Glaciecola lipolytica sp. nov., isolated from seawater near Tianjin city, China

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A Gram-negative, non-spore-forming, rod-shaped bacterium, designated strain E3^T, which was motile with a polar flagellum, was isolated from coastal surface seawater near Tianjin city, China. The taxonomy of strain E3^T was studied by phenotypic, chemotaxonomic and phylogenetic methods. Strain E3^T forms non-pigmented colonies with a butyrous–mucoid consistency on marine 2216 agar. It grows optimally at 30 °C, in the presence of 2.0 % (w/v) NaCl and at pH 7.6. Strain E3^T is positive for catalase and oxidase and hydrolysis of lipid and reduces nitrate to N₂ gas. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain E3^T belongs to the *Gammaproteobacteria* and is grouped in the genus *Glaciecola*, with highest sequence similarity to the type strains of *Glaciecola chathamensis* (94.6 %), *G. agarilytica* (94.4 %) and *G. mesophila* (94.4 %). Strain E3^T contained summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, 34.5 %), C_{18:1}ω7c (17.3 %) and C_{16:0} (17 %) as the major fatty acids and phosphatidylethanolamine and phosphatidylglycerol as the main phospholipids. The DNA G + C content was 40.8 mol%. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain E3^T is considered to represent a novel species of the genus *Glaciecola*, for which the name *Glaciecola lipolytica* sp. nov. is proposed. The type strain is E3^T (=CGMCC 1.7001^T =JCM 15139^T).

The genus *Glaciecola* was originally described to accommodate aerobic, psychrophilic, slightly halophilic bacteria and initially comprised two species, *Glaciecola punicea* and *Glaciecola pallidula* (Bowman *et al.*, 1998). Both species were isolated from sea-ice diatom assemblage samples collected from coastal areas of eastern Antarctica. Since then, further species of the genus *Glaciecola* have been isolated from marine environments: *Glaciecola mesophila* (Romanenko *et al.*, 2003) from marine invertebrates, *Glaciecola polaris* (Van Trappen *et al.*, 2004) from Arctic Ocean seawater, *Glaciecola nitratireducens* (Baik *et al.*, 2006) from coastal surface seawater, *Glaciecola chathamensis* (Matsuyama *et al.*, 2006) from Pacific Ocean floor sediment and *Glaciecola psychrophila* (Zhang *et al.*, 2006) and *Glaciecola agarilytica* (Yong *et al.*, 2007) from East Sea sediments. Species of the genus *Glaciecola* show diverse physiological and biochemical properties, such as psy-

chrophily (*G. punicea*, *G. pallidula* and *G. psychrophila*), agar digestion (*G. mesophila* and *G. agarilytica*), nitrate reduction (*G. nitratireducens*) and polysaccharide formation (*G. chathamensis*). Their wide distribution in marine environments and diversity of physiological properties suggest that they may play important roles in processes within marine ecosystems such as the degradation of agar and biogeochemical cycles of elements such as carbon and nitrogen.

In the course of our study on microbial diversity of coastal surface seawater near Tianjin city, China, an aerobic and heterotrophic, Gram-negative bacterium, strain E3^T, was obtained that is phylogenetically related to members of the genus *Glaciecola*. In this communication, we describe the isolation and polyphasic taxonomic study of strain E3^T.

A surface seawater sample was collected and diluted with sterilized NaCl solution (2 %, w/v). For isolation, serially diluted samples were spread onto low-organic seawater medium (LOSWM) that contained 1.0 g peptone and 0.50 g yeast extract (1 distilled water)^{−1} with agar (1.5 %, w/v) and incubated at 30 °C for 10 days. Individual colonies were picked up, cultured in LOSWM and stored at −20 °C in 20 % glycerol (w/v). Strain E3^T could also

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain E3^T is EU183316.

Transmission electron micrographs, 2D TLC of polar lipids and a comparison of fatty acid profiles are available as supplementary material with the online version of this paper.

grow in marine broth 2216 (MB) and on marine agar 2216 (MA) (both from Difco).

Cell morphology was examined by transmission electron microscopy (H600; Hitachi) and scanning electron microscopy (FEI QUANTA 200). Gliding motility was investigated as described by Dong & Cai (2001). The Gram reaction was examined by staining cells grown on LOSWM plates at 30 °C for 24 h according to the method described by Gerhardt *et al.* (1994). Endospore formation was determined after malachite green staining (Dong & Cai, 2001) of cells grown on LOSWM plates. Cells of strain E3^T are Gram-negative, non-spore-forming rods (0.4–0.5 µm wide and 1.0–2.5 µm long) and motile with a polar flagellum. Buds and prosthecae can be produced (see Supplementary Fig. S1, available in IJSEM Online), similarly to *G. polaris* (Van Trappen *et al.*, 2004).

The 16S rRNA gene of strain E3^T was amplified using bacterial universal primers 27F and 1492R (Lane, 1991) and 16S rRNA gene sequence alignment was performed with CLUSTAL_X (version 1.64b; Thompson *et al.*, 1997). Database searches by using BLAST (Altschul *et al.*, 1990) at NCBI indicated that strain E3^T was closely related to *G. chathamensis* and *G. agarilytica*, with 94.6 and 94.4 % 16S rRNA gene sequence similarity to the respective type strains. Phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei, 1987) and the maximum-parsimony method (Fitch, 1971) in MEGA version 3.1 (Kumar *et al.*, 2004). Strain E3^T formed a distinct phylogenetic lineage within the genus *Glaciecola* (Fig. 1), indicating that it was likely to represent a novel species of the genus *Glaciecola*.

The pH range for growth was determined in LOSWM that was adjusted to various pH values (initial pH 4.4–10 at intervals of 0.2 pH units) with HCl or Na₂CO₃. Growth at various temperatures (0–40 °C) was measured on LOSWM plates. Sodium ion requirement and tolerance of NaCl were determined in MB with NaCl concentrations of 0–10.0 %

Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of strain E3^T and recognized species of the genus *Glaciecola*. Numbers at nodes represent percentages of bootstrap support of 1000 resampled datasets (values >50 % are shown). The sequence of *Alteromonas macleodii* IAM 12920^T was used as an outgroup. Solid circles indicate that the corresponding nodes were also recovered in a maximum-parsimony tree (not shown). Bar, 0.01 substitutions per nucleotide position.

(w/v, in increments of 0.5 %). Anaerobic growth was determined in LOSWM in an anaerobic test tube gassed with nitrogen. Catalase activity was judged by formation of bubbles after a 3 % H₂O₂ solution was dropped onto a fresh colony. Oxidase activity and hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Dong & Cai (2001) except that artificial seawater (ASW) was used instead of distilled water. The ASW contained (per litre distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). Acid production from carbohydrates was determined using API 50CH strips (bioMérieux), using a medium composed of 50 % CHB/E medium (bioMérieux) with 0.075 % CaCl₂·2H₂O, 1.875 % NaCl and 0.375 % MgCl₂. Carbohydrate assimilation was determined using API 50CH strips, using ASW supplemented with 0.05 % yeast extract for resuspension of cells, and the strips were incubated at 30 °C for 2 weeks. The test strips were set up according to the manufacturer's instructions. Additional biochemical properties and enzyme activities were detected by API 20NE and API ZYM (bioMérieux) according to the manufacturer's instructions. Susceptibility to antibiotics was determined using filter-paper discs (Beijing Pharmaceutical Company) containing various antibiotics as specified in the species description. Phenotypic properties are given in Table 1 and in the species description. Several characteristics, including hydrolysis of gelatin, starch and agar, acid production from glucose and the utilization of several carbohydrates, differentiated strain E3^T from other species of the genus *Glaciecola*.

The cellular fatty acid profile was determined by using the MIDI Sherlock Microbial Identification System (Microbial ID) with cells grown on LOSWM plates at 30 °C for 3 days, according to the manufacturer's instructions. Fatty acids of strain E3^T (>1 %) are summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) (34.5 %), C_{18:1}ω7c (17.3 %), C_{16:0} (17 %), C_{17:1}ω7c (5.0 %), C_{14:0} (4.1 %), C_{12:0} (3.5 %), C_{17:0} (3.4 %), C_{12:0} 3-OH (2.9 %), C_{12:1} 3-OH (2.1 %), C_{15:0} (1.8 %) and C_{18:0} (1.5 %). The fatty acid composition of strain E3^T is similar to those of other species of the genus *Glaciecola* (Supplementary Table S1).

Polar lipids were examined by two-dimensional TLC and characterized with spray reagents specific for α-glycols (periodate–Schiff), sugars (α-naphthol/sulfuric acid, anisaldehyde/sulfuric acid), free amino groups (ninhydrin) and phosphate (Zindzadze) (Ventosa *et al.*, 1993). A 50 % sulfuric acid solution was used to detect spots of all kinds of lipids (Fujii *et al.*, 2003). Strain E3^T contains four polar lipids (Supplementary Fig. S2): large amounts of phosphatidylethanolamine and phosphatidylglycerol as its main phospholipids and small amounts of bis-phosphatidic acid and an unknown glycolipid. Phosphatidylethanolamine and phosphatidylglycerol are the major lipids reported for *G. mesophila* KMM 241^T (Romanenko *et al.*, 2003) and *G. agarilytica* LMG 23762^T (Yong *et al.*, 2007). Strain E3^T differentiated from *G. mesophila* KMM 241^T and *G.*

Table 1. Properties that differentiate strain E3^T from *Glaciecola* species

Species/strain: 1, strain E3^T; 2, *G. mesophila* (data from Romanenko *et al.*, 2003); 3, *G. chathamensis* (Matsuyama *et al.*, 2006); 4, *G. polaris* (Van Trappen *et al.*, 2004); 5, *G. agarilytica* (Yong *et al.*, 2007); 6, *G. punicea* (Bowman *et al.*, 1998); 7, *G. pallidula* (Bowman *et al.*, 1998); 8, *G. nitratreducens* (Baik *et al.*, 2006); 9, *G. psychrophila* (Zhang *et al.*, 2006). +, Positive; –, negative; v, variable between strains, with the reaction for the type strain in parentheses; w, weak or delayed; ND, no data available. All species were positive for motility, sodium ion requirement for growth, oxidase and catalase and growth in 2–6 % (w/v) NaCl.

Characteristic	1	2	3	4	5	6	7	8	9
Flagellation	Polar, 1	Polar, 1	Polar, 1	Polar, 1	Polar, 1	ND	ND	ND	ND
Pigmentation	–	–	–	–	–	Pink–red	Pale pink	–	–
Growth temperature range (°C)	4–36	7–35	4–30	5–30	7–30	–2 to 25	–2 to 20	15–30	4–15
Growth in 10 % NaCl	–	–	+	+	–	–	–	–	–
Hydrolysis of:									
Gelatin	+	–	–	–	ND	–	–	+	ND
Aesculin	+	w	+	+	+	v (+)	–	–	–
Starch	+	+	ND	+	+	–	v (+)	ND	+
Agar	–	+	–	–	+	–	–	–	–
Tween 80	+	+	+	+	ND	v (–)	+	ND	+
Acid from D-glucose	+	w	ND	–	–	w	w	–	–
Utilization of:									
L-Arabinose	+	–	–	–	ND	–	–	+	–
D-Glucose	+	+	+	+	–	–	–	–	–
Maltose	+	+	+	+	–	–	–	+	+
D-Galactose	+	v (+)	+	+	+	–	–	ND	+
Cellobiose	+	v (+)	–	+	+	–	–	ND	–
D-Fructose	+	v (+)	+	+	+	–	–	ND	–
D-Mannose	+	v (w)	+	+	w	–	–	+	–
D-Mannitol	+	+	+	+	+	–	–	–	–
Glycerol	+	–	–	–	ND	–	+	ND	–
N-Acetylglucosamine	+	–	–	–	ND	–	–	+	–
Glycogen	+	+	ND	+	–	–	+	ND	ND
DNA G+C content (mol%)	40.8	44.8	44.8	44	45	44–46	40	44	42.9

agarilytica LMG 23762^T in the absence of diphosphatidylglycerol and the presence of unknown glycolipid GL1.

The G+C content of genomic DNA was determined by thermal denaturation (Marmur & Doty, 1962) with DNA from *Escherichia coli* K-12 used as a control. The G+C content of strain E3^T was 40.8 mol%, a value that is within the range reported for the genus *Glaciecola*.

Based on the phenotypic and chemotaxonomic characteristics and phylogenetic analysis of the 16S rRNA gene sequence, we conclude that strain E3^T represents a novel species of the genus *Glaciecola*, for which we propose the name *Glaciecola lipolytica* sp. nov.

Description of *Glaciecola lipolytica* sp. nov.

Glaciecola lipolytica (li.po.ly'ti.ca. Gr. n. *lipos* fat; N.L. fem. adj. *lytica* from Gr. adj. *lutikos* -ê -on able to loosen, able to dissolve; N.L. fem. adj. *lipolytica* fat-dissolving, referring to the property of being able to hydrolyse lipid).

Exhibits the following properties in addition to those given in Table 1. Aerobic and heterotrophic. Cells are Gram-negative, non-spore-forming rods, 0.4–0.5 µm wide and

1.0–2.5 µm long, motile with a polar flagellum. Forms non-pigmented colonies with a butyrous–mucoid consistency, about 2–4 mm in diameter on LOSWM plates after incubation at 30 °C for 2 days. Grows at 4–36 °C (optimum 30 °C) but not at 0 or 40 °C, at pH 7.0–9.0 (optimum pH 7.6) but not at pH 6.5 or 9.5 and at 0.5–9.0 % (w/v) NaCl (optimum 2 %). NaCl is required for growth. Positive for oxidase, catalase, amylase, urease, nitrate reduction, H₂S production, arginine dihydrolase and hydrolysis of starch and lipid. Negative for indole production. Forms acid from D- and L-arabinose, D-xylose, methyl β-D-xylopyranoside, D-galactose, D-mannitol, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, D-lactose, melibiose, sucrose, trehalose, inulin, raffinose, gentiobiose and D- and L-fucose. Does not form acid from glycerol, erythritol, D-ribose, L-xylose, D-adonitol, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, melezitose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D- or L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. Positive in tests for alkaline phosphatase, β-galactosidase, esterase lipase (C8), leucine

arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase and *N*-acetyl- β -glucosaminidase; tests negative for esterase (C4), lipase (C14), α -chymotrypsin, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and β -fucosidase. Utilizes Tweens 20, 40, 60 and 80, D-ribose, D-xylose, methyl β -D-xylopyranoside, inositol, methyl α -D-glucopyranoside, amygdalin, arbutin, aesculin, salicin, D-lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, gentiobiose, potassium gluconate, potassium 2-ketogluconate, adipic acid, malic acid and trisodium citrate. No growth observed on erythritol, D-arabinose, L-xylose, D-adonitol, L-sorbose, L-rhamnose, dulcitol, D-sorbitol, methyl α -D-mannopyranoside, capric acid, phenylacetic acid, xylitol, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol or potassium 5-ketogluconate. Resistant to (μ g per disc) carbenicillin (100), polymyxin B (300) and benzylpenicillin (10). Sensitive to neomycin (30), streptomycin (10), ampicillin (10), vancomycin (30), ciprofloxacin (5), rifampicin (5), chloramphenicol (30), kanamycin (30), erythromycin (15), norfloxacin (10), tetracycline (30), gentamicin (10) and novobiocin (5). Cells contain C_{16:0}, C_{18:1} ω 7c and summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0}2-OH) as the major fatty acids and phosphatidylethanolamine and phosphatidylglycerol as major polar lipids. The DNA G + C content of the type strain is 40.8 mol%.

The type strain, E3^T (=CGMCC 1.7001^T =JCM 15139^T), was isolated from coastal surface seawater near Tianjin city, China.

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