Parvibaculum indicum sp. nov., isolated from deep-sea water

Qiliang Lai, Liping Wang, Yuhui Liu, Jun Yuan, Fengqin Sun and Zongze Shao

Correspondence
Zongze Shao
shaozz@163.com

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

A taxonomic study was carried out on strain P31^T, which was isolated from a polycyclic aromatic hydrocarbon (PAH)-degrading consortium enriched with deep-sea water of the Indian Ocean. The isolate was Gram-reaction-negative, rod-shaped, motile by means of a polar flagellum and incapable of reducing nitrate to nitrite. Growth was observed at 0.5–8 % NaCl and at 10–41 °C. Strain P31^T was unable to degrade Tween 80 or gelatin. The major respiratory quinone was ubiquinone 11 (Q-11). The dominant fatty acids were C_{18:1}ω7c (39.79 %), 11-methyl C_{18:1}ω7c (17.84 %), C_{19:0} cyclo ω8c (12.05 %) and C_{18:0} (6.09 %). The G+C content of the chromosomal DNA was 62.1 mol%. A phylogenetic tree based on 16S rRNA gene sequence analysis showed that strain P31^T and *Parvibaculum lavamentivorans* DS-1^T formed a distinct lineage in the family *Phyllobacteriaceae*; these two strains showed 95.7 % sequence similarity, while similarities between P31^T and other members of the genus *Parvibaculum* were below 93 %. Based on the genotypic and phenotypic data, strain P31^T represents a novel species of the genus *Parvibaculum*, for which the name *Parvibaculum indicum* sp. nov. is proposed. The type strain is P31^T (=CCTCC AB 208230^T =LMG 24712^T =MCCC 1A01132^T).

While studying the diversity of polycyclic aromatic hydrocarbon (PAH)-degrading bacteria in deep-sea water and marine sediments (Wang *et al.*, 2008; Cui *et al.*, 2008), we isolated a novel bacterium, designated P31^T, from deep-sea water of the Indian Ocean. Based on 16S rRNA gene sequence analysis, it formed a clade with *Parvibaculum lavamentivorans* DS-1^T in the family *Phyllobacteriaceae*. *P. lavamentivorans* was reported as a novel species of a novel genus in the class *Alphaproteobacteria* (Schleheck *et al.*, 2004) and is considered to be a member of the family *Phyllobacteriaceae* in the order *Rhizobiales* (NCBI; <http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Root>). The aim of the present study was to determine the exact taxonomic position of strain P31^T by using a polyphasic approach, including the determination of phenotypic characteristics and detailed phylogenetic analysis based on 16S rRNA gene sequences.

Deep-sea water was collected using Niskin bottles attached to a circular conductivity, temperature and depth (CTD) rosette from a depth of 1068 m (1020 m above the sea floor) at the site IR-CTD9-9 (25.3217° S 70.0405° E) over

the South-west Indian Ridge, during expedition DY-105A of R/V *Da-Yang Yi-Hao* in December 2005. The water sample was enriched with a PAH mixture (final concentrations 200 p.p.m. each) of naphthalene, phenanthrene, anthracene and pyrene dissolved in crude oil. Strains were isolated on 216L marine agar containing (g per litre seawater): sodium acetate (1), tryptone (10), yeast extract (2), sodium citrate (0.5), NH₄NO₃ (0.2) and agar powder (15), pH 7.5, following the method described by Lai *et al.* (2009). For morphological and biochemical characterization, strain P31^T was cultivated on 216L medium.

Genomic DNA was prepared according to the method of Ausubel *et al.* (1995) and the 16S rRNA gene was amplified by PCR using primers described previously (Liu & Shao, 2005a). Sequences of related taxa were obtained from the NCBI GenBank database. After the multiple alignment of sequences by DNAMAN version 5.1 (Lynnon Biosoft), phylogenetic analysis was performed using MEGA version 4 (Tamura *et al.*, 2007). The Kimura two-parameter model distance option was used and bootstrap values were based on 1000 replications. The phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987) and minimum-evolution (Rzhetsky & Nei, 1992, 1993) methods.

The nearly full-length sequence (1447 nt) of the 16S rRNA gene of strain P31^T was determined. As shown in Fig. 1, the neighbour-joining tree based on 16S rRNA gene sequences

Abbreviation: PAH, polycyclic aromatic hydrocarbon.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P31^T is FJ182044.

One supplementary figure and one supplementary table are available with the online version of this paper.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain P31^T and representatives of related taxa. Bootstrap values, expressed as percentages of 1000 replications, are shown at branch points. Bar, 0.005 substitutions per nucleotide position (K_{nuc}).

showed that strain P31^T and *P. lavamentivorans* DS-1^T formed a distinct lineage in the family *Phyllobacteriaceae*. Strain P31^T showed 95.7% sequence similarity to *P. lavamentivorans* DS-1^T but less similarity to other related taxa (<93%). The minimum-evolution tree (not shown) possessed a similar topology to the neighbour-joining tree. In general, a 16S rRNA gene sequence divergence greater than 3% is recommended as a criterion for delineating different species (Stackebrandt & Goebel, 1994). The 16S rRNA gene sequence divergence between strain P31^T and related species was >4.3%; thus the data supports the view that strain P31^T represents a novel species in the genus *Parvibaculum*.

Cell morphology of strain P31^T was studied under an Olympus IX-70 inverted microscope using 2-day-old cultures grown on 216L agar medium. For electron microscopy, exponential-phase cells were harvested, resuspended, absorbed onto a Formvar-carbon-coated grid and then stained with phosphotungstic acid. The Gram reaction and tests for catalase and oxidase activity were carried out according to Dong & Cai (2001). The optimal growth temperature was determined by culturing at 4, 10, 16, 20, 25, 28, 30, 37, 41, 42 and 45 °C on 216L medium. NaCl tolerance was tested by using Luria-Bertani (Sambrook *et al.*, 1989) medium, containing 10 g peptone and 5 g yeast l⁻¹, supplemented with 0, 0.5, 1, 3, 5, 7, 8, 9, 10, 12 or 15% (w/v) NaCl. The chain-length range of n-alkane oxidized by strain P31^T was determined at a concentration of 0.1% (w/v) in artificial seawater medium as described by Liu & Shao (2005b). Antibiotic susceptibility tests were performed by growth on marine agar at 28 °C, for 48 h by using the disc (Oxoid) diffusion method as described by Shieh *et al.* (2003). For strain P31^T and *P. lavamentivorans* DSM 13023^T, all biochemical tests were carried out at 28 °C for 24 h, except for API 20NE tests (72 h). These results are given in the species description and Table 1.

The major respiratory quinone of strain P31^T was determined by HPLC according to the method of Collins

(1985) and found to be ubiquinone 11 (Q-11), a trait that is shared with *P. lavamentivorans* DS-1^T. Fatty acids of whole cells grown on marine agar 2216 (BD Difco) at 28 °C for 72 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, 1990). The fatty acid profile of strain P31^T was determined in parallel with *P. lavamentivorans* DSM 13023^T in this study. The results for both strains are shown in Supplementary Table S1, available in IJSEM Online. The major fatty acids in both strains were C_{18:1}ω7c, C_{18:0}, 11-methyl C_{18:1}ω7c and C_{19:0}ω8c cyclo, which accounted for >75% of the total fatty acids. Using data from this study, strain P31^T and *P. lavamentivorans* DSM 13023^T showed highly similar major fatty acid profiles but differed in the proportions of the following fatty acids: C_{18:1} 2-OH (4.68 and 1.21%, respectively), C_{20:2}ω6,9c (0.73 and 2.95%) and C_{20:0} (4.96 and 0%). In addition, *P. lavamentivorans* DS-1^T was shown by Schleheck *et al.* (2004) to contain C_{19:0} cyclo 2-OH (8.1%); this was not detected in this study but appeared to be replaced by 11-methyl C_{18:1}ω7c (17.94%), which was also present in strain P31^T (17.84%). This may be a result of using a different growth medium.

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 isolate P31^T was 62.1 mol%, which is similar to that of *P. lavamentivorans* DS-1^T (64 mol%).

Strain P31^T was shown to be a Gram-reaction-negative, non-pigmented, rod-shaped bacterium that was motile by means of a polar flagellum (Supplementary Fig. S1). The physiological, biochemical and chemotaxonomic differences between strain P31^T and *P. lavamentivorans* DS-1^T are given in Table 1. On the basis of these differences, together with the results of the 16S rRNA gene sequence phylogenetic analysis described above, strain P31^T represents a novel species of the genus *Parvibaculum*, for which the name *Parvibaculum indicum* sp. nov. is proposed.

Table 1. Comparative characteristics of strain P31^T and *P. lavamentivorans* DS-1^T

Strains: 1, strain P31^T; 2, *P. lavamentivorans* DSM 13023^T. All data are from this study. Both strains were positive for catalase. In the API 20NE test, both strains were positive for utilization of adipic acid, L-arabinose and malic acid but negative for nitrate reduction, indole production, D-glucose fermentation, urease activity and the utilization of capric acid, D-glucose, maltose, D-mannitol, D-mannose, N-acetylglucosamine, phenylacetic acid, potassium gluconate and trisodium citrate. In the API ZYM test, both strains were positive for acid phosphatase, alkaline phosphatase, esterase (C4), leucine aminopeptidase and valine aminopeptidase activities, weakly positive for cystine aminopeptidase and naphthol-AS-BI-phosphoamidase activities and negative for N-acetyl-β-glucosaminidase, α-chymotrypsin, α-fucosidase, α-galactosidase, α-mannosidase and β-glucuronidase activities. In the Biolog GN2 test, both strains utilized acetic acid, methyl pyruvate, propionic acid, Tweens 40 and 80, α-ketobutyric acid, α-ketovaleric acid and β- and γ-hydroxybutyric acid as carbon sources but did not utilize 81 other kinds of carbon sources (except for the five listed in the table). Both strains were sensitive to the following antimicrobials (μg per disc): carbenicillin (100), cefazolin (30), cefobid (30), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), minomycin (30), piperacillin (100), rifampicin (5) and vibramycin (30) and resistant to cefalexin (30), cephradine (30), ciprofloxacin (5), lincomycin (2), metronidazole (5), ofloxacin (5) or oxacillin (1). +, Positive; w, weakly positive; –, negative.

Characteristic	1	2
Oxidase activity	–	+
API 20NE		
Arginine dihydrolase, β-glucosidase, gelatinase, β-galactosidase	–	+
API ZYM		
Esterase lipase (C8), lipase (C14)	w	+
Trypsin, α- and β-glucosidase, β-galactosidase	–	+
Antibiotic susceptibility (μg per disc unless stated otherwise)		
Streptomycin (10), tetracycline (10)	+	–
Ampicillin (10), clindamycin (2), co-trimoxazole (25), furazolidone (15), neomycin (10), norfloxacin (10), penicillin G (10), polymyxin B (30 IU), rocephin (30), vancomycin (30)	–	+
Biolog GN2		
α-Hydroxybutyric acid	–	+
Monomethylsuccinate, succinic acid, L-alanyl glycine, L-aspartic acid	+	–
DNA G + C content (mol%)	62.1	64

Description of *Parvibaculum indicum* sp. nov.

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

Cells are Gram-reaction-negative, rod-shaped, 0.4–0.5 μm wide and 1.3–1.5 μm long and motile by means of a polar flagellum. Positive for catalase activity but negative for oxidase activity. Colonies are smooth, white to non-pigmented, slightly raised in the centre and have regular edges that are 1–2 mm in diameter when grown on 216L marine agar for 72 h at 28 °C. Grows in 0.5–8 % NaCl (optimum 1–5 %) and at 10–42 °C (optimum 25–37 °C) but not at 4 or 45 °C. The major respiratory quinone is Q-11. Principal fatty acids are C_{18:1}ω7c, C_{18:0}, 11-methyl C_{18:1}ω7c and C_{19:0} cyclo ω8c. Grows in artificial seawater medium utilizing n-alkane with chain lengths from C₁₀ to C₁₆ as a carbon source. The DNA G + C content of the type strain is 62.1 mol%. The results of API 20NE, API ZYM, Biolog GN2 and antimicrobial susceptibility tests on strain P31^T are shown in Table 1, as are the characteristics that distinguish strain P31^T from *Parvibaculum lavamentivorans* DS-1^T.

The type strain, P31^T (=CCTCC AB 208230^T =LMG 24712^T =MCCC 1A01132^T), was isolated from deep-sea water of the Indian Ocean.

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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.
- Collins, M. D. (1985). Isoprenoid quinone analysis in classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Cui, Z., Lai, Q., Dong, C. & Shao, Z. (2008). Biodiversity of polycyclic aromatic hydrocarbon-degrading bacteria from deep sea sediments of the Middle Atlantic Ridge. *Environ Microbiol* **10**, 2138–2149.
- Dong, X.-Z. & Cai, M.-Y. (2001). *Determinative Manual for Routine Bacteriology*. Beijing: Scientific Press (English translation).
- Lai, Q., Yuan, J., Wu, C. & Shao, Z. (2009). *Oceanibaculum indicum* gen. nov., sp. nov., isolated from deep seawater of Indian Ocean. *Int J Syst Evol Microbiol* **59**, 1733–1737.
- Liu, C. & Shao, Z. (2005a). *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.

- Liu, C. & Shao, Z. (2005b).** Isolation and characterization of oil-degrading marine microorganisms. *Acta Oceanol Sin* **27**, 114–120.
- 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.
- 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. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Schleheck, D., Tindall, B. J., Rosselló-Mora, R. & Cook, A. M. (2004).** *Parvibaculum lavamentivorans* gen. nov., sp. nov., a novel heterotroph that initiates catabolism of linear alkylbenzenesulfonate. *Int J Syst Evol Microbiol* **54**, 1489–1497.
- 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.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- 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.
- Wang, B., Lai, Q., Cui, Z., Tan, T. & Shao, Z. (2008).** A pyrene-degrading consortium from deep-sea sediment of the West Pacific and its key member *Cycloclasticus* sp. P1. *Environ Microbiol* **10**, 1948–1963.