Planomicrobium glaciei sp. nov., a psychrotolerant bacterium isolated from a glacier

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A novel aerobic, psychrotolerant, yellow-to-orange bacterium (strain 0423^T) was isolated from the China No. 1 glacier. Strain 0423^T displayed phenotypic and chemotaxonomic features of the genus *Planomicrobium*, containing anteiso-C_{15:0} and iso-C_{14:0} as the major fatty acids. The temperature range for growth was 4–28 °C, with optimum growth at 20–21 °C. The genomic DNA G + C content was 49 mol%. Phylogenetic analysis based on 16S rRNA gene sequence similarity showed that strain 0423^T was related to members of the genus *Planomicrobium*, sharing the highest sequence similarities with the type strains of *Planomicrobium chinense*, *P. mcmeekinii*, *P. okeanokoites* and *P. koreense*. On the basis of phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness data, a novel species, *Planomicrobium glaciei* sp. nov., is proposed. The type strain is 0423^T (=CGMCC 1.6846^T =JCM 15088^T).

The genus *Planomicrobium* was proposed by Yoon *et al.* (2001). It currently accommodates aerobic, Gram-positive to Gram-variable, non-spore-forming and yellow-to-orange-pigmented bacteria usually with a DNA G + C content in the range 35–47 mol%. So far, the genus *Planomicrobium* comprises six species: *Planomicrobium okeanokoites* (Nakagawa *et al.*, 1996; Yoon *et al.*, 2001), *P. mcmeekinii* (Junge *et al.*, 1998; Yoon *et al.*, 2001), *P. koreense* (Yoon *et al.*, 2001), *P. alkanoclasticum* (Engelhardt *et al.*, 2001; Dai *et al.*, 2005), *P. psychrophilum* (Reddy *et al.*, 2002; Dai *et al.*, 2005) and *P. chinense* (Dai *et al.*, 2005).

The China No. 1 glacier, located in Xinjiang Uygur Autonomous Region, north-west China, is a relatively simple and closed ecosystem. Many cold-adapted micro-organisms have been isolated from the area by members of our laboratory (Zhu *et al.*, 2003; Zhang *et al.*, 2006, 2007, 2008). In this study, we report the isolation and identification of strain 0423^T. Based on a polyphasic taxonomic approach, strain 0423^T is identified as representing a novel species of the genus *Planomicrobium*.

Strain 0423^T was isolated from frozen soil collected from the China No. 1 glacier using media and methods described elsewhere (Zhu *et al.*, 2003). The strain was routinely

grown aerobically at 20 °C on PYG medium (Zhang *et al.*, 2006). *P. chinense* CGMCC 1.3454^T and *P. okeanokoites* NBRC 12536^T were respectively obtained from the CGMCC and the NBRC and *P. mcmeekinii* DSM 13963^T and *P. koreense* DSM 15895^T were obtained from the DSMZ. These cultures were grown routinely on marine 2216 agar (Difco) and used as reference strains.

DNA from strain 0423^T was extracted and purified as described by Sambrook *et al.* (1989). The gene sequence encoding the 16S rRNA was amplified by PCR with the forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse primer 5'-AAGGAGGTGATCCAGCCGCA-3' (Zhang *et al.*, 2006). The PCR product was sequenced using an ABI BigDye 3.1 sequencing kit (Applied BioSystems) and an automated DNA sequencer (model ABI3730; Applied BioSystems). BLASTN searches with the nearly complete (1465 bp) 16S rRNA gene sequence of strain 0423^T in GenBank and EMBL revealed that the novel isolate shared high sequence similarity (~98.0%) with members of the genus *Planomicrobium*. Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony algorithms with Kimura's two-parameter calculation model (Kimura, 1980) 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. Phylogenetic analysis (Fig. 1) showed that strain 0423^T grouped with members of the genus *Planomicrobium*, including *P. chinense* DX3-12^T (98.0%), *P. mcmeekinii*

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 0423^T is EU036220.

Cellular fatty acid compositions of strain 0423^T and its phylogenetic relatives are available as supplementary material with the online version of this paper.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain 0423^T and related species. The tree was rooted with *Exiguobacterium aurantiacum* NCDO 2321^T. Numbers at nodes represent bootstrap values higher than 50% (based on 1000 resampled datasets). Bar, 1% sequence divergence.

DSM 13963^T (97.7%), *P. okeanoikoites* IFO 12536^T (97.5%) and *P. koreense* JG07^T (97.4%). Very similar tree topologies were obtained using the two different algorithms.

The cell morphology of strain 0423^T was examined with an Axioplan 2 microscope (Zeiss). Colony morphology was observed on PYG agar after incubation at 20 °C. The temperature range for growth was determined with a TN3F temperature-gradient incubator (Advantec). The pH range for growth was determined in PYG adjusted at various pH values with 1 M HCl or NaOH.

General physiological tests were performed using conventional methods (Dong & Cai, 2001). Acid production from carbohydrates was determined as described by Leifson (1963). In addition, biochemical traits were examined using the API 20 E, API 20 NE and API ZYM kits (bioMérieux) according to the manufacturer's instructions. The morphological, cultural, physiological and biochemical characteristics of strain 0423^T are given in the species description and in Table 1.

Respiratory quinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu *et al.*, 1989). Cells of strain 0423^T contained menaquinone 7 (MK-7) and menaquinone 8 (MK-8) as the major respiratory quinones. Cell-wall peptidoglycan was prepared and analysed by the method described by Komagata & Suzuki (1987) and Zhang *et al.* (2007). The cell-wall peptidoglycan of strain 0423^T contained lysine, glutamic acid and alanine, but not ornithine; this is consistent with the A4 α type. For cellular fatty acid analysis, strain 0423^T was grown in PYG at 20 °C for 3 days and *P. chinense* CGMCC 1.3454^T, *P. mcmeekinii* DSM 13963^T, *P. okeanoikoites* NBRC 12536^T and *P. koreense* DSM 15895^T were grown in marine 2216 broth (Difco) at 28 °C for 3 days. Fatty acids were methylated and analysed using the standard Microbial Identification (MIDI) procedure (Sasser, 1990). The cellular fatty acids were anteiso-C_{15:0} (36.97%), iso-

C_{14:0} (15.15%), iso-C_{16:0} (5.80%), C_{16:1} ω 7c alcohol (4.55%), C_{17:0} (3.60%), C_{16:1} ω 11c (3.22%) and anteiso-C_{17:0} (3%). Detailed fatty acid profiles of strain 0423^T and its phylogenetic relatives are shown in Supplementary Table S1, available in IJSEM Online.

The G + C content of the DNA was tested by the thermal denaturation method (Sly *et al.*, 1986) with *Escherichia coli* K-12 as the reference strain. Levels of DNA–DNA hybridization were determined from the initial DNA–DNA liquid reassociation rate as described by De Ley *et al.*

Table 1. Differential phenotypic characteristics between strain 0423^T and type strains of *Planomicrobium* species

Strains: 1, *Planomicrobium glaciei* sp. nov. 0423^T (data from this study); 2, *P. chinense* DX3-12^T (Dai *et al.*, 2005); 3, *P. koreense* JG07^T (Yoon *et al.*, 2001); 4, *P. mcmeekinii* DSM 13963^T (Junge *et al.*, 1998); 5, *P. okeanoikoites* NBRC 12536^T (Nakagawa *et al.*, 1996); 6, *P. alkanoclasticum* NCIMB 13489^T (Engelhardt *et al.*, 2001); 7, *P. psychrophilum* DSM 14507^T (Reddy *et al.*, 2002). +, Positive; –, negative; (+) weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Cell shape*	C/R	C/R	C/R	C/R	R	R	R
Growth at:							
4 °C	+	–	+	+	–	–	+
37 °C	–	+	+	+	+	+	–
NaCl tolerance (%)	11	10	7	7	7	6	12
Nitrate reduction	+	+	–	+	–	–	–
Oxidase	–	–	–	–	(+)	–	+
Degradation of:							
Casein	+	–	+	+	+	ND	ND
Starch	–	–	–	–	–	+	–
DNA G + C content (mol%)	49	34.8	47	35	46.3	45.3	44.5

*C, Cocci; R, rods.

(1970) and modified by Huß *et al.* (1983). The tests were performed on a model Lambda 35 UV/Vis spectrophotometer equipped with a temperature program controller (Perkin-Elmer). The DNA G+C content of strain 0423^T was 49 mol%. The levels of DNA-DNA relatedness between strain 0423^T and *P. chinense* CGMCC 1.3454^T, *P. mcmeekinii* DSM 13963^T, *P. okeanoikoites* NBRC 12536^T and *P. koreense* DSM 15895^T were 45.7, 36.8, 35.3 and 32.1 %, respectively.

Based on phenotypic, chemotaxonomic and molecular data, it is concluded that strain 0423^T represents a novel species of the genus *Planomicrobium*, for which the name *Planomicrobium glaciei* sp. nov. is proposed.

Description of *Planomicrobium glaciei* sp. nov.

Planomicrobium glaciei (gla.ci'ei. L. gen. n. *glaciei* of ice, referring to the isolation source of the type strain, the China No. 1 glacier).

Cells are cocci in young cultures but change to short rods with age. Gram-positive, flagellated and gliding. Colonies are smooth, circular, convex with entire margins and yellow to orange in colour. Catalase-positive and oxidase-negative. Growth occurs at 4–28 °C and pH 5.0–10.0, with optimum growth at 20–21 °C and approximately pH 6.0–7.5. Growth occurs in the presence of 0–11 % (w/v) NaCl. Hydrolyses gelatin, casein and trypsin, but not starch or Tweens 20, 60 or 80. Reduces nitrate. Positive for alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α -chymotrypsin, esterase lipase (C8), cystine arylamidase, leucine arylamidase and valine arylamidase. Negative for indole production, Voges-Proskauer test, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, acid phosphatase, urease, α -galactosidase, β -glucuronidase, β -glucosidase, α -glucosidase and α -fucosidase. The following substrates are utilized as sole carbon sources: cellobiose, trehalose, D-mannose, glucose, sucrose, L-arabinose, ribose, fructose and mannitol. The following substrates are not utilized as sole carbon sources: raffinose, xylose, L-rhamnose, melezitose and lactose. Acid is produced from ribose and glycerol. Cells contain MK-7 and MK-8 as the major respiratory quinones. The peptidoglycan type is A4 α type. The cellular fatty acids are anteiso-C_{15:0}, iso-C_{14:0}, iso-C_{16:0}, C_{16:1} ω 7c alcohol, C_{17:0}, C_{16:1} ω 11c and anteiso-C_{17:0}. The G+C content of the type strain is 49 mol%.

The type strain is 0423^T (=CGMCC 1.6846^T =JCM 15088^T), isolated from the China No. 1 glacier (Xinjiang Uygur Autonomous Region, China).

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