Oceanicola nitratireducens sp. nov., a marine alphaproteobacterium isolated from the South China Sea

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A Gram-negative, non-motile, short-rod-shaped bacterial strain (JLT1210^T) that accumulates poly- β -hydroxybutyrate granules was isolated from the Beibu Gulf in the South China Sea. Cells have polar or subpolar flagella. Phylogenetic analyses based on 16S rRNA gene sequences showed that the strain belongs to the genus *Oceanicola* in the order *Rhodobacterales*, class *Alphaproteobacteria*. The closest neighbours were *Oceanicola nanhaiensis* SS011B1-20^T (96.5% similarity) and *Oceanicola batsensis* HTCC2597^T (96.4%). The predominant respiratory ubiquinone of strain JLT1210^T was Q-10 and the DNA G+C content was 72.8 mol%. Evidence from genotypic, chemotaxonomic and phenotypic data shows that strain JLT1210^T represents a novel species of the genus *Oceanicola*, for which the name *Oceanicola nitratireducens* sp. nov. is proposed; the type strain is JLT1210^T (=LMG 24663^T=CGMCC 1.7292^T).

The genus *Oceanicola*, which belongs to the order *Rhodobacterales* of the class *Alphaproteobacteria*, was first proposed by Cho & Giovannoni (2004). Currently, the genus includes five species, *Oceanicola granulosus* (the type species), *Oceanicola batsensis*, *Oceanicola nanhaiensis*, *Oceanicola marinus* and *Oceanicola pacificus*, which are Gram-negative, non-motile, poly- β -hydroxybutyrate (PHB)-accumulating and contain large amounts of unsaturated cellular fatty acids. *O. granulosus* strains HTCC2516^T and HTCC2523 and *O. batsensis* HTCC2597^T were isolated from the Bermuda Atlantic Time-series Study (BATS) site by means of a high-throughput culturing method based on dilution-to-extinction in an oligotrophic seawater-based medium (Cho & Giovannoni, 2003). *O. nanhaiensis* SS011B1-20^T was isolated from sediment samples from the South China Sea (Gu *et al.*, 2007). *O. marinus* was obtained from seawater collected off the coast of Taiwan (Lin *et al.*, 2007). *O. pacificus* was isolated from a deep-sea pyrene-degrading consortium (Yuan *et al.*, 2009). All strains of this genus were isolated from the ocean. JLT1210^T was isolated from the Beibu Gulf in the South China Sea and was subjected to taxonomic investigation. Polyphasic taxonomic analyses indicated that this marine isolate represents a novel species of the genus *Oceanicola*.

During our surveys of the biodiversity of bacteria in the South China Sea during voyage no. 908 on the ship *Dongfanghong 2* in July 2006, strain JLT1210^T was obtained after direct plating of seawater dilutions. It was incubated in a rich organic (RO) medium (Yurkov *et al.*, 1999) at room temperature (28 °C) for 10 days. RO medium contained the following (g l⁻¹): yeast extract, 1.0; Bacto Peptone, 1.0; sodium acetate, 1.0; KCl, 0.3; MgSO₄·7H₂O, 0.5; CaCl₂·2H₂O, 0.05; NH₄Cl, 0.3; K₂HPO₄, 0.3; and NaCl, 20.0. This medium was supplemented with a mixture of vitamins and trace element solution.

Cell morphology was examined by transmission electron microscopy (JEM 2100 HC). The Gram reaction was determined on cells grown on RO at 25 °C for 24 h according to the method described by Gerhardt *et al.* (1994). Colonies were pale yellow, 0.5–3.0 mm in diameter, uniformly circular, convex and opaque after growth on marine agar RO at 28 °C for 3 days. Cells stained Gram-negative and were short rods, 0.7–2.1 μ m long and 0.4–1.1 μ m wide, with polar or subpolar flagella, dividing by binary fission (Fig. 1).

The salt requirement for growth of strain JLT1210^T was tested by growth on RO medium with final NaCl concentrations of 0, 2, 5, 10, 15, 20, 25 and 30% (w/v) (pH 7.8 at 28 °C). The pH and temperature requirements for growth were determined by adjusting the final pH of RO medium to pH 0, 2, 4, 6, 7, 8, 10, 12 and 14 with HCl and NaOH (2% NaCl, 28 °C), and by incubation at 4–45 °C (4, 10, 15, 25, 28, 37 and 45 °C) (2% NaCl, pH 7.8),

Abbreviation: PHB, poly- β -hydroxybutyrate; RO, rich organic [medium].

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JLT1210^T is EU581832.

Fig. 1. Electron micrographs of negatively stained cells of strain JLT1210^T observed by transmission electron microscopy. Bars, 0.5 μm.

respectively. The temperature range for growth of strain JLT1210^T was 10–45 °C (optimum 28 °C), the pH range for growth was pH 7.0–9.5 (optimum 7.8–8.5) and the NaCl concentration range for growth was 0.5–7.0 % (w/v) (optimum 1.5–3.0 %).

Oxidase activity of strain JLT1210^T was tested according to the method of Smibert & Krieg (1994) and catalase activity was determined by using 3 % (v/v) hydrogen peroxide solution (Dong & Cai, 2001). Nitrite and nitrate reduction were tested in RO medium by growing the strain in the presence of NO₂⁻ and NO₃⁻, respectively (Dong & Cai, 2001). Denitrification was tested by growing the strain anaerobically in the presence of NO₃⁻ (Zumft, 1992). Hydrolysis of starch, gelatin and Tween 80, and urease activity were determined as described by Dong & Cai (2001). H₂S production was tested in RO medium supplemented with 0.01 % L-cysteine, the indicator being a strip of paper impregnated with lead acetate placed in the neck of the tube (Clarke, 1953; Mata *et al.*, 2002). Methyl red and Voges–Proskauer tests were performed by using methyl red and Barritt's reagent (Barritt, 1936; Mata *et al.*, 2002). Accumulation of PHB was determined by the Sudan Black staining method (Smibert & Krieg, 1994). Growth on sole carbon and nitrogen sources was examined according to the procedures described by Williams *et al.* (1983) on RO medium without organic compounds at 28 °C for 7–14 days.

The isolate was positive for oxidase and catalase activities. Biochemical tests were positive for aerobic nitrate reduction, and PHB and H₂S production. Gelatin, starch, Tween 80 and casein hydrolysis, indole production, aerobic nitrite reduction, anaerobic nitrate reduction, methyl red and Voges–Proskauer tests, and urease activity were negative.

The strain utilized a wide range of substrates, including hexoses, oligosaccharides, organic acids and amino acids, as sole carbon sources. Antimicrobial susceptibility tests were performed by using the disc-diffusion plate (Kirby–Bauer) method according to Fraser & Jorgensen (1997) and Andrews (2008), and with the antimicrobial compounds suggested by Mata *et al.* (2002).

Table 1. Fatty acid composition (%) of strain JLT1210^T and type strains of related *Oceanicola* species

Taxa: 1, JLT1210^T; 2, *Oceanicola nanhaiensis* (Gu *et al.*, 2007); 3, *Oceanicola granulatus* (Cho & Giovannoni, 2004); 4, *Oceanicola batsensis* (Cho & Giovannoni, 2004); 5, *Oceanicola marinus* (Lin *et al.*, 2007); 6, *Oceanicola pacificus* (Yuan *et al.*, 2009).

Fatty acid	1	2	3	4	5	6
10:0	—	0.2	0.1	—	—	—
10:0 3-OH	0.1	1.0	1.5	0.4	0.1	—
11:0 3-OH	—	—	—	—	—	0.4
12:0	2.4	0.1	—	2.0	—	—
12:1ω11c	—	—	—	4.9	—	—
12:0 2-OH	—	—	—	—	—	0.4
12:0 3-OH	1.8	—	1.6	—	2.1	—
12:1 3-OH	—	—	—	—	—	1.6
14:0	3.8	0.3	—	1.5	0.2	0.3
14:0 3-OH	—	0.9	—	—	—	—
iso-15:0	0.8	—	—	—	—	—
anteiso-15:0	0.8	—	—	—	—	—
15:0	1.6	—	—	0.9	—	1.0
iso-16:0	0.8	—	—	—	—	—
16:0	16.2	7.0	11.9	15.0	14.7	3.8
anteiso-17:0	0.1	—	0.2	—	—	—
cyclo 17:0	—	—	0.2	—	—	—
17:1ω8c	1.7	0.4	0.3	—	—	1.6
17:1ω6c	0.6	—	—	—	—	1.3
17:0	1.1	0.4	0.4	1.5	—	6.6
18:1ω7c	25.7	81.2	62.8	31.0	49.1	32.1
18:0	0.9	1.3	0.9	2.4	1.0	7.3
Methyl 18:1	—	4.3	8.1	—	6.6	19.5
cyclo 19:1	—	1.1	10.8	40.4	24.6	20.9
20:2ω6,9c	—	—	—	—	0.2	0.4
Summed feature:*						
3	40.5	1.8	1.2	—	1.0	0.7
7	1.1	—	—	—	0.4	—
Total	100	100	100	100	100	99.9

*Summed feature 3 comprises iso-15:0 2-OH and/or 16:1ω7c; summed feature 7 comprises 19:0 cyclo ω10c and/or 19:1ω6c.

The chemotaxonomic study of strain JLT1210^T included analysis of its cellular fatty acids. Cellular fatty acid analysis was carried out as described by Komagata & Suzuki (1987). Isoprenoid quinones were analysed as described by Hiraishi *et al.* (1998) using a UPLC-Q-TOF-MS spectrometer and the electrospray ionization method (Romano *et al.*, 2006). The predominant fatty acid was summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; 40.5 %); significant amounts of C_{18:1}ω7c (25.7 %), C_{16:0} (16.2 %), C_{14:0} (3.8 %), C_{12:0} (2.4 %), C_{12:0} 3-OH (1.8 %), C_{17:1}ω8c (1.7 %), C_{15:0} (1.6 %) and C_{17:0} (1.1 %) were also present. Fatty acid profiles of members of the genus *Oceanicola* are listed in Table 1.

Genomic DNA was extracted using the method of Marmur (1961) from cells grown in RO medium for 2 days at 28 °C, washed and resuspended in TE buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA (pH 8.0)]. Purity was assessed using A₂₈₀/A₂₆₀ and A₂₃₀/A₂₆₀ ratios (Johnson, 1994). Phylogenetic analyses based on 16S rRNA gene sequences were performed as described by Lin *et al.* (2007). The 16S rRNA gene was amplified with universal bacterial primers corresponding to *Escherichia coli* positions 8F (5'-AGA-GTTTGATCCTGGCTCAG) and 1492R (5'-GGTTACCT-TGTTACGACTT) (Embley, 1991). The 16S rRNA gene

sequence of strain JLT1210^T was compared with those available in GenBank using the BLAST program (NCBI) to determine the approximate phylogenetic affiliation. The 16S rRNA gene sequence of strain JLT1210^T was aligned with those of related *Oceanicola* species and phylogenetic trees were constructed using the neighbour-joining and maximum-parsimony methods of the MEGA software (Kumar *et al.*, 2004; Fig. 2). Nearly complete 16S rRNA gene sequences (1386 bp) were determined for strain JLT1210^T and used for phylogenetic analyses. The closest neighbours were *O. nanhaiensis* SS011B1-20^T (96.5 % similarity), *O. batsensis* HTCC2597^T (96.4 %) and *O. granulosus* HTCC2516^T (93.2 %).

The genomic DNA G+C content of strain JLT1210^T was estimated from the midpoint value (*T_m*) of the thermal denaturation profile, as described by Mandel *et al.* (1970). The DNA G+C content of strain JLT1210^T was 72.8 mol% and the predominant respiratory ubiquinone was Q-10.

Description of *Oceanicola nitratireducens* sp. nov.

Oceanicola nitratireducens (ni.tri.ti.re.du'cens. N.L. n. nitras -atis nitrate; L. part. adj. *reducens* leading back, bringing back and in chemistry converting to a different

Fig. 2. Neighbour-joining phylogenetic tree for strain JLT1210^T based on 16S rRNA gene sequence analysis. Bootstrap percentages from both neighbour-joining (values above nodes) and maximum-parsimony (values below nodes) are shown. Bar, 0.01 substitutions per nucleotide position.

Table 2. Differential characteristics of *Oceanicola nitrareducens* sp. nov. strain JLT1210^T and type strains of related species of the genus *Oceanicola*

Taxa: 1, JLT1210^T; 2, *Oceanicola nanhaiensis* (Gu *et al.*, 2007); 3, *Oceanicola granulosus* (Cho & Giovannoni, 2004); 4, *Oceanicola batsensis* (Cho & Giovannoni, 2004); 5, *Oceanicola marinus* (Lin *et al.*, 2007); 6, *Oceanicola pacificus* (Yuan *et al.*, 2009). +, Positive; –, negative; w, weak reaction; ND, not determined.

Characteristic	1	2	3	4	5	6
G + C content (mol%)	72.8	64.7	67.3	71.5	70.9	64.6
Catalase	+	w	–	+	+	+
Urease	–	–	–	+	+	–
Tween 80	–	+	+	–	–	–
Nitrate reduction	+	w	–	–	–	–
Temperature range for growth (°C)	10–37	10–37	4–40	4–40	4–42	10–41
NaCl range for growth (% w/v)	0.5–7.0	0–10	0.25–10.0	0.75–10.0	2–8	0.5–10.0
pH range for growth	7.0–9.5	6.0–9.5	5.5–9.5	6–9	6–9	6.5–11.0
Susceptibility to:						
Gentamicin	+	+	–	+	–	+
Chloramphenicol	–	+	+	–	–	+
Streptomycin	+	+	–	+	–	+
Erythromycin	+	–	+	+	–	+
Utilization of:						
D-Malonic acid	w	–	–	+	–	–
Lactic acid	–	–	w	+	ND	+
Ethanol	+	+	–	–	ND	ND
D-Sorbitol	w	+	–	–	–	–
D-Mannitol	+	+	–	+	+	–
D-Fructose	+	+	–	–	+	–
Cellobiose	+	+	+	–	+	–
Trehalose dihydrate	+	–	+	–	+	–
Maltose	+	–	+	+	+	–
D-Glucose	+	+	+	–	+	–
D-Galactose	+	ND	+	+	+	ND
Sucrose	+	+	+	–	+	–
D-Xylose	w	–	–	–	+	ND
L-Alanine	+	–	+	–	+	w
L-Glycine	w	–	–	+	ND	w

oxidation state; N.L. part adj. *nitrareducens* reducing nitrate).

Cells are Gram-negative, non-motile, short rods with polar or subpolar flagella. Small (about 0.5–3.0 mm) straw yellowish colonies are produced on RO after incubation at 28 °C for 3–5 days. Colonies are smooth, convex and opaque. Growth occurs at 10–37 °C, at pH 7.0–9.5 and in 0.5–7.0% NaCl; optimum growth occurs at 28 °C, at pH 7.8–8.5 and in 1.5–3.0% NaCl. Positive for oxidase, catalase and nitrate reduction. Negative for hydrolysis of gelatin, starch, Tween 80 and casein, aerobic nitrite reduction, anaerobic nitrate reduction, methyl red and Voges–Proskauer tests, and indole production. The utilization of various substrates as sole carbon sources, antimicrobial susceptibility and other differentiating characteristics are listed in Table 2. The following substrates are utilized: maltose, sucrose, D-fructose, D-galactose, dextrin, trehalose dihydrate, cellobiose, citric acid, sodium D-gluconate, glycerol, mannitol, L(–)-malic acid, acetic

acid, ethanol, L-threonine, L-alanine, L-glycine and L-arginine. Sensitive to ciprofloxacin, cephalothin, tetracycline, gentamicin, chloramphenicol and cefotaxime. The predominant fatty acid is summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c); significant amounts of C_{18:1}ω7c, C_{16:0}, C_{14:0}, C_{12:0}, C_{12:0} 3-OH, C_{17:1}ω8c, C_{15:0} and C_{17:0} are also present. The predominant respiratory ubiquinone is Q-10.

The type strain is JLT1210^T (=LMG 24663^T=CGMCC 1.7292^T), isolated from surface water of the Beibu Gulf in the South China Sea. The DNA G + C content of the type strain is 72.8 mol% (*T_m* method).

Acknowledgements

The authors would like to thank Professor Shuang-Jiang Liu, Institute of Microbiology, Chinese Academy of Science and China General Microbiological Culture Collection Center, for valuable help. This work was supported by the MOST project (2007CB815900), the NSFC projects (40632013), the SOA project (200805068), the

National Infrastructure of Natural Resources for Science and Technology Program of China (2005DKA21209), the COMRA project (DYXM-115-02-4-3) and the Technical Innovation Project of Ministry of Education of China (704029).

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