Oceanibaculum indicum gen. nov., sp. nov., isolated from deep seawater of the Indian Ocean

Qiliang Lai, Jun Yuan, Changliang Wu and Zongze Shao

Correspondence

Zongze Shao
shaozz@163.com

Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, Xiamen 361005, PR China

A taxonomic study was carried out on strain P24^T, which was isolated from a polycyclic aromatic hydrocarbon-degrading consortium, enriched from a deep-seawater sample collected from the Indian Ocean. The isolate was Gram-negative, rod-shaped, motile by means of a polar flagellum, moderately halophilic and capable of reducing nitrate to nitrite. Growth was observed at salinities of 0–9‰ and at temperatures of 10–42 °C. The strain was unable to degrade Tween 80 or gelatin. The dominant fatty acids were C_{16:0} (15.2% of the total), C_{18:0} (10.3%), C_{18:1 ω 7c} (52.0%), C_{18:1} 2-OH (4.7%) and C_{19:0 ω 8c} cyclo (4.7%). The G + C content of the chromosomal DNA was 64.8 mol%. 16S rRNA gene sequence comparisons showed that strain P24^T was related most closely to *Thalassobaculum litoreum* CL-GR58^T (92.7% similarity); levels of similarity between strain P24^T and type strains of recognized species in the family *Rhodospirillaceae* were all less than 90.8%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain P24^T formed a distinct evolutionary lineage within the family *Rhodospirillaceae*. Strain P24^T could be distinguished from phylogenetically related genera based on differences in several phenotypic properties. On the basis of the phenotypic and phylogenetic data presented, strain P24^T is considered to represent a novel species of a new genus, for which the name *Oceanibaculum indicum* gen. nov., sp. nov. is proposed. The type strain is P24^T (=CCTCC AB 208226^T=LMG 24626^T=MCCC 1A02083^T).

To investigate polycyclic aromatic hydrocarbon (PAH)-degrading bacteria from deep seawater of the Indian Ocean, many bacterial strains were isolated and characterized taxonomically. The present study focuses on one of these isolates, designated strain P24^T. Comparative 16S rRNA gene sequence analysis indicated that strain P24^T formed a deep branch within the family *Rhodospirillaceae*. Accordingly, the aim was to determine the exact taxonomic position of strain P24^T by using a polyphasic characterization, including determination of phenotypic properties and detailed phylogenetic analysis based on 16S rRNA gene sequences.

Deep seawater was sampled with Niskin bottles attached to a CTD (conductivity, temperature and depth) circular rosette in December 2005 during the course of cruise DY-105A of research vessel *Da-Yang Yi-Hao*. The sampling site (designated IR-CTD9-2) was at 70.0405° E, 25.3217° S on the Southwest Indian Ridge. Water depth at the site is 2488 m; a water sample from 2468 m depth was used for

enrichment of PAH-degrading bacteria with 1% (v/v) sterilized crude oil and a PAH mixture (final concentrations of 200 p.p.m. naphthalene, 200 p.p.m. phenanthrene, 200 p.p.m. anthracene and 200 p.p.m. pyrene; PAHs were dissolved in crude oil) as the carbon and energy source. Enrichment was undertaken on board immediately after sampling. Two months later, at our laboratory, 1 ml of the enrichment culture was transferred into 100 ml fresh medium containing (per litre) 1.0 g NH₄NO₃, 0.5 g KH₂PO₄ and 2.8 mg FeSO₄·7H₂O, with the PAH mixture as the only carbon and energy source. PAH stock solution in chloroform was added to flasks to the same concentrations as above, and the flasks were then shaken at 160 r.p.m. for 2 days to evaporate the chloroform before inoculation. After 3 weeks incubation at 28 °C with shaking at 160 r.p.m., 1 ml culture broth was transferred repeatedly to the same medium for further enrichment. Sequential transfers were performed every 4 weeks three times. Bacteria were isolated by using the plate screening method on 216L medium (per litre seawater: 1.0 g CH₃COONa, 10.0 g tryptone, 2.0 g yeast extract, 0.5 g sodium citrate and 0.2 g NH₄NO₃; pH 7.5). This medium was also used for morphological and biochemical characterization.

Genomic DNA was prepared according to the method of Ausubel *et al.* (1995); the 16S rRNA gene was amplified by

Abbreviation: PAH, polycyclic aromatic hydrocarbon.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P24^T is EU656113.

Transmission electron micrographs of negatively stained cells of strain P24^T and a table giving the cellular fatty acid contents of strain P24^T and related genera are available as supplementary material with the online version of this paper.

Table 1. Differential characteristics between strain P24^T and related taxa

Taxa: 1, strain P24^T (*Oceanibaculum indicum* sp. nov.); 2, *Thalassobaculum litoreum* CL-GR58^T; 3, *Azospirillum*; 4, *Rhodocista*; 5, *Rhodovibrio*; 6, *Defluviicoccus*; 7, *Tistrella*; 8, *Skermanella*; 9, *Inquilinus*. Data for 2–9 were taken from Zhang *et al.* (2008). +, Positive; w, weakly positive; –, negative; v, variable; NA, data not available.

Characteristic	1	2	3	4	5	6	7	8	9
Colony colour	Grey	Cream–yellow	Pink, white	Red, pink	Pink, red	Beige	NA	Apricot	Pink
Cell shape	Rod	Curved and rod	Plump, vibrioid, rod	Vibrioid, spiral	Vibrioid, spiral	Coccus	Rod	Rod	Rod
Flagella*	MP	MP	v	MP	MP, BP	Absent	MP	MP	NA
Oxidase	–	+	+	NA	NA	–	+	+	v
Catalase	+	+	+	NA	NA	+	+	+	+
Gelatinase	–	+	v	NA	NA	w	+	–	v
Temperature range (°C)	10–42	10–35	4–41	25–47	20–47	20–30	20–40	10–37	25–42
Temperature optimum (°C)	25–37	30–35	20–41	31–45	35–42	25–30	30	28	NA
pH range	6–11	7–9	5–8.5	5.7–8	7–8	5–8.5	5–9	NA	NA
pH optimum	7–9	8	5–7.2	6.8–7	7–8	7.5–8	7.4	NA	NA
Salt tolerance (% w/v)	0–9	1–10	<5	NA	3–24	NA	<1	<5	<6
Bacteriochlorophyll <i>a</i>	–	–	–	+	+	+	–	–	NA
Utilization of:									
<i>N</i> -Acetylglucosamine	–	–	v	NA	NA	+	+	–	+
L-Arabinose	–	+	v	NA	NA	+	+	+	–
D-Glucose	–	–	v	–	–	+	NA	+	–
Citrate	–	–	v	–	–	NA	NA	+	–
Inositol	–	–	v	NA	NA	NA	NA	–	–
D-Mannitol	–	–	v	–	–	NA	+	+	–
L-Rhamnose	+	–	v	NA	NA	NA	NA	+	–
Sucrose	–	+	v	–	–	NA	NA	NA	–
DNA G + C content (mol%)	64.8	68	64–71	68.3–69.9	66.2–68.1	66	67.5	67.2	70.9

*BP, Bipolar; MP, monopolar.

PCR by using the primers described by Liu & Shao (2005). Sequences of related taxa were obtained from the GenBank database. Phylogenetic analysis was performed by using MEGA version 4 (Tamura *et al.*, 2007) after multiple alignment of the data by DNAMAN (version 5.1; Lynnon Biosoft). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method of Saitou & Nei (1987) and minimum-evolution method of Rzhetsky & Nei (1992, 1993) were determined by using bootstrap values based on 1000 replications. Data for the minimum-evolution clustering are not shown as the results were similar to those with the neighbour-joining method.

General cell morphology was studied under an Olympus inverted microscope by using 1-day-old cultures on 216L agar. For electron microscopy, exponential-phase cells were harvested, subsequently suspended and absorbed on a Formvar-carbon-coated grid, then stained with phosphotungstic acid (see Supplementary Fig. S1 in IJSEM Online). Tests for the Gram reaction, catalase and oxidase activities, lipase (Tween 80) and amylase were carried out according to Dong & Cai (2001). The optimal growth temperature was determined over the range 4–55 °C on 216L agar. Tolerance to NaCl was tested by using Luria–Bertani (LB) medium (Sambrook *et al.*, 1989) supplemented with NaCl at concentrations of 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 18 and 20 % (w/v). Antibiotic susceptibility tests were performed by the disc diffusion method as described by Shieh *et al.* (2003). Bacteriochlorophyll *a* content was determined as described by Biebl *et al.* (2005). Other biochemical tests were carried out by using API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions, and by using the Biolog GN2 microplate system according to Ivanova *et al.* (1998). The G + C content of the chromosomal DNA was determined according to the method described by Mesbah & Whitman (1989) by using reversed-phase HPLC. Fatty acids in whole cells grown on 216L plate medium at 28 °C for 48 h were extracted, saponified and esterified; this was followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1997). These results are given in the species description below and in Table 1.

A nearly full-length 16S rRNA gene sequence (1451 nt) of strain P24^T was determined. Phylogenetic analysis indicated that strain P24^T belonged to the family *Rhodospirillaceae* (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, strain P24^T joined the clade comprising *Thalassobaculum litoreum* CL-GR58^T, with which it shared highest 16S rRNA gene sequence similarity (92.7 %). Strain P24^T showed 90.8 % 16S rRNA gene sequence similarity to *Skermanella aerolata* DSM 18479^T and 79.3–89.1 % to the type strains of other type species in the family *Rhodospirillaceae* used in the phylogenetic analysis (Fig. 1). In addition, BLASTN results showed that strain P24^T shared highest 16S rRNA gene sequence similarity (98.7 %) with a bacterium, designated strain SL3.14, from the Silver Lake throughflow play in California, USA (Navarro *et al.*, 2009).

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain P24^T and representatives of some related taxa, based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.02 nucleotide substitution rate (K_{nuc}) units.

Although strain P24^T and *Thalassobaculum litoreum* CL-GR58^T form a separate clade in the family *Rhodospirillaceae* with high bootstrap support (98 %, Fig. 1), the novel strain was not classified within the genus *Thalassobaculum* as it showed important differences in several phenotypic properties. Strain P24^T was negative for oxidase, gelatinase and amylase, in contrast to the results for *Thalassobaculum litoreum* CL-GR58^T (see Table 1). In addition, these two strains differed in ten enzyme reactions in API ZYM tests and in sensitivity to seven antibiotics (see Table 2). Moreover, strain P24^T showed differences from *Thalassobaculum litoreum* CL-GR58^T in fatty acid composition, such as the proportion of C_{17:0} (0.4 vs. 12.2 %, respectively) and C_{18:0} (10.3 vs. 2 %), and the presence of C_{16:0} 3-OH, C_{18:0} 3-OH and C_{18:1} 2-OH (see Supplementary Table S1 in IJSEM Online). Phenotypically, strain P24^T could be differentiated from members of the genera *Azospirillum*, *Tistrella* and *Skermanella*, which are positive for oxidase and have low salt tolerance (<5 %, w/v); it could also be differentiated from members of the genera *Rhodocista*, *Rhodovibrio* and *Defluviicoccus* based on several phenotypic features (colony colour, cell shape and temperature range for growth) and

Table 2. Differential characteristics between strain P24^T and *Thalassobaculum litoreum*

Strains: 1, P24^T (*Oceanibaculum indicum* sp. nov.); 2, *Thalassobaculum litoreum* CL-GR58^T. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2
Reaction to antimicrobial agents		
Ampicillin (10 µg)	–	+
Gentamicin (10 µg)	–	+
Erythromycin (15 µg)	–	+
Penicillin G (10 µg)	–	+
Tetracycline (30 µg)	–	+
Vancomycin (30 µg)	–	+
Polymyxin B (30 IU)	+	–
API ZYM data		
Esterase (C4)	w	+
Cystine aminopeptidase	w	–
Naphthol-AS-BI-phosphoamidase	w	+
α-Galactosidase	–	w
β-Galactosidase	–	w
α-Glucosidase	–	+
β-Glucosidase	–	+
N-Acetyl-β-glucosaminidase	–	+
α-Mannosidase	–	w
α-Fucosidase	–	w

from members of the genus *Inquilinus* by its pink colony colour, DNA G + C content difference (>6 mol%) and low salt tolerance (<6%, w/v) (Table 1).

The low levels of 16S rRNA gene sequence similarity between strain P24^T and all other members of the family *Rhodospirillaceae*, together with differential phenotypic properties (Table 1), suggest that strain P24^T represents a novel species of a new genus within the family *Rhodospirillaceae*, for which the name *Oceanibaculum indicum* gen. nov., sp. nov. is proposed.

Description of *Oceanibaculum* gen. nov.

Oceanibaculum (O.ce.a'ni.ba'cu.lum. Gr. n. *oceanus* ocean; L. neut. n. *baculum* stick; N.L. neut. n. *Oceanibaculum* rod-shaped bacterium from the ocean).

Cells are rod shaped, motile by means of a single polar flagellum, Gram-negative, oxidase-negative, catalase-positive, capable of reducing nitrate to nitrite, but not capable of denitrification. The dominant fatty acids are C_{16:0}, C_{18:0}, C_{18:1}ω7c, C_{18:1} 2-OH and C_{19:0}ω8c cyclo. Bacteriochlorophyll *a* is not detected. The type species is *Oceanibaculum indicum*.

Description of *Oceanibaculum indicum* sp. nov.

Oceanibaculum indicum (in.di'cum. L. adj. *indicum* referring to the Indian Ocean, from where the type strain was isolated).

Exhibits the following properties in addition to those given in the genus description. Cells are 2.3–2.5 µm long and 0.6–1.5 µm wide. Negative for lipase (Tween 80), amylase, urease, gelatinase, arginine dihydrolase and indole production. On 216L agar, forms smooth grey colonies with regular edges that are 1–2 mm in diameter after 72 h incubation at 28 °C, non-pigmented and slightly raised in the centre. Moderately halophilic; grows in the presence of 0–9% NaCl (optimum, 0.5–7%). Grows at 10–42 °C (optimum, 25–37 °C), but no growth is observed after 7 days at 4 or 45 °C. Unable to ferment glucose. Sensitive to chloramphenicol (30 µg per disc; Oxoid), ciprofloxacin (5 µg), co-trimoxazole (25 µg), kanamycin (30 µg), neomycin (10 µg), polymyxin B (30 IU) and streptomycin (10 µg), but resistant to ampicillin (10 µg), carbenicillin (100 µg), cefalexin (30 µg), cefazolin (30 µg), cefoperazone (30 µg), cephradine (30 µg), clindamycin (2 µg), erythromycin (15 µg), furazolidone (15 µg), gentamicin (10 µg), lincomycin (2 µg), metronidazole (5 µg), minocycline (30 µg), norfloxacin (10 µg), ofloxacin (5 µg), oxacillin (1 µg), penicillin G (10 µg), piperacillin (100 µg), rifampicin (5 µg), ceftriaxone (30 µg), tetracycline (30 µg), vancomycin (30 µg) and doxycycline (30 µg). In API ZYM tests, positive for acid phosphatase, alkaline phosphatase, leucine aminopeptidase and valine aminopeptidase, weakly positive for cystine aminopeptidase, esterase (C4), esterase lipase (C8), lipase (C14) and naphthol-AS-BI-phosphoamidase, but negative for trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Among 95 substrates in the Biolog GN2 system, positive for bromosuccinic acid, D-arabitol, D-saccharic acid, glycyl L-glutamic acid, L-alanine, L-glutamic acid, L-proline, L-pyroglutamic acid, L-rhamnose, succinamic acid, Tween 40 and α-ketoglutaric acid. The DNA G + C content of the type strain is 64.8 mol%. Table 1 shows characteristics that can be used to distinguish the type strain from related species.

The type strain, P24^T (=CCTCC AB 208226^T=LMG 24626^T=MCCC 1A02083^T), was isolated from deep seawater of the Indian Ocean.

Acknowledgements

This work was financially supported by the National Infrastructure of Natural Resources for Science and Technology Program of China (No. 2005DKA21209), COMRA program (No. DYXM115-02-2-05), National Basic Research Program of China (No. 2004CB719601) and National Natural Science Foundation of China (30670051; 40376041).

References

- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (editors) (1995). *Short Protocols in Molecular Biology: a Compendium of Methods from Current Protocols in Molecular Biology*, 3rd edn. New York: Wiley.
- Biebl, H., Allgaier, M., Tindall, B. J., Kobliczek, M., Lünsdorf, H., Pukall, R. & Wagner-Döbler, I. (2005). *Dinoroseobacter shibae* gen.

nov., sp. nov., a new aerobic phototrophic bacterium isolated from dinoflagellates. *Int J Syst Evol Microbiol* **55**, 1089–1096.

Dong, X.-Z. & Cai, M.-Y. (2001). *Determinative Manual for Routine Bacteriology*. Beijing: Scientific Press (English translation).

Ivanova, E. P., Kiprianova, E. A., Mikhailov, V. V., Levanova, G. F., Garagulya, A. D., Gorshkova, N. M., Vysotskii, M. V., Nicolau, D. V., Yumoto, N. & other authors (1998). Phenotypic diversity of *Pseudoalteromonas citrea* from different marine habitats and emendation of the description. *Int J Syst Bacteriol* **48**, 247–256.

Liu, C. & Shao, Z. (2005). *Alcanivorax dieselolei* sp. nov., a novel alkane-degrading bacterium isolated from sea water and deep-sea sediment. *Int J Syst Evol Microbiol* **55**, 1181–1186.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* **479**, 297–306.

Navarro, J. B., Moser, D. P., Flores, A., Ross, C., Rosen, M. R., Dong, H., Zhang, G. & Hedlund, B. P. (2009). Bacterial succession within an ephemeral hypereutrophic Mojave Desert playa Lake. *Microb Ecol* **57**, 307–320.

Rzhetsky, A. & Nei, M. (1992). A simple method for estimating and testing minimum evolution trees. *Mol Biol Evol* **9**, 945–967.

Rzhetsky, A. & Nei, M. (1993). Theoretical foundation of the minimum-evolution method of phylogenetic inference. *Mol Biol Evol* **10**, 1073–1095.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sasser, M. (1997). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Shieh, W. Y., Chen, Y.-W., Chaw, S.-M. & Chiu, H.-H. (2003). *Vibrio ruber* sp. nov., a red, facultatively anaerobic, marine bacterium isolated from sea water. *Int J Syst Evol Microbiol* **53**, 479–484.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Zhang, G. I., Hwang, C. Y. & Cho, B. C. (2008). *Thalassobaculum litoreum* gen. nov., sp. nov., a member of the family Rhodospirillaceae isolated from coastal seawater. *Int J Syst Evol Microbiol* **58**, 479–485.