Streptomonospora amylyolytica sp. nov. and *Streptomonospora flavalba* sp. nov., two novel halophilic actinomycetes isolated from a salt lake

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Two novel halophilic, aerobic, catalase-positive but oxidase-negative, Gram-positive actinomycetes, designated YIM 91353^T and YIM 91394^T, were isolated from a salt lake in the north-west of China. Phylogenetic analyses based on 16S rRNA gene sequences indicated that the novel isolates should be assigned to the genus *Streptomonospora*. The phenotypic and chemotaxonomic characteristics of the isolates also matched those described for members of the genus *Streptomonospora*. The predominant menaquinones were MK-10(H₈), MK-10(H₆) and MK-9(H₈), and meso-diaminopimelic acid was the diagnostic amino acid in the cell walls. The phospholipids of the isolates consisted of diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. The major fatty acids of strain YIM 91353^T were anteiso-C_{17:0} and C_{18:0}, and of strain YIM 91394^T were anteiso-C_{17:0} and iso-C_{16:0}. The DNA G + C contents were 71.2 and 72.5 mol%, respectively. The combination of phylogenetic analysis, DNA–DNA hybridization data, phenotypic characteristics and chemotaxonomic differences supported the view that strains YIM 91353^T and YIM 91394^T each represent a novel species of the genus *Streptomonospora*, for which the names *Streptomonospora amylyolytica* sp. nov. and *Streptomonospora flavalba* sp. nov. are proposed, with type strains YIM 91353^T (=DSM 45171^T=CCTCC AA 208048^T) and YIM 91394^T (=DSM 45155^T=CCTCC AA 208047^T), respectively.

The genus *Streptomonospora*, belonging to the family *Nocardiopsaceae* (Stackebrandt *et al.*, 1997), was first described by Cui *et al.* (2001) and emended by Li *et al.* (2003). Currently, the genus contains three recognized species: *Streptomonospora salina* (Cui *et al.*, 2001), *Streptomonospora alba* (Li *et al.*, 2003) and *Streptomonospora halophila* (Cai *et al.*, 2008b). In order to obtain more extremophilic actinomycetal resources, abundant actinomycetes have been isolated with many

different media. For the rapid identification of *Streptomonospora* species, a genus-specific PCR amplification method has been introduced, according to the method described by Zhi *et al.* (2006), and rapid screening of potential novel taxa of the genus *Streptomonospora* is possible with PCR-single-strand conformation polymorphism analysis (Cai *et al.*, 2008a). As a result, two *Streptomonospora*-like strains, YIM 91353^T and YIM 91394^T, were isolated from a salt lake in Xinjiang Province, north-west China.

Strains YIM 91353^T and YIM 91394^T were isolated from a saline soil sample by plating 1:10 serial dilutions of the sample on starch-casein agar medium (pH 7.0) supplemented with 10% (w/v) NaCl at 37 °C for 3 weeks. The composition of the starch-casein agar medium was (l⁻¹): 10 g starch, 0.3 g casein, 2 g KNO₃, 0.05 g MgSO₄·7H₂O, 2 g K₂HPO₄, 0.02 g CaCO₃, 0.01 g FeSO₄ and 15 g agar.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIM 91353^T and YIM 91394^T are EU442551 and EU442553, respectively.

A figure showing minimum-evolution and maximum-parsimony 16S rRNA gene sequence phylogenies and a table showing the fatty acid compositions of strains YIM 91353^T and YIM 91394^T and other members of the genus *Streptomonospora* are available as supplementary material with the online version of this paper.

After primary isolation and purification, the isolates were preserved both on slants of International *Streptomyces* Project medium 4 agar (ISP 4; Shirling & Gottlieb, 1966) supplemented with 10 % (w/v) NaCl at 4 °C and in 20 % (v/v) glycerol at –80 °C. Three reference *Streptomonospora* strains, *S. salina* YIM 90002^T, *S. alba* YIM 90003^T and *S. halophila* YIM 91355^T, were obtained from Yunnan Institute of Microbiology.

Unless otherwise indicated, all tests were carried out with 10 % NaCl (w/v) at pH 7.0 and incubated at 37 °C. Morphological characteristics were observed by using light microscopy (BH2; Olympus) and scanning electron microscopy (JSM-5600LV; JEOL) after growth for 2 and 4 weeks on ISP 4 supplemented with 10 % (w/v) NaCl. Anaerobic growth was detected as described previously (Chen *et al.*, 2007). Cultural characteristics were examined using standard growth media according to Shirling & Gottlieb (1966). The colours of substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). The media and procedures used to determine physiological and biochemical features and carbon-source utilization patterns were those described by Shirling & Gottlieb (1966) and Williams *et al.* (1989). Strains YIM 91353^T and YIM 91394^T had the typical macroscopic and microscopic appearance of most species of the genus *Streptomonospora* (Cui *et al.*, 2001; Li *et al.*, 2003; Cai *et al.*, 2008b). The detailed results of the phenotypic tests are given in the species description and in Tables 1 and 2.

Genomic DNA was prepared from cells lysed with microwaves. PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Li *et al.*, 2007), and the G+C content was determined using the HPLC method (Mesbah *et al.*, 1989). The resulting 16S rRNA gene sequences were compared with sequences obtained from public databases (GenBank/EMBL/DDBJ) to find the most closely related strains. Phylogenetic analyses were performed using the software package MEGA version 4.1 (Tamura *et al.*, 2007) after multiple alignment of sequence data by CLUSTAL X (Thompson *et al.*, 1997). Distances were calculated using distance options according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and minimum-evolution (Felsenstein, 1997) algorithms by means of methods contained within the software package MEGA version 4.1 (Tamura *et al.*, 2007). Bootstrap analysis was used to evaluate the tree topology by means of 1000 resamplings (Felsenstein, 1985). DNA–DNA hybridizations were carried out using photobiotin-labelled DNA probes and microdilution wells as described by Ezaki *et al.* (1989). A microplate spectrofluorimeter (Gemini XPS; Molecular Devices) was employed for fluorescence measurements.

The almost-complete 16S rRNA gene sequences of strains YIM 91353^T (1532 bp) and YIM 91394^T (1560 bp) were

Table 1. Phenotypic characteristics that differentiate strains YIM 91353^T and YIM 91394^T from each other and recognized *Streptomonospora* species

Strains: 1, *S. amylolytica* sp. nov. YIM 91353^T; 2, *S. flavalba* sp. nov. YIM 91394^T; 3, *S. salina* YIM 90002^T; 4, *S. alba* YIM 90003^T; 5, *S. halophila* YIM 91355^T. Data were taken from this study. All strains were positive for catalase production. All strains were negative for gelatin liquefaction, hydrolysis of urea and utilization of acetate, adonitol, citrate, dextrin, dulcitol, erythrose, fructose, galactose, gluconate, lactose, malonate, mannitol, mannose, melibiose, melezitose, raffinose, ribose, salicin, sorbose, sucrose, tartrate, turanose, xylitol, adenine, asparagine, glutamic acid, methionine and valine. +, Positive; –, negative.

Characteristic	1	2	3	4	5
Hydrolysis of:					
Cellulose	–	–	–	–	+
Starch	+	+	+	–	–
Milk coagulation	+	+	–	–	–
Milk peptonization	+	+	–	–	+
Nitrate reduction	–	–	–	+	–
Production of:					
H ₂ S	+	–	–	–	–
Melanin	–	+	+	–	–
Oxidase	–	–	+	–	–
Utilization of:					
Arabinose	+	+	+	–	–
Cellobiose	+	–	–	–	–
Glucose	+	+	+	+	–
Inositol	–	–	–	–	+
Maltose	–	+	+	–	+
Rhamnose	+	+	–	–	+
Sorbitol	+	–	–	–	–
Alanine	–	+	–	–	+
Arginine	+	+	–	–	–
Glycine	–	+	–	–	–
Histidine	–	+	+	–	+
Hydroxyproline	+	–	–	–	–
Lysine	+	–	–	–	–
Phenylalanine	–	+	–	–	+
Proline	+	+	–	–	+
Serine	–	+	–	–	–
Threonine	+	+	–	–	+
Tryptophan	+	+	–	–	–
Tyrosine	–	+	–	–	–

determined. Preliminary comparison of the sequences against the GenBank database indicated that these isolates were closely related to the members of the genus *Streptomonospora*. In all of the three phylogenetic trees, strains YIM 91353^T and YIM 91394^T formed a stable clade with the three recognized *Streptomonospora* species (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online). The levels of 16S rRNA gene sequence similarities for *S. alba* YIM 90003^T, *S. salina* YIM 90002^T and *S. halophila* YIM 91355^T with strain YIM 91353^T were 99.3, 98.8 and 96.7 %, respectively, and with strain YIM 91394^T were 99.6,

Table 2. Cultural characteristics of strains YIM 91353^T and YIM 91394^T

All media were adjusted to pH 7.0 and supplemented with 10 % (w/v) NaCl. Diffusible pigments were not produced on any of the media tested except for strain YIM 91394^T, which produced deep yellow diffusible pigments on potato extract agar. —, No growth.

Medium	Growth		Aerial mycelium		Substrate mycelium	
	YIM 91353 ^T	YIM 91394 ^T	YIM 91353 ^T	YIM 91394 ^T	YIM 91353 ^T	YIM 91394 ^T
Yeast extract-malt extract agar (ISP 2)	Moderate	Good	Yellow-white	Yellow-white	Yellow-grey	Pale yellow
Oatmeal agar (ISP 3)	Good	Moderate	White	Yellow-white	Yellow-grey	Pale yellow
Inorganic salt-starch agar (ISP 4)	Moderate	Moderate	Yellow-white	Yellow-white	Yellow-grey	Pale yellow
Glycerol-asparagine agar (ISP 5)	—	Poor	—	Yellow-white	—	Pale yellow
Czapek's agar	—	Poor	—	Yellow-white	—	Yellow-white
Nutrient agar	Good	Good	Yellow-white	Yellow-white	Ivory-olive-brown	Pale yellow
Potato extract agar	Moderate	Moderate	—	Yellow-white	Pale yellow	Ivory-olive-brown

98.9 and 96.9 %, respectively. The similarity between the two novel isolates was 98.7 %.

To establish the precise taxonomic positions of the novel isolates, DNA–DNA hybridizations were performed. The levels of DNA–DNA relatedness for *S. salina* YIM 90002^T and *S. alba* YIM 90003^T with strain YIM 91353^T were 47.3 and 45.7 %, respectively, and with YIM 91394^T were 46.8 and 25.1 %, respectively. The DNA–DNA relatedness value for strains YIM 91353^T and YIM 91394^T was 37.4 %. These values were significantly lower than the threshold of 70 % for the delineation of genomic species (Wayne *et al.*, 1987). It is therefore evident, based on the phylogenetic analysis and DNA–DNA hybridization data, that strains YIM 91353^T and YIM 91394^T represent two novel species of the genus *Streptomonospora*.

Amino acids and sugars of whole-cell hydrolysates were analysed on the basis of the methods established by Lechevalier & Lechevalier (1980) and Stanek & Roberts (1974), respectively. Polar lipids were extracted, examined by using two-dimensional TLC and identified using described procedures (Minnikin *et al.*, 1979). Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). Fatty acids were determined for the isolates as well as for the three reference strains, *S. salina* YIM 90002^T,

S. alba YIM 90003^T and *S. halophila* YIM 91355^T, as described by Sasser (1990) using the Microbial Identification System (MIDI) with cells grown in trypticase soy broth (BBL) supplemented with 10 % (w/v) NaCl (pH 7.0) in flasks on a rotary shaker (200 r.p.m.) at 37 °C for 3 days. Chemotaxonomic data for the novel isolates were consistent with their assignment to the genus *Streptomonospora* (Cui *et al.*, 2001; Li *et al.*, 2003; Cai *et al.*, 2008a). The cell-wall diamino acid in the peptidoglycan layer of both strains was *meso*-diaminopimelic acid and the whole-cell sugars were galactose and glucose with a trace of mannose. The phospholipids of the two isolates consisted of diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. The predominant menaquinones were MK-10(H₈) (35.8 %), MK-10(H₆) (16.4 %) and MK-9(H₈) (15.3 %) for strain YIM 91353^T, and MK-10(H₈) (41.5 %), MK-9(H₈) (27.7 %) and MK-10(H₆) (12.6 %) for strain YIM 91394^T. The fatty acid profiles of the novel isolates were similar to those of the type strains of species of the genus *Streptomonospora* (Supplementary Table S1, in IJSEM Online).

The results of the 16S rRNA gene sequence analysis clearly demonstrated that strains YIM 91353^T and YIM 91394^T belong to the genus *Streptomonospora*. Both organisms

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence analysis, reconstructed from evolutionary distances with the neighbour-joining method, showing the phylogenetic positions of strains YIM 91353^T and YIM 91394^T and related taxa. Bootstrap values based on 1000 replicates are shown at branch nodes. *Streptosporangium album* DSM 43023^T was used as the outgroup. Bar, 1 substitution per 100 nucleotide positions.

contained MK-10(H₈) and MK-10(H₆) as the predominant menaquinones and *meso*-diaminopimelic acid as the diagnostic amino acid in the peptidoglycan and, together with their similar fatty acid profiles, these properties also supported the inclusion of strains YIM 91353^T and YIM 91394^T within the genus *Streptomonospora*. However, the two novel isolates could be distinguished from each other and from recognized *Streptomonospora* species with a range of phenotypic and chemotaxonomic differences (Tables 1 and 2 and Supplementary Table S1). On the basis of the results of the phylogenetic analysis, the DNA–DNA hybridization data and the phenotypic and chemotaxonomic properties presented above, we propose that strains YIM 91353^T and YIM 91394^T represent two novel species of the genus *Streptomonospora*, *Streptomonospora amylolytica* sp. nov. and *Streptomonospora flavalba* sp. nov., respectively.

Description of *Streptomonospora amylolytica* sp. nov.

Streptomonospora amylolytica (a.my.lo.ly.ti'ca. Gr. n. *amylōs* starch; Gr. adj. *lutikos* dissolving; N.L. fem. adj. *amylolytica* producing lysis of starch).

Gram-positive, aerobic, catalase-positive, oxidase-negative. Grows well on oatmeal agar and nutrient agar media supplemented with 10 % (w/v) NaCl, but not on Czapek's agar or glycerol/asparagine agar supplemented with 10 % (w/v) NaCl. Diffusible pigments are not produced on all media tested. Aerial and substrate mycelia are not fragmented. White to yellow–white aerial mycelia form spore chains at maturity, with non-motile, oval to cylindrical spores (0.3–0.4 × 0.4–0.6 µm). Single, oval spores are borne on sporophores of substrate mycelium. Substrate mycelia are yellow–grey on inorganic salt–starch agar, yeast extract–malt extract agar and oatmeal agar, pale yellow on potato extract agar and ivory–olive–brown on nutrient agar. Growth occurs with 5–20 % (w/v) NaCl (optimum, 10 %), at 20–45 °C (optimum, 37 °C) and at pH 5.0–9.0 (optimum, pH 7.0). Utilizes arabinose, cellobiose, glucose, rhamnose and sorbitol as sole carbon and energy sources, and arginine, hydroxyproline, lysine, proline, threonine and tryptophan as sole nitrogen, carbon and energy sources. Positive for hydrolysis of starch, H₂S production and milk coagulation and peptonization, but negative for gelatin liquefaction, hydrolysis of cellulose and urea, melanin production and nitrate reduction. The diagnostic amino acid of the cell-wall peptidoglycan is *meso*-diaminopimelic acid, and galactose is the major diagnostic sugar in whole-cell hydrolysates. The predominant menaquinones are MK-10(H₈), MK-10(H₆) and MK-9(H₈). Polar lipids consist of diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. Major fatty acids are anteiso-C_{17:0} and C_{18:0}. Other phenotypic properties are given in Table 1. The DNA G + C content of the type strain is 71.2 mol% (HPLC).

The type strain, YIM 91353^T (=DSM 45171^T=CCTCC AA 208048^T), was isolated from saline soil collected from a salt lake in Xinjiang Province, north-west China.

Description of *Streptomonospora flavalba* sp. nov.

Streptomonospora flavalba (fla.val'ba. L. adj. *flavus* yellow; L. adj. *albus* white; N.L. fem. adj. *flavalba* yellow–white, referring to the colour of aerial mycelium).

Gram-positive, aerobic, catalase-positive, oxidase-negative. Grows well on yeast extract–malt extract agar and nutrient agar media supplemented with 10 % (w/v) NaCl, but grows poorly on Czapek's agar or glycerol/asparagine agar supplemented with 10 % (w/v) NaCl. Diffusible pigments are not produced on most media tested, except for potato extract agar (pH 7.0) supplemented with 10 % (w/v) NaCl, on which deep yellow diffusible pigments are produced. Aerial and substrate mycelia are not fragmented. Yellow–white aerial mycelia form spore chains at maturity, with non-motile, oval to cylindrical spores (0.5–0.6 × 0.7–0.9 µm). Single, oval spores are borne on sporophores of substrate mycelium. Substrate mycelia are pale yellow on inorganic salt–starch agar, oatmeal agar, yeast extract–malt extract agar, glycerol/asparagine agar and nutrient agar and yellow–white on Czapek's agar and ivory–olive–brown on potato extract agar. Growth occurs with 5–25 % (w/v) NaCl (optimum, 10 %), at 20–45 °C (optimum, 37 °C) and pH 5.0–9.0 (optimum, pH 7.0). Utilizes arabinose, glucose, maltose and rhamnose as sole carbon and energy sources, and alanine, arginine, glycine, histidine, phenylalanine, proline, serine, threonine, tryptophan and tyrosine as sole nitrogen, carbon and energy sources. Positive for hydrolysis of starch, melanin production and milk coagulation and peptonization, but negative for gelatin liquefaction, hydrolysis of cellulose and urea, H₂S production and nitrate reduction. The diagnostic amino acid of the cell-wall peptidoglycan is *meso*-diaminopimelic acid, and galactose is the major diagnostic sugar in whole-cell hydrolysates. The predominant menaquinones are MK-10(H₈), MK-9(H₈) and MK-10(H₆). Polar lipids consist of diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. Major fatty acids are anteiso-C_{17:0} and iso-C_{16:0}. Other phenotypic properties are given in Table 1. The DNA G + C content of the type strain is 72.5 mol% (HPLC).

The type strain, YIM 91394^T (=DSM 45155^T=CCTCC AA 208047^T), was isolated from saline soil collected from a salt lake in Xinjiang Province, north-west China.

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