Isoptericola halotolerans sp. nov., a novel actinobacterium isolated from saline soil from Qinghai Province, north-west China

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A Gram-positive, non-motile actinobacterium, designated strain YIM 70177^T, was isolated from a saline soil sample from Qinghai Province, north-west China. Phylogenetic analysis of this organism based on 16S rRNA gene sequences revealed 98·3 % similarity to *Isoptericola variabilis* DSM 10177^T. Chemotaxonomic data determined for the isolate, such as the peptidoglycan type A4α, variation L-Lys–D-Asp, supported the placement of strain YIM 70177^T within the genus *Isoptericola*. Galactose was detected as cell wall sugar. Phospholipids identified were phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol, and the predominant menaquinones were MK-9(H_4) and MK-9(H_2). The major fatty acids were ai-$C_{15:0}$, $C_{16:0}$, ai-$C_{17:0}$ and i-$C_{15:0}$. The DNA G+C content was 72·8 mol%. The low level of DNA–DNA relatedness (27·4 %) to *I. variabilis* DSM 10177^T in combination with differentiating chemotaxonomic and physiological data demonstrated that the isolate YIM 70177^T should be classified as representing a novel species of the genus *Isoptericola*. The name *Isoptericola halotolerans* sp. nov. is proposed, with strain YIM 70177^T (=DSM 16376^T=KCTC 19046^T) as the type strain.

Cellulosimicrobium variabile Bakalidou *et al.* 2002 was reclassified as *Isoptericola variabilis* by Stackebrandt *et al.* (2004) because of differences in the 16S rRNA gene sequence (96·6 % similarity), cell-wall sugar (glucose rather than fucose) and in the amino acid composition of the peptidoglycan (L-Lys–Asp versus L-Lys–Ser–Asp) in comparison with *Cellulosimicrobium cellulans* (Schumann *et al.*, 2001). Isolate YIM 70177^T from saline soil sampled in China is here proposed as the type strain of a novel species of the genus *Isoptericola*, *Isoptericola halotolerans* sp. nov.

Strain YIM 70177^T was isolated by using the dilution plating method on a modified Horikoshi medium (Horikoshi & Grant, 1998). This medium contained (l^{-1}) 10·0 g glucose, 5·0 g peptone, 5·0 g yeast extract, 1·0 g $K_2HPO_4.3H_2O$, 0·2 g $MgSO_4.7H_2O$, 200 g NaCl, 10·0 g Na_2CO_3 and 15·0 g agar. NaCl and sodium carbonate were sterilized separately before addition to the medium. The pH of the medium was adjusted to 10·0–10·5 using $NaHCO_3/Na_2CO_3$ buffer. Strain YIM 70177^T was maintained on ISP 5 agar slants containing 10 % (w/v) NaCl at 4 °C and as 20 % (w/v) glycerol suspensions at −20 °C. Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (about 150 r.p.m.) using modified ISP 5 broth [10 % (w/v) NaCl, pH 7·0] at 28 °C for 1 week.

Gram staining was carried out by the standard Gram reaction and was confirmed by using the KOH lysis test method (Cerny, 1978). Morphology and motility were examined by light microscopy (model BH 2; Olympus) and electron microscopy (JEM-1010 electron microscope) using cells from exponentially growing cultures. Colony morphology was observed on marine agar (MA), ISP 5 medium containing 10 % NaCl and trypticase soy agar (TSA) containing 10 % NaCl after incubation at 28 °C for 3 days. Colony colour was determined by comparing the cultures with the most suitable colour chips from the ISCC-NBS colour charts (Kelly, 1964). Growth temperature was tested at 4, 10, 28, 37, 40, 45 and 55 °C on the same medium. pH and NaCl tolerances were examined as described by Tang *et al.* (2003). Metabolic properties were determined using API ID 32 E

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test kits (bioMérieux) according to the manufacturer's instructions. Other physiological and biochemical tests were performed as described by Gonzalez *et al.* (1978).

Growth of strain YIM 70177^T was aerobic and cells stained Gram-positive. Coccoid and rod-shaped cells were observed, but neither flagella nor spores were found. Pale-yellow colonies with a smooth surface and a maximal diameter of approximately 1 mm were formed on MA and TSA containing 10 % NaCl after incubation for 48 h at 28 °C.

Strain YIM 70177^T grew well at 28 and 37 °C; only slow growth occurred at 4 and 45 °C. Growth was observed at initial pH values between 6 and 10 and on TSA containing 0–25 % NaCl. The strain grew optimally at 28 °C, at pH 8–9 and in the presence of 10 % NaCl. Detailed physiological and biochemical characteristics of the strain are given in Table 1 and in the species description below.

The chemotaxonomic properties of strain YIM 70177^T, including peptidoglycan type, purified cell-wall sugars, phospholipids, menaquinones and whole-cell fatty acid pattern, were analysed as described by Li *et al.* (2004). The peptidoglycan type was A4α, variation L-Lys–D-Asp. Galactose was detected in the purified cell wall. Phospholipids identified were phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol. The menaquinones were MK-9(H_4), MK-9(H_2), MK-8(H_4) and MK-9 (ratio of peak areas, 15 : 10 : 2 : 1). The major fatty acids were ai-$C_{15:0}$, $C_{16:0}$, ai-$C_{17:0}$ and i-$C_{15:0}$; the detailed profile is given in the species description.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Xu *et al.* (2003). Multiple alignments with sequences of the most closely related actinobacteria and calculations of levels of 16S rRNA gene sequence similarity were carried out using CLUSTAL X (Thompson *et al.*, 1997). A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980, 1983) using MEGA version 2.1 (Kumar *et al.*, 2001). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The 16S rRNA gene sequence (1382 bp) for strain YIM 70177^T was determined. Phylogenetic analysis of strain YIM 70177^T revealed that it was most closely related to *I. variabilis* DSM 10177^T with a 16S rRNA gene sequence similarity of 98·3 %, and the two strains formed a distinct subclade in the family *Promicromonosporaceae* (Fig. 1).

The G+C content of the DNA of strain YIM 70177^T was 72·8 mol% as determined by reversed-phase HPLC of

Table 1. Differentiating phenotypic characteristics of strain YIM 70177^T and *I. variabilis* DSM 10177^T

The following phenotypic characteristics are the same for both strains. Gram-positive, non-motile, coccoid or rod-shaped cells. Catalase-positive and oxidase-negative. Milk coagulation, melanin production, H_2S, Voges–Proskauer and indole production are negative; Tweens 20 and 80, casein and starch are not decomposed. Activity for α-galactosidase, β-glucosidase, β-galactosidase, α-maltosidase and lipase and growth on cellulose are positive. Urease, arginine dihydrolase and ornithine decarboxylase are negative. The following substrates are utilized as sole carbon sources for growth: maltose, sucrose, mannose, fructose, salicin and galactose; rhamnose, acetamide, inositol, mannitol, adonitol and sorbitol are not utilized.

Characteristic	YIM 70177^T	DSM 10177^T
Colony pigmentation (PYGV medium)	Pale-yellow	Yellow
Methyl red test	+	−
Acid production from maltose	+	−
Utilization of trehalose	−	+
Enzyme activities		
Lysine decarboxylase	−	+
N-Acetyl-glucosaminidase	+	−
L-Aspartic arylamidase	−	+
Cell-wall sugar(s)*	Gal	Rha, Gal, Glc
Major menaquinone(s)	MK-9(H_4), MK-9(H_2)	MK-9(H_4)
Phospholipids†	PI, PG, DPG	PI, PG, DPG, PL
Major fatty acids	ai-$C_{15:0}$, i-$C_{15:0}$, $C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{15:0}$, $C_{14:0}$, $C_{16:0}$, i-$C_{16:0}$, ai-$C_{17:0}$
DNA G+C content (mol%)	72·8	70–72

*Gal, Galactose; Glc, glucose; Rha, rhamnose.

†DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipids.

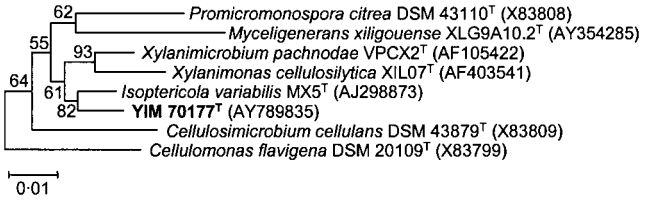

Fig. 1. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 70177^T and of its phylogenetic neighbours. Numbers on branch nodes are bootstrap percentages (1000 resamplings; only values over 50 % are given). The sequence of *Streptomyces megasporus* DSM 41476^T (Z68100) was used as the root. Bar, 1 % sequence divergence.

nucleosides according to Mesbah *et al.* (1989). DNA–DNA relatedness was studied using the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992) by using a UV-VIS spectrophotometer model UV1601 (Shimadzu). The level of DNA–DNA relatedness between YIM 70177^T and *I. variabilis* DSM 10177^T was 27·4 % (repeated twice; the original recorded values were 25·8 and 29·0 %).

The results of 16S rRNA gene sequence analysis clearly demonstrate that strain YIM 70177^T represents a member of the genus *Isoptericola*. Similarities in morphological characteristics, peptidoglycan type and DNA G+C content to *I. variabilis* DSM 10177^T support the inclusion of strain YIM70177^T within the genus *Isoptericola*. However, strain YIM 70177^T differs from *I. variabilis* DSM 10177^T in components of the cell-wall sugars, phospholipids, menaquinones, cellular fatty acids and some physiological and enzymic properties (see Table 1). The level of DNA–DNA relatedness of 27·4 % between YIM 70177^T and *I. variabilis* DSM 10177^T is significantly lower than 70 %, the threshold value considered for the delineation of genomic species (Wayne *et al.*, 1987). These results support the proposal of a novel species, for which the name *Isoptericola halotolerans* sp. nov. is proposed.

Description of *Isoptericola halotolerans* sp. nov.

Isoptericola halotolerans (ha.lo.to′le.rans. Gr. n. *hals* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans* salt-tolerating, referring to the organism's ability to tolerate high salt concentrations).

Cells are Gram-positive, coccoid or rod-shaped, non-motile and do not form spores. Primary mycelium is formed. Colonies are pale-yellow, circular, opaque and approximately 1·0 mm in diameter after 24 h growth at 28 °C. Optimal growth occurs at 10 % NaCl, pH 8·0–9·0 and 28 °C. In addition to the properties listed in Table 1, the strain is negative for gelatin liquefaction, ammonia production, milk peptonization and starch hydrolysis, but positive for methyl red test and nitrate reduction. The following substrates are utilized: glucose, ribose, arabinose, maltose, cellobiose, trehalose, sorbitol, lactose, xylose and dextrin. The peptidoglycan type is A4α, variation L-Lys–D-Asp. The cell-wall sugar is galactose. Phospholipids are phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol. The predominant menaquinones are MK-9(H_4) and MK-9(H_2). The cellular fatty acids are ai-$C_{15:0}$ (54·46 %), $C_{16:0}$ (20·05 %), ai-$C_{17:0}$ (10·69 %), i-$C_{15:0}$ (7·02 %), $C_{14:0}$ (2·33 %), i-$C_{16:0}$ (1·44 %), $C_{15:0}$ (0·98 %), $C_{17:0}$ (0·61 %), $C_{18:0}$ (0·36 %), i-$C_{14:0}$ (0·64 %), i-$C_{17:0}$ (0·51 %) and ai-$C_{15:1}$ (0·51 %). The G+C content of the DNA of the type strain is 72·8 mol%.

The type strain, YIM 70177^T (=DSM 16376^T=KCTC 19046^T), was isolated from a saline soil sample collected from Qinghai Province, north-west China.

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