Saccharomonospora amisosensis sp. nov., isolated from deep marine sediment

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A novel actinomycete, strain DS3030^T, was isolated from a deep sediment sample, collected from the southern Black Sea coast, Turkey, and was examined using a polyphasic approach. On the basis of 16S rRNA gene sequence analysis, strain DS3030^T was shown to belong to the genus *Saccharomonospora* and to be related most closely to *Saccharomonospora marina* XMU15^T (99.6 % similarity). Sequence similarities with other strains of the genus *Saccharomonospora* were lower than 97.0 %. The organism had chemical and morphological features typical of the genus *Saccharomonospora*. The cell wall of the novel strain contained *meso*-diaminopimelic acid, arabinose and galactose as diagnostic sugars. The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside. The predominant menaquinone was MK-9(H_4). Major fatty acids were iso-$C_{16:0}$, iso-$C_{16:0}$ 2-OH and $C_{16:1}$*cis* 9. Phenotypic data clearly distinguished the new isolate from its closest relative, *S. marina* XMU15^T. The combined genotypic and phenotypic data and low DNA–DNA relatedness with its closest related strain reveal that strain DS3030^T represents a novel species of the genus, for which the name *Saccharomonospora amisosensis* sp. nov. is proposed. The type strain is DS3030^T (=DSM 45685^T=KCTC 29069^T=NRRL B-24885^T).

The genus *Saccharomonospora* was proposed by Nonomura & Ohara (1971), with the type species *Saccharomonospora viridis*, for actinomycetes producing predominantly single spores, sometimes spores in pairs and short chains, on aerial hyphae. *Saccharomonospora* strains are also characterized by a number of chemical markers, including the presence of *meso*-diaminopimelic acid in the whole-cell hydrolysate, and contain arabinose and galactose as characteristic sugars (wall chemotype IV; Lechevalier & Lechevalier, 1970), MK-8(H_4) and MK-9(H_4) as the predominant menaquinones, and phosphatidylethanolamine, hydroxy-phosphatidylethanolamine and lyso-phosphatidylethanolamine as the main phospholipids (Kroppenstedt, 1985; Embley *et al.*, 1985; Greiner-Mai *et al.*, 1987; Al-Zarban *et al.*, 2002; Li *et al.*, 2003; Syed *et al.*, 2008; Liu *et al.*, 2010).

The aim of the present study was to determine the taxonomic status of an actinomycete, strain DS3030^T, isolated from sediment offshore from the southern Black Sea coast, Turkey. The organism was the subject of a polyphasic taxonomic study which showed that it represents a novel species of the genus *Saccharomonospora*.

Strain DS3030^T was isolated from a sediment sample collected by a dredge at a depth of 60 m, offshore of the Melet River (GPS coordinates for the sampling site are 41° 00.353′ N 37° 57.489′ E). Sediment samples were subsampled aseptically and stored at −20 °C until use. Strain DS3030^T was isolated from the sediment sample by using SM3 medium (Tan *et al.*, 2006), supplemented with filter-sterilized cycloheximide (50 μg ml^{-1}), nalidixic acid (10 μg ml^{-1}), novobiocin (10 μg ml^{-1}) and nystatin (50 μg ml^{-1}), incubated at 28 °C for 30 days. The strain was maintained on yeast extract-malt extract (ISP medium 2; Shirling & Gottlieb, 1966) agar slopes at room temperature and as glycerol suspensions (20 %, v/v) at −20 °C.

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product were carried out following Chun & Goodfellow (1995). The almost-complete (1477 bp) 16S rRNA gene sequence of strain DS3030^T was determined using an ABI PRISM 3730 XL automatic sequencer. The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Saccharomonospora amisosensis* DS3030^T is JN989292.

Four supplementary figures and two supplementary tables are available with the online version of this paper.

server (http://eztaxon-e.ezbiocloud.net; Kim *et al.*, 2012). Multiple alignment with sequences from closely related species was performed by using the program CLUSTAL W in MEGA 5.0 (Tamura *et al.*, 2011). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms in MEGA 5.0 (Tamura *et al.*, 2011). Evolutionary distances were calculated using the model of Jukes & Cantor (1969). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The phylogenetic tree based on the neighbour-joining algorithm showed that strain DS3030^T formed a cluster with the type strain of the marine species *Saccharomonospora marina* within members of the genus *Saccharomonospora* (Fig. 1). The other two tree-making algorithms (maximum-likelihood and maximum-parsinomy) resulted in trees showing similar topologies (Figs S1 and S2 available in IJSEM Online). Strain DS3030^T shared a 16S rRNA gene sequence similarity of 99.6 % (6 nt differences at 1475 locations) with its nearest relative, *S. marina* XMU15^T. Sequence similarities with all other members of the genus *Saccharomonospora* were <97.0 %.

DNA–DNA relatedness values between strain DS3030^T and its closest phylogenetic neighbour, *S. marina* XMU15^T, were determined by the Identification Service at the Deutsche Sammlung von Mikroorganismen und Zelkulturen (DSMZ), Braunschweig, Germany. DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) following the modifications described by Huss *et al.* (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian).

The taxonomic integrity of the test strain was supported by DNA relatedness data. Strain DS3030^T had 56.1 ± 0.1 % genomic DNA–DNA relatedness (mean ± SD of duplicate determinations in 2 × SSC and 10 % formamide at 70 °C) to *S. marina* XMU15^T, the phylogenetically closest related species within the genus *Saccharomonospora*, which is below the 70 % relatedness threshold proposed by Wayne *et al.* (1987) for the delineation of separate species.

The freeze-dried cell biomass used for the analysis of polar lipids, quinones and cell sugars was produced from cultures grown for 10 days in ISP 2 broth under aerobic conditions at 28 °C with shaking at 160 r.p.m.; cells were harvested by centrifugation, washed twice in distilled water and recentrifuged and freeze-dried. Amino acid and sugar analysis of whole-cell hydrolysates was performed according to methods described by Staneck & Roberts (1974). Analyses of polar lipids and respiratory quinones were carried out by the Identification Service of the DSMZ. Respiratory quinones were extracted from 100 mg of freeze-dried cells based on the two-stage method described by Tindall (1990a, b). Respiratory quinones were separated into their different classes by TLC on silica gel (Macherey-Nagel Art. no. 805 023), using hexane/tert-butylmethylether (9 : 1, v/v) as solvent. UV absorbing bands corresponding to menaquinones were removed from the plate and further analysed by HPLC. This step was carried out by HPLC with a reversed-phase column (Macherey-Nagel, 2 mm × 125 mm, 3 μm, RP18) using methanol as the eluant. Respiratory quinones were detected at 269 nm.

A starter culture for fatty acid analyses was prepared in a flask containing 20 ml trypticase soy broth (TSB; Difco), which was shaken at 150 r.p.m. at 28 °C for 5 days. Five

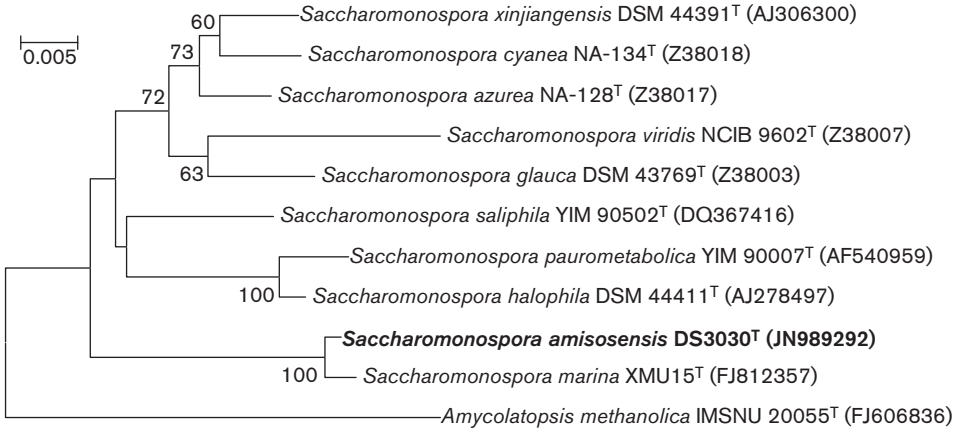

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16 rRNA gene sequences showing the position of strain DS3030^T amongst its phylogenetic neighbours. *Amycolatopsis methanolica* IMSNU 20055^T was used as an outgroup. Numbers at nodes indicate levels of bootstrap support (%); only values ⩾50 % are shown. GenBank accession numbers are given in parentheses. Bar, 0.005 substitutions per site.

millilitres of the resultant culture was used to inoculate 50 ml TSB, which was incubated under the same conditions, the biomass harvested by cellulose filtration (pore size 0.45 μm) and the wet cells (200 mg) placed in an extraction tube. Cellular fatty acids were extracted, methylated and separated by GC using an Agilent Technologies 6890N, according to the standard protocol of the Sherlock Microbial Identification (MIDI) System (Sasser, 1990; Kämpfer & Kroppenstedt, 1996); the fatty acid methyl ester peaks were quantified via the TSBA 5.0 database. The DNA G+C content of the isolate was determined following the procedure of Gonzalez & Saiz-Jimenez (2005).

The cell-wall diamino acid of strain DS3030^T was *meso*-diaminopimelic acid (type III; Lechevalier & Lechevalier, 1970), and the whole-cell hydrolysates contained galactose, arabinose (major components), glucose and small amounts of xylose. The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside, two unidentified aminolipids and two unidentified aminophosphates (Fig. S3). The predominant menaquinone of strain DS3030^T was MK-9(H_4) (62.0 %); MK-8(H_4) (22.0 %) and MK-7(H_4) (10.0 %) were also detected. The major cellular fatty acids were iso-$C_{16:0}$ (35.8 %), iso-$C_{16:0}$ 2-OH (18.2 %), $C_{16:1}$ *cis* 9 (9.8 %), $C_{16:0}$ (8.4 %) and iso-$C_{16:1}$ H (7.5 %). Comparative cellular fatty acid compositions of strain DS3030^T and *S. marina* XMU15^T are shown in Table S1. The G+C content of the DNA of strain DS3030^T was 68.9 mol%.

The morphological and physiological characteristics of strains DS3030^T and *S. marina* XMU15^T were studied after incubation for 14 days at 28 °C on various media described by Shirling & Gottlieb (1966): yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salt-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract-iron agar (ISP 6), tyrosine agar (ISP 7), modified Bennett's agar (MBA; Jones, 1949) and nutrient agar (NA; Difco). Colony morphology and micromorphological properties of isolate DS3030^T were determined by examined gold-coated dehydrated specimens of 45 day cultures from ISP 4 medium using a JEOL JSM 6060 instrument. Growth was tested at various temperatures (4, 10, 20, 28, 30, 37, 45, 50 and 55 °C), different pH values (4, 5, 6, 7, 8, 9, 10 and 11) and NaCl concentrations (1–15 %, w/v) using ISP 2 as the basal medium. The test strains were examined for a range of phenotypic properties using well-established procedures (Gordon & Mihm, 1957; Shirling & Gottlieb, 1966; Gordon *et al.*, 1974; Williams *et al.*, 1983). Tests in the commercial systems API CORYNE and API ZYM (bioMérieux) were performed according to the manufacturer's instructions.

Cells of strain DS3030^T were aerobic, Gram-reaction-positive, non-motile actinomycetes which formed branched substrate hyphae that produced single spores, and pairs and short chains of spores (Fig. S4) when grown on ISP 4 medium for 45 days at 28 °C. Growth was moderate on ISP 2, ISP 4, ISP 5, ISP 7 and modified Bennett's agar but weak growth was observed on ISP 3 and Czapek's agar (Table S2). The aerial mycelium was white and the vegetative mycelium was yellowish to pale orange yellow. Moderate reddish orange diffusible pigments were produced on tyrosine agar (ISP 7). Melanoid pigments were not produced on ISP 6 or ISP 7 medium. Sporulation was not observed on ISP 2, ISP 3, ISP 5, ISP 6, ISP 7, modified Bennett's or Czapek's agar. The detailed morphological and physiological characteristics of strain DS3030^T are given in Table 1 and in the species description.

Based on data from the present polyphasic study, strain DS3030^T could be readily differentiated from other members of the genus *Saccharomonospora* and is considered to represent a novel species, for which the name *Saccharomonospora amisosensis* sp. nov. is proposed.

Description of *Saccharomonospora amisosensis* sp. nov.

Saccharomonospora amisosensis (a.mi.sos.en′sis. N.L. fem. adj. *amisosensis* of or belonging to Amisos, Samsun, Turkey).

Aerobic, Gram-stain-positive, non-motile actinomycetes which form branched substrate hyphae that produce single spores, or pairs and short chains of spores. Positive for aesculin hydrolysis. Negative for arbutin, allantoin and urea hydrolysis, nitrate reduction, H_2S production and catalase activity. Growth occurs at pH 5.0–10.0 and at 28–45 °C, but not at pH 4.0 or 11.0, or at 4, 10, 20, 50 or 55 °C. Optimal growth is at 30 °C and pH 7.0. Growth is observed in the presence of 0–10 % (w/v) NaCl. Elastin, guanine, hypoxanthine, L-tyrosine, and Tweens 20 and 80 are not degraded. The following carbon sources are utilized: lactose, cellobiose, D-galactose, D-mannitol, D-mannose, L-rhamnose, dextrin and starch. Adonitol, L-arabinose, inulin, maltose, melezitose, D-sorbitol, D-glucose, sucrose and xylose are not utilized as sole carbon and energy sources. Utilizes L-cysteine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine and L-valine as sole nitrogen sources but not α-isoleucine, L-alanine, L-arginine, glycine, D-phenylalanine or L-hydroxyproline. Positive for (in API ZYM and API CORYNE strips) alkaline phosphatase, leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, pyrazinecarboxamide and potassium nitrate, and hydrolysis of gelatin and 2-naphthyl-phosphate; negative for esterase, esterase lipase, lipase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase (ONPG), β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase, and hydrolysis of pyroglutamic acid-β-naphthylamide, naphthol-AS-BI-glucuronic acid, 2-naphthyl-α-D-glucopyranoside, 1-naphthyl-*N*-acetyl-β-D-glucosaminide and glycogen. The predominant menaquinone is MK-9(H_4). The polar lipid profile contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside. Major fatty acids are iso-$C_{16:0}$, iso-$C_{16:0}$ 2-OH and $C_{16:1}$*cis* 9.

Table 1. Phenotypic properties of strain DS3030^T and *Saccharomonospora marina* XMU15^T

Strains were positive for growth with cellobiose, D-galactose, D-mannose, D-mannitol, dextrin and L-rhamnose as sole carbon sources (1.0 %), L-cysteine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine and L-valine as sole nitrogen sources (0.1 %), for alkaline phosphatase, leucine arylamidase, trypsin, acid phosphatase, potassium nitrate, pyrazinecarboxamide, and hydrolysis of 2-naphthyl-phosphate and aesculin. Negative for hydrolysis of allantoin, H_2S production, degradation of guanine (0.05 %), L-tyrosine (0.5 %), Tween 20 (1.0 %), Tween 80 (1.0 %) and hypoxanthine (0.4 %), growth with D-sorbitol, melezitose, inulin and L-arabinose as sole carbon sources (1.0 %), and presence of esterase, lipase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, pyroglutamic acid-β-naphthylamide, naphthol-AS-BI-glucuronic acid, 1-naphthyl-*N*-acetyl-β-D-glucosaminide, urea and glycogen activity. All data are from this study.

Characteristic	DS3030^T	*S. marina* XMU15^T
Biochemical tests		
Arbutin hydrolysis	−	+
Nitrate reduction	−	+
Degradation of:		
Elastin (0.3 %)	−	+
Carbon source utilization (1.0 %)		
Adonitol	−	+
Maltose	−	+
Starch	+	−
Sucrose	−	+
D-Glucose	−	+
Lactose	+	−
Xylose	−	+
Nitrogen source utilization (0.1 %)		
α-Isoleucine	−	+
D-Phenylalanine	−	+
Glycine	−	+
L-Alanine	−	+
L-Arginine	−	+
L-Hydroxyproline	−	+
API ZYM results		
Esterase lipase	−	+
Valine arylamidase	+	−
Cystine arylamidase	+	−
α-Chymotrypsin	+	−
Naphthol-AS-BI-phosphohydrolase	−	+
α-Glucosidase	−	+
API CORYNE results		
2-Naphthyl-α-D-glucopyranoside	−	+
Hydrolysis of gelatin	+	−
Catalase	−	+

The type strain, DS3030^T (=DSM 45685^T=KCTC 29069^T=NRRLB-24885^T), was isolated from deep sediment collected from the southern Black Sea coast, Turkey. The DNA G+C content of the type strain is 68.9 mol%.

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