

Saccharopolyspora halophila sp. nov., a novel halophilic actinomycete isolated from a saline lake in China

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A halophilic actinomycete strain, designated YIM 90500^T, was isolated from a saline lake in Xinjiang province, north-west China and subjected to a polyphasic taxonomic study. Good growth of the novel isolate occurred at 28–37 °C, pH 7–8 and with 10–15% (w/v) NaCl; no growth occurred without any salts. Strain YIM 90500^T contained *meso*-diaminopimelic acid with glucose, arabinose and ribose as the whole-cell sugars. The major phospholipids were phosphatidylcholine and diphosphatidylglycerol. MK-9(H₄) was the predominant menaquinone. The major fatty acids were iso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. The chemotaxonomic data together with the morphological properties of strain YIM 90500^T consistently assigned the strain as belonging to the genus *Saccharopolyspora*. Phylogenetic analysis based on 16S rRNA gene sequences further revealed that strain YIM 90500^T formed a distinct phyletic lineage in the genus *Saccharopolyspora* and showed low 16S rRNA gene similarities (<96.4%) with other species of the genus. On the basis of the evidence from the polyphasic study, a novel species, *Saccharopolyspora halophila* sp. nov., is proposed. The type strain is YIM 90500^T (=DSM 45007^T=KCTC 19162^T).

The genus *Saccharopolyspora* was first described by Lacey & Goodfellow (1975) and it currently comprises 13 species with validly published names: *S. hirsuta* (Lacey & Goodfellow, 1975), *S. erythraea* (Labeda, 1987), *S. taberi* (Labeda, 1987; Korn-Wendisch *et al.*, 1989), *S. gregorii* (Goodfellow *et al.*, 1989), *S. hordei* (Goodfellow *et al.*, 1989), *S. rectivirgula* (Korn-Wendisch *et al.*, 1989), *S. spinosa* (Mertz & Yao, 1990), *S. spinosporotrichia* (Zhou *et al.*, 1998), *S. flava* (Lu *et al.*, 2001), *S. thermophila* (Lu *et al.*, 2001), *S. antimicrobica* (Yuan *et al.*, 2008), *S. cebuensis* (Pimentel-Elardo *et al.*, 2008) and *S. shandongensis* (Zhang *et al.*, 2008). All the above-mentioned species of the genus *Saccharopolyspora* are non-halophilic actinomycetes.

Strain YIM 90500^T was isolated from a hypersaline sample collected from a saline lake in Xinjiang, after 3 weeks incubation at 37 °C on cellulose-casein multi-salt (CCMS) medium. The composition of the CCMS medium was (l⁻¹ distilled water): 10 g microcrystalline cellulose (MCC),

0.3 g casein, 0.2 g KNO₃, 0.5 g K₂HPO₄, 0.02 g CaCO₃, 0.01 g FeSO₄, 150 g NaCl, 30 g MgCl₂·6H₂O, 20 g KCl and 15 g agar. Multi-salts, including NaCl, KCl and MgCl₂·6H₂O, was sterilized separately before being added to the medium. The pH of the medium was adjusted to pH 7.5 with 1 M NaOH. The strain was maintained on ISP medium 4 (Shirling & Gottlieb, 1966) agar slants containing 10% (w/v) NaCl at 4 °C and as suspensions of mycelium fragments in glycerol (20%, v/v). Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (about 150 r.p.m.) using ISP medium 4 [10% (w/v) NaCl, pH 7.5] at 37 °C for 2 weeks.

Morphological characteristics of strain YIM 90500^T were observed by light microscopy (model BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 21 days growth on ISP 4 agar medium containing 10% (w/v) NaCl. Cultural characteristics were determined after 3–4 weeks according to the methods used in the *International Streptomyces Project* (ISP) (Shirling & Gottlieb, 1966). All media were supplemented with 10% (w/v) NaCl for growth. The colours of substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the ISCC-NBS

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 90500^T is DQ923129.

A scanning electron micrograph of spore chains of strain YIM 90500^T is available as supplementary material with the online version of this paper.

colour charts (Kelly, 1964). Media and procedures used to determine physiological features and carbon source utilization were those described by Williams *et al.* (1989). Acid production from carbohydrates was assessed using media and methods described by Williams *et al.* (1983). The growth temperature was tested at 4, 10, 20, 28, 37, 45, 55 and 65 °C on ISP medium 4 containing 10% (w/v) NaCl. For NaCl tolerance experiments, ISP medium 4 was used as the basal medium. The following NaCl concentrations (w/v) were used: 0, 1, 3, 5, 10, 15, 20, 25 and 30%. The pH range for growth was investigated between 4.0–10.0 at intervals of 1 pH unit, using the buffer system: pH 4.0–5.0: 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0: 0.1 M KH₂PO₄/0.1 M NaOH; pH 9.0–10.0: 0.1 M NaHCO₃/0.1 M Na₂CO₃.

Strain YIM 90500^T developed well on most media; the detailed results are given in the species description. Substrate mycelia were long and well developed. Aerial mycelia were well developed and long spore chains were borne on the aerial mycelium. All spores were non-motile, smooth-surfaced, oval or spherical and 0.6–0.7 × 0.6–1.1 µm in size (Supplementary Fig. S1, available in IJSEM Online). Good growth occurred at 28–37 °C and at pH 7–8 with 10–15% (w/v) NaCl. The detailed physiological and biochemical characteristics of the strain are given in Table 1 and in the species description.

Isomers of diaminopimelic acid and whole-cell sugars were analysed according to the procedures developed by Hasegawa *et al.* (1983). Polar lipids were extracted and examined by using two-dimensional TLC and identified

using previously described procedures (Minnikin *et al.*, 1984). Menaquinones were isolated according to Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). Cellular fatty acids analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI). Strain YIM 90500^T contained *meso*-diaminopimelic acid as the cell wall diamino acid, with glucose, arabinose and ribose as the major whole-cell sugars. The major cellular fatty acids were iso-C_{15:0} (20.8%), iso-C_{16:0} (19.1%) and anteiso-C_{17:0} (21.0%); minor fatty acids iso-C_{14:0} (1.2%), anteiso-C_{15:0} (6.2%), C_{16:1}ω7*c*/iso-C_{15:0} 2-OH (3.4%), C_{16:0} (3.5%), 10-methyl C_{16:0} (6.3%), iso-C_{17:0} (7.0%), C_{17:1}ω8*c* (2.3%), C_{17:0} (1.2%), 10-methyl C_{17:0} (1.8%) and C_{18:1}ω9*c* (1.3%) were also detected. Detailed data for phospholipids and menaquinones are given in the species description.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li *et al.* (2007). Multiple alignments with sequences of the most closely related *Saccharopolyspora* species and calculations of levels of sequence similarity were carried out using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analyses were performed using three tree-making algorithms, neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971). A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) from *K*_{nuc} values (Kimura, 1980) using MEGA version 2.1 (Kumar *et al.*, 2001). The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method of

Table 1. Differential phenotypic and chemotaxonomic characteristics between strain YIM 90500^T and its closest neighbours of the genus *Saccharopolyspora*

Strains: 1, YIM 90500^T (*S. halophila* sp. nov.); 2, *S. flava* AS4.1520^T; 3, *S. thermophila* AS4.1511^T; 4, *S. spinosa* DSM 44228^T. Data for organisms other than strain YIM 90500^T are from Mertz & Yao (1990) and Lu *et al.* (2001). +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4
Fragments of substrate mycelium	–	+	+	+
Colour of*:				
Aerial mycelia	WY	W	W	YP
Substrate mycelia	Y-OY	Y	C-Bf	Y-YBr
Soluble pigment	–	–	–	+
Degradation of:				
Adenine	–	+	+	–
Casein	+	–	–	+
Starch	–	+	+	–
Tyrosine	+	–	+	+
Reduction of nitrate	+	+	–	+
NaCl range for growth (% w/v)	3–20	0–7	0–7	0–11
Temperature range for growth (°C)	10–45	28–37	45–55	15–37
Menaquinones	MK-9(H ₂), MK-9(H ₄), MK-9(H ₆)	MK-9(H ₄)	MK-9(H ₆), MK-9(H ₈)	MK-9(H ₄), MK-9(H ₆)
Polar lipids†	DPG, PC, PI	PC	ND	DPG, PC
DNA G + C content (mol%)	66.3	67	73.1	ND

*Bf, buff; Br, brown; C, colourless; O, orange; P, pink; W, white; Y, yellow.

†DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol.

Felsenstein (1985) with 1000 replicates. For the determination of the G + C content, the genomic DNA of strain YIM 90500^T was prepared according to the method of Marmur (1961). The G + C content of the DNA was determined by using reversed-phase HPLC of nucleosides, according to Mesbah *et al.* (1989).

The results of the 16S rRNA gene sequence comparison clearly demonstrated that strain YIM 90500^T is a member of the genus *Saccharopolyspora*. In the phylogenetic tree based on the neighbour-joining algorithm, strain YIM 90500^T formed a distinct subclade with *S. flava*, and both shared the same branch with a high bootstrap value of 97 % (Fig. 1). Topologies of phylogenetic trees constructed using the maximum-likelihood and maximum-parsimony algorithms were similar to those of the tree constructed by using neighbour-joining analysis (data not shown). The 16S rRNA gene sequence similarities between strain YIM 90500^T and its closest neighbours, *S. spinosa* and *S. flava*, were 96.3 and 96.2 %, respectively. The G + C content of the DNA was 66.3 mol%.

On the basis of the differential phenotypic and chemotaxonomic characteristics (Table 1) and the phylogenetic data of strain YIM 90500^T and its closest neighbours in the genus *Saccharopolyspora*, strain YIM 90500^T merits recognition as representing a novel species within the genus, for which we propose the name *Saccharopolyspora halophila* sp. nov.

Description of *Saccharopolyspora halophila* sp. nov.

Saccharopolyspora 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).

Aerobic, Gram-positive, moderately halophilic filamentous actinomycete. Substrate mycelia are well-developed and no fragments are observed. Aerial mycelia form long chains of

spores; spores are non-motile, smooth-surfaced, oval or spherical and $0.6\text{--}0.7 \times 0.6\text{--}1.1\text{ }\mu\text{m}$ in size. Good growth occurs on Czapek agar, yeast extract-malt extract, potato agar, glycerol/asparagine agar and inorganic salts-starch agar. Moderate growth occurs on nutrient agar and oatmeal agar. Aerial mycelia are white-yellow in colour and substrate mycelia are yellow to moderate orange-yellow. No diffusible pigments are produced. Casein and tyrosine are degraded, but adenine, cellulose and chitin are not. Tests for gelatin liquefaction, nitrate reduction and milk peptonization and coagulation are positive and tests for H₂S and melanin production and starch hydrolysis are negative. L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, D-lactose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, sucrose, sodium acetate, D-xylitol and D-xylose are utilized as carbon sources, whereas D-sorbitol and trehalose are not. Acid production occurs on L-arabinose, D-glucose, *myo*-inositol, L-rhamnose and D-xylose. Growth occurs on alanine, asparagine, arginine, cystine, glycine, histidine, homocysteine, hypoxanthine, lysine, praline, threonine, tyrosine, valine and urea as nitrogen sources, but not on adenine, hydroxyproline or glutamate. Major phospholipids are phosphatidylcholine and diphosphatidylglycerol; minor amounts of phosphatidylinositol are also detected. Menaquinones are MK-9(H₂), MK-9(H₄) and MK-9(H₆) (ratio of peak areas, 13.1:82.9:3.9). Major cellular fatty acids are iso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}; minor fatty acids iso-C_{14:0}, anteiso-C_{15:0}, C_{16:1} ω 7c/iso-C_{15:0} 2-OH, C_{16:0}, 10-methyl C_{16:0}, iso-C_{17:0}, C_{17:1} ω 8c, C_{17:0}, 10-methyl C_{17:0} and C_{18:1} ω 9c are also detected. Temperature, pH and NaCl tolerance ranges are 10–45 °C, pH 6–8.5 and 3–20 % (w/v), respectively. Good growth occurs at 28–37 °C and pH 7–8 and with 10–15 % (w/v) NaCl. The G + C content of the DNA of the type strain is 66.3 mol%.

The type strain, YIM 90500^T (=DSM 45007^T=KCTC 19162^T), was isolated from a saline lake in Xinjiang, north-west China.

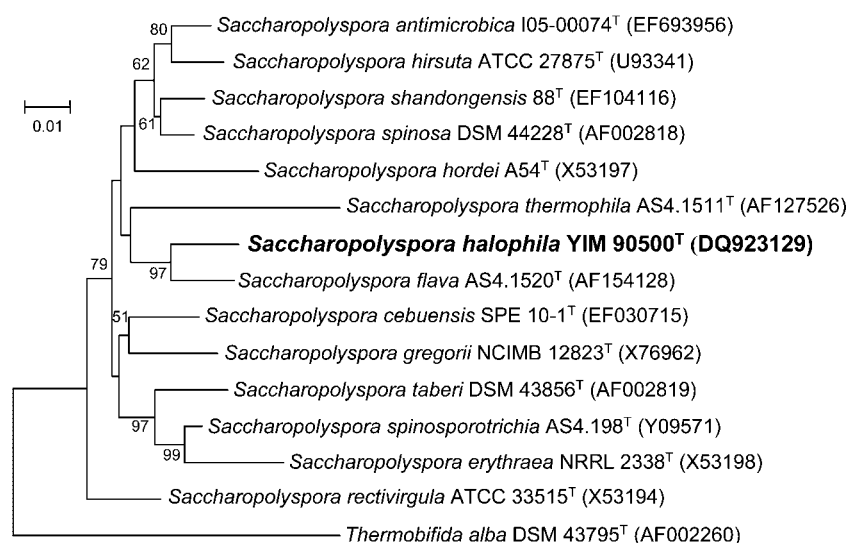


Fig. 1. Phylogenetic dendrogram obtained by using distance matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 90500^T and related phylogenetic neighbours. Numbers at branch nodes are bootstrap values (1000 resamplings; only values over 50 % are given). The sequence of *Thermobifida alba* DSM 43795^T (AF002260) was used as the outgroup. Bar, 1 % sequence divergence.

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