Paracoccus homiensis sp. nov., isolated from a sea-sand sample

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Strain DD-R11^T, isolated from a sea-sand sample from Homi Cape, Pohang city, South Korea, was a Gram-negative, aerobic, motile, non-spore-forming, rod- to ovoid-shaped bacterium. Colonies grown on marine agar were circular, convex and colourless to creamy white. Growth occurred between 10 and 40 °C (optimum 25–30 °C) and at pH 5.0–9.0 (optimum pH 6.0–8.0). The strain could grow in up to 15 % NaCl (optimum 3–5 % NaCl). According to 16S rRNA gene sequence analysis, the strain was a member of the genus *Paracoccus* in the *Alphaproteobacteria*. Sequence similarities to type strains of the genus *Paracoccus* were between 94.6 and 98.3 %, showing the highest sequence similarity to *Paracoccus zeaxanthinifaciens* ATCC 21588^T. The DNA–DNA relatedness value of strain DD-R11^T and *P. zeaxanthinifaciens* ATCC 21588^T was 27 %. Strain DD-R11^T was characterized by having ubiquinone 10 as the major respiratory quinone and C_{18:1}ω7c as the predominant fatty acid. The DNA G + C content was 63.0 mol%. On the basis of its phenotypic and genotypic characteristics, it is suggested that DD-R11^T represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus homiensis* sp. nov. is proposed, with DD-R11^T (=KACC 11518^T = DSM 17862^T) as the type strain.

The genus *Paracoccus* was proposed by Davis *et al.* (1969). At the time of writing, the genus contains 19 recognized species with validly published names. Phylogenetic analyses based on 16S rRNA gene sequences have shown that the genus *Paracoccus* falls within the α-3 subgroup of the *Proteobacteria*. Species of this genus are characterized as Gram-negative and catalase- and oxidase-positive, with a high metabolic versatility and a large amount of C_{18:1}ω7c and having ubiquinone 10 as the major isoprenoid quinone.

In this study, a moderately halophilic strain, designated DD-R11^T, was characterized on the basis of a polyphasic approach. The strain was isolated from a sea-sand sample collected in Pohang city, Korea. The sample was suspended in a solution of sea salts (Sigma), spread on marine agar 2216 (MA; Difco) and incubated at 28 °C. The isolate was routinely cultured on MA and maintained as a glycerol suspension (15 %, v/v) at –80 °C.

The isolate was cultivated on MA at 28 °C for the investigation of morphological and physiological characteristics.

For observation of cell morphology by transmission electron microscopy, cells were grown for 48 h on MA, negatively stained with 0.5 % (w/v) uranyl acetate and examined with a LEO model 912AB electron microscope. Tolerance of pH and temperature was recorded following growth in marine broth at 28 °C for 21 days. The requirement for NaCl for growth was tested using nutrient broth supplemented with 0, 1, 3, 5, 7, 10, 15, 20 and 25 % (w/v) NaCl. Phenotypic characteristics such as Gram staining, catalase and oxidase production and hydrolysis of alginic acid, carboxymethyl-cellulose, casein, gelatin, pectin, tyrosine and starch were performed using the methods of Smibert & Krieg (1994). Growth under anaerobic conditions was tested in GasPak (BBL) jars at 28 °C for 21 days. The commercially available API 20NE and API ZYM kits (bioMérieux) were used to determine biochemical properties, utilization of carbohydrates and enzymic activities according to the manufacturer's instructions. Utilization of organic substrates was tested using Biolog GN2 microplates. Colonies suspended in half-strength sea salts (Sigma) solution were used as the inoculum.

The 16S rRNA gene was amplified by PCR using two universal primers as described by Kwon *et al.* (2003). The

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DD-R11^T is DQ342239.

Fig. 1. Transmission electron micrograph of strain DD-R11^T. Bar, 500 nm.

sequences of DD-R11^T and related species were aligned using CLUSTAL X (Thompson *et al.*, 1997). The evolutionary distances of 1394 aligned positions were computed using the Kimura two-parameter model (Kimura, 1980) and a phylogenetic tree was generated with MEGA version 2.1 (Kumar *et al.*, 2001) using the neighbour-joining method (Saitou & Nei, 1987) and evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

DNA–DNA hybridization was carried out as described by Seldin & Dubnau (1985). Probe labelling was conducted using the non-radioactive DIG-High Prime system (Roche); hybridized DNA was visualized using the DIG Luminescent Detection kit (Roche). DNA–DNA relatedness was quantified using a densitometer (Bio-Rad). Isoprenoid quinone analysis was performed as described by Groth *et al.* (1996). The whole-cell fatty acid methyl ester profile was determined using the MIDI Sherlock Microbial Identification system (Microbial ID) with cells of DD-R11^T grown at 28 °C for 24 h in MA. The DNA G+C content was determined according to the method of Mesbah *et al.* (1989) using a reversed-phase HPLC system with a C18 column.

Cells of strain DD-R11^T were Gram-negative, non-spore-forming, aerobic and catalase- and oxidase-positive. Cells were rod- to ovoid-shaped (0.7–2.5 × 0.5–0.7 µm) and motile with one or more flagella (Fig. 1). They were able to grow on MA. However, they grew weakly on nutrient agar (Difco) and did not grow on trypticase soy agar (Difco) or MacConkey agar (Difco). The strain grew optimally on nutrient broth containing 3–5 % NaCl, grew weakly without NaCl and did not grow in the presence of more than 15 % (w/v) NaCl. After 48 h on MA, cells formed circular, convex, colourless to creamy white colonies. A

phenotypic comparison of DD-R11^T and closely related species of the genus *Paracoccus* is shown in Table 1.

The major fatty acid for strain DD-R11^T was C_{18:1}ω7c (78.9%). Small amounts of other fatty acids were detected as follows: 11-methyl C_{18:1}ω7c (5.8%), C_{18:0} (4.5%), C_{10:0} 3-OH (2.6%), unidentified ECL 11.799 (2.4%), C_{12:0} ALDE (1.8%) and C_{18:0} 3-OH (1.1%). This fatty acid profile was similar to those of members of the genus *Paracoccus* (Kelly *et al.*, 2000) (Table 2).

16S rRNA gene sequencing showed that the sequence of strain DD-R11^T exhibited 94.6–98.3 % sequence similarity to type strains of the genus *Paracoccus*. Strain DD-R11^T showed the highest sequence similarity to *Paracoccus zeaxanthinifaciens* ATCC 21588^T (98.3%), followed by *Paracoccus seriniphilus* DSM 14827^T (96.7%). The phylogenetic tree showing the position of strain DD-R11^T relative to members of the genus *Paracoccus* is shown in Fig. 2. Strain DD-R11^T and *P. zeaxanthinifaciens* ATCC 21588^T formed a compact cluster (100 % bootstrap value), which was loosely related to another cluster including *Paracoccus*

Table 1. Phenotypic comparison of strain DD-R11^T with closely related species within the genus *Paracoccus*

Taxa: 1, DD-R11^T; 2, *P. zeaxanthinifaciens* (three strains); 3, *P. carotinifaciens* IFO 16121^T; 4, *P. haeundaensis* KCCM 10460^T; 5, *P. marcusii* DSM 11574^T; 6, *P. seriniphilus* DSM 14827^T. Data from Berry *et al.* (2003), Pukall *et al.* (2003), Lee *et al.* (2004) and this study. +, Positive; –, negative; w, weak; ND, not determined.

Characteristic	1	2	3	4	5	6
Motility	+	–	+	–	–	–
Orange to red pigment	–	+	+	+	+	–
Growth at 40 °C	+	+	–	–	–	–
Growth in 6 % NaCl	+	+	w	+	w	+
Nitrate reduction	–	–	–	+	–	+
Urease	–	+	–	–	–	–
Growth on:						
Acetic acid	+	–	ND	ND	–	+
Adonitol	–	–	+	–	+	+
D-Alanine	+	–	ND	ND	–	+
L-Aspartate	–	+	+	ND	–	+
L-Asparagine	+	+	+	–	–	+
iso-Erythritol	+	–	+	ND	+	+
Gentiobiose	–	–	+	ND	+	–
L-Phenylalanine	+	–	ND	ND	–	–
Propionic acid	+	–	ND	ND	+	+
Quinic acid	–	–	+	ND	+	–
L-Rhamnose	+	–	ND	–	–	–
D-Sorbitol	+	–	+	–	+	+
Succinic acid	+	–	ND	ND	+	–
Xylitol	–	–	+	ND	+	+
DNA G+C content (mol%)	63.0	66.9–67.7	67	66.9	66	63.3

Table 2. Fatty acid composition of DD-R11^T and its nearest phylogenetic neighbours within the genus *Paracoccus*

Taxa: 1, DD-R11^T; 2, *P. zeaxanthinifaciens* (mean values from three strains); 3, *P. carotinifaciens* IFO 16121^T; 4, *P. haeundaensis* KCCM 10460^T; 5, *P. marcusii* DSM 11574^T; 6, *P. seriniphilus* DSM 14827^T. Data from Berry *et al.* (2003), Pukall *et al.* (2003), Lee *et al.* (2004) and this study. Values are percentages of total fatty acids; —, not detected or <1%.

Fatty acid	1	2	3	4	5	6
C _{10:0} 3-OH	2.6	4.9	3.4	2.1	6.2	7.9
Unidentified ECL 11.799	2.4	3.6	2.8	—	4.9	—
Unidentified ECL 15.275	—	1.6	1.1	—	2.9	—
C _{12:1} ω7c	—	—	—	2.0	—	—
C _{12:0} ALDE	1.8	—	—	—	—	—
C _{14:0} 3-OH	—	—	—	1.5	—	—
C _{18:1} ω7c	78.9	80.2	84.0	84.3	80.3	83.5
C _{18:0}	4.5	3.6	5.2	7.8	2.6	2.5
C _{18:0} 3-OH	1.1	—	—	—	—	—
11-Methyl C _{18:1} ω7c	5.8	—	—	—	—	1.5
Summed feature 2*	—	2.9	2.1	—	3.0	—

*Summed feature 2 comprises C_{14:0} 3-OH and/or C_{16:1} iso I.

seriniphilus DSM 14827^T, *Paracoccus carotinifaciens* IFO 16121^T, *Paracoccus marcusii* DSM 11574^T and *Paracoccus haeundaensis* KCCM 10460^T.

According to DNA–DNA hybridization, strain DD-R11^T showed a DNA–DNA relatedness value of 27% to *P. zeaxanthinifaciens* ATCC 21588^T. The levels obtained for 16S rRNA gene similarity (<97%) and DNA–DNA hybridization (<70%) support the genomic distinction of strain DD-R11^T from other species in the genus *Paracoccus* (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). On the basis of the phylogenetic, chemotaxonomic and phenotypic data, strain DD-R11^T should be classified as the type strain of a novel species of the genus *Paracoccus*, for which we propose the name *Paracoccus homiensis* sp. nov.

Description of *Paracoccus homiensis* sp. nov.

Paracoccus homiensis (ho.mi.en'sis. N.L. masc. adj. *homiensis* pertaining to Homi Cape, South Korea, where the type strain was isolated).

Cells are Gram-negative, aerobic, motile, non-spore-forming and rod- to ovoid-shaped (0.7–2.5 × 0.5–0.7 μm). Colonies on MA are circular, convex and colourless to creamy white. Growth occurs between 10 and 40 °C (optimum 25–30 °C) and at pH 5.0–9.0 (optimum pH 6.0–8.0). Grows optimally at 3–5% NaCl and weakly at 15% (w/v) NaCl. Positive for catalase, oxidase and hydrolysis of aesculin, gelatin, Tween 80 and tyrosine. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, hydrolysis of alginic acid, casein, carboxymethylcellulose, pectin, starch and urea. Enzymic activity is observed for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase and α-glucosidase, but no activity is observed for lipase (C14), valine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Utilizes Tween 80, D-arabitol, D-cellobiose, iso-erythritol, D-fructose, D-galactose, α-D-glucose, myo-inositol, α-D-lactose, D-mannitol, D-mannose, D-melibiose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, pyruvic acid methyl ester, succinic acid mono-methyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-gluconic acid, D-glucuronic acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, DL-lactic acid, propionic acid, D-saccharic acid, succinic acid, D-alanine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, phenylethylamine and glycerol. Does not utilize α-cyclodextrin, dextrin, glycogen, Tween 40, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, L-fucose, gentiobiose, lactulose, maltose, methyl β-D-glucoside, D-psicose, xylitol, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, α-hydroxybutyric acid,

Fig. 2. Neighbour-joining tree showing the relationship of strain DD-R11^T to other members of the genus *Paracoccus* based on 16S rRNA gene sequences. Only bootstrap percentages above 50% are shown (1000 replications). The sequence of *Rhodobacter capsulatus* ATCC 11166^T was used as the outgroup. Bar, 0.5% sequence divergence.

γ -hydroxybutyric acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, malonic acid, quinic acid, sebacic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, L-alanyl glycine, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-pyroglutamic acid, D-serine, L-threonine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, putrescine, 2-aminoethanol, 2,3-butanediol, DL- α -glycerol phosphate, α -D-glucose 1-phosphate and D-glucose 6-phosphate. Ubiquinone Q-10 is the major isoprenoid quinone. The major fatty acid is C_{18:1} ω 7c. The G + C content is 63.0 mol%.

The type strain, DD-R11^T (=KACC 11518^T=DSM 17862^T), was isolated from a sea-sand sample collected from Homi Cape, Pohang city, South Korea.

Acknowledgements

This work was supported by a grant (203068-03-2-SB010) from the Agricultural R&D Promotion Center, South Korea.

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