Paracoccus aestuarii sp. nov., isolated from tidal flat sediment

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A Gram-negative micro-organism, designated strain B7^T, was isolated from tidal flat sediment and subjected to a polyphasic taxonomic study involving morphological, physiological, biochemical and 16S rRNA gene sequence analyses. A phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain B7^T belonged to the genus *Paracoccus* and was closely related phylogenetically to *Paracoccus marcusii* MH1^T (97.5 % sequence similarity), *Paracoccus marinus* KKL-A5^T (97.5 %), *Paracoccus haeundaensis* BC74171^T (97.3 %), *Paracoccus carotinifaciens* E-396^T (97.3 %), *Paracoccus homiensis* DD-R11^T (97.2 %), *Paracoccus seriniphilus* MBT-A4^T (96.9 %) and other type strains of the genus *Paracoccus* (95.2–96.7 %). The G+C content of the genomic DNA and the major isoprenoid quinone of the type strain were 62.0 mol% and ubiquinone-10, respectively. The major fatty acid components were $C_{18:1}\omega7c$ (68.9 %) and $C_{18:0}$ (18.1 %); this profile, with $C_{18:1}\omega7c$ as the predominant fatty acid, was characteristic of members of the genus *Paracoccus*. The 16S rRNA gene sequence analysis, DNA–DNA hybridization studies and physiological and biochemical tests identified genotypic and phenotypic differences between strain B7^T and recognized *Paracoccus* species. On the basis of these data, therefore, strain B7^T represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus aestuarii* sp. nov. is proposed. The type strain is B7^T (=KCTC 22049^T=DSM 19484^T=JCM 15119^T).

The genus *Paracoccus* belongs to the *Alphaproteobacteria* and was first proposed by Davis *et al.* (1969) as comprising a Gram-negative, catalase-positive, oxidase-positive bacterium, *Paracoccus denitrificans*. Members of this genus contain $C_{18:1}\omega7c$ as a major component of the cellular fatty acids and are metabolically versatile. At the time of writing, since this genus was proposed in 1969, the names of 24 species belonging to the genus *Paracoccus* have been validly published, having been isolated from various environments including soil, sediment and activated sludge. The purpose of this paper was to establish the taxonomic position of strain B7^T, which was isolated from tidal flat sediment, on the basis of data from phenotypic, genetic and chemotaxonomic analyses.

Strain B7^T was isolated from tidal flat sediment in Yeosu (34° 47′ 26″ N 127° 34′ 01″ E), South Korea. This novel strain was isolated on TSBA (tryptic soy broth solidified with 20.0 g agar l^{-1}; Difco) and a pure culture was obtained by using repeated restreaking. Cell biomass for analysis of cellular composition and for DNA extraction was taken from TSBA plates that had been incubated at 30 °C for 2 days.

A DNA-extraction kit (G-spin, iNtRON Biotechnology) was used to obtain chromosomal DNA. Two universal bacterial primers: forward primer 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492r (5′-TACGGYTACCTTGTTACGACTT-3′) (Lane, 1991) were used to PCR-amplify the 16S rRNA gene from the chromosomal DNA. After purification with a PCR purification kit (Solgent), the PCR product was sequenced as described previously (Roh *et al.*, 2008). SeqMan software (DNASTAR) was used to assemble full-length 16S rRNA gene sequences. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007). 16S rRNA gene sequences from the novel isolate and related taxa (NCBI database) were aligned using the multiple sequence alignment program CLUSTAL_X (version 1.8)

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B7^T is EF660757.

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(Thompson *et al.*, 1997). Phylogenetic relationships between representative *Paracoccus* species were determined using the MEGA3 software program (Kumar *et al.*, 2004). Dendrograms were elaborated by means of distance matrices (Kimura, 1980), using the neighbour-joining method (Saitou & Nei, 1987). To evaluate the stability of the trees, a bootstrap analysis was performed using a consensus tree that was based on 1000 randomly generated trees. DNA–DNA hybridization was performed using the fluorometric method of Ezaki *et al.* (1989). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain B7^T falls within the cluster of *Paracoccus* species (Fig. 1) and is closely related phylogenetically to *Paracoccus marcusii* MH1^T (97.5 % sequence similarity), *Paracoccus marinus* KKL-A5^T (97.5 %), *Paracoccus haeundaensis* BC74171^T (97.3 %), *Paracoccus carotinifaciens* E-396^T (97.3 %), *Paracoccus homiensis* DD-R11^T (97.2 %), *Paracoccus seriniphilus* MBT-A4^T (96.9 %) and other type strains of the genus *Paracoccus* (95.2–96.7 %). A DNA–DNA hybridization study revealed the following mean levels of DNA–DNA relatedness between strain B7^T and the type strains of its closest relatives in the phylogenetic tree: *P. marcusii* (9.4 %), *P. homiensis* (13.9 %) and *P. seriniphilus* (11.3 %). *P. haeundaensis* BC74171^T and *P. carotinifaciens* E-396^T are patent strains that are not publicly available, so a DNA–DNA relatedness study could not be conducted with these two strains. Species definitions can be established with DNA–DNA reassociation values below 70 % (Wayne *et al.*, 1987), and 16S rRNA gene sequence similarity values that are less than 98.7 % between two strain pairs have DNA–DNA reassociation values of less than 70 % (Stackebrandt & Ebers, 2006); therefore, strain B7^T can be considered to represent a distinct genospecies.

The DNA G+C content was determined using HPLC as described by Mesbah & Whitman (1989), with *Escherichia coli* B (Sigma-Aldrich) as the calibration reference. The G+C content of the genomic DNA of the type strain was 62.0 mol%. The lowest genomic DNA G+C content among species belonging to the genus *Paracoccus* is 61.3 mol% (for the type strain of *Paracoccus sulfuroxidans*; Liu *et al.*, 2006) and the highest value is 71.0 mol% (for the

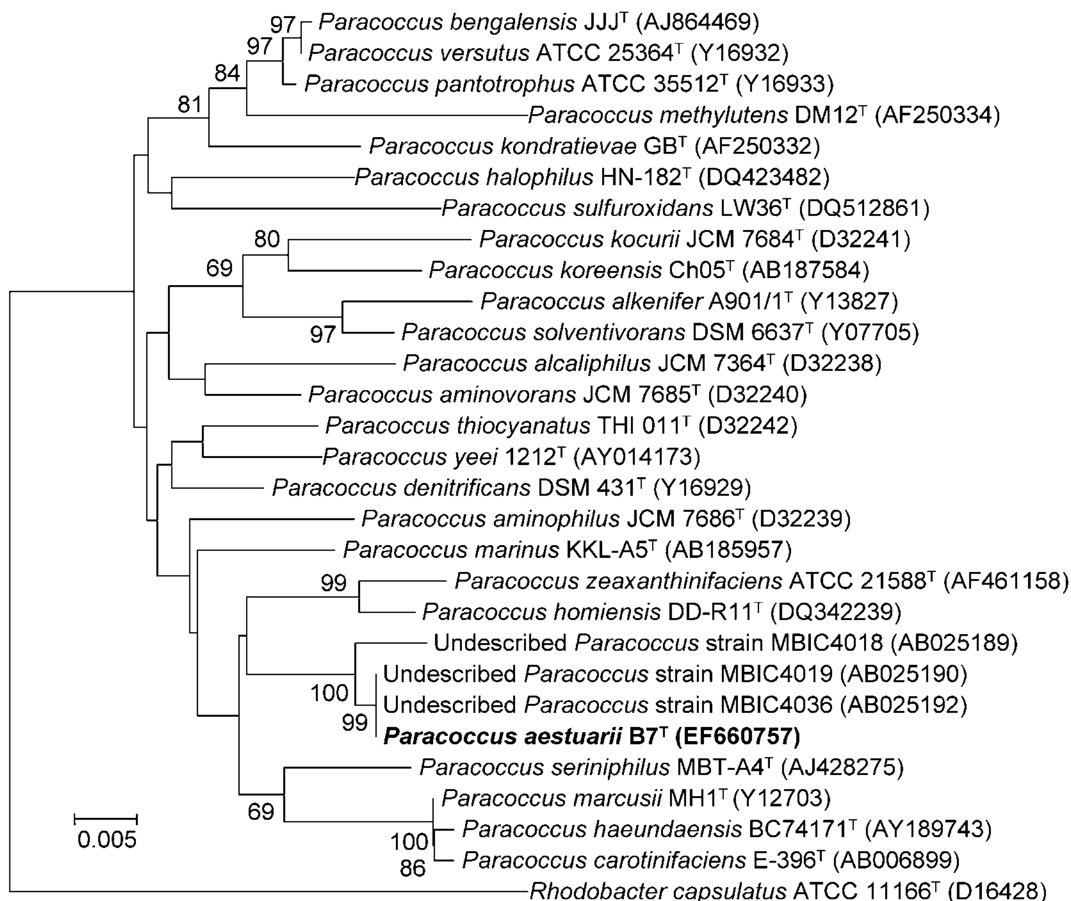

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain B7^T with respect to species in the genus *Paracoccus*. Numbers at nodes indicate bootstrap percentages (based on 1000 replications), where greater than 50 %. Bar, 0.005 accumulated changes per nucleotide.

type strain of *Paracoccus kocurii*; Ohara *et al.*, 1990). The G+C content of the genomic DNA of strain B7^T is therefore within the range for recognized *Paracoccus* species.

The quantitative analysis of the cellular fatty acid composition was performed according to the instructions for the Sherlock Microbial Identification System (MIDI). Gas chromatography (6890; Hewlett Packard) together with the Microbial Identification software package (Sasser, 1990) were used to identify the fatty acids. The cellular fatty acids of strain B7^T were $C_{18:1}\omega7c$ (68.9 %), $C_{18:0}$ (18.1 %), $C_{10:0}$ 3-OH (2.5 %), $C_{17:0}$ (2.5 %), $C_{16:0}$ (1.3 %), summed feature 2 (comprising $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I; 2.4 %) and some others (<1 %). This profile, with $C_{18:1}\omega7c$ as the major fatty acid, is characteristic of members of the genus *Paracoccus*. However, the $C_{18:1}\omega7c$ (68.9 %) and $C_{18:0}$ (18.1 %) contents of strain B7^T differed from the ranges (78.9–84.3 % and 2.5–7.8 %, respectively) found among the *Paracoccus* species (*P. marcusii*, *P. haeundaensis*, *P. carotinifaciens*, *P. seriniphilus*, *P. homiensis* and *P. zeaxanthinifaciens*; Kim *et al.*, 2006) that clustered with the novel strain in the phylogenetic tree. The differences in fatty acid composition may be the result of differences in experimental conditions.

Quinones were characterized as described by Collins (1985) and Wu *et al.* (1989). *Paracoccus* species are characterized as possessing ubiquinone-10 (Q-10). Strain B7^T also possessed Q-10 as the sole respiratory quinone. This result, in combination with the 16S rRNA gene sequence data, the G+C content and the major fatty acid components, confirmed that the novel strain belongs to the genus *Paracoccus*.

Polar lipids were extracted and then examined by means of two-dimensional TLC followed by spray reagents (Collins *et al.*, 1980). The polar lipid analysis indicated that strain B7^T contained phosphatidylcholine, phosphatidylglycerol and diphosphatidylglycerol.

To determine the optimum growth temperature for strain B7^T, cells were grown on TSBA plates at 4–45 °C. The optimum NaCl concentration and pH were determined in TSB medium supplemented with NaCl (0–30 %, w/v) and with HCl or NaOH (pH 4.0–13.0). Cell morphology was examined under light microscopy (ECLIPSE 80*i*; Nikon). Motility was observed using the wet-mount method (Murray *et al.*, 1994) and Gram staining was performed with the non-staining method described by Buck (1982). Anaerobic growth was tested on TSBA plates using the BBL GasPak Pouch System (Becton Dickinson). Enzyme activities and substrate utilization from sole carbon sources were determined using API ZYM, API 20NE and API 50 CH test strips (bioMérieux). Catalase activity was investigated by assessing bubble production in a 3 % (v/v) hydrogen peroxide solution. A detailed species description is presented below and Table 1 shows a comparison between the characteristics of strain B7^T and those of six closely related strains (selected on the basis of 16S rRNA gene sequence similarity and phylogenetic tree topology). Our detailed investigation has revealed genotypic and phenotypic differences between strain B7^T and recognized *Paracoccus* species.

Table 1. Characteristics that serve to differentiate strain B7^T from closely related species

Strains: 1, B7^T (*P. aestuarii* sp. nov.); 2, *P. marcusii* MH1^T (patent strain; data from Harker *et al.*, 1998); 3, *P. haeundaensis* BC74171^T (patent strain; Lee *et al.*, 2004); 4, *P. carotinifaciens* E-396^T (patent strain; Tsubokura *et al.*, 1999); 5. *P. seriniphilus* MBT-A4^T (Pukall *et al.*, 2003); 6. *P. homiensis* DD-R11^T (Kim *et al.*, 2006); 7. *P. zeaxanthinifaciens* R-1512^T (Berry *et al.*, 2003). +, Positive; −, negative; W, weakly positive; NR, not reported.

Characteristic	1	2	3	4	5	6	7
Motility	−	−	−	+	−	+	−
Orange to red pigment	+	+	+	+	−	−	+
Growth at 40 °C	−	−	−	−	−	+	+
Growth with 6 % NaCl	−	W	+	W	+	+	+
Reduction of nitrate to nitrite	−	−	+	−	+	−	−
Urease activity	−	−	−	−	−	−	+
β-Glucosidase activity	+	+	NR	+	−	+	+
Growth on:							
Adonitol	+	+	−	+	+	−	−
Erythritol	−	+	NR	+	+	+	−
Gentiobiose	−	+	NR	+	−	−	−
L-Rhamnose	−	−	−	NR	−	+	−
D-Sorbitol	−	+	−	+	+	+	−
Xylitol	−	+	NR	+	+	−	−
DNA G+C content (mol%)	62.0	66	66.9	67	63.3	63	67.6

Thus, on the basis of genetic, chemotaxonomic and phenotypic comparisons with previously described taxa, strain B7^T represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus aestuarii* sp. nov. is proposed.

Description of *Paracoccus aestuarii* sp. nov.

Paracoccus aestuarii (a.es.tu.a.ri′i. L. gen. n. *aestuarii* of a tidal flat).

Cells are aerobic, non-motile, rod-shaped (0.8–1.0 × 1.5–2.0 μm), Gram-negative, catalase-positive and oxidase-positive. Colonies are orange-coloured, circular and approximately 1.0–2.5 mm in diameter after growth for 1 day on TSBA at 30 °C. No growth occurs at NaCl concentrations greater than 5 %. The temperature range for growth is 15–37 °C. The pH range for growth is 7.5–9.5 (optimum, pH 9.0). Nitrate is not reduced to nitrite or nitrogen, indole is not produced and glucose fermentation does not occur. Negative for arginine dihydrolase and urease. Aesculin and PNPG (*p*-nitrophenyl-*β*-D-galactopyranoside) hydrolysis occurs, but not gelatin hydrolysis. D-Glucose, D-mannose, D-mannitol, *N*-acetylglucosamine,

maltose, D-malate, trisodium citrate, D-ribose, D-xylose, D-adonitol, inositol, arbutin, aesculin, D-arabitol, L-arabitol, 2-ketogluconate and 5-ketogluconate are assimilated, but potassium gluconate, capric acid, adipic acid, phenylacetic acid, glycerol, erythritol, D-arabinose, L-arabinose, L-xylose, methyl β-D-xyloside, D-galactose, D-fructose, L-sorbose, L-rhamnose, dulcitol, D-sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, salicin, cellobiose, D-lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose and L-fucose are not assimilated. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase. Negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Predominant fatty acids are $C_{18:1}\omega7c$, $C_{18:0}$, $C_{10:0}$ 3-OH, $C_{17:0}$, $C_{16:0}$ and summed feature 2 (comprising $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I). The polar lipid fraction consists of phosphatidylcholine, phosphatidylglycerol and diphosphatidylglycerol. The major isoprenoid quinone is Q-10. The G+C content of genomic DNA of the type strain is 62.0 mol%.

The type strain, $B7^T$ (=KCTC 22049^T=DSM 19484^T=JCM 15119^T), was isolated from tidal flat sediment in Yeosu (34° 47′ 26″ N 127° 34′ 01″ E), South Korea.

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