Glaciecola agarilytica sp. nov., an agar-digesting marine bacterium from the East Sea, Korea

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A taxonomic study was carried out on an isolate, strain NO2^T, from marine sediment collected from the East Sea, Korea. Comparative 16S rRNA gene sequence studies showed that this strain belonged to the *Gammaproteobacteria* and was most closely related to *Glaciecola mesophila* KMM 241^T and *Glaciecola polaris* LMG 21857^T (98.6 and 98.0% 16S rRNA gene sequence similarity, respectively). The isolate was Gram-negative, aerobic and slightly halophilic and grew in 2–8% NaCl and at 7–30 °C. Strain NO2^T shared some physiological and biochemical properties with *G. mesophila* KMM 241^T and *G. polaris* LMG 21857^T. The G + C content of the genomic DNA of strain NO2^T was 45 mol%. Strain NO2^T possessed C_{16:0}, summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and summed feature 7 (C_{18:1}ω9c/ω12t/ω7c) as the major cellular fatty acids. DNA–DNA relatedness data indicated that strain NO2^T represents a distinct species that is separate from *G. mesophila* and *G. polaris*. On the basis of polyphasic evidence, it is proposed that strain NO2^T (=KCTC 12755^T=LMG 23762^T) represents the type strain of a novel species, *Glaciecola agarilytica* sp. nov.

The genus *Glaciecola* accommodates aerobic, slightly halophilic bacteria and, at the time of writing, includes four species, *Glaciecola punicea* and *Glaciecola pallidula* (Bowman *et al.*, 1998), *Glaciecola mesophila* (Romanenko *et al.*, 2003) and *Glaciecola polaris* (Van Trappen *et al.*, 2004). During screening, a novel agar-digesting bacterial strain, NO2^T, detected by the occurrence of pitting around colonies, was isolated from marine sediment of the East Sea, Korea, and selected for further characterization by a polyphasic approach. Strain NO2^T was isolated using a natural seawater agar medium, prepared using seawater of the East Sea. For solidification, 1.5% (w/v) agarose was added to seawater before autoclaving. A sediment sample was placed in a sterile conical tube and serially diluted with filter-sterilized (0.22 µm pore size, Millipore) natural seawater. An aliquot of each dilution was spread onto the agar medium and incubated at 17 °C for 4 weeks. Agar-digesting colonies were transferred onto new plates and subjected to an additional incubation for 2 days at 25 °C. Cultured strains were preserved at –70 °C as suspensions in marine broth (Difco) containing 20% (w/v) glycerol.

Bacterial genomic DNA was extracted using a commercial genomic DNA extraction kit (Bioneer). The 16S rRNA gene was amplified from chromosomal DNA using the universal bacterial primer set 9F and 1512R and the purified PCR products were sequenced by Solgent Co. Ltd (Daejeon, Korea) (Yoon *et al.*, 1998). The full 16S rRNA gene sequences were compiled using SeqMan software (DNASTAR). 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Sequence alignments were performed using the CLUSTAL_X program (Thompson *et al.*, 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the two-parameter model of Kimura (1983). Phylogenetic trees were constructed by using the neighbour-joining method (Saitou & Nei, 1987) using the MEGA3 program (Kumar *et al.*, 2004) with bootstrap values based on 500 replications (Felsenstein, 1985). In the analysis of phylogeny, strain NO2^T was determined to belong to the *Gammaproteobacteria*, and the highest degrees of sequence similarity were found with *G. mesophila* KMM 241^T (98.6% sequence similarity) and *G. polaris* LMG 21857^T (98.0% sequence similarity) (Fig. 1).

The Gram reaction was examined by the non-staining method described by Buck (1982). Cell morphology was examined by light microscopy (Nikon) and transmission electron microscopy (Carl Zeiss) after negative staining with 1% (w/v) phosphotungstic acid. Catalase activity was

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NO2^T is DQ784575.

A transmission electron micrograph and a comparison of the fatty acid profile of strain NO2^T and related strains are available as supplementary material in IJSEM Online.

Fig. 1. Neighbour-joining analysis of 16S rRNA gene sequences showing the position of strain NO2^T among species of genus *Glaciecola*. Percentages at branching points refer to bootstrap analyses (500 resamplings). Bar, 5 inferred nucleotide substitutions per 1000 nucleotides.

determined by bubble production in 3% (v/v) H₂O₂ and oxidase activity was determined using 1% (w/v) tetramethyl-*p*-phenylenediamine. Strain NO2^T is Gram-negative, aerobic and forms curved cells. Cells possess a polar flagellum (Supplementary Fig. S1 available in IJSEM Online) and form aggregates when cultivated in liquid media. Colonies are white, circular and convex, with regular edges and a diameter of 2–3 mm when grown on marine agar (Difco) plates for 2 days at 25 °C.

Cellular fatty acids were analysed from the isolate and reference organisms (*G. mesophila* KMM 241^T and *G. polaris* LMG 21857^T) grown on trypticase soy agar (Difco) for 2 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI, 1999). The fatty acids were analysed by GC (Hewlett Packard 6890) and identified using the Microbial Identification software package. Chromosomal DNA extracted for 16S rRNA gene amplification was also used for determination of G+C content. RNA in the DNA solution was removed by incubation with a mixture of RNase A and RNase T1 (each at 20 U ml⁻¹) at 30 °C for 1 h. The G+C content of the chromosomal DNA was analysed as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Phospholipid analysis was performed as described previously (Komagata & Suzuki, 1987; Vaskovsky *et al.*, 1975).

The major cellular fatty acids of strain NO2^T were C_{16:0}, summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and summed feature 7 (C_{18:1}ω9c/ω12t/ω7c). This fatty acid profile is similar to those of *G. mesophila* KMM 241^T and *G. polaris* LMG 21857^T (Supplementary Table S1). The G+C content of genomic DNA of strain NO2^T was 45 mol%. This value is consistent with the G+C content of the genus *Glaciecola*, which ranges between 40 and 47 mol% (Bowman *et al.*, 1998; Romanenko *et al.*, 2003; Van Trappen *et al.*, 2004). The phospholipid composition of strain NO2^T included phosphatidylethanolamine (71.8%) and phosphatidylglycerol (23.2%) as the main phospholipids. The polar lipid composition of NO2^T was similar to that reported for *G. mesophila* KMM 241^T (Romanenko *et al.*, 2003).

Utilization of various substrates as sole carbon sources and some physiological characteristics were determined with API 20NE, API 32GN and API ZYM galleries according to the instructions of the manufacturer (bioMérieux). Bacterial suspensions were made in sterile, chilled artificial seawater medium (Levring, 1946). After inoculation, the galleries were incubated for 2 days at 25 °C and reactions were read. Growth at different temperatures was assessed after 5 days incubation on marine agar (Difco). Strain NO2^T was able to grow at 7–30 °C, but not at 4 or above 37 °C. Salt tolerance was tested on R2A broth supplemented with 0–12% (w/v) NaCl after 3 days incubation at 25 °C. Strain NO2^T required sodium ions for growth and grew in 2–8% NaCl. A comparison of selected physiological characteristics with *G. mesophila*, *G. polaris*, *G. punicea* and *G. pallidula* is shown in Table 1.

DNA–DNA hybridization experiments were carried out with NO2^T, *G. mesophila* KMM 241^T and *G. polaris* LMG 21857^T using the method described by Ezaki *et al.* (1989). Hybridization levels of strain NO2^T with *G. mesophila* KMM 241^T and *G. polaris* LMG 21857^T were respectively 27.7 and 28.4%.

Table 1. Differential phenotypic characteristics of strain NO2^T and species of *Glaciecola*

Strains/species: 1, NO2^T (*G. agarilytica* sp. nov.); 2, *G. mesophila* (data for two strains from Romanenko *et al.*, 2003); 3, *G. polaris* (four strains; Bowman *et al.*, 1998); 4, *G. punicea* (15 strains; Van Trappen *et al.*, 2004); 5, *G. pallidula* (three strains; Van Trappen *et al.*, 2004). All taxa were positive for following tests: sodium ion requirement for growth, oxidase, catalase, growth at 7–30 °C and in 2–6% (w/v) NaCl, degradation of starch, aesculin and DNA and utilization of dextrin, cellobiose, D-fructose, D-galactose, D-mannitol and D-mannose. All taxa were negative for growth at 4 and 37 °C, arginine dihydrolase, utilization of citrate and acid production from glucose, inositol, sorbitol, rhamnose, sucrose, melibiose and arabinose. Characteristics are scored as: w, weak; +, positive; –, negative; v–, variable between strains, type strain negative; v+, variable between strains, type strain positive.

Characteristic	1	2	3	4	5
Growth in 10% NaCl	–	v–	+	–	–
Hydrolysis of egg yolk	–	–	+	–	–
β-Galactosidase	–	+	+	+	–
Nitrate reduction	–	+	–	–	–
Utilization of:					
D-Glucose, D-mannitol	–	+	+	–	–
Sucrose, maltose	–	v+	+	–	–
D-Mannose	w	v+	+	–	–
Acetate	+	–	+	–	+
Glycogen	–	+	+	–	+
Propionate	–	–	+	–	–
L-Glutamate	–	v+	+	–	+
L-Malate	w	–	–	+	–
DNA G+C content (mol%)	45	44	44	44–46	40

On the basis of morphological, physiological and chemotaxonomic characteristics, together with the 16S rRNA gene sequence comparison described above, strain NO2^T should be placed into a novel species, for which we propose the name *Glaciecola agarilytica* sp. nov.

Description of *Glaciecola agarilytica* sp. nov.

Glaciecola agarilytica (a.ga.ri.ly'ti.ca. N.L. n. *agarum* agar-agar, algal polysaccharide; N.L. adj. *lyticus* -a -um from Gr. adj. *lutikos* loosening, dissolving; N.L. fem. adj. *agarilytica* agar-dissolving).

Cells are Gram-negative, oxidase-positive, catalase-positive, agar-digesting and ovoid or curved rod-shaped (1.0–1.5 µm) and possess a single polar flagellum. Slightly halophilic and grows in 2–8 % NaCl at 7–30 °C. Favourable growth occurs aerobically, with circular colonies with regular edges formed within 2 days, with diameters of approximately 2.0–3.0 mm. Requires Na⁺ for growth. Negative for arginine dihydrolase and utilization of citrate and positive for degradation of starch, aesculin and DNA and utilization of dextrin, cellobiose, D-fructose, D-galactose, D-mannitol and D-mannose. No acid is produced from glucose, inositol, sorbitol, rhamnose, sucrose, melibiose or arabinose. Other phenotypic and biochemical tests are listed in Table 1. The major cellular fatty acids are C_{16:0}, summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and summed feature 7 (C_{18:1}ω9c/ω12t/ω7c). Polar lipids include phosphatidylethanolamine (71.8 %) and phosphatidylglycerol (23.2 %). DNA G+C content is 45 mol% (as determined by HPLC).

The type strain is NO2^T (=KCTC 12755^T = LMG 23762^T), isolated from marine sediment of the East Sea, Korea.

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