-glucosamine and phenylacetic acid	+	_	_
Trisodium citrate	+	+	_
Acid production from:			
D-Galactose, glucose, lactose, maltose, D-mannose, L-rhamnose, sucrose and trehalose	_	+	+
Ribitol	-	_	+
Susceptibility to (ug per disc):			
Amoxicillin (20), ampicillin (10), carbenicillin (100), penicillin (10) and polymyxin B (300 IU)	+	+	-
DNA G+C content (mol%)	64.6	62.0*	70.9†

strains (Fig. S2). One unidentified glycolipid and three unidentified lipids were present in strain 157^{T} as moderate or minor amounts, while these were not detected in the reference strains. In addition, one unidentified lipid was present in the reference strains, while it was absent in strain 157^{T} .

In conclusion, strain 157^{T} exhibits the typical characteristics of the genus *Pseudooceanicola*, such as having Q-10 as the sole respiratory quinone and summed feature 8 as the major fatty acid. However, strain 157^{T} could also be distinguished from the type strains of its closely related species by phenotypic characteristic differences, such as NaCl tolerance, temperature range, optimum pH, hydrolysis of aesculin and Tweens 20, 40 and 80, enzyme activities in API tests, carbohydrate utilization and acid production

(Table 1). For instance, strain 157^{T} could not grow at 10° C, while its close relatives were able to grow at 4° C. Strain 157^{T} hydrolysed Tweens 40 and 80, while the reference strains could not. Oppositely, the reference strains produced acid from D-galactose, glucose, lactose, maltose, D-mannose, L-rhamnose, sucrose and trehalose, but strain 157^{T} did not. Other differences are given in Table 1.

On the basis of the phylogenetic analysis, chemotaxonomic results, as well as phenotypic characteristics, strain 157^{T} represents a new species of the genus *Pseudooceanicola*, for which the name *Pseudooceanicola lipolyticus* is proposed. In addition, *Oceanicola flagellatus* is reclassified as *Pseudooceanicola flagellatus* comb. nov., with the type strain DY470^T (=CGMCC 1.12664^T=LMG 27871^T) based on the results from Lai *et al.* [1].

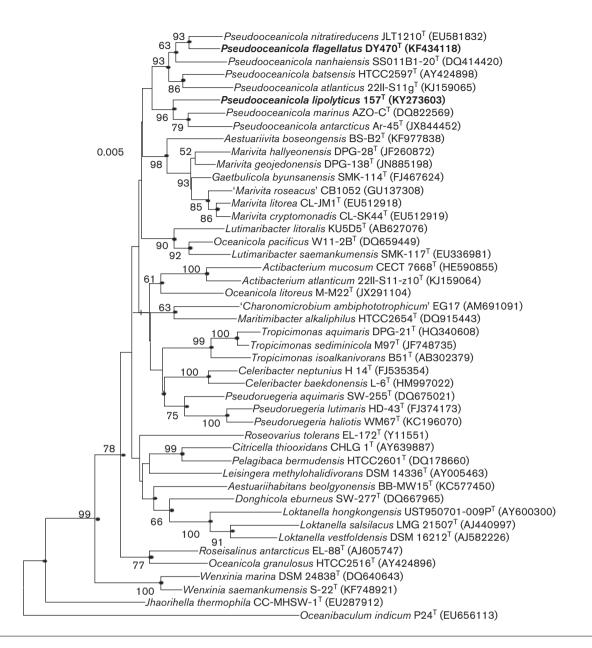


Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationships of the isolate and its related taxa. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and minimum-evolution algorithms. Bar, 0.005 substitutions per nucleotide position.

EMENDED DESCRIPTION OF THE PSEUDOOCEANICOLA LAI ET AL. 2015

In addition to the characteristics reported by Lai *et al.* [1], the following properties are observed. Cells of most species are non-motile, and some species are motile based on the presence of flagella [2]. The major fatty acids include summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), 11-methyl $C_{18:1}\omega7c$, $C_{19:0}$ cyclo $\omega8c$, $C_{16:0}$, $C_{12:0}$ 3-OH or $C_{16:0}$ 2-OH.

DESCRIPTION OF *PSEUDOOCEANICOLA LIPOLYTICUS* SP. NOV.

Pseudooceanicola lipolyticus (li.po.ly'ti.cus. Gr. n. *lipos*, animal fat; N.L. adj. *lyticus -a -um* (from Gr. adj. *lytikos* $-\hat{e} - on$), able to loosen, able to dissolve; N.L. masc. adj. *lipolyticus*, fat-dissolving).

Cells are Gram-stain-negative, non-spore-forming, rodshaped, non-motile, $0.6-1.0 \,\mu\text{m}$ wide and $1.5-2.5 \,\mu\text{m}$ long. Colonies are cream, circular, smooth, flat and 1 mm in

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IP: 183.**413**.162.147 On: Fri. 11 Jan 2019 06:57:55 **Table 2.** Comparison of cellular fatty acid composition of strain 157^{T} and related *Pseudooceanicola* species

Strains: 1, strain 157^{T} ; 2, *P. antarcticus* Ar- 45^{T} ; 3, *P. marinus* LMG 23705^{T} ; All data was obtained from this study. Fatty acids representing less than 0.5% in all strains were omitted. –, Not detected; TR, traces (<0.5%).

Fatty acid (%)	1	2	3
Straight-chain:			
C _{10:0}	TR	-	0.7
C _{16:0}	5.3	16.3	21.1
C _{18:0}	1.1	1.3	1.6
Unsaturated:			
11-methyl $C_{18:1}\omega7c$	3.1	5.6	5.9
$C_{19:0}$ cyclo $\omega 8c$	19.5	22.9	36.5
$C_{20:2}\omega 6,9c$	0.5	0.6	1.4
Hydroxy:			
C _{10:0} 3-OH	3.1	0.7	0.5
С _{12:1} 3-ОН	3.4	7.1	4.8
C _{16:0} 2-OH	6.0	-	-
Summed features*			
3	TR	1.7	1.7
8	55.5	43.1	25.0

*Summed features represent groups of two fatty acids that could not be separated by gas-liquid chromatography with the MIDI system. Summed feature 3 contained $C_{16:1}\omega^7c$ and/or $C_{16:1}\omega\delta c$; summed feature 8 contained $C_{18:1}\omega^7c$ and/or $C_{18:1}\omega\delta c$.

diameter after incubation for 3 days at 30 °C on MA. Grow on NaCl-free MB supplemented with 0.5-10.0% (w/v) NaCl (optimum 3.0%). The pH and temperature ranges for growth are pH 6.0-8.5 and 15-40 °C (optimum at pH 7.0 and 30 °C). No anaerobic growth occurs on MA supplemented with sodium nitrate or sodium nitrite. Positive for oxidase and catalase activities. Negative for nitrate reduction, indole production and arginine dihydrolase. Tweens 20, 40 and 80 and urea are hydrolysed, while aesculin, gelatin and starch not. The following substances are utilised as single carbon resource for growth: adipic acid, L-alanine, malate, D-mannitol, N-acetyl-glucosamine, peptone, phenylacetic acid, sodium acetate, trisodium citrate, yeast extract and tryptone, but not aesculin, L-arabinose, capric acid, ethanol, D-glucose, lactose, maltose, D-mannose, potassium, raffinose, sodium gluconate, sodium malate, L-sorbose and sucrose. L-Cysteine, DL-isoleucine and trehalose are weakly used. Acid is produced from D-mannitol. Acid is not produced from ethanol, D-galactose, glucose, D-inositol, lactose, maltose, D-mannose, melezitose, raffinose, L-rhamnose, ribitol, D-sorbitol, L-sorbose, sucrose and trehalose. Acid and alkaline phosphatases, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BIphosphohydrolase and valine arylamidase activities are present, whereas α -chymotrypsin, α -fucosidase, α - and β galactosidases, α - and β -glucosidases, β -glucuronidase, lipase (C14), α -mannosidase and N-acetyl- β -glucosaminidase activities are absent. The sole respiratory quinone is Q-10. The major fatty acid (>5%) is summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), $C_{19:0}$ cyclo $\omega8c$, $C_{16:0}$ 2-OH and $C_{16:0}$. The major polar lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, one unidentified aminolipid and one unidentified glycolipid. In addition, moderate to minor amounts of four unidentified glycolipids, one unidentified phospholipid and five unidentified lipids are observed.

The type strain 157^{T} (=KCTC 52654^{T} =MCCC 1K03317^T), was isolated from seawater from the Philippine Sea. The DNA G+C content is 64.6 mol% (by genome).

DESCRIPTION OF *PSEUDOOCEANICOLA FLAGELLATUS* COMB. NOV.

Pseudooceanicola flagellatus (fla.gel.la'tus. L. n. *flagellum* a whip; L. masc. suff. *-atus* suffix denoting provided with; N. L. masc. adj. *flagellatus* flagellated).

Basonym: Oceanicola flagellatus Huo et al. (2014) [2].

The description is as given for *Oceanicola flagellatus* by Huo *et al.* [2].

The type strain is DY470^T (=CGMCC 1.12664^{T} =LMG 27871^{T}).

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References

- Lai Q, Li G, Liu X, Du Y, Sun F et al. Pseudooceanicola atlanticus gen. nov. sp. nov., isolated from surface seawater of the Atlantic Ocean and reclassification of Oceanicola batsensis, Oceanicola marinus, Oceanicola nitratireducens, Oceanicola nanhaiensis, Oceanicola antarcticus and Oceanicola flagellatus, as Pseudooceanicola batsensis comb. nov., Pseudooceanicola marinus comb. nov., Pseudooceanicola nitratireducens comb. nov., Pseudooceanicola nanhaiensis comb. nov., Pseudooceanicola antarcticus comb. nov., and Pseudooceanicola flagellatus comb. nov. Antonie van Leeuwenhoek 2015;107:1065–1074.
- Huo YY, Li ZY, You H, Wang CS, Post AF et al. Oceanicola antarcticus sp. nov. and Oceanicola flagellatus sp. nov., moderately halophilic bacteria isolated from seawater. Int J Syst Evol Microbiol 2014;64:2975–2979.
- Lin KY, Sheu SY, Chang PS, Cho JC, Chen WM. Oceanicola marinus sp. nov., a marine alphaproteobacterium isolated from seawater collected off Taiwan. Int J Syst Evol Microbiol 2007;57:1625–1629.
- Cho JC, Giovannoni SJ. Oceanicola granulosus gen. nov., sp. nov. and Oceanicola batsensis sp. nov., poly-beta-hydroxybutyrateproducing marine bacteria in the order 'Rhodobacterales'. Int J Syst Evol Microbiol 2004;54:1129–1136.
- Gu J, Guo B, Wang YN, Yu SL, Inamori R et al. Oceanicola nanhaiensis sp. nov., isolated from sediments of the South China Sea. Int J Syst Evol Microbiol 2007;57:157–160.
- Zheng Q, Chen C, Wang YN, Jiao N. Oceanicola nitratireducens sp. nov., a marine alphaproteobacterium isolated from the South China Sea. Int J Syst Evol Microbiol 2010;60:1655–1659.

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- Dong X, Cai M. Determinative Manual for Routine Bacteriology. Beijing: Scientific Press; 2001.
- Farmer III JJ, Janda JM, Brenner FW, Cameron DN, Birkhead KM et al. Genus I. Vibrio Pacini 1854, 411^{AL}. In: Garrity GM, Brenner DJ, Krieg NR and Staley JT (editors). Bergey's Manual of Systematic Bacteriology, 2nd ed, vol. 2, The Proteobacteria, Part B, The Gammaproteobacteria. New York: Springer; 2005. pp. 494.
- 9. Leifson E. Determination of carbohydrate metabolism of marine bacteria. J Bacteriol 1963;85:1183.
- Wu YH, Xu L, Zhou P, Wang CS, Oren A et al. Brevirhabdus pacifica gen. nov., sp. nov., isolated from deep-sea sediment in a hydrothermal vent field. Int J Syst Evol Microbiol 2015;64:3645–3651.
- Chen C, Su Y, Tao T, Fu G, Zhang C et al. Maripseudobacter aurantiacus gen. nov., sp. nov., a novel member of the family Flavobacteriaceae isolated from a sedimentation basin. Int J Syst Evol Microbiol 2017;67:778–783.
- Cheng H, Zhang S, Huo YY, Jiang XW, Zhang XQ et al. Gilvimarinus polysaccharolyticus sp. nov., an agar-digesting bacterium isolated from seaweed, and emended description of the genus Gilvimarinus. Int J Syst Evol Microbiol 2015;65:562–569.
- Kim OS, Cho YJ, Lee K, Yoon SH, Kim M et al. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 2012;62:716–721.
- Ludwig W, Strunk O, Westram R, Richter L, Meier H et al. ARB: a software environment for sequence data. *Nucleic Acids Res* 2004; 32:1363–1371.
- Yarza P, Richter M, Peplies J, Euzeby J, Amann R et al. The All-Species Living Tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. Syst Appl Microbiol 2008;31:241– 250.
- Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W et al. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 2007;35:7188–7196.
- Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;22:4673–4680.

- Tamura K, Peterson D, Peterson N, Stecher G, Nei M et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28:2731–2739.
- Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406– 425.
- Rzhetsky A, Nei M. Statistical properties of the ordinary leastsquares, generalized least-squares, and minimum-evolution methods of phylogenetic inference. J Mol Evol 1992;35:367–375.
- Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 1981;17:368–376.
- Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980;16:111–120.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–477.
- Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ et al. ABySS: a parallel assembler for short read sequence data. *Genome Res* 2009;19:1117–1123.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015; 25:1043–1055.
- Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–1103.
- Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
- Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
- Moore L, Moore E, Murray R, Stackebrandt E, Starr M. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 1987;37:463–464.

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