Paracoccus fistulariae sp. nov., a lipolytic bacterium isolated from bluespotted cornetfish, *Fistularia commersonii*

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A Gram-stain-negative, non-motile, non-spore-forming and short rod- or rod-shaped bacterial strain, designated 22-5^T, was isolated from a bluespotted cornetfish, *Fistularia commersonii*, and subjected to taxonomic study. Strain 22-5^T grew optimally at 30 °C and in the presence of 2–5 % (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain 22-5^T belonged to the genus *Paracoccus* and joined the cluster comprising *Paracoccus homiensis* DD-R11^T and *Paracoccus zeaxanthinifaciens* ATCC 21588^T, with which strain 22-5^T exhibited 97.4 and 96.9 % 16S rRNA gene sequence similarity, respectively. Strain 22-5^T exhibited 94.0–96.6 % 16S rRNA gene sequence similarity with the other type strains of species of the genus *Paracoccus*. Strain 22-5^T contained Q-10 as the predominant menaquinone and C_{18:1}ω7c as the predominant fatty acid. In this study, *P. zeaxanthinifaciens* KCTC 22688^T also contained Q-10 as the predominant isoprenoid quinone. The DNA G + C content of strain 22-5^T was 63.6 mol%. Strain 22-5^T exhibited 44 and 32 % DNA–DNA relatedness to *P. homiensis* KACC 11518^T and *P. zeaxanthinifaciens* KCTC 22688^T, respectively. On the basis of phenotypic, phylogenetic and genetic data, strain 22-5^T is considered to represent a novel species of the genus *Paracoccus*, for which the name *Paracoccus fistulariae* sp. nov. is proposed. The type strain is 22-5^T (=KCTC 22803^T =CCUG 58401^T).

The genus *Paracoccus* was first described by Davis *et al.* (1969) to accommodate a Gram-negative, catalase- and oxidase-positive, coccoid-shaped bacterium, *Paracoccus denitrificans*. Subsequently, at least 26 species with validly published names have been proposed as members of the genus *Paracoccus* (Euzéby, 2009). Phylogenetic analyses based on 16S rRNA gene sequences have shown that the genus *Paracoccus* falls within the class *Alphaproteobacteria* (Anzai *et al.*, 2000; Lee *et al.*, 2005). Members of the genus *Paracoccus* are chemotaxonomically characterized as having ubiquinone-10 as the predominant isoprenoid quinone and C_{18:1} as the major fatty acid (Kelly *et al.*, 2006; Roh *et al.*, 2009). Early described species of the genus *Paracoccus* were isolated from various terrestrial environments (Kelly *et al.*, 2006) and some recently described species have been isolated from marine environments, including intertidal sediment and seawater (Khan *et al.*,

2008; Kim *et al.*, 2006; Lee *et al.*, 2004; Liu *et al.*, 2008; Pukall *et al.*, 2003; Roh *et al.*, 2009). In this study, we describe a *Paracoccus*-like bacterial strain, 22-5^T, which was isolated from the intestine of a bluespotted cornetfish, *Fistularia commersonii*.

Strain 22-5^T was isolated by means of the standard dilution-plating technique on marine agar 2216 (MA; Difco) at 25 °C. The type strains of *Paracoccus homiensis* and *Paracoccus zeaxanthinifaciens* were used as reference strains for phenotypic characterization, fatty acid analysis and DNA–DNA hybridization: *P. homiensis* KACC 11518^T was obtained from the Korean Agricultural Culture Collection and *P. zeaxanthinifaciens* KCTC 22688^T was obtained from the Korean Collection for Type Cultures. For DNA and isoprenoid quinone extraction, cultures were prepared in marine broth 2216 (MB; Difco) at 30 °C for 3 days. For fatty acid extraction and morphological, physiological and biochemical characterization, cultures were prepared on MA at 30 °C for 3 days.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 22-5^T is GQ260189.

Table 1. Differential phenotypic characteristics of strain 22-5^T and the type strains of the two most closely related species of the genus *Paracoccus*

Strains: 1, *Paracoccus fistulariae* sp. nov. 22-5^T; 2, *P. homiensis* KACC 11518^T; 3, *P. zeaxanthinifaciens* KCTC 22688^T. All data from this study unless otherwise indicated. All strains were Gram-negative, rod-shaped and positive for catalase*, oxidase*, hydrolysis of tyrosine, Tweens 20 and 40 and hypoxanthine, utilization of cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, sucrose, acetate, citrate, L-malate, pyruvate and succinate, activity of alkaline phosphatase, esterase (C4) and α -glucosidase and susceptibility to carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. All strains were negative for production of H₂S and indole, hydrolysis of casein, starch and urea, utilization of benzoate, formate and L-glutamate, acid production from D-mannitol, melezitose, raffinose and trehalose, activity of lipase (C14), arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, valine arylamidase, trypsin, α -chymotrypsin, α -fucosidase, β -glucuronidase, β -glucosidase, N-acetyl- β -glucosaminidase and α -mannosidase and susceptibility to lincomycin and oleandomycin. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3
Motility	–	+	–
NaCl range for growth (%)	0–14†	0–14*	3–8*
Nitrate reduction	+	–	–
Lipolytic activity	+	–	–
Hydrolysis of:			
Aesculin	+	–	+
Gelatin	+	+	–
Tween 60	+	–	+
Tween 80	+	–	+
Xanthine	+	+	–
Utilization of:			
L-Arabinose	+	–	–
Salicin	+	–	–
Trehalose	+	–	–
D-Xylose	–	+	–
Acid production from:			
L-Arabinose	+	+	w
Cellobiose	+	–	–
D-Fructose	+	–	+
D-Galactose	+	–	+
D-Glucose	+	–	+
myo-Inositol	–	+	+
Lactose	+	–	–
Maltose	+	w	+
D-Mannose	+	–	+
Melibiose	+	–	w
L-Rhamnose	+	+	–
D-Ribose	+	–	+
D-Sorbitol	–	w	–
Sucrose	+	–	+
D-Xylose	+	+	–
Enzyme activity (API ZYM)			
Acid phosphatase	w	+	–
Esterase lipase (C8)	–	+	–
Leucine arylamidase	w	+	w

Table 1. cont.

Characteristic	1	2	3
Cystine arylamidase	–	+	–
Naphthol-AS-BI-phosphohydrolase	w	+	–
α -Galactosidase	–	+	+
β -Galactosidase	–	+	+
Susceptibility to:			
Ampicillin	+	–	–
Novobiocin	+	+	–
Penicillin G	+	+	–
Tetracycline	+	–	–
DNA G+C (mol%)	63.6	63*	66.9–67.7*

*Data were taken from Kim *et al.* (2006) and Berry *et al.* (2003) for columns 2 and 3, respectively.

†Growth with 0% NaCl occurs when Mg²⁺ ions are added.

Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy. Flagellation was determined by using a Philips CM-20 transmission electron microscope with cells from an exponentially growing culture. For this purpose, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined by using a Gram-stain kit (bioMérieux), according to the manufacturer's instructions. Growth at 4, 10, 20, 25, 28, 30, 35, 37, 40 and 45 °C was determined on MA. Growth with 0, 0.5, 1.0, 2.0 and 3.0% (w/v) NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except for the exclusion of NaCl and inclusion of 0.45% (w/v) MgCl₂·6H₂O or 0.06% (w/v) KCl. Growth with 2.0–15.0% (w/v) NaCl (in increments of 1.0%) was investigated in MB. The pH range for growth was determined in MB adjusted to pH 4.5–9.5 (in increments of 0.5 pH unit) with sodium acetate/acetic acid and Na₂CO₃ buffers. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with potassium nitrate (0.1%, w/v), both prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Lipolytic activity was investigated on tricaprillin-MA, which was prepared as follows: 5 ml tricaprillin was emulsified in 45 ml gum arabic solution (200 mM NaCl, 10 mM CaCl₂ and 5%, w/v, gum arabic) for 2 min using a Waring blender and 50 ml tricaprillin emulsion was mixed with 450 ml MA. Hydrolysis of casein, starch, hypoxanthine, tyrosine and xanthine was tested on MA using the substrate concentrations described by Cowan & Steel (1965). Nitrate reduction and hydrolysis of aesculin, gelatin, urea and Tweens 20, 40, 60 and 80 were investigated as described by Lányi (1987) with the modification that artificial seawater was used for preparation of media. The artificial seawater contained (l^{–1} distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g

MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). H₂S production was tested as described by Bruns *et al.* (2001). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981) using supplementation with 2 % (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1 % (v/v) vitamin solution (Staley, 1968). Susceptibility to antibiotics was tested on MA plates using antibiotic discs containing the following (µg per disc unless otherwise stated): polymyxin B (100 U), streptomycin (50), penicillin G (20 U), chloramphenicol (100), ampicillin (10), cephalothin (30), gentamicin (30), novobiocin (5), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), neomycin (30) and carbenicillin (100). Other physiological and biochemical tests were performed with the API 20E and API ZYM systems (bioMérieux). The morphological, cultural, physiological and biochemical characteristics of strain 22-5^T are given in the species description and Table 1.

Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplification products and phylogenetic analysis were performed as described previously (Yoon *et al.*, 2003). The identification of phylogenetic neighbours was achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). The almost-complete

16S rRNA gene sequence of strain 22-5^T determined in this study comprised 1383 nt. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain 22-5^T fell within the clade comprising species of the genus *Paracoccus* and formed a coherent cluster with *P. homiensis* DD-R11^T and *P. zeaxanthinifaciens* ATCC 21588^T (Fig. 1). The same cluster was found in the trees reconstructed using the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain 22-5^T exhibited 97.4 and 96.6 % 16S rRNA gene sequence similarity with *P. homiensis* DD-R11^T and *P. zeaxanthinifaciens* ATCC 21588^T, respectively, and 94.0–96.6 % with the type strains of the other species of the genus *Paracoccus*. The DNA G + C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The DNA G + C content of strain 22-5^T was 63.6 mol%.

Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. The predominant isoprenoid quinone detected in strain 22-5^T was ubiquinone-10 (Q-10), which was in line with those for other members of the genus *Paracoccus* (Kelly *et al.*, 2006; Roh *et al.*, 2009). In this study, *P. zeaxanthinifaciens* KCTC 22688^T was also found to have Q-10 as the predominant isoprenoid quinone. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the Hewlett Packard Microbial Identification System (MIDI; Sasser, 1990). The fatty acid profile of strain 22-5^T is shown in Table 2 with those of

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain 22-5^T and members of the genus *Paracoccus*. 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 maximum-parsimony algorithms. *Rhodobacter capsulatus* ATCC 11166^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid compositions of strain 22-5^T and the type strains of the two most closely related species of the genus *Paracoccus*

Strains: 1, *Paracoccus fistulariae* sp. nov. 22-5^T; 2, *P. homiensis* KACC 11518^T; 3, *P. zeaxanthinifaciens* KCTC 22688^T. All data from this study. Fatty acids that represented <0.5 % in all strains are omitted. ECL, Equivalent chain-length.

Fatty acid (%)	1	2	3
Straight-chain			
C _{16:0}	0.7	0.6	0.4
C _{18:0}	10.1	6.5	4.5
Unsaturated			
C _{17:1} ω7c	0.3	1.1	0.7
C _{18:1} ω7c	73.8	72.1	82.9
Hydroxy			
C _{11:0} 2-OH	3.1	3.1	3.5
C _{12:0} 3-OH	0.6	0.5	0.6
11-Methyl C _{18:1} ω7c	3.6	10.2	—
Summed feature 2*	3.5	2.0	3.2
ECL 11.799	2.9	2.9	3.3

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

P. homiensis KACC 11518^T and *P. zeaxanthinifaciens* KCTC 22688^T also analysed in this study. Strain 22-5^T contained large amounts of unsaturated, straight-chain, hydroxy and 11-methyl fatty acids; the major components (>10 % of total fatty acids) were C_{18:1}ω7c and C_{18:0}. The fatty acid profiles of strain 22-5^T, *P. homiensis* KACC 11518^T and *P. zeaxanthinifaciens* KCTC 22688^T were essentially similar in that C_{18:1}ω7c was the predominant fatty acid, although there were differences in the proportions of some fatty acids. The results obtained from chemotaxonomic analyses confirmed the result of phylogenetic analysis, i.e. that strain 22-5^T belongs to the genus *Paracoccus* (Kelly *et al.*, 2006; Roh *et al.*, 2009; Tables 1 and 2).

DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values. Strain 22-5^T exhibited 44 and 32 % mean DNA–DNA relatedness with *P. homiensis* KACC 11518^T and *P. zeaxanthinifaciens* KCTC 22688^T, respectively, which indicated that it differed genetically from the two reference strains (Wayne *et al.*, 1987).

Strain 22-5^T was distinguishable from the two reference strains by differences in several phenotypic characteristics, most of which were determined in this study using the same conditions and methods (Table 1). The phylogenetic

and genetic distinctiveness and the phenotypic properties of strain 22-5^T are sufficient to show that this strain is separate from recognized species of the genus *Paracoccus* (Stackebrandt & Goebel, 1994). Therefore, strain 22-5^T is considered to represent a novel species of the genus *Paracoccus*, for which the name *Paracoccus fistulariae* sp. nov. is proposed.

Description of *Paracoccus fistulariae* sp. nov.

Paracoccus fistulariae (f.i.stu.la'ri.ae. N.L. gen. n. *fistulariae* of *Fistularia*, named after the generic name of the bluespotted cornetfish, *Fistularia commersonii*, from which the type strain was isolated).

Cells are Gram-stain-negative, non-spore-forming, non-flagellated, short rods or rods (0.3–0.6 × 0.7–2.5 µm). Colonies on MA after 3 days at 30 °C are circular, slightly convex, smooth, glistening, greyish-yellow and 2.0–3.0 mm in diameter. Grows at 10–40 °C (optimum 30 °C), but not at 4 or 45 °C. Grows at pH 5.0 (optimum pH 7.0–8.0), but not at pH 4.5. Grows with 0–14.0 % (w/v) NaCl (optimum 2–5 % NaCl). Mg²⁺ ions are required for growth. Growth does not occur under anaerobic conditions on MA or on MA supplemented with nitrate. Catalase- and oxidase-positive. Nitrate reduction is positive. Shows lipolytic activity. The predominant ubiquinone is Q-10. The major fatty acids (>10 % of total fatty acids) are C_{18:1}ω7c and C_{18:0}. Other phenotypic properties are shown in Table 1. The DNA G+C content of the type strain is 63.6 mol% (HPLC).

The type strain, 22-5^T (=KCTC 22803^T =CCUG 58401^T), was isolated from the intestine of the bluespotted cornetfish, *Fistularia commersonii*.

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