Nitratireductor indicus sp. nov., isolated from deep-sea water

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A taxonomic study was carried out on a novel bacterial strain, designated C115^T, isolated from a crude-oil-degrading consortium, enriched from deep-sea water of the Indian Ocean. Cells were Gram-negative short rods, mobile by means of a monopolar flagellum. Growth was observed at salinities of 0–7 ‰ and at 10–43 °C. It was unable to degrade Tween 80 or gelatin. 16S rRNA gene sequence analysis showed that strain C115^T was related most closely to *Nitratireductor aquibiodomus* NL21^T (96.5 % similarity), *Nitratireductor kimnyeongensis* KY 101^T (96.4 %) and *Nitratireductor basaltis* J3^T (96.2 %). The predominant fatty acids were summed feature 8 (C_{18:1}ω7c and/or C_{18:1}ω6c, 81.8 %) and C_{18:0} (7.0 %). The G + C content of the chromosomal DNA of strain C115^T was 59 mol%. Based on its morphology, physiology and fatty acid composition together with 16S rRNA gene sequence comparisons, the novel strain most appropriately belongs to the genus *Nitratireductor*, but can be distinguished readily from recognized species of the genus. Strain C115^T is therefore considered to represent a novel species of the genus *Nitratireductor*, for which the name *Nitratireductor indicus* sp. nov. is proposed. The type strain is C115^T (=RC92-7^T =CCTCC AB 209298^T =LMG 25540^T =MCCC 1A01260^T).

The genus *Nitratireductor*, belonging to the family *Phyllobacteriaceae*, was proposed by Labbé *et al.* (2004) and, at the time of writing, comprises three recognized species, *Nitratireductor aquibiodomus* (Labbé *et al.*, 2004), *Nitratireductor kimnyeongensis* (Kang *et al.*, 2009) and *Nitratireductor basaltis* (Kim *et al.*, 2009). In this study, we isolated a novel strain, designated C115^T, from a crude-oil-degrading consortium enriched from a deep-sea water sample collected from the Indian Ocean. The strain was found to be related phylogenetically to members of the genus *Nitratireductor*. Characterization and classification of strain C115^T were based on data from the present polyphasic study.

Deep-sea water was sampled with Niskin bottles attached to a CTD (conductivity, temperature and depth) sampler in December 2005 during cruise DY-105A of R/V *Da-Yang Yi-Hao*. The sampling site was located at 25.3217° S 70.0405° E in the south-western part of the Indian Ridge, at a water depth of 2488 m. The seawater was enriched with crude oil, and bacteria in the community were isolated on marine agar 216L according to Lai *et al.* (2009). Strain C115^T was obtained as a novel bacterium. It was further

cultivated for morphological and biochemical characterization on marine agar 2216 (BD).

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 previously (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 Kimura's 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 (1448 nt) of strain C115^T was obtained. Phylogenetic analysis of strain C115^T indicated that it belonged to the family *Phyllobacteriaceae*, forming a robust clade with members of the genus *Nitratireductor* in the neighbour-joining tree (Fig. 1); a similar tree topology was obtained with the minimum-evolution method. Strain C115^T was related most closely to *N. aquibiodomus* NL21^T (96.5 % 16S rRNA gene sequence similarity), *N. kimnyeongensis* KY 101^T (96.4 %) and *N. basaltis* J3^T (96.2 %). Levels of 16S rRNA gene sequence divergence between strain C115^T and the type strains of recognized *Nitratireductor* species were

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain C115^T is GU447302.

A supplementary figure and a supplementary table are available with the online version of this paper.

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain C115^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.

$\geq 3.5\%$, and thus the data support the view that strain C115^T represents a novel species.

Gram staining, catalase, oxidase and lipase (Tween 80) activities, hydrolysis of aesculin and starch, optimal growth temperature and pH, tolerance of NaCl and general cell morphology were studied as described previously (Lai *et al.*, 2009). Other biochemical tests were carried out by using API 20NE and API ZYM strips (bioMérieux) and Biolog GN2 microplates according to the manufacturers' instructions, except that the NaCl concentration was adjusted in all tests to 3.0%. *N. aquibiodomus* DSM 15645^T, *N. kimnyeongensis* KCTC 22759^T and *N. basaltis* KCTC 22119^T were tested at the same time for comparison. The results of these tests are given in the species description 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 C115^T was 59 mol%, a value close to those given for the type strains of the three recognized *Nitrateductor* species (56.7–60.4 mol%).

Fatty acids in whole cells grown on marine agar 2216 at 28 °C for 48 h were extracted, saponified and esterified,

followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1990). The fatty acid profiles of *N. aquibiodomus* DSM 15645^T, *N. kimnyeongensis* KCTC 22759^T and *N. basaltis* KCTC 22119^T were determined in parallel with that for strain C115^T in this study. The detailed results are shown in Supplementary Table S1, available in IJSEM Online. The major fatty acids of strain C115^T were summed feature 8 ($C_{18:1\omega 7c}$ and/or $C_{18:1\omega 6c}$; 81.8%) and $C_{18:0}$ (7.0%), similar to the data for *N. basaltis* KCTC 22119^T. Strain C115^T could be differentiated from the type strains of *N. aquibiodomus* and *N. kimnyeongensis* based on the proportion of summed feature 8 and $C_{19:0}$ cyclo $\omega 8c$ ($>15\%$ difference). The proportions of the individual fatty acids of the three reference type strains were slightly different from those given in the original publications.

Antibiotic susceptibility tests were performed by the disc diffusion method according to Lai *et al.* (2009); antibiotic discs were obtained from Oxoid. Strain C115^T and the type strains of *N. aquibiodomus*, *N. kimnyeongensis* and *N. basaltis* were tested at the same time in this study. All four strains were sensitive to (μg per disc) cefalexin (30), cefobid (30), chloromycetin (30), erythromycin (15), rifampicin (5) and rocephin (30), but resistant to

Table 1. Differential characteristics between strain C115^T and the type strains of recognized *Nitrateductor* species

Strains: 1, C115^T; 2, *N. aquibiodomus* NL21^T (unless indicated, data from Labbé *et al.*, 2004); 3, *N. kimnyeongensis* KY 101^T (Kang *et al.*, 2009); 4, *N. basaltis* J3^T (Kim *et al.*, 2009). In API 20NE tests in our study, all strains were negative for indole production, D-glucose fermentation and gelatin hydrolysis. In API ZYM tests in our study, all strains were positive for acid phosphatase, alkaline phosphatase, esterase (C4), leucine aminopeptidase, trypsin and valine aminopeptidase and negative for α -fucosidase, α -mannosidase, β -glucosidase and β -glucuronidase. +, Positive; w, weakly positive; –, negative. ND, No data available.

Characteristic	1	2	3	4
Isolation source	Deep-sea water	Denitrification system	Seaweed	Black sand
Cell shape	Rods	Rods	Rods	Cocci, rods
Cell size (μ m)	1.3 $\times$ 3	1 $\times$ 3	0.4–0.5 $\times$ 1.2–2.7	0.6–0.7 $\times$ 0.6–2.0
Motility	+	+	+	–
NaCl concentration for growth (% w/v)				
Range	0–7	0–5	0–7	0–8
Optimum	3	1	ND	ND
Temperature for growth ($^{\circ}$ C)				
Range	10–37	ND	10–45	15–45
Optimum	25–30	30–35	30	ND
API 20NE results*				
Arginine dihydrolase	+	w	w	–
Urease	+	w	w	–
Aesculin hydrolysis	+	+	+	w
β -Galactosidase	w	w	–	+
Capric acid	–	–	w	–
Maltose, L-arabinose	–	w	–	–
D-Mannitol	–	w	w	+
Potassium gluconate	–	w	–	+
Adipic acid	+	–	–	+
D-Glucose, N-acetylglucosamine	+	+	w	+
D-Mannose	+	+	+	–
Malic acid, phenylacetic acid	+	–	–	–
Trisodium citrate	+	+	–	–
API ZYM results*				
Esterase lipase (C8)	–	+	+	+
N-Acetyl- β -glucosaminidase	–	+	–	–
α -Galactosidase, β -galactosidase	–	–	–	+
Lipase (C14)	+	w	w	+
Cystine aminopeptidase, naphthol-AS-BI-phosphoamidase, α -chymotrypsin, α -glucosidase	+	+	+	w
Susceptibility to antibiotics*				
Clindamycin, co-trimoxazole, gentamicin, norfloxacin, ofloxacin, oxacillin, polymyxin B, vancomycin	–	–	–	+
Ciprofloxacin, minomycin	–	–	+	+
Cephadrin, streptomycin, tetracycline, vibramycin	–	+	+	+
Ampicillin, carbenicillin, piperacillin	+	–	–	+
Cefazolin, penicillin G	+	–	+	+
DNA G + C content (mol%)	59	57	60.4	56.7

*Data from this study using the strains listed in the text.

kanamycin (30), lincomycin (2) and metronidazole (5). Detailed results of antibiotic susceptibility tests are given in Table 1.

On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence comparisons, strain C115^T is considered to represent a novel species of the genus

Nitrateductor, for which the name *Nitrateductor indicus* sp. nov. is proposed.

Description of *Nitrateductor indicus* sp. nov.

Nitrateductor indicus (in'di.cus. L. masc. adj. *indicus* Indian, referring to the Indian Ocean, from where the type strain was isolated).

Cells are rods, about 3.0 µm long and 1.3 µm wide, mobile by means of at least one polar flagellum (Supplementary Fig. S1). Positive for catalase, oxidase, urease, arginine dihydrolase, β-galactosidase, β-glucosidase (aesculin hydrolysis) and nitrate reduction, but negative for the Gram reaction, indole production, D-glucose fermentation, lipase (Tween 80), amylase, gelatinase and denitrification. After 3 days of incubation at 28 °C on marine agar, produces smooth grey colonies with regular edges that are 2–3 mm in diameter, non-pigmented and slightly raised in the centre. Grows in the presence of 0–7 % NaCl (optimum 3 %) and at 10–43 °C (optimum 25–30 °C); no growth is observed at 45 °C after 1 week. The major fatty acids are C_{18:0} and summed feature 8 (C_{18:1}ω7c/ω6c). Sensitive to (µg per disc, unless otherwise indicated) ampicillin (10), carbenicillin (100), cefalexin (30), cefazolin (30), cefobid (30), chloromycetin (30), erythromycin (15), penicillin G (10), piperacillin (100), rifampicin (5), rocephin (30) and vibramycin (30), but resistant to cephradine (30), ciprofloxacin (5), clindamycin (2), co-trimoxazole (25), gentamicin (10), kanamycin (30), lincomycin (2), metronidazole (5), minomycin (30), norfloxacin (10), ofloxacin (5), oxacillin (1), polymyxin B (30 IU), streptomycin (10), tetracycline (30) and vancomycin (30). In the API ZYM system, positive for acid phosphatase, alkaline phosphatase, cystine aminopeptidase, esterase (C4), leucine aminopeptidase, lipase (C14), naphthol-AS-BI-phosphoamidase, trypsin, valine aminopeptidase, α-chymotrypsin and α-glucosidase, but negative for esterase lipase (C8), N-acetyl-β-glucosaminidase, α-fucosidase, α-galactosidase, α-mannosidase, β-galactosidase, β-glucosidase and β-glucuronidase. In the API 20NE system, utilizes adipic acid, D-glucose, D-mannose, malic acid, N-acetylglucosamine, phenylacetic acid and trisodium citrate, but not capric acid, maltose, D-mannitol, L-arabinose or potassium gluconate. Of the 95 substrates in the Biolog GN2 system, positive for acetic acid, cis-aconitic acid, citric acid, DL-lactic acid, D-alanine, D-mannose, trehalose, glycerol, glycyl L-glutamic acid, hydroxy-L-proline, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, L-leucine, L-ornithine, L-proline, L-pyrroglutamic acid, L-threonine, malonic acid, methyl pyruvate, myo-inositol, monomethyl succinate, N-acetyl-D-glucosamine, propionic acid, succinamic acid, sucrose, turanose, uronic acid, α-D-glucose, α-hydroxybutyric acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, β-hydroxybutyric acid and γ-aminobutyric acid, weakly positive for DL-α-glycerol phosphate, glycogen, glycyl L-aspartic acid, maltose, succinic acid, Tween 80 and γ-hydroxybutyric acid; negative for the remainder. The DNA G + C content of the type strain is 59 mol%. Table 1 shows

the characteristics used to distinguish the type strain from those of related species.

The type strain, C115^T (=RC92-7^T =CCTCC AB 209298^T =LMG 25540^T =MCCC 1A01260^T), was isolated from deep-sea water of the Indian Ocean.

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