Roseovarius indicus sp. nov., isolated from deep-sea water of the Indian Ocean

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A taxonomic study was carried out on a novel bacterial strain, designated B108^T, which was isolated from a polycyclic aromatic hydrocarbon (PAH)-degrading consortium, enriched from deep-sea water of the Indian Ocean. The isolate was Gram-reaction-negative, rod-shaped and non-motile. Growth of strain B108^T was observed in 1–15 % (w/v) NaCl and at 10–39 °C and it was unable to degrade Tween 80 or gelatin. 16S rRNA gene sequence comparisons showed that strain B108^T was most closely related to *Roseovarius halotolerans* HJ50^T (97.1 % sequence similarity), followed by *Roseovarius pacificus* 81-2^T (96.6 %) and *Roseovarius aestuarii* SMK-122^T (95.2 %); other species shared <95.0 % sequence similarity. DNA–DNA hybridization tests showed that strain B108^T had a low DNA–DNA relatedness to *R. halotolerans* HJ50^T and *R. pacificus* 81-2^T (48 ± 4 % and 44 ± 5 %, respectively). The predominant fatty acids were C_{16:0}, C_{16:0} 2-OH, summed feature 8 (C_{18:1ω7c/ω6c}) and C_{19:0ω8c} cyclo, which accounted for 84.2 % of the total cellular fatty acids. The G + C content of the chromosomal DNA was 63.6 mol%. The major respiratory quinone was ubiquinone 10 (Q10). Phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and some unidentified compounds were detected. These characteristics were in good agreement with those of members of the genus *Roseovarius*. The *pufLM* gene was also detected. According to its morphology, physiology, fatty acid composition and phylogenetic position based on 16S rRNA sequence data, the novel strain most appropriately belongs to the genus *Roseovarius* but can be readily distinguished from known species of this genus. Therefore, strain B108^T represents a novel species, of the genus *Roseovarius*, for which the name *Roseovarius indicus* sp. nov. is proposed. The type strain is B108^T (=2PR52-14^T =CCTCC AB 208233^T =LMG 24622^T =MCCC 1A01227^T).

In a study investigating polycyclic aromatic hydrocarbon (PAH)-degrading bacteria in deep-sea water of the Indian Ocean, many bacterial strains were isolated and characterized taxonomically. One of these isolates, designated strain B108^T, was chosen for further investigation. Comparative 16S rRNA gene sequence analysis indicated that strain B108^T formed a clade with the genus *Roseovarius* within the family *Rhodobacteraceae*. The genus *Roseovarius* was

proposed by Labrenz *et al.* (1999) and, at the time of writing, included eight type strains: *Roseovarius tolerans* (Labrenz *et al.*, 1999), *Roseovarius nubinhibens* (González *et al.*, 2003), *Roseovarius crassostreae* (Boettcher *et al.*, 2005), *Roseovarius mucosus* (Biebl *et al.*, 2005), *Roseovarius aestuarii* (Yoon *et al.*, 2008), *Roseovarius pacificus* (Wang *et al.*, 2009), *Roseovarius halotolerans* (Oh *et al.*, 2009) and *Roseovarius nanhaiticus* (Wang *et al.*, 2010). Strain B108^T was characterized by using a polyphasic taxonomic approach including the determination of phenotypic properties and a detailed phylogenetic analysis based on 16S rRNA gene sequences.

Deep-sea water was sampled from a depth of 4696 m (50 m above the sea floor) at the site of IR-CTD5-2 (31.0710° S 58.9945° E) on the South-west Indian Ridge during the

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Abbreviation: PAH, polycyclic aromatic hydrocarbon.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B108^T is EU742628.

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

cruise of DY-105A of R/V 'Da-Yang Yi-Hao' in December 2005. The sample was enriched with PAHs (100 p.p.m. phenanthrene, 10 p.p.m. anthracene, 10 p.p.m. fluoranthene and 10 p.p.m. pyrene) and the strains were isolated on 216L marine agar medium, comprising (g per l seawater) CH₃COONa (1.0), Tryptone (10.0), yeast extract (2.0), sodium citrate (0.5), NH₄NO₃ (0.2) and agar (15) (pH 7.5), following the method described by Lai *et al.* (2010). For morphological and biochemical characterization, strain B108^T was cultivated on marine agar (MA) 2216 medium (BD).

Genomic DNA was prepared according to the method of Ausubel *et al.* (1995) and the 16S rRNA gene was amplified by PCR using primers that had been described previously (Liu & Shao, 2005). Sequences of related taxa were obtained from the GenBank database. Phylogenetic analysis was performed using MEGA version 4 (Tamura *et al.*, 2007) after multiple alignment of data by DNAMAN (version 5.1; Lynnon Biosoft). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony and minimum-evolution (Rzhetsky & Nei, 1992, 1993) methods were determined by using bootstrap calculations based on 1000 replications.

The nearly full-length 16S rRNA gene sequence (1430 nt) of strain B108^T was determined. Phylogenetic analysis of strain B108^T indicated that it belonged to the class *Alpha-proteobacteria*, forming a robust clade within the genus *Roseovarius* in the neighbour-joining tree (Fig. 1). The resultant maximum-parsimony tree is shown in Supplementary Fig. S1, available in IJSEM Online. Strain B108^T formed a group with *R. halotolerans* HJ50^T and *R. pacificus* 81-2^T in both trees. The closest related species was *R. halotolerans* HJ50^T (97.1 % 16S rRNA sequence similarity), followed by *R. pacificus* 81-2^T (96.6 %) and *R. aestuarii* SMK-122^T (95.2 %); other species shared <95.0 % sequence similarity. In general, a 16S rRNA gene sequence divergence

>2 % is accepted as the recommended criterion for delineating different species (Stackebrandt & Goebel, 1994). 16S rRNA gene sequence divergences between strain B108^T and recognized species of genus *Roseovarius* were ≥2.9 %, and thus the data supported the view that strain B108^T represented a novel species. The signature nucleotides detected for strain B108^T were: helical region 240–242/284–286 UAG/CUA, position 113–115/312–314 GUG/CAC and position 577–580/764–767 GCGC/GUGC.

DNA–DNA hybridization experiments were performed as previously described (Liu & Shao, 2005) with genomic DNA of strain B108^T, *R. halotolerans* HJ50^T and *R. pacificus* 81-2^T. Strain B108^T showed low DNA–DNA relatedness to *R. halotolerans* HJ50^T and *R. pacificus* 81-2^T (48 ± 4 % and 44 ± 5 %, respectively), demonstrating its affiliation to a novel species in accordance with the cut-off value of 70 % recognized by Wayne *et al.* (1987) for the discrimination of bacterial species.

Gram reaction, activities of catalase, oxidase and lipase (Tween 80), hydrolysis of aesculin and starch, optimal temperature and pH for growth, tolerance to NaCl, antibiotic susceptibility and general cell morphology via electron microscopy were determined as described previously (Lai *et al.*, 2010). Other biochemical tests were carried out on API 20 NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions but with media adjusted to contain 3 % (w/v) NaCl. The results are given in the species description and in Table 1. Eight type species of the genus *Roseovarius* were, in most cases, tested in parallel for comparison (Table 1).

The G + C content of the chromosomal DNA was determined according to the methods described by Mesbah & Whitman (1989) using reversed-phase HPLC. The DNA G + C content of strain B108^T was 63.6 mol%, which was in the range previously reported for species of the genus *Roseovarius* (58.6–66.0 mol%).

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain B108^T and representatives of related taxa, based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.005 substitutions per nucleotide position (K_{nuc}).

Table 1. Characteristics that differentiate B108^T from related species of the genus *Roseovarius*

Strains: 1, B108^T (results from this study); 2, *R. halotolerans* HJ50^T (Oh *et al.*, 2009); 3, *R. pacificus* 81-2^T (Wang *et al.*, 2009); 4, *R. aestuarii* SMK-122^T (Yoon *et al.*, 2008); 5, *R. nubinihibens* DSM 15170^T (González *et al.*, 2003); 6, *R. tolerans* EL-172^T (Labrenz *et al.*, 1999); 7, *R. nanhaiticus* NH52J^T (Wang *et al.*, 2010); 8, *R. mucosus* DFL-24^T (Biebl *et al.*, 2005); 9, *R. crassostreae* DSM 16950^T (Boettcher *et al.*, 2005). Catalase, oxidase, API 20 NE and API ZYM tests were performed for all type strains at the same time. All strains were positive for catalase and oxidase activities. In the API 20 NE system, all strains were negative for indole production, D-glucose fermentation, β -glucosidase, gelatin hydrolysis, β -galactosidase activity and utilization of adipic acid, capric acid, D-glucose, maltose, D-mannitol, D-mannose, L-arabinose, malic acid, N-acetylglucosamine, phenylacetic acid, potassium gluconate and trisodium citrate. In the API ZYM system, all strains were positive for alkaline phosphatase, esterase (C4) and leucine aminopeptidase activities but were negative for N-acetyl- β -glucosaminidase, trypsin, α -chymotrypsin, α -fucosidase, α - and β -galactosidase, α - and β -glucosidase, α -mannosidase and β -glucuronidase. All strains were sensitive to carbenicillin, gentamicin and penicillin G but resistant to lincomycin, polymyxin B and tetracycline.

Characteristic	1*	2	3	4	5	6	7	8	9
Motility	—	—	+	+	+	+	+	—	+
Flagella	—	—	Subpolar	Polar	ND	—	—	—	Subpolar-lateral
Tolerance of NaCl (% w/v)	1–15	0.5–20	1–15	≤7	0.8–4.7	1–15	0.5–10	0.3–10	ND
Optimal salinity (%)	3–5	3–4	2–12	ND	1.1–2.3	1–8	2–4	1–7	1–1.5
Temperature range for growth (°C)	10–39	10–45	ND	10–37	10–40	3.0–43.5	ND	20–40	19–40
Optimum temperature (°C)	25	35	25	30	30	8.5–33.5	30	31	34
Photosynthetic reaction centre genes (<i>pufLM</i>)*	+	—	—	—	—	+	—	+	—
API 20 NE*									
Nitrate reduction	+	+	—	+	—	—	—	—	+
Arginine dihydrolase	+	+	w	—	—	—	+	w	—
Urease	+	+	+	—	—	w	+	+	—
API ZYM*									
Esterase lipase (C8)	+	+	+	+	w	+	+	+	+
Lipase (C14)	w	w	w	w	w	+	w	+	w
Valine aminopeptidase	+	+	+	w	w	+	+	+	w
Acid phosphatase	+	+	w	+	+	+	+	+	+
Naphthol-AS-B1-phosphohydrolase	+	+	—	+	+	+	w	+	+
Cystine aminopeptidase	—	—	—	—	—	—	—	w	—
Susceptible to:									
Ampicillin, chloramphenicol	+	—*	+	+	+	+	+	+	+
Kanamycin	+	+	—	+	+	+	+	+	+
Streptomycin	—	+	+	+	+	+	+	+	+
DNA G + C content (mol%)	63.6	59.0	62.3	58.6	66	63.3–63.4	60.9	60.9–62.9	59

*Results obtained in this study.

Whole-cell fatty acids from cells grown on MA 2216 medium at 28 °C for 48 h were extracted, saponified and esterified, followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1990). Type strains of eight species of the genus *Roseovarius* were tested under the same conditions in this study along with strain B108^T. As shown in Supplementary Table S1, the major fatty acids of strain B108^T were C_{16:0} (5.66%), C_{16:0} 2-OH (6.75%), summed feature 8 (C_{18:1}ω7d/ω6c) (63.15%) and C_{19:0}ω8c cyclo (8.64%), which accounted for 84.2% of the total cellular fatty acids. In addition, the major fatty acids of the other species of the genus *Roseovarius* tested were C_{16:0} (5.66–17.63%) and summed feature 8 (C_{18:1}ω7d/ω6c) (48.04–70.70%). The fatty acid profile of strain B108^T was in good agreement with those of members of the genus *Roseovarius* but could be

differentiated from other species by the percentage of C_{16:0} 2-OH and C_{19:0}ω8c cyclo.

Additionally, the genetic potential for anoxygenic phototrophy was determined by PCR amplification of the photosynthetic reaction centre genes (*pufLM*) using the primers *pufLf* (5'-CTKTTCGACTTCTGGGTSGG-3') and *pufMr* (5'-CCCATSGTCCAGCGCCAGAA-3') (Béjà *et al.*, 2002). The specific 1.5 kb products were clearly amplified from strain B108^T, *R. tolerans* EL-172^T and *R. mucosus* DFL-24^T, but not from the other six type strains tested. These results were similar to those of Oh *et al.* (2009), who only detected the specific PCR product in *R. tolerans* EL-172^T and *R. mucosus* DFL-24^T using the primers *pufM.557F* and *pufM.750R*.

The major respiratory quinone of strain B108^T was ubiquinone 10 (Q10), as determined by HPLC using the

method of Collins (1985). This trait was in accordance with the properties of members of the genus *Roseovarius* except that three type strains, *R. pacificus* 81-2^T, *R. nubinhibens* DSM 15170^T and *R. nanhaiticus* NH52^T, have no available data. Polar lipids of strain B108^T were extracted and separated according to the methods described by Tindall (1990). The results are shown in Supplementary Fig. S2. The polar lipids of strain B108^T were phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and some unidentified compounds. These results were similar to the polar lipid patterns of *R. halotolerans* HJ50^T, *R. mucosus* DFL-24^T and *R. tolerans* EL-172^T.

On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence analysis and DNA–DNA hybridization tests described above, strain B108^T represents a novel species of the genus *Roseovarius*, for which the name *Roseovarius indicus* sp. nov. is proposed.

Description of *Roseovarius indicus* sp. nov.

Roseovarius indicus (in'di.cus. L. adj. *indicus* Indian, referring to the Indian Ocean, where the strain was isolated).

Cells are Gram-reaction-negative, non-motile, short rods, $1.7\text{--}2.4 \times 1.0\text{--}1.1\ \mu\text{m}$. Positive for catalase and oxidase activities and nitrate reduction, but negative for indole production, urease, lipase (Tween 80), gelatinase, β -glucosidase, β -galactosidase and arginine dihydrolase activities, glucose fermentation, denitrification and hydrolysis of aesculin. On 216L agar, strain B108^T produces smooth colonies with regular edges that are 2–3 mm in diameter after 72 h of incubation at 28 °C. Colonies are opaque, white to faintly pink in the centre, non-pigmented and slightly raised in the centre. Moderately halophilic. Grows in 1–15 % (w/v) NaCl (optimum 3–5 %) and at 10–39 °C (optimum 25 °C), but not at 4 or 40 °C after 1 week. Principal fatty acids were C_{16:0}, C_{16:0} 2-OH, summed feature 8 (C_{18:1}ω7c/ω6c) and C_{19:0}ω8c cyclo. The major respiratory quinone was ubiquinone 10 (Q10). Phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and some unidentified compounds were detected. The *pufLM* gene was detected. Sensitive to [μg per disk (OXOID), unless stated otherwise]: ampicillin (10), carbenicillin (100), cefalexin (30), cefazolin (30), cefobid (30), cephradine (30), chloramphenicol (30), ciprofloxacin (5), furazolidone (15), gentamicin (10), kanamycin (30), minomycin (30), norfloxacin (10), penicillin G (10), piperacillin (100), rifampicin (5), rocephin (30), vancomycin (30); resistant to clindamycin (2), Cotrimoxazole (25), erythromycin (15), lincomycin (2), metronidazole (5), neomycin (10), ofloxacin (5), oxacillin (1), polymyxin B (30 IU), streptomycin (10), tetracycline (30) and vibramycin (30). In API ZYM tests: positive for acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine aminopeptidase, naphthol-AS-BI-phosphohydrolase and valine aminopeptidase; weakly positive for lipase (C14); negative for cystine aminopeptidase,

N-acetyl- β -glucosaminidase, trypsin, α -chymotrypsin, α -fucosidase, α - and β -galactosidase, α - and β -glucosidase, α -mannosidase and β -glucuronidase. Negative for utilization of all 12 carbon sources in API 20 NE tests. Characteristics used to distinguish strain B108^T from related species are shown in Table 1.

The type strain, B108^T (=2PR52-14^T =CCTCC AB 208233^T =LMG 24622^T =MCCC 1A01227^T), was isolated from deep-sea water of the Indian Ocean. The DNA G+C content of the type strain is 63.6 mol%.

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