

Saccharomonospora paurometabolica sp. nov., a moderately halophilic actinomycete isolated from soil in China

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A novel, moderately halophilic actinomycete, strain YIM 90007^T, was isolated from a soil sample collected from the Xinjiang Province, China, and characterized. The optimum growth temperature of the strain was between 35 and 37 °C and growth occurred optimally in 10 % (w/v) NaCl. The cell wall of strain YIM 90007^T contained *meso*-diaminopimelic acid. Whole-cell sugars were galactose, arabinose and ribose. The principal menaquinone was MK-9(H₄), while MK-9(H₂) was found in smaller amounts. The phospholipids were phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and hydroxyphosphatidylethanolamine. The predominant cellular fatty acids were of the iso- and anteiso-branched and unbranched types; significant amounts of 2-hydroxy fatty acids were also found but 10-methyl-branched fatty acids were missing. The DNA G + C content of strain YIM 90007^T was 71 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed strain YIM 90007^T to be closely related to *Saccharomonospora halophila* (98.7 % similarity). DNA–DNA hybridization revealed a relatedness of 53.8 % between strain YIM 90007^T and *S. halophila* DSM 44411^T. Based on physiological and biochemical characteristics, phylogenetic analysis (based on 16S rRNA gene sequences) and DNA–DNA relatedness, it is concluded that strain YIM 90007^T represents a novel species of the genus *Saccharomonospora*, for which the name *Saccharomonospora paurometabolica* (type strain YIM 90007^T = CCTCC AA001018^T = CCRC 16315^T = DSM 44619^T) is proposed.

The genus *Saccharomonospora* (Nonomura & Ohara, 1971) was created for actinomycetes producing predominantly single spores on aerial hyphae. The cell wall contains *meso*-diaminopimelic acid together with the sugars arabinose and galactose. At the time of writing, the genus embraces six species, namely, *Saccharomonospora viridis* (Nonomura & Ohara, 1971), *Saccharomonospora azurea* (Hu, 1987), *Saccharomonospora glauca* (Greiner-Mai *et al.*, 1988), *Saccharomonospora cyanea* (Hu *et al.*, 1988), *Saccharomonospora xinjiangensis* (Jin *et al.*, 1998) and *Saccharomonospora halophila* (Al-Zarban *et al.*, 2002); only the latter species is halophilic. During a study on halophilic actinomycetes, a

halophilic strain, YIM 90007^T, was isolated from one soil sample from the Xinjiang Province, in the west of China.

Strain YIM 90007^T was isolated on modified glycerol/asparagine agar (Shirling & Gottlieb, 1966) [ISP5 medium containing 20 % (w/v) NaCl] and incubated at 28 °C for about 4 weeks. The strain was maintained on ISP2 and ISP5 agar slants containing 10 % NaCl at 4 °C and as glycerol suspensions (20 %, v/v) at –20 °C. Biomass for chemical and molecular systematic studies was obtained by growing in shake flasks (about 150 r.p.m.) of ISP2 medium broth containing 10 % NaCl at 28 °C for 1 week. Cultural characteristics were determined after 4 weeks at 28 °C by methods used in the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966). Morphological properties, spores and mycelia were examined by light microscopy (Olympus microscope BH-2) and scanning electron microscopy with a JEOL, model JSM5600LV. Media and procedures used for

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The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of *Saccharomonospora paurometabolica* YIM 90007^T is AF540959.

Table 1. Characteristics that differentiate strain YIM 90007^T from other *Saccharomonospora* species

Species: 1, strain YIM 90007^T; 2, *S. halophila* DSM 44411^T (Al-Zarban *et al.*, 2002); 3, *S. azurea* JCM 7551^T (Embley, 1992); 4, *S. cyanea* JCM 7552^T (Embley, 1992); 5, *S. glauca* JCM 7444^T (Embley, 1992); 6, *S. viridis* JCM 3036^T (Embley, 1992); 7, *S. xinjiangensis* CCTCC AA97021^T (Jin *et al.*, 1998). +, Property present; –, property absent; D, presence of property doubtful; ND, not determined.

Characteristics	1	2	3	4	5	6	7
Aerial mycelium colour	White	Light-blue to greenish	Azure	Dark-blue	Light- to bluish-green	Green	Yellow-white
Spore ornamentation	Smooth or wrinkled	Warty	Smooth	Warty	Warty	Warty	Smooth
Growth on sole carbon source (1 %, w/v):							
L-Arabinose	–	+	–	ND	+	–	ND
Galactose	–	+	–	+	ND	–	ND
Glucose	–	+	+	–	+	D	ND
Mannitol	–	+	–	–	+	D	ND
Melibiose	–	+	+	–	ND	ND	ND
Rhamnose	–	+	–	ND	–	–	+
Ribose	–	D	+	+	ND	ND	ND
Xylose	–	–	+	D	+	–	+
Growth in the presence of NaCl (%):							
0	–	–	+	+	+	+	+
5	+	–	+	–	–	–	–
7	+	–	+	–	–	–	–
10	+	+	–	–	–	–	–
20	+	+	–	–	–	–	–
30	–	+	–	–	–	–	–
Optimum growth temperature (°C)	35–37	28–30	28–30	28–30	45–50	45–50	37

determination of physiological features and carbon source utilization were those described by Shirling & Gottlieb (1966) and Williams *et al.* (1989). Some results are indicated in comparison to those of other *Saccharomonospora* species (Table 1). Colour determination was done with colour chips from the ISCC–NBS Color-Name Charts Standard Samples no. 2106 (Kelly, 1964). Strain YIM 90007^T developed well on most media tested (see species description). No diffusible pigments were produced.

Morphological features were observed on glycerol/asparagine agar (ISP5 medium) and yeast extract/malt extract (ISP2 medium) [10 % (w/v) NaCl]. Incubation time was for 4 weeks at 28 °C. Strain YIM 90007^T had the typical characteristics of the genus *Saccharomonospora*. Aerial mycelium and substrate mycelium were well developed (Fig. 1). Single spores were borne on aerial mycelium, while some single spores were sporadically borne on the substrate mycelium. Spores were smooth or wrinkled.

The amino acid and sugar analysis of whole-cell hydrolysates followed procedures described by Stanek & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1984). Menaquinones were isolated using the methods of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt *et al.*, 1981; Kroppenstedt, 1982). Cellular fatty acid composition was determined as described by Sasser (1990). Cell walls of strain YIM 90007^T

contained *meso*-diaminopimelic acid. Whole-cell hydrolysates contained mainly galactose, arabinose and ribose. The predominant menaquinones were MK-9(H₄) (90 %) and MK-9(H₂) (10 %). The polar lipid extract contained phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and hydroxy-phosphatidylethanolamine. Cellular fatty acids are indicated in the species description.

Extraction of genomic DNA and amplification of 16S rDNA were done as described by Cui *et al.* (2001). Multiple alignments with sequences of a broad selection of

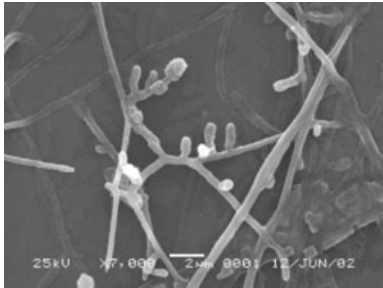


Fig. 1. Scanning electron micrograph of the spore chains of *Saccharomonospora paurometabolica* YIM 90007^T grown on yeast extract/malt extract agar (ISP2 medium) containing 10 % (w/v) NaCl for 28 days at 28 °C. Bar, 2 μm.

Actinobacteria and calculations of levels of sequence similarity were carried out using CLUSTAL W 1.8 (Thompson *et al.*, 1994). A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980, 1983). The topology of the phylogenetic tree (Fig. 2) was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The almost-complete 16S rDNA sequence of strain YIM 90007^T was 1474 bp long. The highest similarity values were found with sequences of members of the genus *Saccharomonospora*: except for *S. halophila* DSM 44411^T, displaying 98.7 % similarity, none of the other type strains shared higher than 97.0 % sequence similarity with strain YIM 90007^T.

DNA for renaturation studies and determination of the base content of strain YIM 90007^T was prepared following the method of Marmur (1961). The G + C content was determined using the thermal denaturation method of Marmur & Doty (1962). DNA–DNA hybridization was carried out according to described methods (De Ley *et al.*, 1970; Huss *et al.*, 1983; Jahnke, 1992). The G + C contents of the genomic DNA from strain YIM 90007^T and *S. halophila* DSM 44411^T were 71 and 70 mol%, respectively. DNA–DNA reassociation similarity between strain YIM 90007^T and *S. halophila* DSM 44411^T indicated a moderate value of 53.8 % (repeated).

Based on phylogenetic, morphological and chemical data, strain YIM 90007^T should be considered a member of the genus *Saccharomonospora*. It is only moderately related to *S. halophila* DSM 44411^T, as measured by DNA–DNA relatedness, and shows differences in chemotaxonomic properties, such as the presence of MK-8(H₄) and the different fatty acid patterns in strain DSM 44411^T (Al-Zarban *et al.*, 2002). It also shows sufficient physiological and cultural differences to other *Saccharomonospora*

species to justify the description of a new species, *Saccharomonospora paurometabolica* (type strain YIM 90007^T).

Description of *Saccharomonospora paurometabolica* sp. nov.

Saccharomonospora paurometabolica (pau.ro.me.ta.bo'li.ca. Gr. adj. *pauros* little; Gr. adj. *metabolikos* changeable; N.L. fem. adj. *paurometabolica* little changeable, referring to the poor utilization of carbon sources).

Aerial mycelium is well developed on yeast extract/malt extract agar (ISP2 medium), glycerol/asparagine agar (ISP5 medium), nutrient agar and Czapek's agar; moderate on oatmeal agar (ISP3 medium) and poor on inorganic salt/starch agar (ISP4 medium) and potato agar. White aerial mycelium on all media, except for a green-yellow mycelium on nutrient agar. Sporulation is good on ISP2, ISP5, nutrient agar and Czapek's agar media, moderate on ISP3 medium and poor on ISP4 medium. Substrate mycelium is well developed on most test media. Colour is deep orange-yellow (ISP2), light yellow-brown (nutrient agar), light yellow-orange (potato agar) or white (ISP4, ISP5 and Czapek's agar). Non-motile single spores with smooth or wrinkled surface, borne on aerial mycelium; some single spores borne on substrate mycelium. Optimum growth temperature is between 35 and 37 °C. Optimum growth concentration of NaCl is 10 % (w/v). Positive only for nitrate reduction. Negative for milk peptonization and coagulation, gelatin liquefaction, growth in cellulose, H₂S and melanin production, starch hydrolysis and urease production. The range of carbon utilization could not be determined because of negative reactions caused by extremely poor growth in basal media. The cell wall contains *meso*-diaminopimelic acid. Whole-cell hydrolysates contain mainly galactose, arabinose and ribose. The only menaquinones are MK-9(H₂) (10 %) and MK-9(H₄) (90 %), and the phospholipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and hydroxyphosphatidylethanolamine. Predominant cellular fatty acids are C_{16:0} (20.7 %), iso-C_{16:0} (11.2 %) and C_{18:1} (44.3 %); smaller amounts (>1 %) are iso-C_{15:0} (1.0 %), iso-C_{16:1} (1.1 %), iso-C_{17:0} (1.2 %), iso-C_{17:1} (1.4 %), anteiso-C_{17:0} (3.1 %), C_{16:1} (4.4 %), C_{18:0} (3.0 %), 2-hydroxy-anteiso-C_{15:0} (4.8 %) and 2-hydroxy-iso-C_{16:0} (1.7 %).

Type strain is YIM 90007^T (= CCTCC AA001018^T = CCRC 16315^T = DSM 44619^T). Its DNA G + C content is 71 mol%. Isolated from saline soil collected from the Xinjiang Province, in the west of China.

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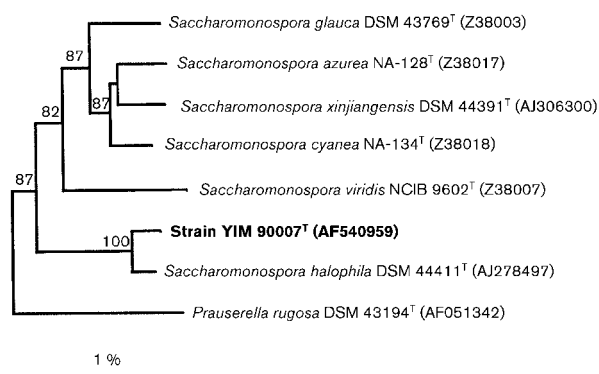


Fig. 2. Phylogenetic dendrogram, obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strain YIM 90007^T among its phylogenetic neighbours. Numbers at the branch nodes are bootstrap values, expressed as a percentage of 1000 replicates. Sequences of *Amycolatopsis* type strains were used as the root. Bar, 1 % sequence divergence.

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