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NOTE

Saccharomonospora halophila sp. nov., a novel halophilic actinomycete isolated from marsh soil in Kuwait

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An actinomycete, strain 8^T, was isolated from marsh soil in Kuwait. The strain was aerobic, Gram-positive, halophilic and produced light blue to greyish aerial mycelium. The warty spores were sessile, occurring singly or in pairs on aerial mycelium. The mycelium was stable and did not fragment during ageing. Chemotaxonomic markers of the isolate were consistent with its classification as *Saccharomonospora*. The strain possessed *meso*-diaminopimelic acid as the diagnostic amino acid in the peptidoglycan. The diagnostic sugars were arabinose and galactose; polar lipids were phosphatidyl inositol, phosphatidyl ethanolamine, hydroxy-phosphatidyl ethanolamine, lyso-phosphatidyl ethanolamine and diphosphatidyl glycerol; the principal menaquinone was MK-9(H_4); and the iso/anteiso-branched fatty acid pattern was combined with 10-methyl-branched and 2-hydroxy-branched fatty acids. *Saccharomonospora cyanea* DSM 44106^T was the closest phylogenetic neighbour of strain 8^T, showing 96·8% 16S rDNA sequence similarity. These data, together with distinct physiological traits, led to the conclusion that the novel isolate represents a new species within the genus *Saccharomonospora* for which the name *Saccharomonospora halophila* sp. nov. is proposed. The type strain is strain 8^T (= DSM 44411^T = NRRL B-24125^T).

Keywords: *Saccharomonospora halophila* sp. nov., polyphasic taxonomy

Strains of thermophilic *Saccharomonospora* species are usually isolated from natural high-temperature habitats such as leaf litter, manure and compost (Embley, 1992; Greiner-Mai *et al.*, 1988), whereas mesophilic species are frequently found in soil samples (Hu, 1987; Hu *et al.*, 1988; Jin *et al.*, 1998). Until now, there have been no reports on the isolation of a halophilic *Saccharomonospora* strain. The aim of this study was to clarify the taxonomic position of a novel halophilic *Saccharomonospora* isolate by morphological, physiological and chemotaxonomic means and to elucidate its phylogenetic position by comparing the 16S rDNA sequences of strain 8^T with members of the class *Actinobacteria*.

Strain 8^T was isolated from marsh soil in Kuwait and was deposited as *Saccharomonospora halophila* DSM 44411^T in the DSMZ database. Determination of morphological traits, colour of the aerial and substrate mycelium as well as that of soluble pigments were performed as described previously (Shirling & Gottlieb, 1966). Biochemical tests were done as described by Lechevalier *et al.* (1986). Strain 8^T showed the typical macroscopic and microscopic appearance of *Saccharomonospora* with light blue, branched aerial mycelium that does not fragment during ageing. Sessile warty spores were produced on the branched aerial mycelium singly or in pairs (Fig. 1). Optimum growth and sporulation was obtained on starch-nitrate agar supplemented with 10% NaCl. The medium was prepared by adding the following components to 1 l deionized water: starch, 20·0 g; K_2HPO_4, 1·0 g; KNO_3, 2·0 g; $MgSO_4$, 0·5 g; $CaCO_3$, 3·0 g; NaCl, 100·0 g; and 1 ml trace element solution. Trace element solution was composed of: $FeSO_4.7H_2O$, 0·1 g l^{-1}, $MnCl_2.4H_2O$, 0·1 g l^{-1}; and $ZnSO_4.7H_2O$, 0·1 g l^{-1}.

Abbreviation: FAMEs; fatty acid methyl esters.

The EMBL accession number for the 16S rDNA sequence of strain 8^T is AJ278497.

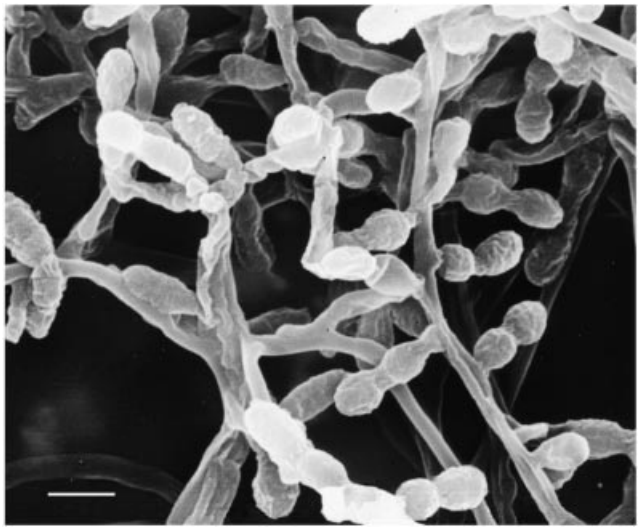

Fig. 1. SEM of spores of strain 8^T. Bar, 1 μm.

Cell material used for chemotaxonomic analyses was obtained from cultures grown in starch-nitrate broth for 1 week at 28 °C, aerated on a rotary shaker. After sedimentation of the starch, the liquid phase was collected and the suspended cells were harvested by centrifugation and washed twice with distilled water. Analyses of amino acids and sugars were carried out using the methods of Staneck & Roberts (1974). Polar lipids and menaquinones were extracted following the procedure of Minnikin *et al*. (1984). Polar lipids and menaquinones were analysed by TLC (Minnikin *et al*., 1977) and HPLC (Kroppenstedt, 1982, 1985), respectively. Whole-cell fatty acids were transmethylated and extracted by the method of Miller (1982). Fatty acid methyl esters (FAMEs) were analysed by GLC (Sasser, 1990) using the microbial identification system (MIDI). The presence of mycolic acids was checked by the method of Minnikin *et al*. (1975).

The chemotaxonomic properties of strain 8^T were consistent with their classification into the genus *Saccharomonospora* (Greiner-Mai *et al*., 1987, 1988). Whole-cell hydrolysates of strain 8^T contained *meso*-diaminopimelic acid as the peptidoglycan diamino acid, and galactose and arabinose as the major sugars of the cell. Menaquinone with a tetra-hydrogenated isoprenoid chain of nine isoprene units [MK-9(H_4)] was the dominant isoprenoid quinone (88%); MK-8(H_4) was found in minor amounts (12%). The polar lipids were made up of diphosphatidyl glycerol, phosphatidyl inositol, phosphatidyl ethanolamine, hydroxy-phosphatidyl ethanolamine and lyso-phosphatidyl ethanolamine. This pattern matched quite well with those of the *Saccharomonospora* spp. reported by Greiner-Mai *et al*. (1987). The fatty acid composition comprised iso/anteiso-branched fatty acids in combination with 2-hydroxy-branched and 10-methyl-branched fatty acids (fatty acid pattern 3c *sensu* Kroppenstedt, 1992), i.e. branched FAMEs iso-$C_{15:0}$ (1·8%), iso-$C_{16:0}$ (22·5%), iso-$C_{16:0}$ (5·3%), iso-$C_{17:0}$ (3·6%), iso-$C_{18:0}$ (0·8%), anteiso-$C_{17:0}$ (5·5%), anteiso-$C_{17:1}$ (0·5%), 2-hydroxy-iso-$C_{16:0}$ (10·9%), 2-hydroxy-iso-$C_{17:0}$ (0·7%), 2-hydroxy-anteiso-$C_{15:0}$ (3·4%), 2-hydroxy-anteiso-$C_{17:0}$ (2·4%) and 10-methyl-$C_{16:0}$ (6·1%), and unbranched FAMEs $C_{14:0}$ (0·9%), $C_{15:0}$ (0·3%), $C_{16:0}$ (15·8%), $C_{16:1}$ (14·1%), $C_{17:0}$ (0·7%), $C_{17:1}$ (0·7%), $C_{18:0}$ (1·6%), $C_{18:1cis9}$ (1·7%) and 2-hydroxy-$C_{16:0}$ (0·4%). This pattern is similar to those found in other *Saccharomonospora* species, but differed with respect to the 10-methyl branched fatty acids which are missing in the other *Saccharomonospora* species. The occurrence of this branched fatty acid might be a response to the high salt concentrations in the growth medium of strain 8^T and possibly in the halophilic marsh, the isolation site of

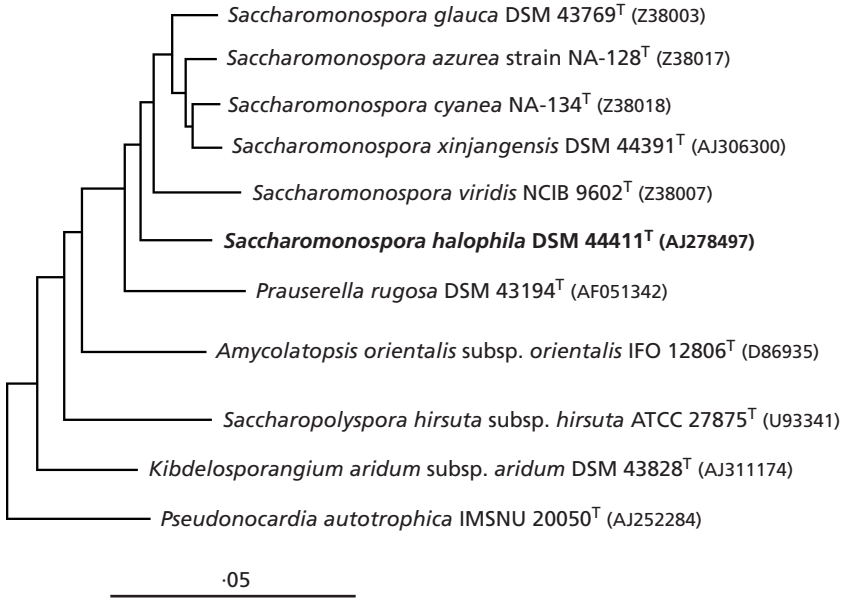

Fig. 2. Phylogenetic dendrogram obtained by distance matrix analysis showing the position of *Saccharomonospora halophila* DSM 44411^T among species of the genus *Saccharomonospora* and related genera. *Pseudonocardia autotrophica* IMSNU 20050^T served as a root. Bar, 1 nt substitution per 100 nt.

Table 1. Characters that differentiate strain 8^T from other *Saccharomonospora* species

Strain: 1, strain 8^T; 2, *S. azurea*; 3, '*S. caesia*'; 4, *S. cyanea*; 5, *S. glauca*; 6, *S. viridis*; 7, *S. xinjiangensis*. +, Property present; −, property absent; D, property doubtful; ND, not determined.

Character	1	2*	3*	4*	5*	6*	7†
Aerial mycelium colour	Light blue to greenish	Azure	Green	Dark blue	Light to bluish green	Green	Yellow-white
Spore ornamentation	Warty	Smooth	Warty	Warty	Warty	Warty	Smooth
Growth on sole carbon source (1 %, w/v):							
L-Arabinose	+	−	+	ND	+	−	ND
Galactose	+	−	+	+	ND	−	ND
Glucose	+	+	D	−	+	D†	ND
Mannitol	+	−	+	−	+	D	ND
Mannose	+	+	+	+	ND	−	+
Melibiose	+	+	−	−	ND	ND	ND
Rhamnose	+	−	−	ND	−	−	+
Ribose	D	+	−	+	ND	ND	ND
Xylose	−	+	D	D	+	−	+
Growth in the presence of NaCl (%):							
0	−	+	+	+	+	+	+
5	−	+	−	−	−	−	−
7	−	+	−	−	−	−	−
10	+	−	−	−	−	−	−
20	+	−	−	−	−	−	−
30	+	−	−	−	−	−	−
Growth temperature range (°C)	28–30	24–40	28–50	24–40	37–60	28–60	28–40

* Adapted from Embley (1992).

† Adapted from Jin *et al*. (1998).

the organism. As expected, mycolic acids could not be detected.

Determination of the 16S rDNA sequence (Rainey *et al*., 1996) and phylogenetic analyses (De Soete, 1983; Felsenstein, 1993) followed previously described methods. The almost complete 16S rDNA sequence of strain 8^T consisting of 1439 nt was compared to sequences of members of the class *Actinobacteria*. Strain 8^T was most closely related to *Saccharomonospora cyanea*, showing a sequence similarity of 96·9 %. Strain numbers and 16S rDNA accession numbers of reference organisms are indicated in Fig. 2.

Except for xylose, strain 8^T was able to utilize all tested sugars as sole source of carbon. For growth, a high salt concentration in the medium of at least 10 % was essential. The strain was able to grow at NaCl concentrations up to 30 % and could use feathers (keratin) as sole C and N source in the presence of 10 % NaCl. The optimal growth temperature was 28–30 °C. Physiological properties separated strain 8^T clearly from all known *Saccharomonospora* species (Table 1).

Based on phenotypic and genotypic data, it is concluded that strain 8^T merits species status in the genus *Saccharomonospora*. The name *Saccharomonospora halophila* sp. nov. is therefore proposed for strain 8^T (= DSM 44411^T = NRRL B-24125^T).

Description of *Saccharomonospora halophila* sp. nov.

Saccharomonospora halophila (ha.lo.phi′la. M.L. gen. adj. *halophila* salt-loving, referring to the ability to grow at high NaCl concentration).

Aerobic, Gram-positive, non-motile actinomycete that forms light blue aerial mycelium. No specific endo- or exopigments are produced. Optimal growth is obtained on starch-nitrate agar supplemented with 10 % NaCl at 28 °C. Halophilic and grows in 10–30 % NaCl. Can use feathers as sole C and N source in the presence of 10 % NaCl. The following carbon sources are utilized: L(+)-arabinose, D-galactose, D-glucose, mannitol, mannose, melibiose and L-rhamnose. The utilization of D-ribose is doubtful. D-Xylose is not used. Whole-cell hydrolysates contain *meso*-diaminopimelic acid, arabinose and galactose. The predominant menaquinone is MK-9(H_4). The polar lipids are diphosphatidyl glycerol, phosphatidyl ethanolamine, hydroxy-phosphatidyl ethanolamine, and phosphatidyl inositol. Fatty acids are iso/anteiso-branched

and 10-methyl branched fatty acids and significant amounts of hydroxy fatty acids are also found, i.e. iso-$C_{15:0}$ (1·8 %), iso-$C_{16:0}$ (22·5 %), iso-$C_{16:0}$ (5·3 %), iso-$C_{17:0}$ (3·6 %), iso-$C_{18:0}$ (0·8 %), anteiso-$C_{17:0}$ (5·5 %), anteiso-$C_{17:1}$ (0·5 %), 2-hydroxy-iso-$C_{16:0}$ (10·9 %), 2-hydroxy-iso-$C_{17:0}$ (0·7 %), 2-hydroxy-anteiso-$C_{15:0}$ (3·4 %), 2-hydroxy-anteiso-$C_{17:0}$ (2·4 %) and 10-methyl-$C_{16:0}$ (6·1 %), and unbranched FAMEs $C_{14:0}$ (0·9 %), $C_{15:0}$ (0·3 %), $C_{16:0}$ (15·8 %), $C_{16:1}$ (14·1 %), $C_{17:0}$ (0·7 %), $C_{17:1}$ (0·7 %), $C_{18:0}$ (1·6 %), $C_{18:1cis9}$ (1·7 %) and 2-hydroxy-$C_{16:0}$ (0·4 %). Mycolic acids are not synthesized. Strain 8^T (= DSM 44411^T = NRRL B-24125^T) was isolated from salt marsh soil in Kuwait.

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