Saccharomonospora marina sp. nov., isolated from an ocean sediment of the East China Sea

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A novel, aerobic, Gram-positive actinomycete strain, designated XMU15^T, was isolated from an ocean sediment collected from Zhaoan Bay in the East China Sea and was subjected to a polyphasic approach to determine its taxonomic position. The isolate grew optimally at 28 °C and at pH 7.0 in the presence of 3 % (w/v) NaCl on ISP medium 2. Gelatin liquefaction, milk coagulation and nitrate reduction were positive. Cellulose and starch hydrolysis, hydrogen sulfide and melanin production, and catalase, urease and oxidase activities were negative. The predominant menaquinone of the isolate was MK-9 (H₄), and *meso*-diaminopimelic acid was the diagnostic amino acid in the cell wall. The phospholipids of the isolate comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and a minor amount of phosphatidylethanolamine. The major fatty acids of the strain were iso-C_{16:0} (26.36 %), C_{17:1ω6c} (16.80 %), C_{15:0} (16.2 %), C_{16:0} (8.90 %), C_{17:1ω8c} (7.69 %) and iso-C_{16:1} H (5.95 %). The DNA G + C content of the genomic DNA was 68.1 mol%. A phylogenetic tree based on 16S rRNA gene sequences showed that the isolate fell within the evolutionary radiation encompassed by the genus *Saccharomonospora* and showed the highest 16S rRNA gene sequence similarity (96.7 %) to *Saccharomonospora xinjiangensis* DSM 44391^T. Based on the results of 16S rRNA gene sequence analysis and phenotypic and genotypic characterization, strain XMU15^T (=KCTC 19701^T =CCTCC AA 209048^T) represents a novel species of the genus *Saccharomonospora*, for which the name *Saccharomonospora marina* sp. nov. is proposed.

The genus *Saccharomonospora*, which belongs to the family *Pseudonocardiaceae*, was established by Nonomura & Ohara (1971). At the time of writing, the genus comprises eight recognized species, *Saccharomonospora viridis* (Nonomura & Ohara, 1971), *Saccharomonospora azurea* (Hu, 1987), *Saccharomonospora glauca* (Greiner-Mai *et al.*, 1988), *Saccharomonospora cyanea* (Runmao *et al.*, 1988), *Saccharomonospora xinjiangensis* (Jin *et al.*, 1998), *Saccharomonospora halophila* (Al-Zarban *et al.*, 2002),

Saccharomonospora paurometabolica (Li *et al.*, 2003) and *Saccharomonospora saliphila* (Syed *et al.*, 2008). The members of the genus are aerobic, Gram-positive and non-acid-fast. The hydrolysates of the whole cells contain *meso*-diaminopimelic acid, major amounts of galactose and arabinose (some strains also have ribose) and MK-9(H₄) as the predominant menaquinone.

Strain XMU15^T was isolated from an ocean sediment sample collected from Zhaoan Bay in the East China Sea (GPS coordinates for the sample site are 117° 17.64' E 23° 66.5' N, at a depth of 4 m) after being incubated on PLA-emulsified agar (Pranamuda & Tokiwa, 1999) at 28 °C for 4 weeks. The isolate was preserved on International *Streptomyces* Project medium 2 (ISP 2, Shirling & Gottlieb, 1966) slants at 4 °C and in 20 % (v/v) glycerol at –80 °C. Morphology was observed by light microscopy

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain XMU15^T is FJ812357.

A scanning electron micrograph showing spores of strain XMU15^T, and tables detailing cultural characteristics and signature nucleotide analysis results are available with the online version of this paper.

(BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 14 days of growth on ISP medium 2 agar at 28 °C. Substrate and aerial mycelia were well developed. Smooth or wrinkled spores were produced on the branched aerial mycelium singly, in pairs or occasionally in short spore-chains (See Supplementary Fig. S1 available in IJSEM online). The media used for cultural and physiological characterization were those described by Shirling & Gottlieb (1966) and Williams *et al.* (1989). Cultural characteristics were determined after 4 weeks at 28 °C by methods used in the ISP (Shirling & Gottlieb, 1966). Colours of substrate and aerial mycelia were determined by comparison with colour chips from the ISCC-NBS colour charts standard sample no. 2106 (Kelly, 1964). The isolate could be distinguished from the most closely related species of the genus *Saccharomonospora*, *S. xinjiangensis* DSM 44391^T, by comparing a battery of cultural characteristics (Supplementary Table S1).

Growth at different temperatures (4, 10, 15, 20, 28, 37, 40, 45, 50, 55, 60 and 65 °C) was tested with ISP medium 2 (pH 7.0) plates. Growth at different NaCl concentrations was tested by using ISP medium 2 (pH 7.0) as the basal medium with NaCl concentrations ranging from 0 to 15 % (w/v), at intervals of 1 %, and incubating at 28 °C for 4 weeks. Growth was investigated between pH 4.0 and 10.0 at intervals of 1 pH unit, using the following buffer system in ISP 2 broth medium: 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. Methods for determining catalase and oxidase activity were as described by Rivas *et al.* (2003). Biomass for chemical and molecular studies was obtained by cultivation in ISP medium 2 (pH 7.0) in shaking flasks (150 r.p.m.) at 28 °C for one week. The isolate showed phenotypic differences from its closest relative, *S. xinjiangensis* DSM 44391^T, when comparing a battery of morphological and physiological properties (Table 1) after being incubated at 28 °C for 4 weeks and observed once a week. The detailed morphological and physiological properties of strain XMU15^T are given in the species description.

The amino acids and sugars in cell wall hydrolysates were analysed by using the methods established by Hasegawa *et al.* (1983). Polar lipids were extracted, examined by using two-dimensional TLC and identified on the basis of procedures described by Minnikin *et al.* (1984). Isoprenoid quinones were analysed by HPLC as described by Minnikin *et al.* (1984) and Kroppenstedt (1982). Cellular fatty acids were determined for the novel isolate and for *S. xinjiangensis* DSM 44391^T by means of the Microbial Identification System (MIDI; Microbial ID; Sasser, 1990) with cells grown in TSB (trypticase soy broth) medium (pH 7.0) in flasks on a rotary shaker at 200 r.p.m. and at 28 °C for 3 days. Chemotaxonomic data for the new isolate was consistent with its assignment to the genus *Saccharomonospora* (Nonomura & Ohara, 1971; Hu, 1987; Greiner-Mai *et al.*, 1988; Hu *et al.*, 1988; Jin *et al.*, 1998; Al-Zarban *et al.*, 2002; Li *et al.*, 2003; Syed *et al.*,

2008). The cell wall diamino acid in the peptidoglycan layer of both strains was *meso*-diaminopimelic acid, and the whole-cell sugars mainly comprised galactose and arabinose. The phospholipids of the isolate consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and a minor amount of phosphatidylethanolamine. The menaquinones were MK-9(H₄) (89.6 %) and MK-8(H₄) (10.4 %). Cellular fatty acids comprised iso-C_{16:0} (26.36 %), C_{17:1}ω6c (16.80 %), C_{15:0} (16.2 %), C_{16:0} (8.90 %), C_{17:1}ω8c (7.69 %), iso-C_{16:1} H (5.95 %), C_{17:0}

Table 1. Phenotypic characteristics that differentiate the novel isolate from its closest relative, *Saccharomonospora xinjiangensis* DSM 44391^T

Strains: 1, XMU15^T; 2, *S. xinjiangensis* DSM 44391^T. +, Positive; –, negative; R, resistant; S, sensitive. All data are from this study.

Characteristic	1	2
Degradation of:		
Cellulose	–	+
Starch	–	+
Carbon source utilization		
D-Glucose	+	–
myo-Inositol	+	–
Mannose	+	–
Melibiose	+	–
L-Rhamnose	+	–
Ribose	–	+
Nitrogen source utilization		
L-Histidine	–	+
Enzyme production		
Autolysis	–	+
Catalase	–	+
Antibiotic assay		
Carbenicillin (10 µg)	R	S
Cephalothin (V) (30 µg)	R	S
Cephalothin (VI) (30 µg)	R	S
Furoxone (30 µg)	R	S
Piperacillin (10 µg)	R	S
Menaquinones	MK-9 (H ₄) (89.6 %), MK-8 (H ₄) (10.4 %)	MK-9 (H ₄) (85.1 %), MK-8 (H ₄) (13.0 %), MK-7(H ₂) (1.9 %)
Major fatty acids (>5.0 %)	iso-C _{16:0} (26.36 %), C _{17:1} ω6c (16.80 %), C _{15:0} (16.20 %), C _{16:0} (8.90 %), C _{17:1} ω8c (7.69 %), iso-C _{16:1} H (5.95 %)	C _{17:1} ω8c (19.69 %), C _{17:1} ω6c (17.38 %), iso-C _{16:0} (14.56 %), C _{16:0} (12.31 %), C _{17:0} (6.26 %), C _{15:0} (5.91 %)
DNA G + C mol%	68.1	67.7

(2.14%), anteiso- $C_{17:0}$ (1.87%), $C_{15:1\omega6c}$ (1.32%) and $C_{18:1\omega9c}$ (1.24%), which is similar to those of strains of the genus *Saccharomonospora*.

Methods used for extraction of genomic DNA and PCR amplification of the 16S rRNA gene were as described by Li *et al.* (2007). Multiple alignments with sequences of the most closely related type strains of the genus *Saccharomonospora* and calculations of levels of sequence similarity were carried out by using the EzTaxon server 2.0 (Chun *et al.*, 2007). Phylogenetic analysis was performed by using three tree-making algorithms, namely the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was reconstructed by using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980) using MEGA version 4.0 (Tamura *et al.*, 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The genomic DNA of the isolate for the determination of the G+C content was prepared according to the method of Marmur (1961). The G+C content of the DNA was determined by reverse-phase HPLC of nucleosides, according to Mesbah *et al.* (1989), to be 68.1 mol%.

The 16S rRNA gene sequence of strain XMU15^T (1529 bp) was analysed by preliminary comparison with sequences from the GenBank database. The results indicated that the new isolate had the highest sequence similarity to members of the genus *Saccharomonospora*. Phylogenetic analysis showed that strain XMU15^T fell into a separate subclade between the genera *Prauserella* and *Saccharomonospora* (Fig. 1). The topology of the neighbour-joining tree was similar to those of the phylogenetic trees reconstructed by using the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain XMU15^T showed the highest 16S rRNA gene sequence similarity (96.7%) to *S. xinjiangensis* DSM 44391^T and its morphology and chemotaxonomic data were similar to those of members of the genus *Saccharomonospora*. Thus, strain XMU15^T should be assigned to the genus *Saccharomonospora*.

Despite its phylogenetic position between the genera *Saccharomonospora* and *Prauserella*, the new isolate had highest 16S rRNA gene sequence similarity to *S. xinjiangensis* DSM 44391^T, from which it could be distinguished by using a battery of cultural characteristics (Supplementary Table S1) and other phenotypic characteristics (Table 1). In addition, 16S rRNA gene signature nucleotides of strain XMU15^T were most similar to those of the genus *Saccharomonospora* (Supplementary Table S2), rather than to those of the genus *Prauserella*. Thus, we consider strain XMU15^T to represent a novel species of the genus *Saccharomonospora*, for which we propose the name *Saccharomonospora marina* sp. nov.

Description of *Saccharomonospora marina* sp. nov.

Saccharomonospora marina (ma.ri'na. L. fem. adj. *marina* of the sea).

Aerobic, Gram-positive, non-motile actinomycete. Non-motile smooth or wrinkled spores are produced on the branched aerial mycelium singly, in pairs and occasionally in short chains. Optimal growth occurs at 28–37 °C and at pH 7.0 on ISP medium 2. NaCl is tolerated up to 5% (w/v), with an optimum concentration of 0–3% (w/v). D-Arabinose, cellobiose, D-galactose, D-glucose, *myo*-inositol, maltose, D-mannitol, D-mannose, melibiose, L-rhamnose, sucrose and D-xylose can be utilized as carbon sources, but lactose, sorbitol, L-sorbose, raffinose and ribose cannot. L-Alanine, L-arginine, L-cystine, L-glutamate, glycine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine can be used as nitrogen sources, but DL-asparagine, L-histidine, L-hydroxyproline and DL-tryptophan cannot. Gelatin liquefaction, milk coagulation and nitrate reduction are positive; cellulose and starch hydrolysis, hydrogen sulfide and melanin production, and catalase, urease and oxidase activities are negative. Resistant to ampicillin (10 µg), carbenicillin (10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), cephalothin (V) (30 µg), cephalothin (VI) (30 µg), chloramphenicol (30 µg), furoxone (30 µg), penicillin (10 µg), piperacillin (10 µg) and oxacillin (1 µg), but not to cephalothin (IV) (300 µg) and fortum

Fig. 1. Neighbour-joining phylogenetic tree derived from 16S rRNA gene sequences, showing the position of strain XMU15^T among species of the genus *Saccharomonospora* and the related genus *Prauserella*. Asterisks indicate branches recovered with all three methods: neighbour-joining, maximum-likelihood and maximum-parsimony. Bootstrap values (only those above 50%) are shown as percentages of 1000 replicates. The sequence of *Amycolatopsis methanolica* IMSNU 20055^T (AJ249135) was used as the outgroup. Bar, 0.5 nucleotide substitutions per 100 nt.

(30 µg). Whole cell hydrolysates contain *meso*-diaminopimelic acid and arabinose, galactose and ribose. The menaquinones are MK-9(H₄) (89.6 %) and MK-8(H₄) (10.4 %) and the phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylethanolamine. Fatty acids are iso-C_{16:0}, C_{17:1}ω6c, C_{15:0}, C_{16:0}, C_{17:1}ω8c, iso-C_{16:1} H, C_{17:0}, anteiso-C_{17:0}, C_{15:1}ω6c and C_{18:1}ω9c. The DNA G+C content of the type strain is 68.1 mol%.

Strain XMU15^T (=KCTC 19701^T =CCTCC AA 209048^T) was isolated from an ocean sediment sample collected from Zhaoan Bay in the East China Sea, Xiamen, Fujian province, China.

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