Frigoribacterium mesophilum sp. nov., a mesophilic actinobacterium isolated from Bigeum Island, Korea

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A taxonomic study was performed on strain MSL-08^T, which was isolated from a soil sample collected from Bigeum Island. The novel isolate was aerobic and Gram-positive. Cells were short and motile rods. Growth temperature ranged from 20 to 28 °C and the pH for growth ranged from 6.5 to 12.0. The optimum growth temperature and pH were 28 °C and 7.3, respectively. The predominant menaquinone was MK-9. Cell wall analysis showed B-type peptidoglycan containing 2,4-diaminobutyric acid, alanine, glycine, glutamate and lysine. The diagnostic phospholipids were diphosphatidylglycerol and phosphatidylglycerol. The major fatty acids were ai-C_{15:0}, i-C_{16:0}, C_{18:1ω7c} and ai-C_{17:0}. The DNA G + C content was 67.5 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain MSL-08^T had less than 97% similarity to any recognized species of the genus *Frigoribacterium*. *Frigoribacterium faeni* DSM 10309^T was found to be the closest neighbour (96.95%) to the novel strain. Based on the 16S rRNA gene sequence analysis and phenotypic characteristics, it is proposed that strain MSL-08^T represents a novel member of the genus *Frigoribacterium* for which the name *Frigoribacterium mesophilum* sp. nov. is proposed. The type strain is MSL-08^T (=DSM 19442^T=KCTC 19311^T).

The genus *Frigoribacterium*, with the type species *Frigoribacterium faeni*, was proposed by Kämpfer *et al.* (2000) for a psychrophilic strain isolated from airborne hay dust and air inside a museum. In this study, we report the isolation and identification of strain MSL-08^T, the first mesophilic organism of this genus, with an optimum growth temperature of 26–28 °C.

During the isolation of industrially important micro-organisms from diverse soil samples in Korea, strain MSL-08^T was isolated from a soil sample collected from Bigeum Island, Republic of Korea, after incubation for 2 weeks at 28 °C on R2A medium (10-fold dilution). The isolate was maintained on modified R2A agar slants and as glycerol suspensions (20%, w/v) at –70 °C. Biomass for chemical and molecular systematic studies was obtained from cultures grown in 2-fold diluted R2A medium incubated at 28 °C for 7–10 days.

Morphology and motility of cells were examined by using light microscopy and scanning electron microscopy. Gram staining was carried out using the standard Gram reaction combined with the KOH lysis test method (Cerny, 1978). Growth at different temperatures, salt concentrations (w/v) and pH values was investigated as described by Tang *et al.* (2003). The pH was regulated by using autoclaved Na₂CO₃.

The following buffers were used to control the pH range; pH 6.0, 7.0 and 8.0, 0.1 M KH₂PO₄/0.1 M NaOH; pH 9.0 and 10.0, 0.1 M NaHCO₃/0.1 M Na₂CO₃; pH 11.0, 0.05 M Na₂HPO₄/0.1 M NaOH, and pH 12.0, 0.2 M KCl/0.2 M NaOH, with modified R2A used as the basic medium. Metabolic properties were determined using API ZYM test kits (bioMérieux) according to the manufacturer's instructions. Other physiological and biochemical tests such as cell morphology, motility, acid production and assimilation of carbon sources were performed as described previously (Kämpfer *et al.*, 2000).

The procedures for the identification of cell-wall amino acids and sugars followed those described by Stanek & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures of Minnikin *et al.* (1984). Menaquinones were extracted using the methods of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). Cellular fatty acid content was determined as described by Sasser (1990) using the Microbial Identification System (MIDI, Inc.). The amino acids found in the peptidoglycan layer of strain MSL-08^T were 2,4-diaminobutyric acid, alanine, glycine, glutamate and lysine instead of ornithine indicating a B2β-type wall chemotype according to the classification of Schleifer & Kandler (1972). The predominant menaquinone was MK-9. Phospholipids present in strain MSL-08^T were diphosphatidylglycerol, phosphatidylglycerol and some

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MSL-08^T is EF466126.

glycolipids. The cellular fatty acid content of strain MSL-08^T is given in the species description.

Chromosomal DNA from strain MSL-08^T was prepared following the method of Marmur (1961). The G+C content of the DNA was determined using the thermal denaturation method of Marmur & Doty (1962).

Amplification of the 16S rRNA gene sequence was performed as described by Cui *et al.* (2001). The identification of the phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity was conducted using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007) and sequences were aligned using the CLUSTAL_X program (Thompson *et al.*, 1997). A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980, 1983). The reliability of the phylogenetic tree was evaluated using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Phylogenetic analysis of the 16S rRNA gene sequence (1476 nucleotides) revealed that the novel isolate fell within the cluster with the genus *Frigoribacterium* (Fig. 1). Strain MSL-08^T formed a monophyletic clade with *Frigoribacterium faeni* at a low nucleotide sequence similarity (96.95%). The relationship was confirmed in all three tree making analyses; least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Fig. 1).

Most of the physiological properties of strain MSL-08^T were consistent with those of *F. faeni* DSM 10309^T

(GenBank accession no. Y18807). The temperature range for growth was 20–28 °C which distinguished the novel strain from *F. faeni* (2–25 °C). In contrast to *F. faeni* DSM 10309^T, strain MSL-08^T could utilize malate, α -melibiose and D-ribose. Fructose and N-acetylglucosamine were not utilized by strain MSL-08^T but were utilized by *F. faeni* DSM 10309^T (Table 1). The amino acid composition of the peptidoglycan determined for strain MSL-08^T differed from that obtained for *F. faeni* DSM 10309^T. Alanine, glycine, homoserine and lysine were found in *F. faeni* DSM 10309^T whereas homoserine was replaced by glutamate in strain MSL-08^T. The fatty acid profile of strain MSL-08^T was distinguishable from that of *F. faeni* DSM 10309^T. The fatty acid profile of the novel strain is given in the species description. The G+C content of the genomic DNA of strain MSL-08^T was 67.5 mol%.

Phylogenetic analyses based on 16S rRNA gene sequences and chemotaxonomic data revealed that strain MSL-08^T could be assigned to the genus *Frigoribacterium* (Kämpfer *et al.*, 2000). On the basis of the phenotypic and chemotaxonomic properties shown in Table 1, strain MSL-08^T should be classified in the genus *Frigoribacterium* as a representative of a novel species, for which the name *Frigoribacterium mesophilum* sp. nov. is proposed.

Description of *Frigoribacterium mesophilum* sp. nov.

Frigoribacterium mesophilum [me.so.phi'lum. Gr. adj. *mesos* middle; Gr. adj. *philos* loving; N.L. neut. adj. *mesophilum* middle (temperature)-loving, mesophilic].

Fig. 1. Neighbour-joining tree showing the phylogenetic position of strain MSL-08^T. Asterisks indicate branches that were recovered using least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) algorithms. *Agromyces albus* DSM 15934^T (GenBank accession no. AF503917) served as an outgroup. Numbers at the nodes represent bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets (only values >50 % are indicated). Bar, 0.005 nucleotide substitutions per nucleotide position.

Table 1. Characteristics that differentiate *Frigoribacterium mesophilum* sp. nov. MSL-08^T from its nearest phylogenetic neighbours

+, Positive; −, negative; w, weak; ai, anteiso branched; i, iso branched; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; GL, glycolipids. Acid formation from the carbohydrates lactose, sucrose, D-mannitol, dulcitol, salicin, inositol, sorbitol, L-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, melibiose and D-mannose was negative for both strains. Both strains utilized the following substrates as sole source of carbon: L-arabinose, cellobiose, D-galactose, D-glucose, D-mannose, maltose, sucrose, salicin, trehalose, D-xylose and D-mannitol. Both strains had MK-9 as the major menaquinone. Phenylacetate utilization was negative for both strains. Data for *F. faeni* DSM 10309^T are from Kämpfer *et al.* (2000).

Characteristic	MSL-08 ^T	<i>F. faeni</i> DSM 10309 ^T
Colony colour	Cream	Yellow
Catalase	w	+
H ₂ S	−	−
Hydrolysis of aesculin	−	+
Growth temp. range (°C)	20–37	2–25
Growth on sole carbon sources:		
<i>N</i> -acetylglucosamine	−	+
Citrate	−	+
Fructose	−	+
Malate	+	−
α-Melibiose	+	w
D-Ribose	+	−
Major fatty acids	ai-C _{15:0} , i-C _{16:0} , C _{18:1ω7c} and ai-C _{17:0}	ai-C _{15:0} , i-C _{16:0} , C _{16:0} and ai-C _{17:0}
Polar lipids	DPG, PG and GL	PG and DPG
DNA G + C content (mol%)	67.5	71.0

Cells are aerobic, Gram-positive, circular, convex, smooth, glistening, cream coloured and non-endospore-forming with a diameter of 0.2–0.4 mm. Neither substrate mycelium nor aerial mycelium is formed. Growth occurs at 20–37 °C. No growth is observed below 20 °C. Growth occurs over a wide range of pH values, pH 6.5–12.0. The optimum pH value is 7.3 ± 0.2. Growth is also observed in the presence of 0–3 % (w/v) NaCl. Cells are weakly positive for catalase activity but negative for oxidase activity. Tween 80 is hydrolysed but aesculin is not hydrolysed. Utilizes the following: L-arabinose, cellobiose, D-galactose, D-glucose, D-mannose, maltose, sucrose, salicin, trehalose, D-xylose and D-mannitol, but does not utilize fructose or *N*-acetylglucosamine. Positive in tests for starch hydrolysis. Negative results in tests for the production of H₂S and for nitrate reduction. The cell-wall peptidoglycan contains 2,4-diaminobutyric acid with glycine and lysine as the diagnostic diamino acids. The predominant menaquinone is MK-9. The phospholipids present are diphosphatidylglycerol, phosphatidylglycerol and some glycolipids. The fatty acids present are ai-C_{15:0} (39.57 %), i-C_{16:0} (18.04 %), C_{18:1ω7c} (15.42 %), ai-C_{17:0} (10.80 %), i-C_{15:0} (5.84 %), i-C_{14:0} (3.84 %), C_{16:1ω11c} (2.39 %) and C_{16:0} (2.21 %). Other phenotypic characteristics are given in Table 1. The DNA G + C content is 67.5 mol%.

The type strain, MSL-08^T (=DSM 19442^T=KCTC 19311^T), was isolated from a soil sample from Bigeum Island, Republic of Korea.

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