Glaciecola nitratireducens sp. nov., isolated from seawater

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A marine bacterial strain, FR1064^T, was isolated from a surface seawater sample collected off Jeju Island, South Korea. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate belonged to the *Gammaproteobacteria* and was related to the genus *Glaciecola* with 97.6 % sequence similarity to *Glaciecola pallidula*, its nearest phylogenetic neighbour. DNA–DNA relatedness between strain FR1064^T and *G. pallidula* ACAM 615^T was 55 %. Cells of the novel isolate were Gram-negative, aerobic, rod-shaped, motile and halophilic, with an optimum sea salts concentration of 4–7 %. The major fatty acids were straight-chain saturated (C_{16:0}), summed feature 3 and monounsaturated fatty acid C_{18:1}. The DNA G + C content was 44 mol%. Several phenotypic characteristics differentiated the novel isolate from all previously described members of the genus *Glaciecola*. The polyphasic data obtained in this study clearly demonstrate that strain FR1064^T represents a novel species of the genus *Glaciecola*. The name *Glaciecola nitratireducens* sp. nov. is therefore proposed, with strain FR1064^T (=KCTC 12276^T =JCM 12485^T) as the type strain.

The genus *Glaciecola* was originally created to accommodate aerobic, psychrophilic, halophilic bacteria and initially comprised two species, *Glaciecola punicea* and *Glaciecola pallidula*. These species were isolated from sea-ice diatom assemblage samples collected from coastal areas of eastern Antarctica (Bowman *et al.*, 1998). Recently, two further species of the genus, namely *Glaciecola mesophila* and *Glaciecola polaris*, have been described, isolated from marine invertebrate specimens and Arctic Ocean seawater, respectively (Romanenko *et al.*, 2003; Van Trappen *et al.*, 2004). In the course of our study on marine microbial diversity, a *Glaciecola*-like strain, designated strain FR1064^T, was isolated from surface seawater and was the subject of a comprehensive taxonomic investigation. In this study, the polyphasic taxonomic properties of strain FR1064^T are presented.

Strain FR1064^T was isolated from a coastal surface seawater sample collected off Jeju Island, Republic of Korea. The

sample was diluted with sterilized artificial seawater (ASW; Lyman & Fleming, 1940), spread onto a plate containing marine agar 2216 (MA; Difco) and incubated at 25 °C for 3 weeks. The isolate was routinely cultured on MA and maintained as a glycerol suspension (20 %, w/v) at –80 °C. *G. pallidula* ACAM 615^T, cultured on MA at 15 °C, was used as a reference strain.

Bacterial DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). The resulting gene sequence of strain FR1064^T was aligned manually against sequences obtained from GenBank. Phylogenetic trees were inferred from the regions available for all sequences (positions 28–1433; *Escherichia coli* numbering system) using the Fitch–Margoliash (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant tree topologies were evaluated in bootstrap analyses (Felsenstein, 1985) of the neighbour-joining methods

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain FR1064^T is AY787042.

based on 1000 resamplings. The alignment and phylogenetic analyses were carried out using the jPHYDIT program (Jeon *et al.*, 2005; <http://chunlab.snu.ac.kr/jphydit>) and PAUP 4.0 (Swofford, 1998) as described previously (Kim *et al.*, 2005; Yi & Chun, 2006).

Preliminary sequence comparisons with the 16S rRNA gene sequences held in GenBank indicated that the novel isolate belonged to the class *Gammaproteobacteria*. The resultant sequence was then aligned manually based on 16S rRNA secondary structure (Gutell, 1994) with representative sequences of the *Gammaproteobacteria* obtained from GenBank. Strain FR1064^T showed the highest 16S rRNA gene sequence similarity to *G. pallidula* ACAM 615^T (97.6%), followed by *G. polaris* LMG 21857^T (95.4%), *G. mesophila* LMG 21855^T (95.2%) and *G. punicea* ACAM 611^T (94.2%). To elucidate the phylogenetic relationship between the novel isolate and the other species of the genus *Glaciecola*, phylogenetic trees were constructed using four different tree-making algorithms. The neighbour-joining tree (Fig. 1) showed that strain FR1064^T formed a monophyletic clade with *G. pallidula* ACAM 615^T with 100% bootstrap support. This relationship was confirmed by all other tree-inferring methods used in this study.

The taxonomic relationship between strain FR1064^T and *G. pallidula* ACAM 615^T was further examined using DNA–DNA hybridization. Genomic relatedness was determined using a membrane filter technique (Seldin & Dubnau, 1985) using a DIG High Prime DNA labelling and detection starter kit II (Roche). When DNAs were used individually as labelled DNA probes for reciprocal hybridization experiments and conducted in duplicate, the DNA–DNA relatedness value between strain FR1064^T and *G. pallidula* ACAM

615^T was 55%. It is clear from 16S rRNA gene sequence and DNA–DNA hybridization data that strain FR1064^T represents a novel species in the genus *Glaciecola* (Wayne *et al.*, 1987).

For phenotypic characterization, strain FR1064^T was grown on MA at 25 °C unless otherwise specified. Cellular morphology was observed by differential interference microscopy (Axioskop 40; Zeiss) and scanning electron microscopy (JSM-6400; JEOL) using cells grown at 25 °C for 3 days. Motility was examined using wet mounts. Growth under anaerobic conditions was checked in an anaerobic chamber (CO₂/H₂/N₂, 10:10:80; Sheldon Manufacturing). The pH range (pH 4–12) for growth was determined using MA. The requirement for sea salts (0–11%, Sigma) for growth was tested using synthetic ZoBell medium (ZoBell, 1941; 15 g Bacto agar, 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate in 1 l distilled water). Growth at various temperatures was examined on MA at 4–50 °C. Biochemical tests were performed using the API 20E, API 20NE and API ZYM kits (bioMérieux). Strips were inoculated with a heavy bacterial suspension in ASW or AUX medium (bioMérieux) supplemented with 2% (w/v) sea salts. Catalase and oxidase activities were determined using 3% (v/v) hydrogen peroxide and Kovacs' reagent (Kovacs, 1956), respectively. The novel isolate is halophilic as it required 2–9% (w/v) artificial sea salts for growth (optimum 4–7%). The results of biochemical and physiological tests are given in the species description and Table 1.

Cellular fatty acids of the novel isolate were analysed as methyl esters by GLC by the MIDI system according to the manufacturer's instructions. Fatty acid methyl esters were prepared from biomass grown on MA at 25 °C for 2 days.

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing relationships between strain FR1064^T and members of the class *Gammaproteobacteria*. Bootstrap values, expressed as percentages of 1000 replications, are given at the nodes. Solid circles indicate that the corresponding nodes were also recovered in Fitch–Margoliash, maximum-likelihood and maximum-parsimony trees. *Helicobacter pylori* ATCC 43504^T was used as an outgroup. Bar, 0.1 nucleotide substitutions per position.

Table 1. Phenotypic characteristics that differentiate strain FR1064^T from its nearest phylogenetic neighbours

Species: 1, FR1064^T; 2, *Glaciecola polaris* LMG 21857^T; 3, *G. mesophila* LMG 21855^T; 4, *G. punicea* ACAM 611^T; 5, *G. pallidula* ACAM 615^T. Data from Bowman *et al.* (1998), Romanenko *et al.* (2003), Van Trappen *et al.* (2004) and this study. +, Positive; –, negative; W, weakly positive; ND, not determined.

Characteristic	1	2	3	4	5
Pigmentation	–	–	–	Pink–red	Pale pink
Temperature range for growth (°C)	15–30	5–30	7–35	–2–25	–2–20
Growth at:					
0 and 4 °C	–	–	–	+	+
7 °C	–	+	+	+	+
25 °C	+	+	+	+	–
35 °C	–	–	+	–	–
Nitrate reduction	+	–	–	–	–
Acid production from glucose	–	–	W	W	W
Hydrolysis of:					
Aesculin	–	+	W	+	–
Gelatin	+	+	–	–	–
Utilization of:					
<i>N</i> -Acetylglucosamine	+	–	–	–	–
Arabinose	+	–	+	–	–
Citrate	–	–	+	–	–
Gluconate	W	ND	–	–	–
Glucose	–	–	+	–	–
Malate	–	–	–	+	–
Maltose	+	+	+	–	–
Mannitol	–	+	+	–	–
Mannose	+	+	W	–	–
Sucrose	–	+	+	–	–
Enzyme activities (API ZYM):					
α-Galactosidase	–	W	+	+	+
β-Galactosidase	–	+	+	+	+
DNA G + C content (mol%)	44	44	44	44–46	44

DNA G + C content (mol%) was determined by the thermal denaturation method of Marmur & Doty (1962). The DNA G + C content for strain FR1064^T was 44 mol% and the cellular fatty acid profile is given in Table 2.

On the basis of phylogenetic analysis and DNA–DNA hybridization, it is evident that strain FR1064^T should be assigned as representing a novel species of the genus *Glaciecola*. In addition, a number of physiological and chemotaxonomic characteristics clearly distinguish the novel isolate from other phylogenetically related species (Tables 1 and 2). Therefore, we propose that strain FR1064^T be classified as a representative of a novel species within the genus *Glaciecola*, for which the name *Glaciecola nitratreducens* sp. nov. is proposed.

Description of *Glaciecola nitratreducens* sp. nov.

Glaciecola nitratreducens (ni.tra.ti.re.du'cens. N.L. n. *nitratreducens* nitrate; L. part. adj. *reducens* converting to a different state; N.L. part. adj. *nitratreducens* reducing nitrate).

Gram-negative, aerobic and halophilic. Catalase-positive. Colonies on MA are circular, convex with an entire margin, slightly cream-coloured and approximately 1.5 mm in diameter after 5 days at 25 °C. Cells are motile, oval- or rod-shaped and 0.5–0.6 × 1.0–1.5 µm. Spores are not formed. Does not grow without sea salts. Growth occurs in 2–9 % (w/v) sea salts (optimum of 4–7 %). Growth occurs at pH 6–9 (optimum pH of 8) and at temperatures between 15 and 30 °C (optimum of 25 °C). Does not utilize citrate, caprate, adipate or phenylacetate. Produces cytochrome oxidase, but not ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase, urease, indole, acetoin, H₂S or tryptophan deaminase. Negative in tests for the fermentation of inositol, sorbitol, rhamnose, melibiose and amygdalin. Produces alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and trypsin, but not lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, β-glucosidase, α-mannosidase or α-fucosidase. Other physiological and biochemical characteristics are given in Table 1. Major fatty acids are summed feature 3 (38.2 %),

Table 2. Cellular fatty acid content (%) of strain FR1064^T and *Glaciecola* species

Strains: 1, FR1064^T; 2, *G. polaris* LMG 21857^T; 3, *G. mesophila* LMG 21855^T; 4, *G. punicea* ACAM 611^T; 5, *G. pallidula* ACAM 615^T. Data from Bowman *et al.* (1998), Romanenko *et al.* (2003), Trappen *et al.* (2004) and this study. Values are percentages of total fatty acids. ND, Not detected; tr, <1.0%.

Fatty acid	1	2	3	4	5
Saturated fatty acids:					
C _{12:0}	1.6	3.1	2.6	ND	ND
C _{14:0}	7.0	4.1	1.2	1.2	3.6
C _{15:0}	1.0	2.0	tr	1.0	1.6
C _{16:0}	20.5	23.3	33.0	10.0	9.4
C _{17:0}	1.5	ND	2.1	tr	1.3
C _{18:0}	1.9	ND	1.9	tr	tr
Branched fatty acids:					
iso-C _{16:0}	1.0	ND	2.3	1.8	2.5
anteiso-C _{17:0}	tr	ND	2.0	ND	ND
Unsaturated fatty acids:					
C _{14:1} ω7c	ND	ND	tr	tr	3.7
C _{15:1} ω8c	tr	ND	tr	tr	3.4
C _{17:1} ω8c	2.6	2.6	3.2	4.6	5.0
C _{18:1} ω7c	7.7	4.8	5.2	16.3	12.6
C _{18:1} ω9c	tr	ND	tr	tr	tr
Hydroxy fatty acids:					
C _{12:0} 3-OH	2.8	5.5	tr	ND	ND
C _{16:0} 2-OH	ND	1.7	ND	ND	ND
C _{16:1} 2-OH	ND	2.0	ND	ND	ND
Methyl fatty acids:					
C _{18:0} 10-methyl	ND	1.4	ND	ND	ND
C _{18:1} ω7c 11-methyl	1.4	ND	ND	ND	ND
Summed feature 3*	38.2	41.7	43.0	60.7	54.2
Unknown fatty acid 11.799 (ECL)	4.2	ND	ND	ND	ND

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

C_{16:0} (20.5%), C_{18:1}ω7c (7.7%) and C_{14:0} (7.0%). The DNA G+C content of the type strain is 44 mol%.

The type strain, FR1064^T (= KCTC 12276^T = JCM 12485^T), was isolated from seawater off Jeju Island, Republic of Korea.

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