Saccharomonospora saliphila sp. nov., a halophilic actinomycete from an Indian soil

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A Gram-positive, moderately halophilic actinomycete strain, designated YIM 90502^T, was isolated from a sample of muddy soil collected from Gulbarga, Karnataka Province, India, and subjected to a polyphasic taxonomical analysis. The isolate grew optimally at 28 °C and in the presence of 10 % (w/v) NaCl. The isolate was characterized chemotaxonomically as having *meso*-diaminopimelic acid in the cell-wall peptidoglycan and galactose and arabinose as whole-cell sugars. The predominant menaquinone was MK-9(H₄), while MK-8(H₄) was found in smaller amounts. The phospholipids were phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine. The major fatty acids were iso-C_{16:0} (49.2 %) and C_{17:1ω6c} (9.1 %). The DNA G + C content of the isolate was 71.8 mol%. A phylogenetic tree based on 16S rRNA gene sequences showed that the isolate fell within the evolutionary radiation encompassed by the genus *Saccharomonospora*. 16S rRNA gene sequence similarity between strain YIM 90502^T and the type strains of *Saccharomonospora* species ranged from 92.42 % (with *Saccharomonospora xinjiangensis* CCTCC AA 97021^T) to 97.45 % (with *Saccharomonospora azurea* KCTC 9693^T). Levels of DNA–DNA relatedness between strain YIM 90502^T and *S. azurea* KCTC 9693^T, *Saccharomonospora halophila* DSM 44411^T and *Saccharomonospora paurometabolica* DSM 44619^T were 46.0, 41.0 and 42.5 %, respectively. On the basis of phenotypic, phylogenetic and genotypic data, strain YIM 90502^T was classified in the genus *Saccharomonospora* as a member of a novel species, for which the name *Saccharomonospora saliphila* sp. nov. is proposed, with YIM 90502^T (=KCTC 19234^T =DSM 45087^T) as the type strain.

Nonomura & Ohara (1971) described the genus *Saccharomonospora* in the actinomycete family *Pseudonocardiaceae* for organisms that produce predominantly single spores, and occasionally spores in pairs and short chains, on aerial hyphae. Members of the genus contain *meso*-diaminopimelic acid together with the sugars arabinose and galactose. To date, seven species with validly published names are described in the genus: *Saccharomonospora viridis* Nonomura and 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, *Saccharomonospora halophila* Al-Zarban *et al.* 2002 and *Saccharomonospora paurometabolica* Li *et al.* 2003. Of these, only the last two are halophilic. During a study on halophilic actinomycetes, a halophilic strain YIM 90502^T was isolated from a sample of muddy soil from Gulbarga, Karnataka Province, in the southern part of India. This is the first report of a *Saccharomonospora* species from Indian soil.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 90502^T is DQ367416.

A scanning electron micrograph of spores of strain YIM 90502^T and an extended 16S rRNA gene sequence-based phylogenetic tree are available as supplementary material with the online version of this paper.

Strain YIM 90502^T was isolated by using modified glycerol/asparagine agar (Shirling & Gottlieb, 1966) [International *Streptomyces* Project medium 5 (ISP 5) containing 20 % (w/v) NaCl] and incubated at 28 °C for about 4 weeks. The strain was maintained on yeast extract/malt extract (ISP 2)

and ISP 5 agar slants containing 10 % NaCl at 4 °C and as glycerol suspensions (20 %, v/v) at –80 °C. Biomass for chemical and molecular systematic studies was obtained by growing in shake flasks (about 150 r.p.m.) of ISP 2 broth medium containing 10 % NaCl at 28 °C for 1 week. Cultural characteristics were determined after 4 weeks at 28 °C by methods used in the ISP (Shirling & Gottlieb, 1966), but all media were supplemented with 10 % NaCl (w/v). Morphological properties, spores and mycelia were examined by light microscopy (Olympus microscope BH-2) and scanning electron microscopy (JEOL model JSM5600LV). Media and procedures used for determination of physiological features and carbon source utilization were those described by Shirling & Gottlieb (1966) and Williams *et al.* (1989). Colour determination was done with colour chips from the ISCC–NBS Color Name Charts Standard Samples no. 2106 (Kelly, 1964). Strain YIM 90502^T developed well on most media tested. No diffusible pigments were produced. The results are indicated in detail in the species description and in Table 1.

Morphological features were observed on ISP 5 and ISP 2 medium [10 % (w/v) NaCl] incubated for 4 weeks at 28 °C. Strain YIM 90502^T had characteristics typical of the genus *Saccharomonospora*. Aerial mycelium and substrate mycelium were well developed. Smooth or wrinkled spores were produced on the branched aerial mycelium singly or

in pairs (Supplementary Fig. S1, available in IJSEM Online).

Amino acid and sugar analysis of whole-cell hydrolysates was performed according to the 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 extracted using the methods of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt *et al.*, 1981; Kroppenstedt, 1982). For fatty acid methyl ester analysis, cell mass was harvested from trypticase soy broth after incubation for 7 days at 28 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The cell wall of strain YIM 90502^T contained *meso*-diaminopimelic acid. Whole-cell hydrolysates contained mainly galactose and arabinose. The menaquinones were MK-9(H₄) (90 %) and MK-8(H₄) (10 %). The polar lipid extract contained phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine. Major cellular fatty acids are indicated in detail in the species description.

Extraction of genomic DNA and amplification of the 16S rRNA gene were done as described by Li *et al.* (2007). The almost-complete 16S rRNA gene sequence (1497 nt) of strain YIM 90502^T was obtained (positions 29–1542 in the

Table 1. Characteristics that differentiate strain YIM 90502^T from type strains of other *Saccharomonospora* species

Strains: 1, YIM 90502^T; 2, *S. halophila* DSM 44411^T; 3, *S. paurometabolica* DSM 44619^T; 4, *S. azurea* KCTC 9693^T; 5, *S. cyanea* NA-134^T; 6, *S. glauca* DSM 43769^T; 7, *S. viridis* NCIB 9602^T; 8, *S. xinjiangensis* DSM 44391^T. +, Present; –, absent; D, doubtful; ND, no data available. Data for reference strains were taken from Al-Zarban *et al.* (2002) and Li *et al.* (2003).

Characteristic	1	2	3	4	5	6	7	8
Aerial mycelium colour	Grey–red	Light blue–greenish	White	Azure	Light bluish green	Green	Green	Yellow–white
Spore ornamentation	Smooth or wrinkled	Warty	Smooth or wrinkled	Smooth	Warty	Warty	Warty	Smooth
Utilization of carbon sources (1 %, w/v)								
L-Arabinose	–	+	–	–	+	+	–	ND
Galactose	+	+	–	–	ND	ND	–	ND
Glucose	–	+	–	+	+	+	D	ND
Mannitol	+	+	–	–	+	+	D	+
Mannose	–	+	–	+	+	ND	–	+
Melibiose	+	+	–	+	–	ND	ND	ND
Rhamnose	+	+	–	–	–	–	–	+
Xylose	+	–	–	+	+	+	–	+
Growth in the presence of NaCl (%):								
0	+	–	–	+	+	+	+	+
5	+	–	+	+	–	–	–	–
7	+	–	+	+	–	–	–	–
10	+	+	+	–	–	–	–	–
20	+	+	+	–	–	–	–	–
30	–	+	–	–	–	–	–	–
Optimal growth temperature (°C)	28–30	28–30	35–37	28–30	28–30	45–50	45–50	28–40

Escherichia coli numbering) and BLAST search comparisons were made against the GenBank/EMBL/DDBJ databases. Phylogenetic analysis was performed using software packages PHYLIP (Felsenstein, 1993) and MEGA version 2.1 (Kumar *et al.*, 2001) following the multiple alignment of data using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analysis was performed by using different tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Evolutionary distances (Kimura's two-parameter model; Kimura, 1980, 1983) and clustering were calculated with the neighbour-joining method of Saitou & Nei (1987). The topology of the phylogenetic tree (Fig. 1) was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The highest similarity values were found with sequences of members of the genus *Saccharomonospora*: *S. azurea* KCTC 9693^T (97.45 %), *S. halophila* DSM 44411^T (97.41 %) and *S. paurometabolica* DSM 44619^T (97.27 %). A neighbour-joining tree showing the phylogenetic relationships between strain YIM 90502^T and other members of the family *Pseudonocardiaceae* is shown in Supplementary Fig. S2.

DNA for renaturation studies and determination of the base content of strain YIM 90502^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 methods described by He *et al.* (2005). The G+C content of the genomic DNA from strain YIM 90502^T was 71.8 mol%. DNA–DNA reassociation between strain YIM 90502^T and *S. azurea* KCTC 9693^T, *S. halophila* DSM 44411^T and *S. paurometabolica* DSM 44619^T indicated moderate values of 46.0, 41.0 and 42.5 % (mean values, all repeated three times), respectively.

The phylogenetic distinctiveness and DNA–DNA relatedness data were sufficient to categorize strain YIM 90502^T as

Fig. 1. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 90502^T among its phylogenetic neighbours. Numbers at branch nodes are bootstrap values, expressed as percentages of 1000 replicates. The sequence of *Prauserella halophila* YIM 90001^T was used as the root. Bar, 1 % sequence divergence. An extended tree is available as Supplementary Fig. S2 in IJSEM Online.

distinct from previously recognized *Saccharomonospora* species (Hu, 1987; Al-Zarban *et al.*, 2002; Li *et al.*, 2003) and it is supported by sufficient physiological and cultural differences from some phylogenetically related *Saccharomonospora* species (Table 1) to justify the description of a novel species, *Saccharomonospora saliphila* sp. nov.

Description of *Saccharomonospora saliphila* sp. nov.

Saccharomonospora saliphila (sa.li'phi.la. L. n. *sal*, *salis* salt; Gr. adj. *philos* loving; N.L. fem. adj. *saliphila* salt-loving).

Aerial mycelium is well developed on yeast extract/malt extract agar (ISP 2 medium), inorganic salts/starch agar (ISP 4 medium), glycerol/asparagine agar (ISP 5 medium), potato agar and Czapek's agar; no growth on oatmeal agar (ISP 3 medium) or nutrient agar. Greyish to reddish-grey aerial mycelium on all media tested. Sporulation is good on ISP 2, ISP 4, ISP 5, potato agar and Czapek's agar. Substrate mycelium is well developed on most test media. Colour is greyish red (Czapek's agar), dark red (ISP 2 and ISP 5) or blackish red (ISP 4 and potato agar). Non-motile single or paired spores with smooth or wrinkled surfaces are borne on aerial mycelium. Optimum growth temperature is 28 °C; grows well up to 40 °C. Optimum growth is observed at 10 % (w/v) NaCl. Positive for H₂S production. Negative for milk peptonization and coagulation, gelatin liquefaction, growth in cellulose, melanin production, starch hydrolysis and urease production. The following carbon sources are utilized: cellobiose, D-galactose, D-glucose, D-xylose, fructose, maltose, raffinose, sorbitol and sucrose. L-Arabinose, *myo*-inositol, lactose, mannitol, rhamnose, trehalose and xylitol are not utilized. The cell wall contains *meso*-diaminopimelic acid. Whole-cell hydrolysates contain mainly galactose and arabinose. The menaquinones are MK-9(H₄) (90 %) and MK-8(H₄) (10 %) and the phospholipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine. Major cellular fatty acids are iso-C_{16:0} (49.2 %), C_{17:1ω6c} (9.1 %), C_{15:0} (5.42 %) and iso-C_{16:1} OH (5.02 %). Fatty acids present in smaller amounts (>1 %) are iso-C_{15:0} (1.45 %), C_{16:0} (3.61 %), 10-methyl-C_{16:0} (2.51 %), iso-C_{17:0} (3.72 %), C_{17:0} (3.67 %) and iso-C_{18:0} (1.44 %). The DNA G+C content of the type strain is 71.8 mol%.

The type strain is YIM 90502^T (=KCTC 19234^T =DSM 45087^T), isolated from soil collected from Gulbarga, Karnataka Province, southern India.

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