Oceanicola pacificus sp. nov., isolated from a deep-sea pyrene-degrading consortium

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A taxonomic study was carried out on a Gram-negative, rod-shaped, non-motile, halophilic bacterium, designated strain W11-2B^T, which was isolated from a pyrene-degrading consortium that was enriched from sediment from the Pacific Ocean. Growth was observed at salinities of 0.5–10 ‰ and at temperatures of 10–41 °C. Strain W11-2B^T was unable to degrade Tween 80 or gelatin. 16S rRNA gene sequence comparisons showed that strain W11-2B^T was related most closely to *Oceanicola nanhaiensis* SS011B1-20^T (95.8 % similarity) and *Oceanicola batsensis* HTCC2597^T (95.7 %); levels of 16S rRNA gene sequence similarity between strain W11-2B^T and the type strains of other species tested were below 95.2 %. The dominant fatty acids of strain W11-2B^T were C_{18:1}ω7c (32.1 % of the total), C_{19:0} cyclo (20.9 %), C_{18:1}ω7c 11-methyl (19.5 %), C_{18:0} (7.3 %), C_{17:0} (6.6 %) and C_{16:0} (3.8 %). The G + C content of the chromosomal DNA was 64.6 mol%. The above data were in good agreement with those of members of the genus *Oceanicola*. Based on morphology, physiology, fatty acid composition and 16S rRNA gene sequence data, strain W11-2B^T is considered to represent a novel species of the genus *Oceanicola*, for which the name *Oceanicola pacificus* sp. nov. is proposed. The type strain is W11-2B^T (=CCTCC AB 208224^T=LMG 24619^T=MCCC 1A01034^T).

The genus *Oceanicola*, belonging to the order *Rhodobacterales*, class *Alphaproteobacteria*, was proposed by Cho & Giovannoni (2004); and currently comprises four recognized species, namely *Oceanicola granulosus* (Cho & Giovannoni, 2004), *O. batsensis* (Cho & Giovannoni, 2004), *O. nanhaiensis* (Gu *et al.*, 2007) and *O. marinus* (Lin *et al.*, 2007). In the present study, we isolated a novel strain, designated W11-2B^T, from a pyrene-degrading consortium enriched from a deep-sea sediment sample collected from the western Pacific Ocean (Wang *et al.*, 2008). This novel strain is shown to be phylogenetically related to members of the genus *Oceanicola*. Characterization and classification of strain W11-2B^T were based on data from a polyphasic study.

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

A nearly full-length 16S rRNA gene sequence (1427 nt) of strain W11-2B^T was determined. Phylogenetic analysis indicated that this strain belonged to the class *Alphaproteobacteria*, forming a robust clade with members of the genus *Oceanicola* except *O. granulosus* HTCC2516^T (Fig. 1). Strain W11-2B^T was related most closely to *O. nanhaiensis* SS011B1-20^T (95.8 % 16S rRNA gene sequence similarity) and *O. batsensis* HTCC2597^T (95.7 %). It shared 94.1 % 16S rRNA gene sequence similarity with *O. marinus* AZO-C^T and 93.2 % similarity with *O. granulosus* HTCC2516^T; levels of similarity were below 95.2 % to the type strains of other species investigated. Although levels of 16S rRNA gene sequence similarity between strain W11-2B^T and the type strains of species of the genus

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain W11-2B^T is DQ659449.

A transmission electron micrograph of cells of strain W11-2B^T and a table detailing the cellular fatty acid content of strain W11-2B^T are available as supplementary material with the online version of this paper.

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain W11-2B^T and representatives of some other related taxa based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.005 nucleotide substitution rate (K_{nuc}) units.

Thalassobius were in the range 95.2–95.0 %, the novel strain formed a separate clade with robust bootstrap support (≥ 60 %). *O. granulosa* HTCC2516^T formed a clade with *Roseisalinus antarcticus* EL-88^T, and this clade had high bootstrap support (> 99 %). The classification status of *O. granulosa* HTCC2516^T requires further study. In general, a level of 16S rRNA gene sequence divergence of greater than 2 % is accepted as a criterion for delineating different species (Stackebrandt & Goebel, 1994). Levels of 16S rRNA gene sequence divergence between strain W11-2B^T and *O. nanhaiensis*, *O. batsensis*, *O. marinus* and *O. granulosa* were 4.2, 4.3, 6.0 and 6.8 %, respectively, supporting the view that strain W11-2B^T represents a novel species.

General cell morphology was studied by using an Olympus inverted microscope with 1-day-old cultures of the strain grown on 216L (per litre seawater: 10 g peptone, 2 g yeast, 1 g sodium acetate, 0.5 g citrate; pH 7.5) 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, available 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). Optimal growth temperature was determined over the range 4–55 °C on 216L medium. 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 via the disc diffusion method as described by Shieh *et al.* (2003). Other biochemical tests were carried out by using the API 20NE, API ZYM (bioMérieux) and Biolog GN2 MicroPlate panels according to the manufacturers' instructions, with the adjustment that all NaCl concentrations were 3.0 %. The results are given in the species description below and in Table 1.

The G+C content of the chromosomal DNA was determined according to the methods described by Mesbah & Whitman (1989) by using reversed-phase

HPLC. The DNA G+C content of strain W11-2B^T was 64.6 mol%, a value close to the range previously reported for *Oceanicola* species (64.7–71.5 mol%).

Fatty acids in whole cells grown on 216L plate medium at 28 °C for 48 h were extracted, saponified and esterified; this process was followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1990). The major fatty acids of strain W11-2B^T were C_{18:1}ω7c (32.1 % of the total), C_{19:0} cyclo (20.9 %), C_{18:1}ω7c 11-methyl (19.5 %), C_{18:0} (7.3 %), C_{17:0} (6.6 %) and C_{16:0} (3.8 %), which accounted for 90.2 % of the total fatty acids (detailed results are given in Supplementary Table S1). The fatty acid profile of strain W11-2B^T was in good agreement with those of recognized members of the genus *Oceanicola* (Cho & Giovannoni, 2004; Gu *et al.*, 2007; Lin *et al.* 2007), except that *O. nanhaiensis* contains only minor amounts of C_{19:0} cyclo, *O. batsensis* lacks C_{18:1}ω7c 11-methyl and *O. marinus* lacks C_{17:0}.

On the basis of its morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence comparisons, strain W11-2B^T is considered to represent a novel species of the genus *Oceanicola*, for which the name *Oceanicola pacificus* sp. nov. is proposed.

Description of *Oceanicola pacificus* sp. nov.

Oceanicola pacificus (pa.ci'fi.cus. L. masc. adj. *pacificus* pacific, pertaining to the Pacific Ocean).

Cells are non-motile, short rods, 1.0–1.2 µm long and 0.6–0.7 µm wide. Positive for catalase and weakly positive for oxidase, but negative for the Gram reaction, lipase (Tween 80), indole production, urease, gelatinase, β-glucosidase, β-galactosidase, arginine dihydrolase and denitrification. On 216L agar, produces 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 0.5–10 %

Table 1. Characteristics that can be used to differentiate strain W11-2B^T from related species of the genus *Oceanicola*

Strains: 1, W11-2B^T (*Oceanicola pacificus* sp. nov.); 2, *O. nanhaiensis* SS011B1-20^T; 3, *O. batsensis* HTCC2597^T; 4, *O. marinus* AZO-C^T; 5, *O. granulosis* HTCC2516^T. Data for reference strains were obtained from Cho & Giovannoni (2004), Gu *et al.* (2007) and Lin *et al.* (2007). w, Weakly positive; +, positive; –, negative; ND, no data.

Characteristic	1	2	3	4	5
Cell size (µm)	0.6–0.7 × 1.0–1.2	0.4–0.6 × 0.7–0.9	0.7–1.6 × 0.4–0.8	0.5 × 0.9–1.0	1.1–2.1 × 0.5–1.0
DNA G + C content (mol%)	64.6	64.1	67.3	70.9	71.5
Catalase activity	+	+	+	+	–
Oxidase activity	w	+	+	+	+
Urease activity	–	–	+	+	–
Indole production	–	ND	–	+	–
Alkaline phosphatase	w	ND	ND	+	ND
α-Glucosidase	–	ND	ND	+	ND
β-Galactosidase	–	ND	–	–	+
Nitrate reduction	–	w	–	–	–
Temperature range for growth (°C)	10–41	10–37	4–40	4–42	4–40
NaCl range for growth (% w/v)	0.5–10	0–10	0.75–10.0	2–8	0.25–10.0
pH range for growth	6.5–11	6–9.5	6–9	6–9	5.5–9.5
Susceptibility to:					
Chloramphenicol	+	+	–	+	+
Streptomycin	+	+	+	+	–
Erythromycin	+	–	+	+	+
Gentamicin	+	+	+	+	–
Tetracycline	–	ND	ND	+	ND
Penicillin G	+	ND	ND	–	ND
Utilization of:					
Maltose	–	–	+	+	+
D-Sorbitol	–	+	–	–	–
Malonic acid	–	–	+	–	–
Sucrose	–	+	–	+	+
Trehalose	–	–	–	+	+
D-Mannitol	–	–	+	+	–
α-D-Lactose	–	+	ND	–	ND
D-Fructose	–	+	–	+	–
Cellobiose	–	+	–	+	+
Dextrin	–	+	ND	+	ND
Raffinose	–	–	–	+	–
L-Rhamnose	–	–	–	+	–
Melibiose	–	–	–	+	–
Gluconic acid	–	+	+	+	+
Lactic acid	+	–	+	+	–
L-Glutamic acid	+	–	+	+	+
L-Proline	+	–	–	+	–
Succinic acid	w	+	–	+	–
Acetic acid	w	+	ND	+	ND
Propionic acid	w	–	+	+	+
L-Alanine	w	–	–	+	+

NaCl (optimum, 1–4 %). Grows at 10–41 °C (optimum, 25–30 °C); no growth is observed at 4 or 45 °C within 1 week. Does not ferment glucose or reduce nitrate to nitrite. Predominant fatty acids are C_{18:1ω7c}, C_{19:0} cyclo, C_{18:1ω7c} 11-methyl, C_{18:0}, C_{17:0} and C_{16:0}. Sensitive to ampicillin (10 µg per disc; Oxoid), carbenicillin (100 µg), cefalexin (30 µg), cefazolin (30 µg), cefoperazone (30 µg), cephradine (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), kana-

mycin (30 µg), minocycline (30 µg), neomycin (10 µg), norfloxacin (10 µg), ofloxacin (5 µg), oxacillin (1 µg), penicillin G (10 µg), piperacillin (100 µg), polymyxin B (30 U), rifampicin (5 µg), ceftriaxone (30 µg), streptomycin (10 µg) and doxycycline (30 µg). Resistant to clindamycin (2 µg), cotrimoxazole (25 µg), furazolidone (15 µg), lincomycin (2 µg), metronidazole (5 µg), tetracycline (30 µg) and vancomycin (30 µg). In the API ZYM system, positive for esterase (C4), esterase lipase (C8), leucine

aminopeptidase and valine aminopeptidase; weakly positive for acid phosphatase, alkaline phosphatase, cystine aminopeptidase, lipase (C14) and naphthol-AS-BI-phosphamidase; and 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 96 substrates in the Biolog GN2 system, positive for DL-lactic acid, L-aspartic acid, L-glutamic acid, L-proline, L-serine, succinic acid monomethyl ester, succinamic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -aminobutyric acid and γ -hydroxybutyric acid; and weakly positive for acetic acid, D-alanine, D-galacturonic acid, formic acid, glycogen, itaconic acid, L-alanine, L-alanyl glycine, L-asparagine, L-histidine, L-ornithine, *p*-hydroxyphenylacetic acid, propionic acid, succinic acid, thymidine, α -ketobutyric acid and α -ketoglutaric acid. The G + C content of the DNA of the type strain is 64.6 mol%. Characteristics that can be used to distinguish strain W11-2B^T from related species are shown in Table 1.

The type strain, W11-2B^T (=CCTCC AB 208224^T=LMG 24619^T=MCCC 1A01034^T), was isolated from a pyrene-degrading consortium that was enriched from sediment from the Pacific Ocean.

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