

Streptomyces seymenliensis sp. nov., isolated from soil

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Abstract A novel actinomycete, designated strain B1041^T, isolated from soil collected from the Tuz (Salt) Lake in the central Anatolia region, in Turkey, was characterised using a polyphasic taxonomic approach. The isolate was found to have chemical and morphological properties typical of the members of the genus *Streptomyces* and formed a distinct phyletic line in the 16S rRNA gene tree. Strain B1041^T was found to be most closely related to *Streptomyces plumbiresistens* CCNWHX 13-160^T (98.46 % sequence similarity), *Streptomyces pseudovenezuelae* NBRC 12904^T (97.81 %), *Streptomyces novaecaesareae* NBRC 13368^T (97.68 %), *Streptomyces graminifolii* JL-22^T (97.60 %), *Streptomyces phaeoluteigriseus* NRRL ISP-5182^T (97.58 %), *Streptomyces ciscaucasicus* NBRC 12872^T (97.53 %) and *Streptomyces pratensis* ch24^T (97.52 %). Sequence similarities with other strains of the genus

Streptomyces were lower than 97.5 %. The cell wall of the novel strain was found to contain LL-diaminopimelic acid and the whole cell sugars were identified as galactose, glucose and ribose. The major cellular fatty acids were identified as *anteiso* C_{15:0}, *iso* C_{16:0} and *anteiso* C_{17:0}. The predominant menaquinones found were found to be MK-9(H₈) and MK-9(H₆). The polar lipids detected were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, a phosphoglycolipid and two unknown phospholipids. The genomic DNA G+C content was determined to be 70.4 mol%. On the basis of the data from this polyphasic taxonomic study, strain B1041^T can be considered to represent a novel species within the genus *Streptomyces* for which the name *Streptomyces seymenliensis* sp. nov. is proposed (type strain B1041^T = KCTC 29245^T = DSM 42117^T).

Keywords *Streptomycetaceae* · *Streptomyces seymenliensis* · Polyphasic taxonomy

The GenBank accession number for the 16S rRNA gene sequence of *Streptomyces seymenliensis* B1041^T (=KCTC 29245^T = DSM 42117^T) is KC560729.

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Introduction

The members of the genus *Streptomyces* are aerobic and Gram-stain positive actinomycetes with relatively high G+C content. All *Streptomyces* species have LL-diaminopimelic acid in the cell wall peptidoglycan (Otoguro et al. 2009) but no characteristic sugars (wall chemotype I sensu Lechevalier and Lechevalier 1970). Members of

the genus *Streptomyces* have a wide range of metabolic abilities and produce a number of enzymes and chemical compounds, including antibiotics, enzyme inhibitors, vitamins, antitumour agents and antifungal compounds (McCarthy and Williams 1992; Chun et al. 1997; Kim and Hwang 2003; Bérdy 2005; Kim et al. 2006). At the time of writing, more than 600 species with validly published names are known (<http://www.bacterio.cict.fr/index.html>), although there are strong grounds for believing that the taxon is underspeciated (Sembiring et al. 2000; Kim and Goodfellow 2002).

Saline lakes such as Tuz (Salt) Lake, which is the second largest lake of Turkey, have been a valuable source of novel microorganisms (Tatar et al. 2013). Formation of Tuz (Salt) Lake began with tensional movements during the Late Cretaceous, followed by compressional episodes that, during the Late Eocene, resulted in the isolation of the basin from the open sea (Dirik and Erol 2003). Water flows into the Tuz (Salt) Lake through the Melendiz Stream and drainage channels of the Konya plain. The lake dries out and a 30 cm layer of salt forms because of the evaporation of water in the summer. The aim of the present polyphasic study was to clarify the taxonomic position of a novel *Streptomyces* strain, B1041^T, which was isolated from soil collected from Tuz (Salt) Lake.

Materials and methods

Isolation and maintenance of the organism

During research on biodiversity of culturable actinomycetes from a soil sample collected from Tuz (Salt) Lake, Seymenli village, Şereflikoçhisar, Ankara, located in the central Anatolia region, strain B1041^T was isolated on modified Bennett's Agar (MBA; Jones 1949), supplemented with filter sterilized rifampicin (0.5 µg ml⁻¹) and cycloheximide (50 µg ml⁻¹), which had been inoculated with a soil suspension and incubated at 28 °C for 21 days. The organism was maintained on modified Bennett's agar slopes supplemented with cycloheximide (50 µg ml⁻¹) and as glycerol suspensions (20 %, v/v) at -20 °C.

Streptomyces plumbiresistens DSM 42067^T and *Streptomyces pseudovenezuelae* DSM 40212^T were obtained from DSMZ (German Collection of Microorganisms and Cell Cultures GmbH) and cultured under the same conditions for comparative analyses.

Morphological, cultural and physiological characteristics

Cultural characteristics of strain B1041^T were determined after incubation at 28 °C for 14 days on various media as described by Shirling and Gottlieb (1966): yeast extract-malt extract agar (International *Streptomyces* Project [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), MBA and nutrient agar. National Bureau of Standards (NBS) Colour Name Charts (Kelly 1964) were used for determining colour designation and names. Growth at different temperatures (4, 10, 28, 37, 45, 50 and 55 °C) and pH 4.0–12.0 (at intervals of 1.0 pH unit), and in the presence of NaCl (0–10, 15, 20, 30 %; w/v), was determined on ISP 2 (Shirling and Gottlieb 1966). KH₂PO₄/HCl, KH₂PO₄/K₂HPO₄ and K₂HPO₄/NaOH buffer systems were used to maintain the pH values of the media, and a NaCl buffer system for NaCl tolerance. Established methods were used to determine whether the strain degraded Tween 40 and 80 (Nash and Krent 1991); the remaining degradation tests were examined using methods described by Williams et al. (1983). Carbon source utilization was tested using carbon source utilization (ISP 9) medium (Shirling and Gottlieb 1966) supplemented with a final concentration of 1 % (w/v) of the tested carbon sources. Nitrogen source utilization was examined using the basal medium recommended by Williams et al. (1983) supplemented with a final concentration of 0.1 % (w/v) of the tested nitrogen sources. Tests in the commercial system API CORYNE (bioMérieux) were performed according to the manufacturer's instructions.

Antimicrobial activity of strain B1041^T to inhibit the growth of nine microorganisms, including Gram-positive and Gram-negative bacteria as well as fungi, was observed using the agar well method described by Zamanian et al. (2005). Colony morphology and micro-morphological properties of isolate B1041^T were determined by examining gold coated dehydrated specimens of 14 days cultures from ISP 4 (Shirling and Gottlieb 1966) medium using a JEOL JSM 6060 instrument.

Chemotaxonomic characterization

Chemotaxonomic analyses were carried out to support the phylogenetic affiliation of strain B1041^T to genus *Streptomyces*. The strain was grown in ISP 2 broth

under aerobic conditions in flasks on a rotary shaker at 160 rpm and 28 °C for 10 days. Biomass was harvested by centrifugation, washed twice in distilled water and re-centrifuged and freeze-dried. Isomers of diaminopimelic acid in whole-cell hydrolysates and sugars were prepared according to Lechevalier and Lechevalier (1970) and analysed by thin layer chromatography (Staneck and Roberts 1974).

Polar lipid and respiratory quinones analyses were carried out by the Identification Service of the DSMZ, Braunschweig, Germany. 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 (menaquinones and ubiquinones) by thin layer chromatography 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 or ubiquinones were removed from the plate and further analysed by HPLC. This step was carried out on a LDC Analytical (Thermo Separation Products) HPLC fitted with a reverse phase column (Macherey–Nagel, 2 × 125 mm, 3 µm, RP18) using methanol as the eluant. Respiratory lipoquinones were detected at 269 nm.

A starter culture for the fatty acid analyses was prepared in a flask containing 20 ml Trypticase Soy Broth (Difco) which was shaken at 150 rpm at 28 °C for 5 days. Five ml of the resultant culture was used to inoculate 50 ml of 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 gas chromatography using an Agilent Technologies 6890 N instrument, fitted with an autosampler and a 6,783 injector, according to the standard protocol of the Sherlock Microbial identification (MIDI) system (Sasser 1990; Kämpfer and Kroppenstedt 1996); the fatty acid methyl ester peaks were quantified using TSBA 5.0 software.

The DNA G+C content of the isolate was determined following the procedure of Gonzalez and Saiz-Jimenez (2005).

DNA preparation, amplification and determination of 16S rRNA gene sequence

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR

product were carried out by following the method of Chun and Goodfellow (1995). The almost complete (1,480 bp) 16S rRNA gene sequence of strain B1041^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 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 MEGA5.0 (Tamura et al. 2011). Phylogenetic trees were constructed with the neighbour-joining (Saitou and Nei 1987), maximum parsimony (Kluge and Farris 1969) and maximum-likelihood (Felsenstein 1981) algorithms in MEGA5.0 (Tamura et al. 2011). Evolutionary distances were calculated using the model of Jukes and Cantor (1969). Topologies of the resultant trees were evaluated by bootstrap analyses (Felsenstein 1985) based on 1,000 resamplings.

DNA–DNA hybridization

DNA–DNA relatedness values were determined between strains B1041^T and *S. plumbiresistens* DSM 42067^T, 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) under consideration of 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).

Results and discussion

Strain B1041^T was observed to form a branched substrate mycelium and aerial hyphae which differentiated into spiral chains of intermittently spiny-surfaced spores on ISP 4 (Shirling and Gottlieb 1966) (Fig. 1). The organism was found to grow well on ISP 2, 3, 4, 5 and 7, MBA and nutrient agar but only to grow moderately on ISP 6. White aerial mycelia were observed on ISP 4, 5 and 7. Light-yellowish to dark brown substrate mycelia were formed on MBA, nutrient agar, ISP 3, 4, 5, 7 and a light brown soluble

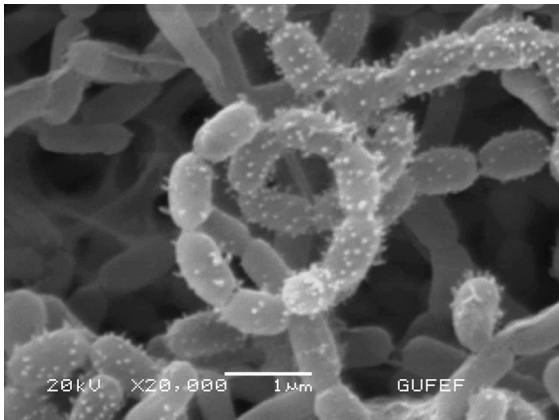


Fig. 1 Scanning electron micrograph of strain B1041^T grown on ISP 4 (Shirling and Gottlieb 1966) medium at 28 °C for 14 days. Bar 1 µm

pigment on ISP 7. The physiological and biochemical properties of strain B1041^T are given in Table 1 and the species description. Strain B1041^T can be distinguished from its most closest phylogenetic neighbour, *S. plumbiresistens* CCNWHX 13-160^T, on the basis of several phenotypic features (Table 1). In addition, the appearance of the spiral chains of spiny-surfaced spores of strain B1041^T is clearly distinguishable from the rectiflexibles spore chains and smooth-surfaced spores of *S. plumbiresistens* DSM 42067^T and *S. pseudovenezuelae* DSM 40212^T. Antimicrobial activity was found against *Aspergillus parasiticus* NRRL-465, *Bacillus subtilis* NRRL B-209, *Staphylococcus aureus* ATCC 29213 and *S. aureus* NRRL B-767.

The cell-wall diamino acid in the peptidoglycan of strain B1041^T was identified as LL-diaminopimelic acid and the whole-cell sugars found to include ribose, glucose, galactose and traces of rhamnose and an unknown sugar. The major polar lipids were identified as diphosphatidylglycerol, phosphatidylinositol, phosphoglycolipid, phosphatidylethanolamine and two unknown phospholipids (Fig. 2). This profile corresponds to phospholipid type 2 (Lechevalier et al. 1977) and similar profiles have been reported for several *Streptomyces* species. The major menaquinones found were identified as MK-9(H₈) (54 %) and MK-9(H₆) (34 %), along with MK-9(H₄) (6 %) and MK-9(H₂) (4 %). The major cellular fatty acids were identified as *anteiso* C_{15:0} (25.1 %), *iso* C_{16:0} (13.2 %) and *anteiso* C_{17:0} (12.1 %) (Table 2). The fatty acid profile of strain B1041^T is consistent with its affiliation to the

Table 1 Phenotypic properties of strain B1041^T and the type strains of the most closely related species

	1	2	3
Use of sole C sources 1.0 % (w/v)			
Sucrose	+	+	–
D-sorbitol	+	–	–
Xylitol	–	+	–
Use of sole N sources 0.1 % (w/v)			
L-tyrosine	+	–	+
L-phenylalanine	–	–	+
Degradation			
Xanthine	–	+	+
pH			
4.0	–	+	–
Temperature (°C)			
10 °C	–	+	–
NaCl % (w/v)			
8.0	–	–	+
API CORYNE results			
Nitrate reduction	–	+	–
Urea hydrolysis	+	–	–
Pyrazinecarboxamide	+	+	–
Pyroglutamic acid-β-naphthylamide	+	–	+
2-naphthyl-α-D-glucopyranoside	–	–	+
1-naphthyl-N-acetyl-β-D-glucosaminide	–	+	–
Gelatin	+	–	–
Catalase	+	–	–

Strains: 1, B1041^T, 2, *S. plumbiresistens* DSM 42067^T, 3, *S. pseudovenezuelae* DSM40212^T. All data were obtained in this study. All strains were positive for allantoin and arbutin hydrolysis, ability of growth at D-arabinose, L-arabinose, D-fructose, D-galactose, D-mannose, L-rhamnose, lactose, maltose, xylose as sole carbon sources 1.0 % (w/v), growth at pH:5.0–12.0, temperatures 28, 37 and 45 °C, and 0–5 % NaCl concentrations, presence of 2-naphthyl-phosphate and 2-naphthyl-β-D-galactopyranoside. But negative for ability of growth at D-ribose as sole carbon sources 1.0 % (w/v), growth at temperatures 4, 50 and 55 °C, and 9, 10, 15, 20 % and 30 % NaCl concentrations, and presence of naphthol-AS-BI-glucuronic acid

genus *Streptomyces* (Kroppenstedt 1985). The G+C content of the DNA was determined to be 70.4 mol%.

The 1,480 bp sequence determined for the 16S rRNA gene (GenBank accession number KC560729) of strain B1041^T was compared with sequences deposited in the public databases. The highest levels of sequence similarity were found with sequences belonging to members of the genus *Streptomyces*, namely *S. plumbiresistens* CCNWHX 13-160^T

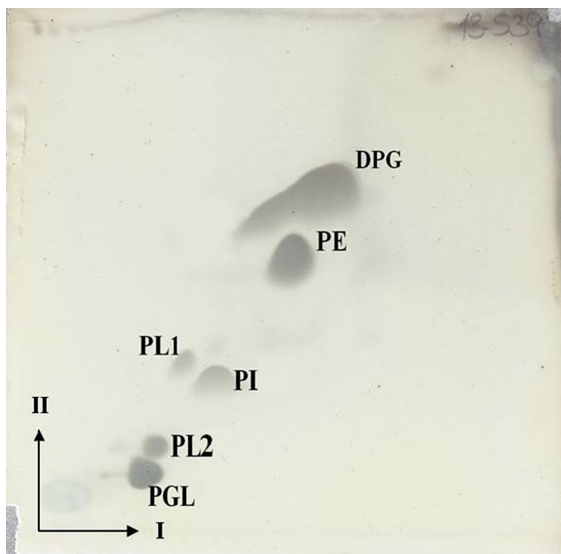


Fig. 2 Molybdophosphoric acid stained two-dimensional TLC of polar lipids from strain B1041^T. *DPG* diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PGL* phosphoglycolipid, *PL 1–2* unknown two phospholipids

(98.46 %), *S. pseudovenezuelae* NBRC 12904^T (97.81 %), *Streptomyces novaecaesareae* NBRC 13368^T (97.68 %), *Streptomyces graminifolii* JL-22^T (97.60 %), *Streptomyces phaeoluteigriseus* NRRL ISP-5182^T (97.58 %), *Streptomyces ciscaucasicus* NBRC 12872^T (97.53 %) and *Streptomyces pratensis* ch24^T (97.52 %). Sequence similarities with other strains of the genus *Streptomyces* were lower than 97.5 %. The phylogenetic tree based on the neighbour-joining algorithm demonstrated that strain B1041^T forms a distinct branch with *S. plumbiresistens* CCNWHX 13-160^T (Fig. 3; Supplementary Figs. S1 and S2).

As *Streptomyces* strains sharing more than 99.5 % 16S rRNA gene sequence similarities with their closest homologues have been reported among novel species (Bouchek-Mechiche et al. 2000; Dastager et al. 2007; Goodfellow et al. 2007; Nimaichand et al. 2012, 2013; Li et al. 2014), strain *S. plumbiresistens* DSM 42067^T was selected as the representative strain to perform DNA–DNA hybridization studies. The taxonomic integrity of the test strains were supported by DNA relatedness data. Strain B1041^T showed DNA relatedness values 48.8 ± 2.2 % to *S. plumbiresistens* DSM 42067^T (based on the average of duplicate determinations in 2X SSC and 10 % formamide at 70 °C), a value below the recommended criterion of

Table 2 Fatty acids profiles of strain B1041^T and *S. plumbiresistens*, the most closely related type strain of the genus *Streptomyces*

Fatty acids	1	2
Saturated		
C _{12:0}	0.5	0.4
C _{13:0}	0.3	–
C _{14:0}	2.0	1.3
C _{15:0}	3.3	3.5
C _{16:0}	11.2	31.3
C _{17:0}	0.5	0.8
C _{18:0}	1.3	0.2
Unsaturated		
C _{16:1 cis9}	7.0	4.0
C _{17:1 cis9}	1.2	0.5
C _{18:1 cis9}	0.6	0.3
Branched		
<i>iso</i> C _{12:0}	0.2	–
<i>iso</i> C _{13:0}	0.2	0.2
<i>iso</i> C _{14:0}	2.5	9.4
<i>iso</i> C _{15:0}	4.8	3.7
<i>iso</i> C _{16:0}	13.2	14.5
<i>iso</i> C _{16:1 H}	2.1	1.6
<i>iso</i> C _{17:0}	1.2	1.4
C _{16:0 9-methyl}	2.3	1.7
<i>anteiso</i> C _{11:0}	0.2	–
<i>anteiso</i> C _{13:0}	0.8	0.7
<i>anteiso</i> C _{15:0}	25.1	14.1
<i>anteiso</i> C _{17:0}	12.1	4.7
<i>anteiso</i> -C _{17:1 C}	5.7	4.5
C _{17:0 3OH}	0.2	–
C _{17:0 cyclo}	0.4	0.6
Summed feature 6	0.2	–
Summed feature 7	0.2	–

All data were obtained in this study

Summed Feature 6 as defined by MIDI comprises C_{18:2 cis 9, 12/anteiso-C_{18:0} or anteiso-C_{18:0/C_{18:2 cis 9, 12}}}

Summed Feature 7 as defined by MIDI comprises C_{18:1 trans 9/t6/c11 or C_{18:1 trans 6/t9/c11 or C_{18:1 cis 11/t9/t6}}}

80 % for species delineation of the genus *Streptomyces* (Labeda 1992).

On the basis of the genotypic and phenotypic data presented, it can be concluded that isolate B1041^T represents a novel species within the genus *Streptomyces*. It is, therefore, proposed that the organism be classified in the genus as *Streptomyces seymenliensis* sp. nov.

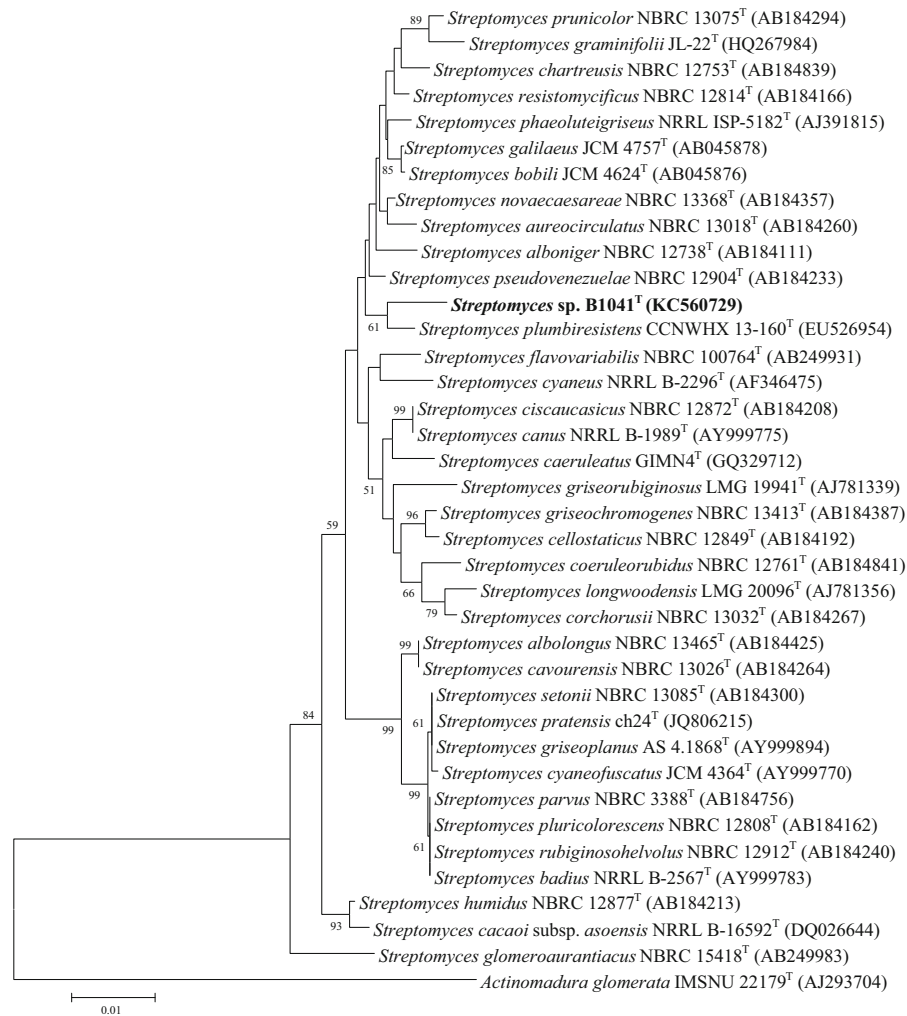


Fig. 3 Neighbour-joining tree (Saitou and Nei 1987) based on almost complete 16S rRNA gene sequences showing the position of strain B1041^T amongst its phylogenetic neighbours. *Actinomadura glomerata* IMSNU 22179^T was used as an out

group. Numbers at the nodes indicate the levels of bootstrap support (%); only values ≥ 50 % are shown. GenBank accession numbers are given in parentheses. Bar 0.01 substitutions per site

Description of *Streptomyces seymenliensis* sp. nov

Streptomyces seymenliensis (sey.men.li.en'sis. N.L. masc. adj. *seymenliensis* of or belonging to Seymenli, Şereflikoçhisar, Ankara, Turkey, from where the type strain was isolated).

Aerobic, Gram-stain positive, non-motile, non-acid-alcohol-fast actinomycete which forms a branched substrate mycelium and aerial hyphae which differentiates into spiral chains of spiny-surfaced spores. Growth occurs at 28–45 °C, pH 5.0–12, and 0–5 % (w/v) NaCl, but not at temperatures of 4, 10, 50

and 55 °C, pH 4.0 and 8–10, and 15, 20 and 30 % (w/v) NaCl. Arbutin, allantoin and urea are hydrolysed. Nitrate reduction is negative. Gelatin is degraded but not xanthine. Utilizes D-arabinose, L-arabinose, D-fructose, D-sorbitol, D-galactose, D-mannose, L-rhamnose, lactose, maltose, sucrose and xylose as sole carbon sources but not D-ribose and xylitol. Utilizes L-tyrosine as sole nitrogen source but not L-phenylalanine. In API CORYNE strips, positive for pyrazine-carboxamide, pyroglutamic acid- β -naphthylamide 2-naphthyl-phosphate, 2-naphthyl- β -D-galactopyranoside and negative for naphthol-AS-BI-glucuronic

acid, 2-naphthyl- α -D-glucopyranoside, 1-naphthyl-N-acetyl- β -D-glucosaminide and glycogen. The predominant menaquinones are MK-9(H₈) and MK-9(H₆). The major fatty acids are *anteiso* C_{15:0}, *iso* C_{16:0} and *anteiso* C_{17:0}. The polar lipid profile contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphoglycolipid and two unknown phospholipids. The G+C content of the genomic DNA of the type strain is 70.4 mol%.

The type strain, B1041^T (=KCTC 29245^T = DSM 42117^T), was isolated from soil samples collected from Tuz Lake, Seymenli village, Şereflikoçhisar, Ankara, Turkey. The GenBank accession number for the 16S rRNA gene sequence of *S. seymenliensis* B1041^T (=KCTC 29245^T = DSM 42117^T) is KC560729.

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