Oceanicola nanhaiensis sp. nov., isolated from sediments of the South China Sea

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A Gram-negative, non-motile, rod-shaped bacterium, strain SS011B1-20^T, was isolated from sediments of the South China Sea. Growth occurred at NaCl concentrations between 0 and 10 % and at temperatures between 10 and 37 °C. Strain SS011B1-20^T contained Q-10 as the major respiratory quinone and C_{18:1}ω7c (81.2 %), C_{16:0} (7.0 %) and C_{18:1} methyl (4.3 %) as the predominant fatty acids. The G + C content of the genomic DNA was 64.7 mol%. A phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain SS011B1-20^T belonged to a clade within the genus *Oceanicola* in the *Alphaproteobacteria*, the highest sequence similarities being found with respect to *Oceanicola batsensis* (96.3 %) and with *Oceanicola granulosus* (94.9 %). Strain SS011B1-20^T could be clearly distinguished from other *Oceanicola* species on the basis of the genotypic, phenotypic and phylogenetic data. Thus, it is proposed that strain SS011B1-20^T represents a novel species of the genus *Oceanicola*, with the name *Oceanicola nanhaiensis* sp. nov. The type strain is SS011B1-20^T (=LMG 23508^T = CGMCC 1.6293^T).

The genus *Oceanicola*, belonging to the order *Rhodobacterales* of the *Alphaproteobacteria*, was proposed by Cho & Giovannoni (2004). The genus currently includes two species, *Oceanicola granulosus* (the type species) and *Oceanicola batsensis*, which are Gram-negative, non-motile, poly-β-hydroxybutyrate-accumulating, contain large amounts of unsaturated cellular fatty acids and have DNA G + C contents of 71.5 and 67.3 mol%, respectively. In this study, we isolated a novel strain, strain SS011B1-20^T, from sediments of the South China Sea, and found that it was phylogenetically related to members of the genus *Oceanicola*. We describe the characterization and classification of strain SS011B1-20^T using polyphasic methods.

Strain SS011B1-20^T was isolated from sediments of the South China Sea (collected at a depth of 1100 m) using a 10-fold dilution plating technique on marine agar 2216 (MA; Nakamura & Silver, 1994) and was purified by restreaking on MA plates incubated at 30 °C for 3–5 days. When grown to the exponential phase on MA, the cell morphology and flagellum type were examined using transmission and scanning electron microscopy. To determine the assimilation

of carbon sources by strain SS011B1-20^T, the mineral medium solution of Shivaji *et al.* (2005) was used (containing: w/v, 0.1 % NH₄Cl, 0.0075 % K₂HPO₄, 0.145 % CaCl₂, 3.0 % NaCl, 0.615 % MgCl₂, 0.075 % KCl and 0.0028 % FeSO₄). Different filter-sterilized carbon sources were added at a concentration of 0.2 % (w/v) after the mineral base solution had been autoclaved. Growth was examined after incubation at 30 °C for 1, 7, 10 and 14 days. The hydrolysis of starch, gelatin and Tween 80 was assessed as described by Smibert & Krieg (1994). Tests for nitrate and nitrite reduction were performed as described by Lanyi (1987). The optimum pH, temperature and salt concentration for growth were tested using marine broth 2216. Sensitivity to various antibiotics was tested by using the disc diffusion (Kirby–Bauer) method.

Cellular fatty acid methyl esters were prepared and analysed using gas chromatography according to the instructions of the Microbial Identification System (MIDI). Fatty acid profiles were analysed using the Sherlock system (Microbial ID). Isoprenoid quinones were extracted from fresh cells with methyl chloride/methanol (2 : 1, v/v) and analysed by reversed-phase HPLC (Shim-pack, VP-ODS; Shimadzu).

Genomic DNA was extracted and purified by using the method of Marmur (1961). The DNA G + C content was determined by thermal denaturation (Marmur & Doty,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SS011B1-20^T is DQ414420.

Electron micrographs of cells of strain SS011B1-20^T are available as supplementary material in IJSEM Online.

1962), using DNA from *Escherichia coli* K-12 as a control. The 16S rRNA gene was amplified using the bacterial universal primer pair 8f (5'-AGAGTTTGTATCCTGGCTC-AG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3') (Lane, 1991); multiple alignments of the almost-complete 16S rRNA gene sequence were performed with the CLUSTAL_X program (version 1.64b; Thompson *et al.*, 1997). A phylogenetic tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987) and was evaluated by bootstrap analysis (based on 1000 resampling replicates) with the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package, version 3.6 (Felsenstein, 2004).

The cells of strain SS011B1-20^T were found to be Gram-negative, having the morphology typical of such micro-organisms, i.e. possessing an outer membrane, being non-motile, and comprising short rods (0.7–0.9 × 0.4–0.6 µm) that divide by binary fission. Neither flagella nor endospores were observed under transmission and scanning electron microscopy (see Supplementary Fig. S1 available in IJSEM Online). Small yellowish colonies (about 1 mm in diameter) were produced on MA agar after incubation at 30 °C for 3–5 days. The colonies were smooth, uniformly circular, convex and opaque. The pH for growth was 6.0–9.5 (optimum, pH 7.5–8.0) and the NaCl concentrations for growth were 0–10 % (w/v) (optimum, 2–3 %). Growth was observed at 10–37 °C, but not at 4 or 40 °C. The isolate was positive for oxidase activity and Tween 80 hydrolysis, and weakly positive for nitrate reduction and catalase activity. Tests for gelatin liquefaction, starch hydrolysis, nitrite-reduction activity and urease activity all gave negative results. The main characteristics that differentiate strain SS011B1-20^T from the two *Oceanicola* species are listed in Table 1.

Analysis of the almost-complete 16S rRNA gene sequence (1427 bp) revealed that strain SS011B1-20^T is a member of the *Alphaproteobacteria* and has a close phylogenetic relationship with the species of the genus *Oceanicola* (Fig. 1). The topology of the phylogenetic tree and the bootstrap values supported the proposed relationship between strain SS011B1-20^T and the species of the genus *Oceanicola*. The 16S rRNA gene sequence of strain SS011B1-20^T showed 96.3 and 94.9 % similarity to those of *O. batsensis* and *O. granulosus*, respectively. In general, a 16S rRNA gene sequence divergence greater than 2 % is accepted as a criterion for delineating different species (Stackebrandt & Goebel, 1994). The 16S rRNA gene sequence divergences between strain SS011B1-20^T and the recognized species *O. batsensis* and *O. granulosus* were 3.7 and 5.1 %, respectively, and thus the data support the view that strain SS011B1-20^T represents a novel species.

The results of the chemotaxonomic analysis are given in Table 2. The main cellular fatty acids of strain SS011B1-20^T were C_{18:1}ω7c (81.2 %), C_{16:0} (7.0 %) and C_{18:1} methyl (4.3 %), while those of *O. batsensis* HTCC2597^T and *O. granulosus* HTCC2516^T were C_{18:1}ω7c (31 and 62.8 %,

Table 1. Characteristics that differentiate *Oceanicola nanhaiensis* sp. nov. strain SS011B1-20^T from related species of the genus *Oceanicola*

Strains: 1, SS011B1-20^T; 2, *O. batsensis* HTCC2597^T; 3, *O. granulosus* HTCC2516^T. Data are from this study and Cho & Giovannoni (2004). All strains are negative for motility, gelatin hydrolysis and utilization of L-isoleucine, D-raffinose, L-proline, L-rhamnose, L-lysine, D-melibiose and D-xylose. All strains are positive for oxidase activity and utilization of gluconic acid. +, Positive; –, negative; W, weak reaction; ND, not determined.

Characteristic	1	2	3
DNA G + C content (mol%)	64.7	67.3	71.5
Catalase activity	W	+	–
Urease activity	–	+	–
Starch hydrolysis	–	ND	ND
Nitrate reduction	W	–	–
Minimum growth temp. (°C)	10	4	4
Maximum growth temp. (°C)	37	40	40
Susceptibility to:			
Chloramphenicol	+	–	+
Streptomycin	+	+	–
Erythromycin	–	+	+
Gentamicin	+	+	–
Utilization of substrates as sole carbon sources			
D-Maltose	–	+	+
L-Arginine	–	+	+
Succinic acid	+	–	–
D-Erythrose	–	ND	ND
Acetic acid	+	ND	ND
L-Sorbose	+	+	–
D-Sorbitol	+	–	–
Malonic acid	–	+	–
Sucrose	+	–	+
myo-Inositol	–	+	–
D-Trehalose	–	–	+
D-Mannitol	+	+	–
α-D-Lactose	+	ND	ND
Pyruvic acid	+	–	+
D-Ribose	+	–	+
D-Fructose	+	–	–
Ethanol	+	–	–
Propionic acid	–	+	+
Glycine	–	+	–
D-Malonic acid	+	+	–
D-Cellobiose	+	–	+
L-Alanine	–	–	+
D-Arabinose	–	+	–
D-Glucose	+	–	+
Lactic acid	–	+	–
L-Glutamic acid	–	+	+
Dextrin	+	ND	ND

respectively), C_{16:0} (15.0 and 11.9 %, respectively) and C_{18:1} methyl (0 and 8.1 %, respectively). The fatty acid C_{19:0} cyclo constituted 1.1, 40.4 and 10.8 % in strain SS011B1-20^T, *O.*

Fig. 1. Neighbour-joining phylogenetic tree for strain SS011B1-20^T, based on 16S rRNA gene sequence analysis. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Bar, 0.01 nucleotide substitutions per nucleotide position.

batsensis and *O. granulosus*, respectively. The major quinone in strain SS011B1-20^T was Q-10 and the DNA G + C content was 64.7 mol% (T_m).

The above results indicate that strain SS011B1-20^T represents a novel marine species within the genus

Oceanicola, for which we propose the name *Oceanicola nanhaiensis* sp. nov.

Description of *Oceanicola nanhaiensis* sp. nov.

Oceanicola nanhaiensis (nan.hai.en'sis. N.L. masc. adj. *nanhaiensis* pertaining to Nan Hai, the Chinese name for the South China Sea, the source of the sediment from which the type strain was isolated).

Cells are Gram-negative, aerobic, non-spore-forming, non-motile rods. Small (about 1–2 mm) yellowish colonies are produced on MA after incubation at 30 °C for 3–5 days. Colonies are smooth, convex and opaque. Growth occurs at 10–37 °C, pH 6.0–9.5 and 0–10 % NaCl; the optimal growth temperature, pH and salinity are 28 °C, pH 7.5–8.0 and 2–3 % NaCl. Positive for oxidase and Tween 80 hydrolysis. Nitrate reduction and catalase activity are weakly positive. Negative for urease activity, denitrification and hydrolysis of starch and gelatin. The utilization of various substrates as sole carbon sources, and other differentiating characteristics, are listed in Table 1. The following substrates are utilized: D-galactose, D-melezitose, succinic acid, acetic acid, L-sorbose, D-sorbitol, sucrose, D-mannitol, α -D-lactose, gluconic acid, pyruvic acid, D-ribose, D-fructose, ethanol, D-malonic acid, D-cellobiose, citric acid, D-glucose and dextrin. Sensitive to chloramphenicol, streptomycin and gentamicin. The predominant cellular fatty acid is C_{18:1} ω 7c (81.2 %). Fatty acids C_{16:0} (7.0 %), C_{18:1} methyl (4.3 %), C_{10:0} 3-OH (1.0 %), C_{18:0} (1.3 %) and C_{19:0} cyclo (1.1 %) are also present. The predominant isoprenoid quinone is Q-10.

The DNA G + C content of the type strain is 64.7 mol% (T_m). The type strain, SS011B1-20^T (= LMG 23508^T = CGMCC 1.6293^T), was isolated from sediments of the South China Sea, China.

Table 2. Cellular fatty acid compositions (%) of strain SS011B1-20^T and related species of the genus *Oceanicola*

Strains: 1, SS011B1-20^T; 2, *O. batsensis* HTCC2597^T; 3, *O. granulosus* HTCC2516^T. Data are from this study and Cho & Giovannoni (2004).

Fatty acid	1	2	3
C _{10:0}	0.2	–	0.1
C _{10:0} 3-OH	1.0	0.4	1.5
C _{12:0}	0.1	2.0	–
C _{12:0} 3-OH	–	–	1.6
C _{12:1} ω 11c	–	4.9	–
C _{14:0}	0.3	1.5	–
C _{14:0} 3-OH	0.9	–	–
C _{15:0}	–	0.9	–
C _{16:0}	7.0	15.0	11.9
C _{17:0}	0.4	1.5	0.4
C _{17:1} ω 8c	0.4	–	0.3
anteiso-C _{17:0}	–	–	0.2
C _{17:0} cyclo	–	–	0.2
C _{18:0}	1.3	2.4	0.9
C _{18:1} ω 7c	81.2	31.0	62.8
C _{18:1} methyl	4.3	–	8.1
C _{19:0} cyclo	1.1	40.4	10.8
iso-C _{15:0} 2-OH + C _{16:1} ω 7c	1.8	–	1.2
Total	100.0	100.0	100.0

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