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Paracoccus xiamenensis sp. nov., isolated from seawater on the Xiamen

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Abstract

Strain 12-3^T was isolated from seawater of the Guanyinshan Coast, Xiamen, Fujian Province, PR China. The bacterium was Gram-stain-negative, rod-shaped, aerobic, oxidase-positive and catalase-negative. Growth of strain 12-3^T occurred at 10–37 °C (optimum, 20–30 °C), at pH 5.0–11.0 (optimum, pH 7.0–8.0) and at a salinity range of 0–10% (optimum, 3–5%). The results of phylogenetic analysis based on its 16S rRNA gene sequence indicated that strain 12-3^T belonged to the genus *Paracoccus* and had the highest sequence similarity to *Paracoccus lutimaris* HDM-25^T (97.4%), followed by *Paracoccus isoporae* SW-3^T (96.9%), *Paracoccus caeni* MJ17^T (96.9%), *Paracoccus pacificus* F14^T (96.8%) and other species in the genus *Paracoccus* (95.3–96.5%). The average nucleotide identity (ANI) and DNA–DNA hybridization (DDH) values between strain 12-3^T and *P. lutimaris* HDM-25^T were 76.1 and 17.0%, respectively. ANI and DDH values between strain 12-3^T and *P. isoporae* SW-3^T were 78.9 and 18.2%, respectively. The principal fatty acid of strain 12-3^T was summed feature 8 ($C_{18:1}$ $\omega6c/\omega7c$) and $C_{18:0}$. The respiratory quinone of strain 12-3^T was Q10. The polar lipids included phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and an unidentified glycolipid. The G+C content of the chromosomal DNA was 63.9 mol%. The combination of the results of the phylogenetic, phenotypic and chemotaxonomic analyses, and its low ANI and DDH values indicate that strain 12-3^T represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus xiamenensis* sp. nov. is proposed. The type strain is 12-3^T (=MCCC 1A16381^T=KCTC 72687^T).

During an investigation of bacterial diversity in seawater of the Guanyinshan Coast, Xiamen, strain 12-3^T was isolated and identified taxonomically along with many other isolates. Comparative 16S rRNA sequence similarity indicated that strain 12-3^T was assigned to the genus *Paracoccus*, a member of the family *Rhodobacteraceae*, class Alphaproteobacteria. The genus *Paracoccus* was first proposed by Davis *et al.* and *Paracoccus denitrificans* is the type species [1]. At the time of writing, the genus *Paracoccus* contained 68 recognized species with validly published names (www.bacterio.net/paracoccus.html) [2]. Species of genus *Paracoccus* were isolated from various environments, including water [3–5], air [6], soil [7, 8], rhizosphere [9, 10], activated sludge [11, 12], sediment [13–15] and fish [16]. This study describes the taxonomic position of strain 12-3^T by a polyphasic approach.

The seawater sample was collected in May 2018 from the intertidal zone of the Guanyinshan Coast, Xiamen, Fujian Province, PR China. The sample was serially diluted with sterilized seawater from 10^{-1} to 10^{-8} and spread on marine agar (MA; BD Difco), followed by aerobic incubation at 28 °C for 3–5 days. Then, colonies with different morphology were picked and purified by repeated restreaking. Finally, strain 12-3^T was obtained from these colonies. In the present study, *Paracoccus pacificus* F14^T (=MCCC 1A09947^T) from the MCCC [4] and *Paracoccus denitrificans* DSM 413^T from the DSMZ [1] were used as reference type strains. Strain 12-3^T and the reference strains were routinely cultured in MA medium at 28 °C in this study unless otherwise indicated.

To conduct the phylogenetic analysis, genomic DNA of strain 12-3^T was extracted using the Bacterial Genomic Extraction Kit (Shanghai SBS GenetechCo., Ltd.) according

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Keywords: *Paracoccus xiamenensis* sp. nov.; taxonomy; seawater; Xiamen.
Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization; DPG, diphosphatidylglycerol; GL, unidentified glycolipid; MA, marine agar; MB, marine broth; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; UBCG, up-to-date bacterial core gene.
The GenBank accession number for the 16S rRNA gene sequence of *Paracoccus xiamenensis* 12-3^T is MT137387. The draft genome accession number for strain 12-3^T is JAAOHY000000000.
Three supplementary figures and one supplementary table are available with the online version of this article.

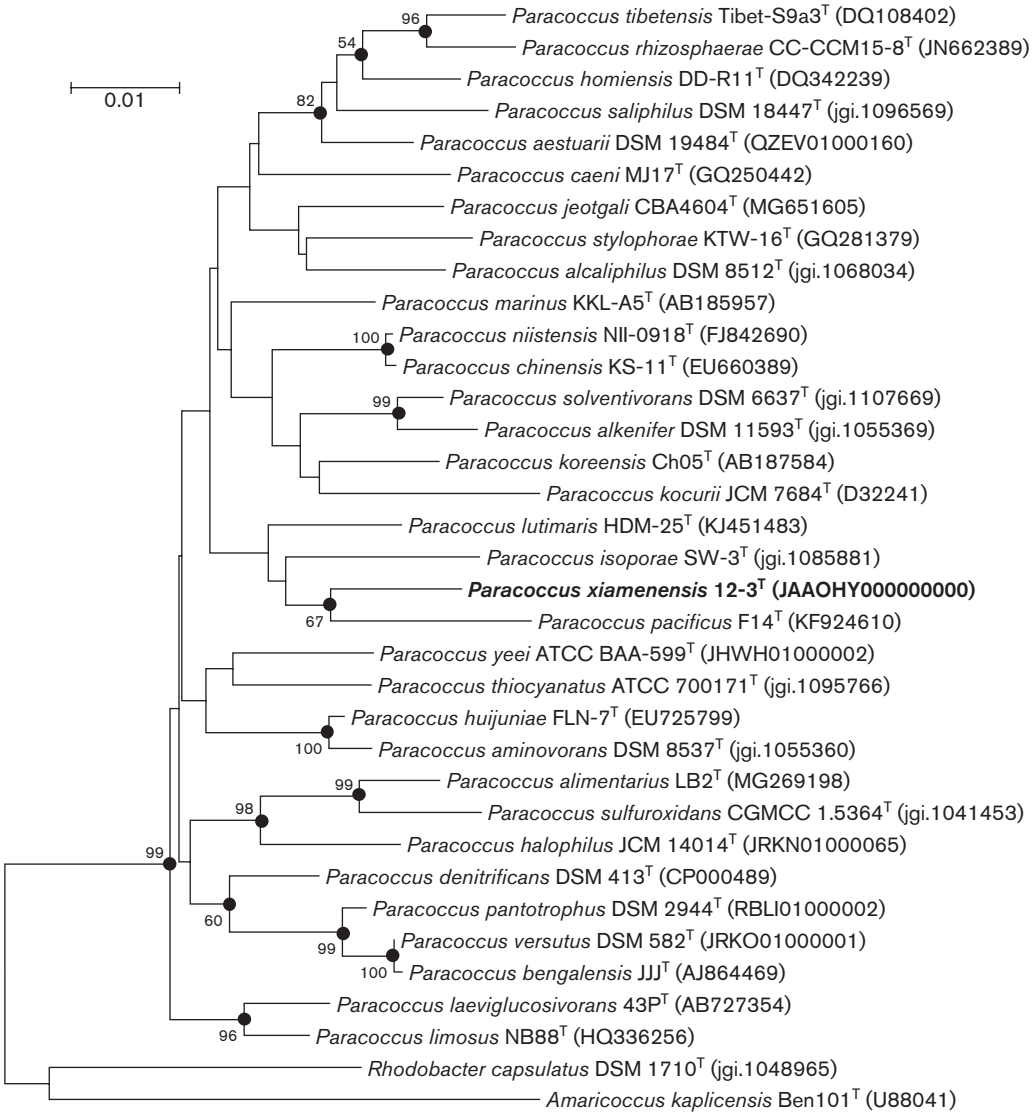

Fig. 1. Neighbour-joining tree showing the phylogenetic position of strain 12-3^T and the related species within the genus Paracoccus based on the 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position. *Rhodobacter capsulatus* DSM 1710^T (jgi.1048965) and *Amaricoccus kaplicensis* Ben101^T (U88041) are used as outgroups.

to the manufacturer's instructions. The 16S rRNA gene sequence was PCR-amplified using 27F and 1492R primers as described previously [17]. Sequence similarity was obtained by comparing the 16S rRNA gene sequence of strain 12-3^T and sequences of the closely related strains in the EzBioCloud database [18, 19]. A phylogenetic tree based on the 16S rRNA gene sequences was reconstructed using MEGA version 7.0 [20] based on the neighbour-joining [21], minimum-evolution [22] and maximum-likelihood [23] algorithms with bootstrap values of 1000 replications [24]. Evolutionary distances of the three phylogenetic trees were calculated based on the Kimura's two-parameter model [25]. The genome-based phylogenetic tree was reconstructed using the UBCG [26].

The 16S rRNA gene sequence of strain 12-3^T (1453 bp) was determined and deposited into GenBank (MT137387). Based on the 16S rRNA gene sequence, strain 12-3^T showed the highest similarity to *Paracoccus lutimaris* HDM-25^T (97.4%), followed by *Paracoccus isoporae* SW-3^T (96.9%), *Paracoccus caeni* MJ17^T (96.9%), *Paracoccus pacificus* F14^T (96.8%) and other species in the genus *Paracoccus* (95.3–96.5%). This indicates that strain 12-3^T represents a novel strain of the genus *Paracoccus* according to the 16S rRNA

gene sequence similarity of 98.65% (the cut-off for definition of the new species) [27]. As shown in phylogenetic trees (Fig. 1), strain 12-3^T formed a branch with *P. pacificus* F14^T, *P. isoporae* SW-3^T and *P. lutimaris* HDM-25^T.

The genome sequence of strain 12-3^T was determined using Solexa paired-end (500 bp library) sequencing technology by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, PR China). A total of 1 Gbp sequence data was retrieved for strain 12-3^T by the Hiseq 2000 platform (Illumina) and assembled by SPAdes (version 3.8.1) with the default settings [19]. There were 21 contigs of the retrieved genomic sequence data for strain 12-3^T. The N50 and L50 values of strain 12-3^T were 330263 bp and 3, respectively. The genome size of strain 12-3^T was 3643702 bp, which was less than *P. lutimaris* HDM-25^T with its genome size of 4206125 bp obtained from the NCBI database. The draft genome sequence of strain 12-3^T was deposited at NCBI to obtain GenBank accession number JAAOHY000000000. According to its draft genome sequence, the chromosomal DNA G+C content of strain 12-3^T was calculated to be 63.9 mol%, which was less than that of *P. lutimaris* HDM-25^T (65.9 mol%) [28] and close to *P. isoporae* SW-3^T (63.7 mol%) [29]. The phylogenomic tree generated with the UBCG showed that strain 12-3^T also formed a branch with *P. isoporae* SW-3^T within the genus *Paracoccus* (Fig. S1, available in the online version of this article).

The average nucleotide identity (ANI) value between two genomes was calculated using the EzBioCloud platform [30]. The ANI values were 76.1 and 78.9% between strain 12-3^T and *P. lutimaris* HDM-25^T, and between strain 12-3^T and *P. isoporae* SW-3^T, respectively. They were below standard ANI criteria for species identity (95–96%) [31]. The DNA–DNA hybridization (DDH) value was calculated using the Genome-to-Genome Distance Calculator 2.1 (GGDC 2.1) with alignment method of BLAST+ [32–34]. The DDH estimate values between strain 12-3^T and *P. lutimaris*, and between strain 12-3^T and *P. isoporae* SW-3^T were 17.0 and 18.2%, which were far below the standard cut-off value (70%) [35]. The ANI and DDH values indicate that strain 12-3^T represents a novel species of the genus *Paracoccus*.

Gram-staining was carried out using a Gram-staining kit (Hangzhou Tianhe Microorganism Reagent Co.) according to the manufacturer's instructions. Cell morphology and flagellation pattern were observed by transmission electron microscopy (JEM-1230, JEOL) after negative staining with phosphotungstic acid. Oxidase activity was evaluated by the oxidation of 1% (w/v) *N*,*N*,*N*′,*N*′-tetramethyl-1,4-phenylenediamine. Catalase activity was tested by observing bubble production in hydrogen peroxide solution (v/v, 3%). The temperature range for growth of strain 12-3^T was determined on MA at 4, 10, 15, 18, 20, 28, 30, 35, 37, 42, 45 and 50 °C, respectively. The pH range for growth was tested in MB (marine broth 2216) medium at various pH values (pH 3.0–12.0, at intervals of 1 pH unit). pH of the MB medium was adjusted with citrate/phosphate (pH 3.0–7.0), Tris/HCl (pH 8.0–9.0) or sodium carbonate/sodium bicarbonate (pH 10.0) buffers. Salt tolerance was examined in Luria–Bertani medium [36] supplemented with NaCl concentrations of 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10% (w/v). API ZYM, 20NE and 20E strip (bioMérieux) tests of strain 12-3^T and the two reference strains were carried out following the manufacturer's instructions. These results are given in the species description and Table 1.

To determine the fatty acids of strain 12-3^T and the two reference strains, their cell biomass was harvested after growing in MA medium for 48 h at 28 °C. Then they were saponified, extracted and methylated based on the standard MIDI protocol (Sherlock Microbial Identification System, version 6.0B) [37]. The fatty acids were determined by gas chromatography (Agilent Technologies 6850) and identified using the TSBA6.0 database of the Microbial Identification System [38]. The cellular fatty acid profiles of strain 12-3^T and the reference strains are shown in Table S1. The principal fatty acids of strain 12-3^T were summed feature 8 ($C_{18:1}$ ω6c/$C_{18:1}$ ω7*c*) and $C_{18:0}$, which are consistent with those of reference strains. The compositions of the fatty acids varied between strain 12-3^T and the reference strains. For example, $C_{17:1}$ ω7*c* and $C_{19:0}$ 10-methyl were found in strain 12-3^T and not in the reference strains. In strain 12-3^T, there was no $C_{17:1}$ ω8*c*, which was detected in the reference strains.

The respiratory quinones in strain 12-3^T were extracted and separated by TLC on silica gel, and further determined by high performance liquid chromatography–mass spectrometry [39, 40]. The respiratory quinone of strain 12-3^T was ubiquinone (Q10, 100%), the major quinone of the genus *Paracoccus* [10] (Table 1). Polar lipids of strain 12-3^T were extracted using a chloroform/methanol system and analysed by one- and two-dimensional TLC on silica gel 60 F254 aluminium-backed thin-layer plates (Merck). The plate dotted with sample was subjected to two-dimensional development, with the first solvent system of chloroform–methanol–water (v/v/v; 65:25:4) and the second solvent of chloroform–methanol–acetic acid–water (v/v/v; 85:12:15:4). The TLC plates were sprayed with molybdatophosphoric acid for determining total lipids [41]. Polar lipids of strain 12-3^T are composed of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and unidentified glycolipid (GL) (Fig. S2). Among them, DPG, PG and PC are also present in other strains of genus *Paracoccus* [4, 29]. However, PE was determined in strain 12-3^T and *P. pacificus* F14^T, but not in *P. denitrificans* DSM 413^T, *P. isoporae* SW-3^T and *P. lutimaris* HDM-25^T. GL was found in strain 12-3^T, *P. isoporae* SW-3^T and *P. lutimaris* HDM-25^T, but not in *P. denitrificans* DSM 413^T and *P. pacificus* F14^T.

The results of phylogenetic, phenotypic and chemotaxonomic analyses mentioned above demonstrate that strain 12-3^T should be classified into the genus *Paracoccus* and has distinguishing characteristics from its related species

Table 1. Differential characteristics of strain 12-3^T and the closely related species of the genus *Paracoccus*

Strains: 1, 12-3^T; 2, *Paracoccus denitrificans* DSM413^T [42]; 3, *Paracoccus pacificus* F14^T [4]; 4, *Paracoccus isoporae* SW-3^T [28]; 5, *Paracoccus lutimaris* HDM-25^T [27]. All data were obtained from this study under the same conditions, except where indicated. All strains were positive for oxidase, catalase, alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase and α-glucosidase; negative for trypsin, α-chymotrypsin and *N*-acetyl-*β*-glucosaminidase. The API 20NE, API 20E and API ZYM tests for strains 1–3 were conducted under the same conditions in present study. Characteristics are scored as: +, positive; –, negative; ND, no data.

Characteristic*	**1**	**2**	**3**	**4**	**5**
Growth temperature (°C):					
Range	10–37	10–40[a]	4–40[b]	4–40[c]	10–37[d]
Optimum	20–30	20–30[a]	28[b]	25–30[c]	30[d]
Growth pH:					
Range	5.0–11.0	6.0–8.0[a]	6.0–10.0[b]	7–10[c]	5.0–9.5[d]
Optimum	7–8	7.0[a]	7.0–8.0[b]	9.0–10.0[c]	7.0–8.0[d]
Growth in NaCl (%, w/v):					
Range	0–10	0–7[a]	0–7[b]	0–12[c]	0–7[d]
Optimum	3–5	2[a]	1–2[b]	3–5[c]	2–3[d]
Quinone	Q-10	ND	Q-10[b]	Q-10[c]	Q-10[d]
DNA G+C content (mol%)	63.9	66.3[a]	61.4[b]	63.7[c]	65.9[d]
Polar lipids	DPG, PG, PC, PE, GL	PG, DPG, PE, PC, L1–L4, AL, PL[a]	DPG, PG, PC, PE, L1–L3[b]	DPG, PG, PC, AL1, GL1, GL2, PL1–PL4[c]	ND
API ZYM results:					
Esterase lipase (C8), valine arylamidase	+	+	+	+[c]	–[d]
Lipase (C14), *α*-galactosidase, *β*-galactosidase, *β*-glucosidase, *α*-mannosidase, *α*-fucosidase	–	–	–	+[c]	–[d]
Cystine arylamidase	+	+	+	–[c]	+[d]
Naphthol-AS-BI- phosphoamidase	–	–	+	+[c]	+[d]
β-Glucuronidase	–	–	–	–[c]	+[d]
API 20NE and 20E results:					
Nitrate reduction, D-glucose	+	+	–	–[c]	+[d]
Indole production	–	–	–	–[c]	ND
Arginine dihydrolase	+	+	+	–[c]	ND
Urease	+	+	+	–[c]	–[d]
Aesculin hydrolysis	+	–	–	+[c]	+[d]
Gelatin hydrolysis	–	+	–	–[c]	–[d]
β-Galactosidase	+	–	–	+[c]	–[d]
D-Arabinose, capric acid, phenylacetic acid, H_2S production, tryptophane deaminase, inositol, sucrose, melibiose, amygdalin	–	–	–	ND	ND
D-Mannose	+	–	–	ND	+[d]
D-Mannitol, maltose	+	+	–	+	+[d]
N-Acetyl-glucosamine, potassium gluconate, malic acid, trisodium citrate	+	+	–	ND	ND
Adipic acid	–	+	–	ND	ND

Continued

Table 1. Continued

Characteristic*	1	2	3	4	5
Lysin decarboxylase, ornithine decarboxylase	+	+	+	ND	ND
Citrate utilization	+	+	+	ND	+[d]
Urease	+	+	−	−	−[d]
Sodium pyruvate/acetoin production	+	−	+	ND	+[d]
Gelatinase	−	−	+	−	−[d]
D-Mannitol	−	−	−	+	+[d]
D-Sorbitol, L-arabinose	−	−	−	ND	+[d]
L-Rhamnose	−	−	−	ND	−[d]

*Data taken from: *a*, Chen *et al.* [42]; *b*, Zhang *et al.* [4]; *c*, Chen *et al.* [28]; *d*, Jung *et al.* [27].

(Tables 1 and S1). These results indicate that strain 12-3^T represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus xiamenensis* sp. nov. is proposed.

DESCRIPTION OF *PARACOCCUS XIAMENENSIS* SP. NOV.

Paracoccus xiamenensis (xia.men.en'sis. N.L. masc. adj. *xiamenensis* pertaining to Xiamen, a district in Fujian, PR China where type strain was isolated).

Cells are Gram-stain-negative, aerobic, rod-shaped (Fig. S3), 0.5–1.0 µm wide, 0.8–2.0 µm long and catalase- and oxidase-positive. Colonies are creamy, circular, smooth, convex and 1–2 mm in diameter with entire edges after 3 days incubation on MA plate at 28 °C. Growth occurs in a salinity range of 0–10% (optimum, 3–5%), at 10–37 °C (optimum, 20–30 °C) and at pH range of pH 5.0–11.0 (optimum, pH 7.0–8.0). Major fatty acid is summed feature 8 ($C_{18:1}\omega6c/\omega7c$) and $C_{18:0}$. The respiratory quinone is Q10. The polar lipids are PC, PE, PG, DPG and GL. In API ZYM test results, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and α-glucosidase; negative for lipase (C14), trypsin, α-chymotrypsin, *N*-acetyl-β-glucosaminidase, α-galactosidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. In API 20NE test strip results, positive for nitrate reduction, arginine dihydrolase, urease, aesculin hydrolysis, β-galactosidase, *N*-acetyl-glucosamine, malic acid, and assimilation of D-glucose, D-mannose, D-mannitol, maltose, potassium gluconate and trisodium citrate; negative for indole production, fermentation of D-glucose, gelatin hydrolysis, assimilation of D-arabinose, capric acid, adipic acid and phenylacetic acid. In API 20E test results, positive for arginine dihydrolase, lysin decarboxylase, ornithin decarboxylase, citrate utilization, β-galactosidase, urease and sodium pyruvate; negative for H_2S production, tryptophane deaminase, indole production, gelatinase, fermentation of D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin and L-arabinose. The DNA G+C content of strain 12-3^T is 66.9 mol%.

The type strain, 12-3^T (=MCCC 1A16381^T=KCTC 72687^T), was isolated from seawater of Guanyinshan Coast, Xiamen, Fujian Province, PR China. The GenBank accession numbers for the 16S rRNA gene sequence and the draft genome sequence of 12-3^T are MT137387 and JAAOHY000000000, respectively.

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Conflicts of interest
The authors declare that there is no conflict of interest.

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