Marinobacter psychrophilus sp. nov., a psychrophilic bacterium isolated from the Arctic

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Strain 20041^T was isolated from sea-ice of the Canadian Basin (78° 23' 14" N 149° 06' 55" W). Phylogenetic analysis based on 16S rRNA gene homology showed that strain 20041^T was related to members of the genus *Marinobacter* and had highest 16S rRNA gene sequence similarity with the type strain of *Marinobacter maritimus*. Cells were Gram-negative, rod-shaped, psychrophilic and motile. The temperature range for growth was 0–22 °C, with optimum growth occurring at 16–18 °C and at approximately pH 6.0–9.0. Strain 20041^T had ubiquinone-9 as the major respiratory quinone and iso-C_{15:0} 2OH and/or C_{16:1}ω7c, C_{16:0}, C_{18:1}ω9c and C_{12:0} 3OH as major fatty acids. The genomic DNA G + C content was 55.4 mol%. On the basis of the phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness data, strain 20041^T is considered to represent a novel species, for which the name *Marinobacter psychrophilus* sp. nov. is proposed. The type strain is 20041^T (=CGMCC 1.6499^T=JCM 14643^T).

The genus *Marinobacter* was proposed by Gauthier *et al.* (1992) to accommodate Gram-negative, aerobic, moderately halophilic gammaproteobacteria that used several hydrocarbons as sole sources of carbon and energy. Recently, many further species, for example *Marinobacter algicola* (Green *et al.*, 2006), *Marinobacter vinifirmus* (Liebgott *et al.*, 2006), *Marinobacter koreensis* (Kim *et al.*, 2006), *Marinobacter gudaonensis* (Gu *et al.*, 2007), *Marinobacter salsuginis* (Antunes *et al.*, 2007), *Marinobacter segnicrescens* (Guo *et al.*, 2007) and *Marinobacter salicampi* (Yoon *et al.*, 2007), have been added to the genus *Marinobacter*, which at the time of writing includes 18 species. In this study, a psychrophilic bacterial strain, 20041^T, isolated from sea-ice samples from the Arctic has been identified as representing a novel species of the genus *Marinobacter* within the Gammaproteobacteria.

Strain 20041^T was isolated from sea-ice samples collected off the Canadian Basin of the Arctic Ocean (78° 23' 14" N

149° 06' 55" W), collected using a MARK II ice auger during the Second Chinese National Arctic Research Expedition cruise of the USCGC icebreaker *Xue Long* into the Canada Basin in August 2003. Sea-ice samples were cut carefully into 10–20 cm sections using a sterile saw and placed in sterile plastic bottles to be melted at 4 °C. The isolation and purification of bacterial strains was done as described elsewhere (Zhang *et al.*, 2006). Strain 20041^T was stored at –80 °C in marine 2216 broth (MB; Difco) supplemented with 30% (v/v) glycerol. *Marinobacter maritimus* JCM 12521^T was obtained from the JCM. The culture was routinely grown on marine 2216 agar (MA) or in MB at 22 °C and used as a reference strain.

DNA was extracted and purified as described by Sambrook *et al.* (1989). The 16S rRNA gene was amplified by PCR with universal primers (Zhang *et al.*, 2006). Purified PCR product was ligated into pMD 18-T (TaKaRa Bio) and cloned according to the manufacturer's instructions. Sequencing reactions were carried out using the ABI BigDye 3.1 Sequencing kit (Applied BioSystems) and an automated DNA sequencer (model ABI3730; Applied BioSystems). The 16S rRNA gene sequence of strain 20041^T was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm. The phylogenetic tree was constructed using Kimura two-parameter and pairwise-deletion model

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Marinobacter psychrophilus* 20041^T is DQ060402.

The fatty acid profiles of strain 20041^T and recognized *Marinobacter* species are available as supplementary material with the online version of this paper.

analyses implemented in the program MEGA version 3.0 (Kumar *et al.*, 2004). The resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates. The nearly complete 16S rRNA gene of strain 20041^T (1506 bp) was PCR-amplified and sequenced. Phylogenetic analysis (Fig. 1) showed that strain 20041^T was grouped with the members of the genus *Marinobacter*, and formed a distinct cluster with *Marinobacter maritimus* CK 47^T (97.4 %) and *Marinobacter salicampi* ISL-40^T (95.7 %). Levels of 16S rRNA gene similarity between strain 20041^T and other strains used in the phylogenetic analysis were less than 96.1 %. Similar tree topologies were found in the tree generated with the maximum-parsimony algorithm (data not shown).

The cell morphology of strain 20041^T was examined with an Axioplan 2 microscope while colony morphology was observed on MA after incubation at 16 °C. Growth temperature was determined with a TN3F temperature-gradient incubator (Advantec). The pH range for growth was determined for the strain in MB at various pH values adjusted with 1 M HCl or 1 M NaOH. General physiological tests were performed using conventional methods (Dong & Cai, 2001). Acid production from carbohydrates was determined as described by Leifson (1963). Biochemical traits were determined using API kits (API 20 E, API 20 NE, API ZYM; bioMérieux) according to the manufacturer's instructions, except that the cultures were suspended in 3 % (w/v) NaCl solution. Cells of strain 20041^T were rod-shaped, Gram-negative and motile. Colonies were smooth, circular and convex with entire margins, at first white and later becoming pink on MA. Strain 20041^T grew aerobically, the optimal growth temperature was 16–18 °C, and growth occurred at 0–22 °C. Furthermore, it could tolerate up to 8 % (w/v)

NaCl but could not grow in the absence of NaCl, indicating that it is halophilic.

Isoprenoid quinones were analysed as described previously (Komagata & Suzuki, 1987) using reversed-phase HPLC, with ubiquinone-9 (Q9) from *Marinobacter maritimus* CK 47^T as a reference standard. For fatty acid methyl ester analysis, cell mass of strain 20041^T was harvested from MA plates following incubation at 16 °C for 5 days. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI (Microbial Identification) system (Sasser, 1990). Cells of strain 20041^T contained Q9 as the major respiratory quinone. The predominant cellular fatty acids of strain 20041^T were iso-C_{15:0} 2OH and/or C_{16:1}ω7c (22.16 %), C_{16:0} (13.98 %), C_{18:1}ω9c (13.36 %), C_{12:0} 3OH (10.17 %), C_{16:1}ω9c (8.75 %), C_{17:1}ω8c (8.56 %), C_{17:0} (7.76 %) and C_{12:0} (4.39 %). The fatty acid profiles resembled those determined for recognized *Marinobacter* species (Romanenko *et al.*, 2005; Shivaji *et al.*, 2005; Yoon *et al.*, 2007). The fatty acid profiles of strain 20041^T and recognized *Marinobacter* species are available as supplementary material in IJSEM Online.

DNA base composition was determined by thermal denaturation (*T*_m) with *Escherichia coli* K-12 as the reference. DNA–DNA hybridizations of strain 20041^T to the type strain of *Marinobacter maritimus* were performed by thermal denaturation and the renaturation method of De Ley *et al.* (1970) as modified by Huß *et al.* (1983). The DNA G + C content of strain 20041^T was 55.4 mol%. The DNA–DNA relatedness between strain 20041^T and *M. maritimus* CK 47^T was 32.7 %.

Strain 20041^T was distinguishable from recognized *Marinobacter* species by several phenotypic characteristics

Fig. 1. Phylogenetic relationship of strain 20041^T and recognized members of the genus *Marinobacter* based on 16S rRNA gene similarity. The tree was constructed using the neighbour-joining method. Numbers at nodes represent levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets. *Oceanospirillum linum* ATCC 11336^T was used as an out-group. GenBank accession numbers of 16S rRNA sequences are given in parentheses. Bar, 1 % sequence divergence.

(Table 1). On the basis of the phenotypic, genotypic and phylogenetic data, it is proposed that strain 20041^T represents a novel species of the genus *Marinobacter*, for which the name *Marinobacter psychrophilus* sp. nov. is proposed.

Description of *Marinobacter psychrophilus* sp. nov.

Marinobacter psychrophilus (psy.chro'phil.us. Gr. adj. *psychros* cold; Gr. adj. *philos* loving; N.L. masc. adj. *psychrophilus* cold loving).

Cells are Gram-negative, rod-shaped, psychrophilic and motile, with a size of 0.3–0.4 µm × 1.0–2.5 µm. Colonies are white in young cultures and can be pink with age, smooth, circular and convex with entire margins. Grows aerobically and produces catalase and cytochrome oxidase. Growth occurs at 0–22 °C and pH 5.0–10.0, with optimum growth at 16–18 °C and approximately pH 6.0–9.0. Growth occurs in the presence of 2–8 % (w/v) NaCl and no growth occurs in the absence of NaCl. Nitrate is reduced to nitrite. Indole production and Voges–Proskauer are negative. Hydrogen sulfide is not produced. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, gelatinase, trypsin, caseinase, agarase, chitinase, amylase, lecithinase, citrate utilization, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-glucosidase, α-chymotrypsin, α-mannosidase, α-fucosidase and naphthol-AS-BI-phosphohydrolase are negative. Weak enzymic activity

is observed for lipase (C14) and cystine arylamidase. Tests for esterase (C4), esterase lipase (C8), alkaline phosphatase, leucine arylamidase, valine arylamidase and *N*-acetyl-β-glucosaminidase are positive. Acid is produced only from glycerol. The following compounds are utilized as sole carbon and energy sources: D-arabinose, glycerol, sucrose and D-serine. The following compounds are not utilized as sole carbon and energy sources: dextrin, lactose, galactose, D-mannose, mannitol, xylose, L-rhamnose, melibiose, erythritol, raffinose, D-sorbitol, sorbinose, melezitose, ribose, trehalose, L-threonine, L-leucine, L-aspartic acid, L-glutamic acid, L-histidine, *N*-acetylglucosamine, trisodium citrate, capric acid, adipic acid and phenylacetic acid. The predominant cellular fatty acids of strain 20041^T are iso-C_{15:0} 2OH and/or C_{16:1}ω7c (22.16 %), C_{16:0} (13.98 %), C_{18:1}ω9c (13.36 %), C_{12:0} 3OH (10.17 %), C_{16:1}ω9c (8.75 %), C_{17:1}ω8c (8.56 %), C_{17:0} (7.76 %), C_{12:0} (4.39 %), C_{16:1}ω5c (1.72 %), C_{18:1}ω7c (1.62 %), C_{11:0} 3OH (1.36 %), C_{18:0} (1.18 %) and C_{15:0} (1.21 %). Q9 is the principal isoprenoid quinone. The G+C content of DNA is 55.4 mol%.

The type strain, 20041^T (=CGMCC 1.6499^T=JCM 14643^T), was collected from the Canadian Basin (78° 23' 14" N 149° 06' 55" W) in the Arctic.

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Table 1. Phenotypic characteristics that differentiate strain 20041^T from related *Marinobacter* species

Species: 1, 20041^T; 2, *Marinobacter maritimus* (Shivaji *et al.*, 2005); 3, *Marinobacter salicampi* (Yoon *et al.*, 2007); 4, *Marinobacter segnicrescens* (Guo *et al.*, 2007); 5, *Marinobacter bryozorum* (Romanenko *et al.*, 2005); 6, *Marinobacter gudaonensis* (Gu *et al.*, 2007). +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6
Cell size (µm)	0.3–0.4 × 1.0–2.5	1.7–2.0 × 6.0–7.2	0.4–0.8 × 1.0–7.0	0.6–0.7 × 0.9–1.2	0.4–0.5 × 1.0–1.3	0.3–0.5 × 1.2–1.8
Growth temperature (°C)						
Minimum	0	4	4	15	7	10
Maximum	22	37	39	45	42	45
NaCl tolerance (%)	8	13	15	15	18	15
Hydrolysis of:						
Starch	–	–	–	–	–	+
Tween 80	–	+	+	+	–	+
Urea	–	+	–	–	–	–
Utilization of:						
Alanine	–	+	ND	–	–	+
Mannitol	–	+	ND	–	–	–
Fructose	–	+	–	w	–	+
Sucrose	+	–	–	w	+	+
Acid production from:						
Fructose	–	+	–	ND	–	ND
Glycerol	+	–	ND	ND	–	ND
Xylose	–	+	–	ND	ND	ND
DNA G+C content (mol%)	55.4	58.0	58.1	62.2	59.6	57.9

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