Streptomonospora halophila sp. nov., a halophilic actinomycete isolated from a hypersaline soil

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An actinomycete strain, designated YIM 91355^T, was isolated from a soil sample collected from a hypersaline soil in Xinjiang Province, China, and its taxonomic position was investigated by using a polyphasic approach. The strain grew well on most media tested and no diffusible pigment was produced. The aerial mycelium of this organism was well developed but not fragmented and, at maturity, formed short chains of spores. Substrate mycelium was branched with non-fragmenting hyphae and single, oval to round spores were formed, borne on sporophores. Strain YIM 91355^T contained *meso*-diaminopimelic acid as the diagnostic amino acid. The purified cell-wall hydrolysate contained galactose. The predominant menaquinones were MK-10(H_8) (43.2 %), MK-10(H_6) (17.7 %) and MK-11(H_8) (10.4 %). Phospholipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol mannosides, phosphatidylcholine, phosphatidylinositol and an unidentified phospholipid. The major fatty acids were i-$C_{16:0}$, ai-$C_{17:0}$, 10-methyl $C_{17:0}$ and 10-methyl $C_{18:0}$. The G+C content of the genomic DNA was 72.1 mol%. Phylogenetic analysis indicated that the isolate belongs to the genus *Streptomonospora* of the family *Nocardiopsaceae*. Based on phenotypic and genotypic data, it is concluded that strain YIM 91355^T represents a novel species of the genus *Streptomonospora*, for which the name *Streptomonospora halophila* sp. nov. (type strain YIM 91355^T =DSM 45075^T =KCTC 19236^T) is proposed.

The genus *Streptomonospora* was first established by Cui *et al.* (2001) with the type species *Streptomonospora salina* and it represents a group of strictly halophilic filamentous actinomycetes that form a distinct branch in the 16S rRNA gene phylogenetic tree adjacent to the genera *Nocardiopsis* and *Thermobifida* of the family *Nocardiopsaceae* (Stackebrandt *et al.*, 1997). Subsequently, Li *et al.* (2003) described another species, *Streptomonospora alba*, and emended the description of the genus *Streptomonospora*. During a biodiversity and taxonomic study on halophilic filamentous actinomycetes, numerous *Streptomonospora*-like or *Nocardiopsis*-like strains have been isolated from the same hypersaline habitat as *S. salina* YIM 90002^T and *S. alba* YIM 90003^T, i.e. close to Aiding Lake in Xinjiang Province, north-west China. For rapid identification of *Streptomonospora* species, genus-specific PCR amplification was introduced according to the method described by Zhi *et al.* (2006). As a result, a third halophilic *Streptomonospora* isolate, strain YIM 91355^T, attracted our attention and was investigated using a polyphasic approach to determine its taxonomic position.

Strain YIM 91355^T was isolated and maintained on modified ISP 5 medium (0.1 % K_2HPO_4, 0.1 % L-asparagine, 1.0 % glycerol, 0.5 % yeast extract, 0.5 % KNO_3, 10.0 % NaCl, 2.0 % agar, pH 7.0). Other media used for examining cultural characteristics were inorganic salts/starch agar, Czapek's agar, potato agar, nutrient agar, yeast extract/malt extract agar and oatmeal agar (Shirling & Gottlieb, 1966), all of which were supplemented with 10 % (w/v) NaCl; see data in Table 1. Strain YIM 91355^T grew well on most media, especially on modified ISP 5 medium. No diffusible pigments were produced on any of the media tested.

Morphological characteristics of the strain were observed by light microscopy (model BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 7 days

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Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 91355^T is EF423989.

Table 1. Cultural characteristics of strain YIM 91355^T

Diffusible pigments were not produced on any of the media listed. All media contained 10 % (w/v) NaCl, pH 7.0. Colours were taken from the ISCC–NBS colour charts (standard samples, no. 2106) (Kelly, 1964).

Medium	Growth	Colour of colonies	
		Aerial mycelium	Substrate mycelium
Czapek's agar	Moderate	Yellow–white	Pale yellow
Nutrient agar	Poor	Yellow–white	Pale yellow
Potato agar	Good	Yellow–white	Pale yellow
Modified ISP 5 medium	Good	Yellow–white	Pale yellow
Inorganic salts/starch agar	Moderate	White	Pale yellow–white
Yeast extract/malt extract agar	Good	Yellow–white	Yellowish brown
Oatmeal agar	Good	Yellow–white	Pale yellow

growth on modified ISP 5 containing 10 % NaCl (w/v) at 37 °C. Investigations of 7-day-old cultures of strain YIM 91355^T revealed that the isolate shared the same morphological characteristics as members of the genus *Streptomonospora*. The aerial mycelium was well developed but not fragmented and, at maturity, it formed spore chains that were straight to flexuous. Spores were oval to cylindrical (0.4–0.5 × 0.8–1.0 μm) and non-motile (Fig. 1a). Substrate mycelium was extensively branched with non-fragmenting hyphae. Single spores, oval to round and 0.8–1.0 μm in diameter, were borne on sporophores of substrate mycelium (Fig. 1b). The colours of substrate and aerial mycelia were determined by comparison with chips from ISCC–NBS colour charts (Kelly, 1964).

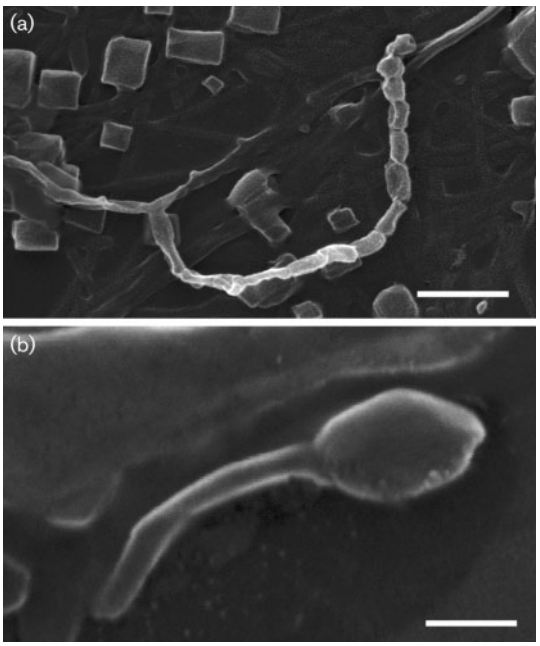

Fig. 1. Scanning electron micrographs of strain YIM 91355^T grown on modified ISP medium 5 for 7 days at 37 °C showing a spore chain on aerial mycelium (a; bar, 2 μm) and a single spore on substrate mycelium (b; bar, 500 nm).

Growth was assessed at 4, 10, 20, 28, 37, 45, 55, 60 and 65 °C; optimum growth was observed at 37 °C. pH and NaCl tolerance was examined as described by Tang *et al.* (2003). Other physiological features and carbon source utilization tests were observed on media commonly used for the characterization of *Streptomyces* species (Shirling & Gottlieb, 1966; Williams *et al.*, 1989) supplemented with 10 % (w/v) NaCl. Detailed physiological and biochemical characteristics of the isolate are given in Table 2 and in the species description.

The cell wall amino acids were analysed by using TLC as described by Lechevalier & Lechevalier (1980). Cell walls contained *meso*-diaminopimelic acid (*meso*-DAP). Sugar analysis of cell walls followed procedures described by Staneck & Roberts (1974); the hydrolysate of purified cell walls of strain YIM 91355^T contained galactose. Polar lipids were extracted, examined by two-dimensional TLC and identified using previously described procedures (Minnikin *et al.*, 1979). Strain YIM 91355^T possessed diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannosides, phosphatidylcholine, phosphatidylinositol and an unidentified phospholipid. The strain was cultivated in modified ISP 5 medium for menaquinone analysis. Menaquinones were extracted as described by Collins *et al.* (1977) and were analysed by HPLC (Kroppenstedt, 1982). The predominant menaquinones were MK-10(H_8) (43.2 %), MK-10(H_6) (17.7 %) and MK-11(H_8) (10.4 %); MK-9(H_4) (1.1 %), MK-9(H_6) (1.7 %), MK-9(H_8) (3.7 %), MK-10 (0.9 %), MK-10(H_2) (2.4 %), MK-10(H_4) (6.8 %), MK-11(H_4) (1.0 %), MK-12 (5.2 %), MK-11(H_6) (4.0 %) and MK-11(H_{10}) (1.8 %) were minor components. Cellular fatty acid analysis was performed as described by Sasser (1990) using the Microbial Identification system (MIDI). The fatty acid profile of strain YIM 91355^T was mainly composed of i-$C_{16:0}$ (39.8 %), ai-$C_{17:0}$ (12.5 %), 10-methyl $C_{17:0}$ (10.2 %) and 10-methyl $C_{18:0}$ (tuberculostearic acid; 11.1 %); for comparison, the fatty acid compositions of the type strains of *S. salina* and *S. alba* are shown in Table 2.

Table 2. Differential phenotypic characteristics of strain YIM 91355^T and the type strains of *Streptomonospora* species

Data are from this study, Cui *et al.* (2001) and Li *et al.* (2003). For all three strains, the optimal growth temperature is 37 °C. They have *meso*-DAP as the diagnostic amino acid in the cell wall. All are negative for gelatin liquefaction, milk coagulation and utilization of arabinose, mannitol, raffinose and sucrose. +, Positive; −, negative; NT, not tested.

Characteristic	*S. halophila* YIM 91355^T	*S. salina* YIM 90002^T	*S. alba* YIM 90003^T
NaCl concentration for growth (%)			
Range	5–20	5–20	5–25
Optimum	10	15	10–15
Milk peptonization	+	−	−
Nitrate reduction	−	−	+
Production of:			
H_2S	−	−	NT
Oxidase	−	+	−
Catalase	+	NT	+
Melanin	−	+	−
Amylase	−	+	−
Cellulase	+	−	−
Carbon-source utilization			
Glucose	−	−	+
Inositol	+	−	−
Maltose	+	−	−
Rhamnose	+	−	−
Cell-wall sugar(s)	Galactose	Galactose	Galactose, arabinose
Predominant menaquinones (>10 %)*	MK-10(H_8) (43.2 %), MK-10(H_6) (17.7 %), MK-11(H_8) (10.4 %)	MK-10(H_8) (39.4 %), MK-10(H_6) (22.6 %), MK-10(H_4) (11.8 %)	MK-10(H_8) (50.6 %), MK-10(H_6) (15.8 %)
Major phospholipids†	DPG, PG, PC, PIM, PI, PL	PG, PI, PC, 2MPE, PL	PG, PE, PI, DPG, MPE, PS, PC, PL
Major fatty acids‡	i-$C_{16:0}$ (39.8 %), ai-$C_{17:0}$ (12.5 %), 10-methyl $C_{17:0}$ (10.2 %), 10-methyl $C_{18:0}$ (11.1 %)	i-$C_{15:0}$ (30.1 %), i-$C_{16:0}$ (16.5 %), 9-methyl $C_{16:0}$ (11.7 %), ai-$C_{17:0}$ (6.7 %)	ai-$C_{17:0}$ (26.0 %), i-$C_{16:0}$ (25.1 %), $C_{16:0}$ (8.6 %), ai-$C_{15:0}$ (8.0 %)
DNA G+C content (mol%)§	72.1	72.9	74.4

*Grown on modified ISP 5 medium.
†DPG, Diphosphatidylglycerol; MPE, methylphosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, PI mannosides; PL, unidentified phospholipid; PS, phosphatidylserine.
‡Grown on modified TSA.
§Determined using the HPLC method.

The DNA G+C content of strain YIM 91355^T, determined by using the HPLC method of Mesbah *et al.* (1989), was 72.1 mol%.

Extraction and amplification of genomic DNA for 16S rRNA gene sequence analysis were carried out as described by Li *et al.* (2007). The nearly full-length 16S rRNA gene sequence of strain YIM 91355^T was determined by direct sequencing of the PCR-amplified 16S rRNA gene. Multiple alignments with sequences of related taxa in the family *Nocardiopsaceae* were implemented by using CLUSTAL_X (Thompson *et al.*, 1997). 16S rRNA gene similarity values were calculated by using a web-based tool as described by Chun *et al.* (2007) (http://www.eztaxon.org). A phylogenetic tree (Fig. 2) was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980). Topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

In the phylogenetic dendrogram, strain YIM 91355^T formed a stable clade (supported by 93 % bootstrap value; see Fig. 2) together with the two species of the genus *Streptomonospora*, family *Nocardiopsaceae*, with validly published names, even though the highest 16S rRNA gene similarity was found between strain YIM 91355^T and *Nocardiopsis halophila* KCTC 9825^T (96.94 %, 1421 bp). The closest phylogenetic relationship was observed between strain YIM 91355^T and members of the genus *Streptomonospora*; this observation was supported by morphological and chemotaxonomic data. For example, the isolate shared the morphological characteristics described for members of the genus *Streptomonospora*, the aerial mycelium was well developed but not fragmented, the substrate mycelium was extensively

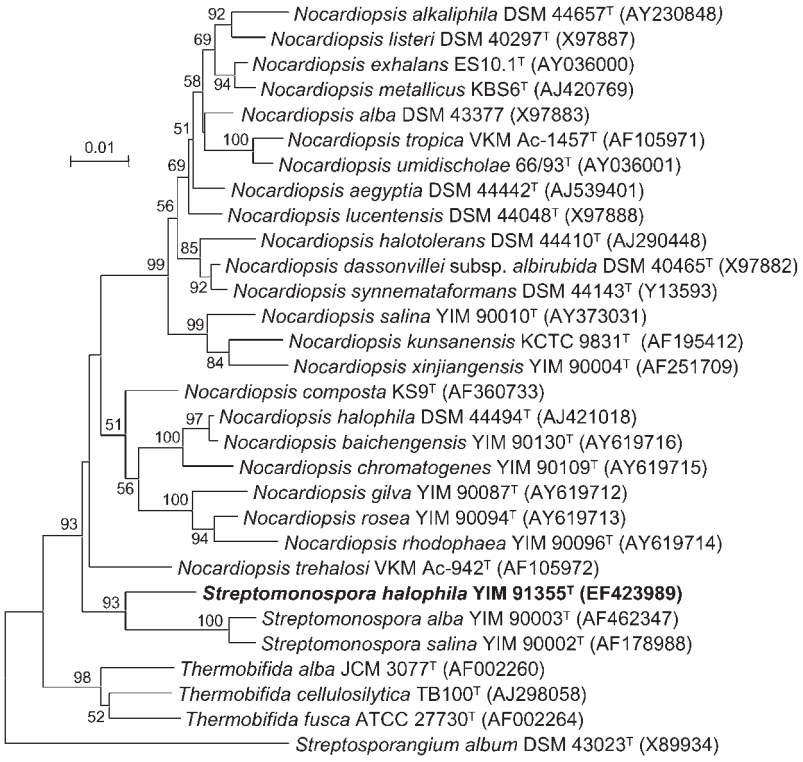

Fig. 2. Phylogenetic dendrogram based on 16S rRNA gene sequence analysis reconstructed from evolutionary distances by using the neighbour-joining method, showing the phylogenetic position of strain YIM 91355^T within the family *Nocardiopsaceae*. Bootstrap values (only above 50 %) of 1000 replicates are shown as percentages. *Streptosporangium album* DSM 43023^T was used as the outgroup. Bar, 1 substitution per 100 nucleotide positions.

branched with non-fragmenting hyphae and single spores were borne on sporophores of substrate mycelium. In addition, the strain contained galactose as the diagnostic sugar, which is consistent with chemotaxonomic characteristics of members of the genus *Streptomonospora*, but not members of the genus *Nocardiopsis*. The 16S rRNA gene sequence similarities between YIM 91355^T and the type strains of *S. salina* and *S. alba* were 96.51 and 96.81 %, respectively. Group-specific signature analysis showed that strain YIM 91355^T and both *Streptomonospora* species shared identical signatures at positions 229 (G), 508 (C), 811 (U), 461–471 (Y–G), 611–629 (U–U) and 1028–1033 (C–G). Thus, all the above data support the inclusion of the isolate in the genus *Streptomonospora* (Cui *et al.*, 2001; Li *et al.*, 2003). However, strain YIM 91355^T differs from the other two species of the genus *Streptomonospora* in many physiological, chemotaxonomic and biochemical properties; details are shown in Table 2.

Furthermore, it is generally recognized that organisms that display sequence divergence values of >3 % do not belong to the same species (Stackebrandt & Goebel, 1994). Thus, based on the above phenotypic and genotypic results, strain YIM 91355^T represents a novel species of the genus *Streptomonospora*, for which the name *Streptomonospora halophila* sp. nov. is proposed.

Description of *Streptomonospora halophila* sp. nov.

Streptomonospora halophila (ha.lo.phi′la. Gr. n. *hals*, *halos* salt; Gr. adj. *philos* loving; N.L. fem. adj. *halophila* salt-loving, referring to the ability to grow at high NaCl concentrations).

Gram-positive and aerobic. Grows well on most test media, but no diffusible pigment is produced. Aerial mycelium and substrate mycelium are well developed but not fragmented on most media. The yellow–white aerial mycelium (white only on inorganic salts/starch agar) forms straight to flexuous spore chains at maturity; spores are oval to cylindrical (0.4–0.5 × 0.8–1.0 μm) and non-motile. Single, oval to round spores (0.8–1.0 μm diameter) are borne on sporophores of substrate mycelium. Colours of the substrate mycelium are pale yellow on Czapek's agar, nutrient agar, potato agar, modified ISP 5 medium and oatmeal agar, pale yellow–white on inorganic salts/starch agar and yellowish brown on yeast extract/malt extract medium. The optimum NaCl concentration, growth pH and temperature are 10 % (w/v), pH 7.0 and 37 °C. Ranges of NaCl concentration, pH and temperature for growth are 5–20 %, pH 6.0–9.0 and 20–45 °C. Inositol, maltose and rhamnose can be utilized as sole carbon sources. Positive for milk peptonization, catalase and cellulose, but negative for oxidase, melanin production, amylase, nitrate reduction and production of H_2S. The diagnostic amino acid is *meso*-DAP; galactose is the main cell wall sugar. The predominant menaquinones are MK-10(H_8), MK-10(H_6) and MK-11(H_8). Phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol mannosides, phosphatidylinositol and an unidentified phospholipid.

The type strain is YIM 91355^T (=DSM 45075^T =KCTC 19236^T), isolated from a hypersaline soil in Xinjiang Province, north-west China. The G+C content of DNA of the type strain is 72.1 mol%.

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