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ORIGINAL PAPER

Saccharopolyspora hattusasensis sp. nov., isolated from soil

Aysel Veyisoglu · **Hayrettin Saygin** · **Demet Tatar** · **Kadriye Inan Bektas** ·
Nevzat Sahin

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Abstract A *Saccharopolyspora* strain, designated CR3506^T, isolated from a soil sample collected from Sungurlu, Corum, Turkey, was examined using a polyphasic approach. Phylogenetic analysis based on an almost-complete 16S rRNA gene sequence analysis showed that the strain is closely related to the type strains of *Saccharopolyspora spinosa* NRRL 18395^T (99.1%), *Saccharopolyspora phatthalungensis* NRRL B-24798^T (98.4%) and *Saccharopolyspora shandongensis* 88^T (98.1%); low levels of DNA–DNA relatedness were found between the isolate and *S. spinosa* and *S. phatthalungensis* (<50%). Strain CR3506^T was found to have chemotaxonomic and phylogenetic properties consistent with its classification in the genus *Saccharopolyspora.* The strain contained *meso*-diaminopimelic acid as the diagnostic diamino acid. Whole-cell hydrolysates contained arabinose and galactose. The polar lipids were identified as phosphatidylmethylethanolamine, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol. The predominant menaquinones (>10%) were MK-9(H_4) and MK-8(H_4). Major fatty acids were (>10%) *iso*-$C_{16:0}$, $C_{15:0}$3OH, $C_{18:0}$ and *iso*-$C_{15:0}$. Further, the morphological, physiological and biochemical characteristics of strain CR3506^T are distinct from *S. spinosa* and other species of the genus *Saccharopolyspora* with which this strain has high 16S rRNA gene sequence similarity (98.0–98.5%). Strain CR3506^T has antimicrobial activity against *Bacillus subtilis* NRRL B-209, *Citrobacter freundi* NRRL B-2643 and *Staphylococcus aureus* ATCC 29213. Consequently, it is proposed that strain CR3506^T

The GenBank accession number for the 16S rRNA gene sequence of Saccharopolyspora hattusasensis CR3506T (=KCTC 29104T = DSM 45715T) is JN989298.

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A. Veyisoglu
Department of Bioengineering, Faculty of Engineering and Architecture, Sinop University, 57000 Sinop, Turkey

H. Saygin · N. Sahin (✉)
Department of Biology, Faculty of Art and Science, Ondokuz Mayis University, 55139 Samsun, Turkey
e-mail: nsahin@omu.edu.tr

D. Tatar
Department of Medical Documentation and Secretary, Osmancık Omer Derindere Vocational Higher School, Hitit University, 19500 Corum, Turkey

K. I. Bektas
Department of Molecular Biology and Genetics, Faculty of Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey

represents a novel *Saccharopolyspora* species for which the name *Saccharopolyspora hattusasensis* sp. nov. is proposed. The type strain is CR3506^T (=KCTC 29104^T = DSM 45715^T).

Keywords *Actinobacteria* · *Saccharopolyspora hattusasensis* sp. nov. · Polyphasic taxonomy · 16S rRNA gene

Introduction

The genus *Saccharopolyspora* was first established by Lacey and Goodfellow (1975) with the description of *Saccharopolyspora hirsuta* as the type species, and it was emended by Warwick et al. (1994). The genus *Saccharopolyspora*, which is a member of the family *Pseudonocardiaceae* (Embley et al. 1988; Stackebrandt et al. 1997; Zhi et al. 2009), currently encompasses 30 validly named taxa (http://www.bacterio.net/saccharopolyspora.html). Members of the genus can be distinguished chemotaxonomically and morphologically, notably by their ability to form aerobic, Gram-positive, non-acid-fast cells which produce extensively branched substrate mycelium that may fragment into rod-shaped elements, and aerial mycelium that may form non-motile, bead-like chains of spores. The cell wall composition is chemotype IV with *meso*-diaminopimelic acid, arabinose and galactose as the characteristic sugars in whole cell hydrolysates, and also *iso*- and *anteiso*-branched fatty acids. The predominant menaquinone type is MK-9(H_4), and mycolic acids are absent (Embley et al. 1987; Goodfellow et al. 1989). The primary reservoir of *Saccharopolyspora* is soil (Zhou et al. 1998; Lu et al. 2001; Yuan et al. 2008). In addition, a strain was isolated from a patient with bronchial carcinoma (Yassin 2009), two species have been isolated from fodder (Goodfellow et al. 1989), an endophytic species was isolated from a coastal salt marsh plant of *Dendranthema indicum* (Linn.) (Zhang et al. 2013), a halophilic species was isolated from a hypersaline lake, Lop Nor Lake, in China (Lv et al. 2014), one strain was isolated from the Swallow Cave in China (Cheng et al. 2013), a strain was isolated from the Dead Sea (Jiang et al. 2016), and a thermophilic species isolated from soil of a sugar cane field (Wu et al. 2016). Members of the genus *Saccharopolyspora* have the ability to produce different bioactive secondary metabolites (Fabre et al. 1993; Kim et al. 2010; Sun et al. 2017). The present polyphasic study was designed to establish the taxonomic status of a putatively novel *Saccharopolyspora* strain isolated from a soil sample collected from Sungurlu, Corum, Turkey. The resultant data showed that isolate CR3506^T represents a new *Saccharopolyspora* species for which the name *Saccharopolyspora hattusasensis* is proposed.

Materials and methods

Isolation and maintenance of strains

Strain CR3506^T was isolated from an arid soil sample collected from Sungurlu, Corum (GPS coordinates for the sampling site are 40°08′18.23″N and 34°22′37.74″E), Turkey. The soil sample was taken from the top layer, dried at room temperature and suspended in 1/4 strength Ringer's solutions (Oxoid) to prepare 10^{-1} dilution. The suspension was heated at 60 °C for 20 min prior to preparing 10^{-2} and 10^{-3} dilutions and aliquots (200 µl) of the dilutions were spread on Stevenson's Medium No. 3 [20 g casamino acids, 20 g soluble starch, 4 g yeast extract, 15 g agar, pH 7.2–7.4 per litre distilled water] (Tan et al. 2006) supplemented with filter sterilised cycloheximide (50 µg/ml), nalidixic acid (10 µg/ml), neomycin sulphate (50 µg/ml), novobiocin (10 µg/ml), and nystatin (50 µg/ml). After 3 weeks incubation at 28 °C, the isolate was purified on yeast extract-malt extract agar [International *Streptomyces* Project medium 2 [ISP 2]; (Shirling and Gottlieb 1966)] slopes at 4 °C and preserved as suspensions of mycelial fragments and spores in glycerol (20%, v/v) at −20 and −80 °C. *Saccharopolyspora spinosa* DSM 44228^T was obtained from DSMZ (German Collection of Microorganisms and Cell Cultures) and *Saccharopolyspora phatthalungensis* NRRL B-24798^T was obtained from ARS Culture Collection (United States Department of Agriculture).

Morphological, cultural and physiological characteristics

Cultural characteristics were determined after 14 days of incubation at 28 °C on Czapek's agar (Waksman 1967), ISP media 2-7 (Shirling and Gottlieb 1966), modified Bennett's agar (MBA; Jones 1949), nutrient agar (Waksman 1961) and tryptic soy agar (TSA; Difco). The colours of aerial and substrate mycelia and production of any diffusible pigments were observed by comparison with the ISCC-NBS colour charts (Kelly 1964). Motility test was performed by according to the method of Tittsler and Sandholzer (1936). The micromorphological properties of the strain were determined by examining gold coated dehydrated specimens of a 21-day culture grown on ISP 3 agar using a JEOL JSM 6060 instrument. Growth at various temperatures (4, 10, 20, 28, 30, 37, 40, 45, 50 and 55 °C) was determined on yeast extract-malt extract (ISP 2) agar after incubation for 14 days at pH 7.2, NaCl tolerance against 1–10% NaCl (w/v) (at intervals of 1.0 NaCl unit) at 30 °C for 14 days and pH tolerance at pH 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 11.0 and 12.0 at 30 °C. In order to maintain pH values of the media, KH_2PO_4/HCl, KH_2PO_4/K_2HPO_4 and K_2HPO_4/NaOH buffer systems were used. Degradation tests for Tweens 40 and 80 were determined by following the methods described by Nash and Krent (1991). The other degradation tests were carried out as described by Williams et al. (1983). Carbon-source utilization was determined according to the methods described by Shirling and Gottlieb (1966). Utilization of amino acids as sole nitrogen sources was determined according to Williams et al. (1983) using a final concentration of 0.1% (w/v) of each nitrogen source. Testing with the commercial system API ZYM (bioMérieux) was performed according to the manufacturer's instructions. Antimicrobial activity of strain CR3506^T to inhibit the growth of 12 microorganisms, including Gram-positive and Gram-negative bacteria as well as fungi, was observed using an overlay technique according to the procedures described by Williams et al. (1983). Spot-inoculated colonies on modified Bennett's agar plates were inverted over 2 ml chloroform for 40 min. Killed colonies were overlaid with 5–7 ml sloppy modified Bennett's broth inoculated with the test organisms. Zones of inhibition were scored as positive results after 24 h incubation at 37 °C.

Chemotaxonomic characterization

Biomass for chemotaxonomic analyses of the isolate was obtained from cultures grown in ISP 2 broth for 2 weeks at 30 °C and 150 rpm, and washed twice with distilled water and freeze-dried. The isomers of diaminopimelic acid and whole-cell sugars were prepared according to Lechevalier and Lechevalier (1970) and analysed by thin layer chromatography (Staneck and Roberts 1974). Polar lipids were extracted and analysed following Minnikin et al. (1984) using the modifications of Kroppenstedt and Goodfellow (2006). Isoprenoid quinones were extracted and purified using the method of Collins et al. (1977) and analysed by HPLC (Kroppenstedt 1982). Cellular fatty acids were extracted, methylated and separated by gas chromatography using an Agilent Technologies 6890 N instrument, fitted with an autosampler and a 6783 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 the TSBA 5.0 database.

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

Genomic DNA extraction, PCR-mediated amplification and 16S rRNA gene sequencing were carried out using established procedures (Chun and Goodfellow 1995) using an ABI PRISM 3730 XL automatic sequencer. The resultant 16S rRNA gene sequence was aligned with corresponding sequences of representative type strains of the genus *Saccharopolyspora* retrieved from the EzBioCloud server (Yoon et al. 2016) using CLUSTAL W in MEGA version 6.0 software (Tamura et al. 2013). Phylogenetic trees were constructed with neighbour-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Kluge and Farris 1969) algorithms using MEGA version 6.0 software. Evolutionary distances were calculated using Kimura two-parameter (Kimura 1980) and topologies of the resultant trees evaluated by bootstrap analysis (Felsenstein 1985) based on 1000 resamplings. *Thermomonospora alba* JCM 3077^T GenBank accession number (AF002260) was used as the outgroup.

DNA base composition and DNA–DNA hybridization

For determination of the DNA G+C content, genomic DNA of strain CR3506^T was extracted and purified according to the method of Marmur (1961), and analysed

Table 1 Differential characteristics of strain CR3506^{T} and type strains of closely related species of the genus *Saccharopolyspora*

Characteristics	1	2	3	4
Spore arrangement	Irregular spirals	Hooks and open loops[b]	Hooks and open loops[a]	Spiral[b]
Spore surface ornamentation	Smooth or irregularly rough	Spiny[b]	Spiny[a]	Spiny[b]
NaCI tolerance (%, w/v)	<7	<11	<7	<7
pH tolerance	5.0–10.0	5.0–10.0	5.0–9.0	5.0–9.0
Temperature range (°C)	28–37	15–37	18–42	15–38
Lysozyme resistance (0.005%)	No growth	No growth	No growth	Growth
Biochemical tests				
Aesculin hydrolysis	–	–	+	+
Arbutin hydrolysis	–	+	–	+
Allantoin hydrolysis	+	+	–	–
Nitrate reduction	–	+	–	+
H_2S production	–	+	+	+
Degradation of (%, w/v)				
Elastin (0.3%)	+	+	–	+
L-Tyrosine (0.5%)	–	–	+	+
Hypoxanthine (0.4%)	–	–	+	+
Starch (1%)	+	–	–	+
Carbon source utilization (1.0%, w/v)				
Adonitol	–	+	+	+
D-Cellobiose	+	–	–	+
D-Galactose	+	–	+	–
D-Mannose	–	+	+	+
Dextrin	–	–	–	+
Inuline	–	+	+	–
Lactose	+	–	–	–
Maltose	–	–	–	+
Sucrose	–	–	–	+
API ZYM				
Esterase (C4)	–	+	ND	ND
Esterase lipase (C8)	–	+	ND	ND
Valine arylamidase	–	+	ND	ND
Major menaquinones (%)	MK-9(H_4), MK-8(H_4)	MK-9(H_4), MK-9(H_6)[c]	MK-9(H_4)[a]	MK-9(H_4)[b]
Polar lipid profile	DPG, PME, PE, PG, PC, PI, 6PL	DPG, PC[c]	PC, PG, PI[a]	PC[b]
Major fatty acids (%)	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{15:0}$ 3OH, $C_{18:0}$	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{15:0}$ 3OH, $C_{18:0}$	*iso*-$C_{16:0}$, $C_{17:0}$ 10-*methyl*	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *anteiso*-$C_{17:0}$, $C_{17:1}w8c$[b]
Whole-cell sugars	ara, gal	ara, gal[c]	ara, gal[a]	ara, gal[b]

Strains: 1 CR3506^{T}; 2 *S. spinosa* DSM 44228^{T}; 3 *S. phatthalungensis* NRRL B-24798^{T}; 4 *S. shandongensis* CGMCC 4.3530^{T}. Symbols: +, positive; −, negative

ND not determined, *DPG* diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PME* phosphatidylmethylethanolamine, *PC* phosphatidylcholine, *PL* unknown phospholipid, *ara* arabinose, *gal* galactose

[a] Duangmal et al. (2010)

[b] Zhang et al. (2008)

[c] Mertz and Yao (1990)

using thermal denaturation (T*m*) method described by Mandel and Marmur (1968). *Escherichia coli* JM109 DNA was used as the reference. DNA–DNA relatedness values were determined between strain CR3506^{T} and its near phylogenetic neighbours, namely *S. spinosa* DSM 44228^{T} and *S. phatthalungensis* NRRL B-24798^{T}.

DNA–DNA hybridization was performed spectrophotometrically by DNA reassociation kinetics as described by De Ley et al. (1970) under consideration of the modifications described by Huss et al. (1983). The experiments were done in triplicate in 2× SSC buffer and 10% formamide at 70 °C.

Results and discussion

Strain CR3506^T showed good growth on ISP 2, ISP 5, ISP 6, ISP 7, modified Bennett's, nutrient and Trypticase soy agar media, and weak growth on ISP 3, ISP 4 and Czapek's agar media. The colour of the aerial mycelia was white on ISP 2 and ISP 7 media. Aerial mycelia appeared like a spider web that differentiated into oval to round, non-motile, smooth surfaced spores (Fig. S1). Extensively branched substrate mycelium was orange–yellow on all tested media. No soluble pigments were produced on any of the tested media. The strain showed mesophilic growth with a temperature range between 28 and 37 °C and optimum at 30 °C. Strain CR3506^T was observed to grow in pH range 5.0–10.0 and tolerate NaCl up to 7% (w/v). The strain showed optimum growth at pH 8.0 and 0–2% NaCl. Strain CR3506^T was found to exhibit antimicrobial activity against *Bacillus subtilis* NRRL B-209, *Citrobacter freundi* NRRL B-2643 and *Staphylococcus aureus* ATCC 29213 but not against *Aspergillus parasiticus* NRRL 465, *E. coli* ATCC 25922, *Micrococcus luteus* NRRL B-1013, *Pseudomonas aeroginosa* NRRL B-2679, *Enterobacter aerogenes* NRRL B-356, *Aspergillus flavus* NRRL 1957, *Candida albicans* ATCC 10231, *Pseudomonas vulgaris* NRRL B-123 and *Bacillus licheniformis* NRRL B-1001. Detailed physiological and biochemical properties are given in the species description and in Table 1.

The assignment of strain CR3506^T to the genus *Saccharopolyspora* was supported by chemotaxonomic properties (Korn-Wendisch et al. 1989). It contained *meso*-diaminopimelic acid in the peptidoglycan, arabinose and galactose in whole-cell hydrolysates, and MK-9(H_4) (66%) and MK-8(H_4) (18%) as the main menaquinones. Polar lipid analysis showed that strain CR3506^T contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylmethylethanolamine, phosphatidylcholine, phosphatidylglycerol and six unidentified polar lipids (Fig. S2). Major cellular fatty acids ($\geq$10%) were *iso* $C_{16:0}$ (21.0%), $C_{15:0}$ 3OH (17.7%), $C_{18:0}$ (16.7%) and *iso* $C_{15:0}$ (12.0%) (Table S1). No mycolic acids were detected. The DNA G+C content of strain CR3506^T was found to be 71.7 ± 0.8 mol%.

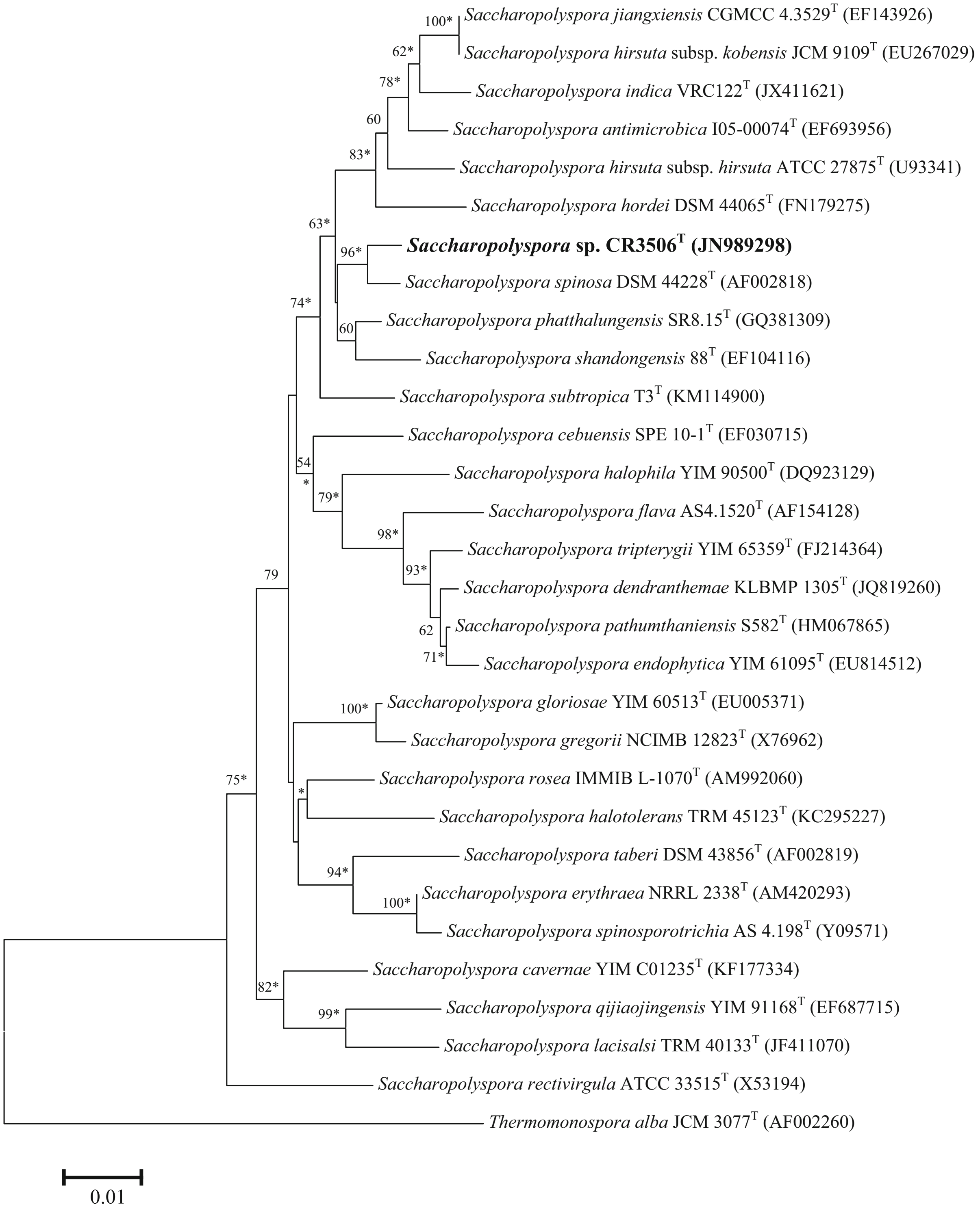
Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain CR3506^T forms a distinct cluster with members of the genus *Saccharopolyspora* (Fig. 1), and a high level of 16S rRNA gene sequence similarity was found between strain CR3506^T and its near phylogenetic neighbour, *S. spinosa* DSM 44228^T. These strains shared a 16S rRNA gene sequence similarity of 99.1%, a value which corresponded to 13 nucleotide (nt) differences at 1444 locations. The corresponding results between strain CR3506^T and *S. phatthalungensis* NRRL B-24798^T and *S. shandongensis* 88^T were 98.4% (23 nt differences at 1444 sites) and 98.1% (27 nt differences at 1417 sites), respectively. Sequence similarities with strains of other species of the genus *Saccharopolyspora* were lower than 97.8%. DNA–DNA relatedness values between strain CR3506^T and the type strains of *S. spinosa* and *S. phatthalungensis* were found to be 53.4 ± 4.4% and 41.7 ± 3.5%, respectively, values well below the cut-off value of 70% recommended for assigning bacterial strains to the same genomic species (Wayne et al. 1987).

It was evident on the basis of genotypic and phenotypic properties that strain CR3506^T can be distinguished from its close phylogenetic neighbours, *S. spinosa* and *S. phatthalungensis*. Therefore, it is proposed that strain CR3506^T be classified in the genus *Saccharopolyspora* as *Saccharopolyspora hattusasensis* sp. nov. The Digital Protologue database TaxonNumber for strain CR3506^T is TA00071.

Description of *Saccharopolyspora hattusasensis* sp. nov.

Saccharopolyspora hattusasensis (hat.tu.sa.sen'sis. N.L. fem. adj. *hattusasensis*, belonging to Hattusas, the Hittite Capital, northern Anatolia, located in the Boğazkale and Sungurlu District of Çorum Province, from where the type strain was isolated).

Aerobic, Gram-stain positive, non-acid-fast, non-motile actinomycete that forms an extensively branched moderate brown substrate mycelia. The aerial mycelium is white, and forms smooth-surfaced spores with irregular spirals. Good growth occurs on ISP 2, ISP 5, ISP 6, ISP 7, nutrient, TSA (tripticate soy agar) and modified Bennett's, weak growth on ISP3, ISP4 and


100*
Saccharopolyspora jiangxiensis CGMCC 4.3529^T (EF143926)
62*
Saccharopolyspora hirsuta subsp. *kobensis* JCM 9109^T (EU267029)
78*
Saccharopolyspora indica VRC122^T (JX411621)
60
Saccharopolyspora antimicrobica I05-00074^T (EF693956)
83*
Saccharopolyspora hirsuta subsp. *hirsuta* ATCC 27875^T (U93341)
Saccharopolyspora hordei DSM 44065^T (FN179275)
63*
96*
Saccharopolyspora sp. CR3506^T (JN989298)
Saccharopolyspora spinosa DSM 44228^T (AF002818)
74*
60
Saccharopolyspora phatthalungensis SR8.15^T (GQ381309)
Saccharopolyspora shandongensis 88^T (EF104116)
Saccharopolyspora subtropica T3^T (KM114900)
Saccharopolyspora cebuensis SPE 10-1^T (EF030715)
54
*
Saccharopolyspora halophila YIM 90500^T (DQ923129)
79*
Saccharopolyspora flava AS4.1520^T (AF154128)
98*
Saccharopolyspora tripterygii YIM 65359^T (FJ214364)
93*
79
Saccharopolyspora dendranthemae KLBMP 1305^T (JQ819260)
62
Saccharopolyspora pathumthaniensis S582^T (HM067865)
71*
Saccharopolyspora endophytica YIM 61095^T (EU814512)
100*
Saccharopolyspora gloriosae YIM 60513^T (EU005371)
Saccharopolyspora gregorii NCIMB 12823^T (X76962)
75*
Saccharopolyspora rosea IMMIB L-1070^T (AM992060)
*
Saccharopolyspora halotolerans TRM 45123^T (KC295227)
Saccharopolyspora taberi DSM 43856^T (AF002819)
94*
Saccharopolyspora erythraea NRRL 2338^T (AM420293)
100*
Saccharopolyspora spinosporotrichia AS 4.198^T (Y09571)
Saccharopolyspora cavernae YIM C01235^T (KF177334)
82*
Saccharopolyspora qijiaojingensis YIM 91168^T (EF687715)
99*
Saccharopolyspora lacisalsi TRM 40133^T (JF411070)
Saccharopolyspora rectivirgula ATCC 33515^T (X53194)
Thermomonospora alba JCM 3077^T (AF002260)
0.01

◀**Fig. 1** Neighbour-joining tree based on almost complete 16S rRNA gene sequences showing the position of strain CR3506^T within the *Saccharopolyspora* gene tree. *Asterisks* indicate branches of the tree that were also recovered using the maximum-likelihood and maximum-parsimony tree-making algorithms. *Numbers* at the nodes indicate levels of bootstrap support (%); only values ≥50% are shown. *Thermomonospora alba* JCM 3077^T (AF002260) was used as the outgroup. GenBank accession numbers are given in *parentheses*. *Bar* 0.01 substitutions per site

Czapek's media. No diffusible pigment is observed on any of the media tested. Melanoid pigments are not produced on ISP 6 or ISP 7 media. Growth occurs at 28–37 °C with an optimum temperature range of 30 °C, and at pH 5.0–10.0 (optimum 8.0) and with 0–7% (w/v) NaCI (optimum 0–2%). Allantoin is hydrolysed, but aesculin, arbutin and urea are not. Negative for the production of H_2S and for nitrate reduction. Elastin and starch are degraded, but guanine and hypoxanthine are not. D-cellobiose, D-galactose, D-mannitol and lactose are utilized as sole carbon and energy sources, but not adonitol, L-arabinose, D-sorbitol, D-melezitose, D-mannose, dextrin, inuline, L-rhamnose, maltose or sucrose. Utilizes alpha-isoleucine, L-alanine, L-arginine, L-proline, L-serine and L-tyrosine as sole nitrogen sources, but not glycine, L-cysteine, L-phenylalanine and L-valine. Positive for acid phosphatase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl-*β*-glucosaminidase and α-mannosidase; negative for alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, *β*-galactosidase (ONPG), *β*-glucuronidase, α-glucosidase, *β*-glucosidase and α-fucosidase in the API ZYM system. MK-9(H_4) and MK-8(H_4) are the major menaquinones. The phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylmethylethanolamine, phosphatidylcholine and six unidentified phospholipids. The whole-cell sugars are arabinose and galactose. Major fatty acids are *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{15:0}$ 3OH, $C_{18:0}$. The G+C content of the genomic DNA of the type strain is 71.7 ± 0.8 mol%.

The type strain, CR3506^T (=DSM 45715^T = KCTC 29104^T) was isolated from soil of Corum, Turkey.

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Conflict of interest The authors declare that they have no conflict of interest.

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