Paracoccus sediminis sp. nov., isolated from Pacific Ocean marine sediment

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Strain CMB17^T was a short rod-shaped bacterium isolated from marine sediment of the Pacific Ocean. Cells were Gram-stain-negative and non-motile. Optimal growth occurred at 25–30 °C, pH 6.5–7 and 0.5–1 % (w/v) NaCl. The major fatty acid was $C_{18:1}\omega7c$ (87.59 %), and ubiquinone-10 was detected as the only isoprenoid quinone. The DNA G+C content of the genomic DNA was 62.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain CMB17^T is most closely related to *Paracoccus stylophorae* KTW-16^T (96.7 %), *P. solventivorans* DSM 6637^T (96.4 %) and *P. saliphilus* YIM 90738^T (96.4 %). Based on phenotypic, genotypic and phylogenetic characteristics, strain CMB17^T is proposed to represent a novel species, denominated *Paracoccus sediminis* sp. nov. (type strain CMB17^T=JCM 18467^T=DSM 26170^T=CGMCC 1.12681^T).

The genus *Paracoccus*, phylogenetically classified in the class *Alphaproteobacteria* (Harker *et al.*, 1998), was proposed by Davis *et al.* (1969). At the time of writing, 35 species of the genus *Paracoccus* with validly published names have been described (Kämpfer *et al.*, 2012; Dastager *et al.*, 2011; Lee *et al.*, 2011; Sheu *et al.*, 2011) and the type species is *Paracoccus denitrificans* (Davis *et al.*, 1969). All strains representing the genus *Paracoccus* have been characterized as Gram-negative, catalase-positive and oxidase-positive. Moreover, $C_{18:1}\omega7c$ and ubiquinone-10 are the predominant fatty acid and isoprenoid quinone of this genus, respectively.

Strain CMB17^T was isolated from a marine sediment sample collected from the East China Sea (125° 59′ 24″ E 30° 58′ 16″ N) at a depth of 70 m (Xu *et al.*, 2011). The sediment was diluted with sterile seawater and spread on modified marine agar 2216 containing the same ingredients as marine agar 2216 (MA; Difco), except that peptone and yeast were reduced to 0.1 g l^{-1} and 0.5 g l^{-1}, respectively. The sample was subsequently cultured at 28 °C until diverse colonies formed. Among the 33 bacteria isolated, strain CMB17^T showed relatively low 16S rRNA gene sequence similarity to known species, prompting its selection for further research. All purified strains were preserved at −80 °C with 25 % (v/v) glycerol. Reference strains used in this paper were *Paracoccus stylophorae* KTW-16^T, *P. homiensis* DD-R11^T, *P. saliphilus* YIM 90738^T and *P. denitrificans* DSM 413^T.

Cell morphology and motility was observed using optical microscopy (BX40; Olympus) and electron microscopy (JEM-1230; JEOL) after incubation on MA at 30 °C for 2 days. Accumulation of poly-β-hydroxybutyrate granules was assessed by staining with Sudan Black and observing with optical microscopy. Optimal growth conditions were determined in marine broth 2216 (MB; Difco) in duplicate. The temperature range for growth was tested at 4–45 °C with intervals of 5 °C. The pH range for growth was measured in the presence of 25 mM MES (pH 4.5–6.0), PIPES (pH 6.5–7.5), Tricine (pH 8.0–8.5) and CAPSO [3-(cyclohexylamino)-2-hydroxy-1-propane sulfonic acid (pH 9.0–9.5)]. The effect of salt was tested at different concentrations of NaCl (0, 0.5, 1, 2, 3, 4, 5, 7, 9, 11, 13 %, w/v). Anaerobic growth was determined in modified MB to which 20 mM sodium thiosulfate, 5 mM sodium sulfite, 20 mM sodium sulfate, 5 mM sodium nitrite, 20 mM sodium nitrate, 5 g L-arginine l^{-1} and 0.5 g cysteine l^{-1} were respectively added as electron acceptors. Hungate tubes filled with N_2 were used for incubation.

Catalase, oxidase, DNase, lipase and hydrolysation of starch and Tweens 20, 40, 60 and 80 were determined as described previously (Gerhardt *et al.*, 1994; MacFaddin, 2000). Hydrolysis of alginate was tested on MA supplemented with 1 % (w/v) sodium alginate (Hosoya *et al.*, 2009). Other biochemical properties and enzyme activities were tested using API ZYM and API 20 NE (BioMérieux). Biolog GN2 microplates were used to detect the utilization of organic substrates according to the manufacturer's

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Three supplementary figures are available with the online version of this paper.

instructions, except that inoculating fluid was replaced with artificial seawater, which contained (l^{-1}) 24 g NaCl, 5.1 g $MgCl_2$, 4 g Na_2SO_4, 1.1 g $CaCl_2$, 0.7 g KCl, 0.2 g $NaHCO_3$, 0.1 g KBr, 0.027 g H_3BO_3, 0.024 g $SrCl_2$ and 0.003 g NaF (Lyman & Fleming, 1940). After incubation at 30 °C for 96 h, data were read out using The Biolog Microbial ID System. Assimilation of mannose and mannitol was tested in modified 2216 medium supplemented with 0.5 % yeast and 2.0 % mannose or mannitol, respectively.

Antibiotic sensitivity was tested on MA with antibiotic discs, each piece of which respectively contained ampicillin (10 μg), chloramphenicol (30 μg), carbenicillin (100 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), novobiocin (30 μg), penicillin G (10 μg), rifampicin (5 μg), streptomycin (10 μg) or tetracycline (30 μg). After pre-incubation at 30 °C for 12 h, antibiotic discs were placed on the surface of the agar plates. Before observation, one more day of incubation was required. Sensitivity to antibiotics was determined by the diameter of inhibition (>13 mm classified as susceptible, 10–12 mm as partly susceptible, <10 mm as resistant) (Nokhal & Schlegel, 1983).

Fatty acid methyl esters were obtained from cells growing on MA at 30 °C for 2 days, as described by Kuykendall *et al.* (1988). Isoprenoid quinones were analysed using reversed-phase HPLC, as described by Komagata & Suzuki (1987). Polar lipids were extracted and separated on a silica gel plate (10 × 10 cm; 5554; Merck), and further analysed as described by Minnikin *et al.* (1984) and Fang *et al.* (2012).

The genomic DNA G+C content of strain CMB17^T was determined using reversed-phase HPLC (Mesbah *et al.*, 1989) with salmon sperm DNA as the calibration standard. The 16S rRNA gene was amplified and cloned into pMD 19-T vector (TaKaRa) for sequencing. Pairwise sequence alignment was performed with the EzTaxon-e server (Kim *et al.*, 2012) while multiple sequences were aligned using

Table 1. Differential phenotypic characteristics of strain CMB17^T, compared with closely related species

Strains: 1, CMB17^T; 2, *P. stylophorae* KTW-16^T; 3, *P. homiensis* DD-R11^T; 4, *P. saliphilus* YIM 90738^T; 5, *P. denitrificans* DSM 413^T. All data were from this study, except DNA G+C content in columns 3 and 5 (taken from Sheu *et al.*, 2011) and column 4 (taken from Wang *et al.*, 2009). All strains are negative for hydrolysis of Tweens 20, 40 and 80. PHB, poly-β-hydroxybutyrate. +, Positive; −, negative; NR, not reported.

Characteristic	1	2	3	4	5
Colony colour*	OR	PY	DY	YB	CW
Motility	−	−	+	−	−
Ranges for growth					
NaCl (%, w/v)	0–11	0–9	0–14	1–15	0–7
pH	5.5–9	6–10	6–10	6–8	6–8
Temperature (°C)	4–40	15–40	10–40	10–55	10–40
PHB accumulation	+	+	−	−	+
Reduction of nitrate to:					
Nitrite	+	+	−	+	−
N_2	−	−	−	−	+
Hydrolysis of:					
Gelatin	−	+	−	−	−
Aesculin	+	+	+	+	−
Urea	−	−	−	+	+
Starch	−	−	+	−	−
DNA	−	−	+	−	+
Assimilation of:					
Glucose	+	+	−	+	−
Arabinose	+	−	+	+	+
β-Galactosidase	+	−	−	−	−
Mannose	+	−	−	−	−
Mannitol	+	−	−	−	−
Maltose	+	−	+	−	+
2-Aminoethanol	−	+	−	−	−
2,3-Butanediol	−	+	−	+	−
Glycerol	−	+	+	+	−
DL-α-Glycerol phosphate	+	+	−	−	−
Glucose 1-phosphate	+	+	−	+	−
Glucose 6-phosphate	+	+	−	+	−
DNA G+C content (mol%)	62.2	67.6	63.0	60.3	66.5

*OR, orange; CW, Creamy white; DY, deep yellow; PY, pale yellow; YB, yellow–brown.

Table 2. Cellular fatty acid profiles of strain CMB17^T and its closest relatives in the genus *Paracoccus*

Strains: 1, CMB17^T; 2, *P. stylophorae* KTW-16^T; 3, *P. homiensis* DD-R11^T; 4, *P. saliphilus* YIM 90738^T; 5, *P. denitrificans* DSM 413^T. All data were from this study. Values are percentages of the total fatty acids. Fatty acids that amounted to less than 0.5 % of the total fatty acids in all strains studied are not shown. –, Not detected; ECL, equivalent chain length.

Fatty acid	1	2	3	4	5
$C_{10:0}$ 3-OH	2.1	2.0	2.7	5.6	2.6
Unidentified ECL 11.799	2.7	2.0	4.0	5.9	3.4
Unidentified ECL 14.959	0.7	0.2	0.5	–	–
$C_{16:0}$	0.4	3.4	1.0	1.1	14.4
$C_{17:0}$	0.6	1.8	–	–	0.8
$C_{17:1}\omega7c$	–	–	–	2.3	–
$C_{18:0}$	4.6	18.2	9.0	7.3	2.0
$C_{18:0}$ 3-OH	–	1.4	1.2	0.2	–
$C_{18:1}\omega7c$	87.6	43.9	73.7	64.8	70.0
11-Methyl $C_{18:1}\omega7c$	–	5.9	5.2	–	–
$C_{19:0}$ cyclo $\omega8c$	–	19.3	–	10.7	3.0
Summed feature 2*	2.9	1.9	3.5	3.0	4.7
Summed feature 3*	–	1.8	0.6	0.4	1.4

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC using the Microbial Identification System. Summed feature 2 consists of $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I. Summed feature 3 consists of $C_{16:1}\omega7c$ and/or $C_{16:1}\omega7c$.

the CLUSTAL_X programme of the MEGA 5 package (Tamura *et al.*, 2011). Phylogenetic trees were reconstructed using the neighbour-joining, maximum-likelihood and maximum-parsimony methods. Bootstrap analysis was based on 1000 replications.

Cells of strain CMB17^T were Gram-negative short rods measuring 0.5–0.9 μm wide by 0.8–1.1 μm long (Fig. S1, available in the online Supplementary Material). Growth was observed in the temperature range of 4–40 °C (optimal, 25–30 °C), pH 5.5–9 (optimal, 6.5–7) and 0–11 % (w/v) NaCl (optimal, 0.5–1 %). Detailed phenotypic properties of strain CMB17^T are shown in Table 1. The major fatty acid and the only isoprenoid quinone of strain CMB17^T were $C_{18:1}\omega7c$ and Q-10, respectively (Table 2). The main polar lipids of strain CMB17^T consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, an unknown aminolipid, three unknown glycolipids and three unknown phospholipids (Fig. S2).

The genomic DNA G+C content of strain CMB17^T was determined as 62.2 mol%. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences was generated, as shown in Figs 1 and S3. According to the analysis, strain CMB17^T shared the highest sequence similarity (96.7 %) with *P. stylophorae* KTW-16^T and clustered with the same type strain on all three kinds of phylogenetic trees. Therefore, strain CMB17^T is proposed to represent a novel species of the genus *Paracoccus*, which is most closely related to *P. stylophorae*.

In conclusion, cells of strain CMB17^T were characterized as Gram-negative short rods (0.5–0.9 μm wide and 0.8–1.1 μm long), heterotrophic, facultatively anaerobic, and catalase- and oxidase-positive. These characteristics, as well as the major fatty acid and isoprenoid quinone are typical of the genus *Paracoccus*. According to the neighbour-joining phylogenetic tree, strain CMB17^T and other species of the genus *Paracoccus* showed high sequence similarity (94.3–96.7 %), with the highest similarity to *P. stylophorae* KTW-16^T, supporting the classification of strain CMB17^T as a distinct genomic species of the genus *Paracoccus*. The novel strain was still distinguishable from the closest phylogenetic neighbours, owing to differences in several physiological and chemotaxonomic properties. As shown in Table 2, strain CMB17^T had the highest level of $C_{18:1}\omega7c$ (87.6 %). Moreover, $C_{18:0}$ 3-OH, 11-methyl $C_{18:1}\omega7c$ and summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega7c$), identified for the other four strains, could not be detected in the new isolate. The polar lipid profile of strain CMB17^T was similar to that of *P. stylophorae* KTW-16^T, except that one phospholipid (PL3) was detected in strain CMB17^T but not *P. stylophorae* KTW-16^T, and one glycolipid (GL4) was detected in *P. stylophorae* KTW-16^T only. As shown in Table 1, strain CMB17^T was positive for mannose and mannitol

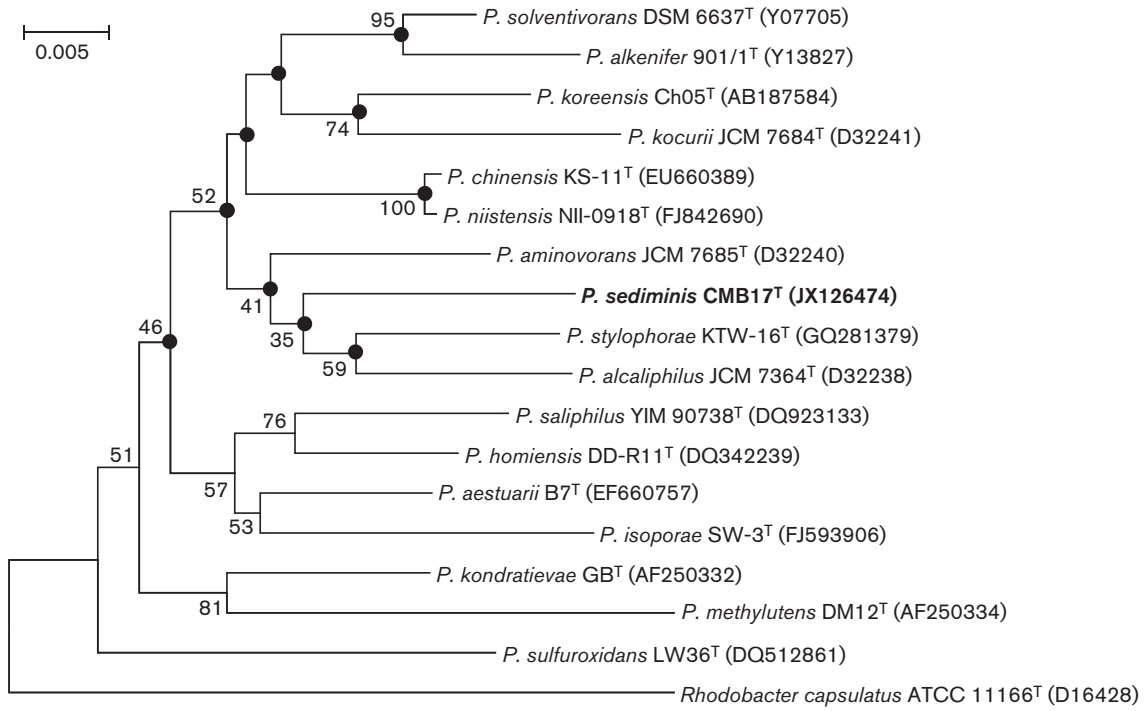

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain CMB17^T with other species of the genus *Paracoccus*. Numbers at branch points are bootstrap values (percentages based on 1000 replications; only values above 35 % are shown). The 16S rRNA gene sequence of *Rhodobacter capsulatus* ATCC 11166^T was used as the outgroup. Bar, 0.5 % substitution per nucleotide position.

fermentation, which was not observed for the other four strains. Moreover, growth at 4 °C was characteristic of strain CMB17^T, which was tested to be negative in all other strains. These findings collectively suggest that strain CMB17 is a representative of a novel species of the genus *Paracoccus* for which we propose the name *Paracoccus sediminis* sp. nov.

Description of *Paracoccus sediminis* sp. nov.

Paracoccus sediminis (se.di′mi.nis. L. gen. n. *sediminis* of a sediment).

Cells are non-motile, Gram-negative, and facultatively anaerobic. The shape ranges from short rods to cocci (0.5–0.9 μm wide and 0.8–1.1 μm long). Colonies are orange, smooth and circular when grown on marine agar 2216 (MA; Difco) at 30 °C for 2 days. Growth occurs at 4–40 °C (optimum, 25–30 °C), pH 5.5–9 (optimum, pH 6.5–7), and with 0–11 % NaCl (optimum, 0.5–1 %). Poly-β-hydroxybutyrate granules accumulate in the cells. Sensitive to ampicillin, chloramphenicol, novobiocin, penicillin G, rifampicin and carbenicillin, but resistant to gentamicin, nalidixic acid, streptomycin, tetracycline and erythromycin. Positive for catalase and oxidase activities, and negative for DNase activity and hydrolysis of starch, alginate and Tweens 20, 40, 60 and 80. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase, but negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. In API 20NE tests, positive for reduction of nitrate, hydrolysis of aesculin, fermentation of D-glucose, β-galactosidase activity, and assimilation of glucose, arabinose, mannose, mannitol, maltose and phenylacetate, but negative for production of indole, fermentation of arginine dihydrolase, urease and gelatin, and assimilation of caprate, adipate, *N*-acetylglucosamine, potassium gluconate, malate and citrate. With Biolog GN2 MicroPlates, utilizes dextrin, Tween 80, L-arabinose, turanose, turanose, citraic acid, itaconic acid, DL-lactic acid, propionic acid, bromosuccinic acid, DL-α-glycerol phosphate, α-D-glucose 1-phosphate and D-glucose 6-phosphate; all other substrates included in Biolog GN2 MicroPlates cannot be utilized. The major fatty acids (>4 %) are $C_{18:1}\omega7c$ and $C_{18:0}$. The polar lipid fraction consists of phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol, unidentified aminolipid, phospholipids and glycolipids. The major isoprenoid quinone is Q-10.

The type strain, CMB17^T (=JCM 18467^T=DSM 26170^T=CGMCC 1.12681^T), was isolated from marine sediment of the Pacific Ocean. The G+C content of genomic DNA of the type strain is 62.2 mol%.

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