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ORIGINAL PAPER

Saccharopolyspora griseoalba sp. nov., a novel actinomycete isolated from the Dead Sea

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Abstract A novel halotolerant actinomycete, designated strain AFM 10238^T, was isolated from a sediment sample collected from the Dead Sea of Israel. The isolate grew at 15–45 °C, pH 6–12 and with 0–15 % (w/v) NaCl. Strain AFM 10238^T contains *meso*-diaminopimelic acid as cell wall diamino acid, and galactose and arabinose as the whole cell sugars. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, and diphosphatidylglycerol. Major fatty acids are iso-$C_{16:0}$, iso-$C_{17:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$ and $C_{17:1}\omega 8c$. MK-9(H_4) is the predominant menaquinone and the DNA G + C content is 72.7 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain AFM10238^T belongs to the genus *Saccharopolyspora*. The 16S rRNA gene sequence similarity between strain AFM 10238^T and its close neighbours, *Saccharopolyspora halophila* YIM 90500^T, *Saccharopolyspora spinosa* DSM 44228^T, *Saccharopolyspora dendranthemae* KLBMP 1305^T and *Saccharopolyspora cebuensis* DSM 45019^T were 98.2, 97.2, 97.1 and 97.0 %, respectively. Sequence similarities to other type strains of this genus were below 97 %. DNA–DNA relatedness data, together with phenotypic and chemotaxonomic differences, clearly distinguished the isolate from its close neighbours. On the basis of the data from this polyphasic analysis, a novel species *Saccharopolyspora griseoalba* sp. nov. is proposed. The type strain is AFM 10238^T (= DSM 46,663 = CGMCC 4.7124).

Keywords Halotolerant actinomycete · *Saccharopolyspora griseoalba* sp. nov. · *Saccharopolyspora* · Polyphasic taxonomy

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Introduction

The genus *Saccharopolyspora* was first described by Lacey and Goodfellow (1975) and was assigned to the family *Pseudonocardiaceae* (Warwick et al. 1994). Members of the genus *Saccharopolyspora* are aerobic, Gram-positive, non-acid-fast actinomycetes. The

substrate hyphae fragment into rod-shaped elements and the aerial hyphae segment into bead-like chains of spores. They contain *meso*-diaminopimelic acid, arabinose and galactose in the cell wall and MK-9(H_4) as the predominant menaquinone. The DNA G + C content is in the range 66–77 mol% (Goodfellow et al. 1989). At the time of writing, the genus comprises 26 species with validly published names(LPSN; http://www.bacterio.net). During a study on the halophilic and halotolerant actinomycetes from a sediment sample collected from the Dead Sea of Israel, a halotolerant actinomycete, designated strain AFM 10238^T, was isolated. Based on the polyphasic taxonomic data, this strain is shown to represent a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora griseoalba* sp. nov. is proposed.

Materials and methods

Organisms

Strain AFM 10238^T was isolated from a sediment sample collected from the Dead Sea in Israel, by using CMKA medium containg 15 % multi-salts, incubated at 28 °C for 30 days. This CMKA medium contained (per liter) 0.5 g Casein hydrolysate, 1.5 g mannitol, 1 g KNO_3, 2 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, 0.5 g $CaCO_3$, 20 g agar. The multi-salts comprised of 49 % (w/w) $MgCl_2{\cdot}6H_2O$, 32 % (w/w) NaCl, 14 % (w/w) $CaCl_2$ and 5 % (w/w) KCl. The strain was maintained on modified PDA (200 g potato, 2.5 g glucose, 18 g agar, 1 L distilled) medium slants containing 15 % (w/v) multi-salts at 4 °C and as suspensions of mycelia fragments in glycerol (20 %, v/v) at −20 °C.

Phenotypic characteristics

Morphological characteristics of strain AFM 10238^T were observed by light microscopy (B5; Motic) and scanning electron microscopy (S-4800; Hitachi) after 28 days incubation on modified PDA medium containing 15 % (w/v) multi-salts at 28 °C. Cultural characteristics were determined after incubation for 2–3 weeks on Czapek's agar (Waksman 1967), nutrient agar, PDA and ISP2-5 (Shirling and Gottlieb 1966) supplemented with 15 % (w/v) multi-salts at 28 °C. The colours of substrate and aerial mycelia and any soluble pigments were determined by comparison with chips from the ISCC-NBS colour charts (Kelly 1964). Growth was tested over a range of temperature (10–55 °C) and pH values (3.0–12.0) as described by Xu et al. (2005) in peptone water medium supplemented with 15 % (w/v) multi-salts, respectively. Tolerance to different NaCl concentrations (0–35 %, w/v) were tested on CMKA medium. Gram staining was carried out by using the standard Gram reaction (Murray et al. 1994). Utilization of carbon and nitrogen sources were examined by using the medium of Smibert and Krieg (1994) supplemented with various substrates and 15 % multi-salts. Catalase activity was determined by assessing bubble production with 3 % (v/v) H_2O_2, according to the methods used by Smibert and Krieg (1994). Oxidase activity was determined by using the Oxidase Reagent (bioMérieux) according to the manufacturer's instructions. Other physiological tests were carried out as described by Gordon et al. (1974).

Chemotaxonomy

Biomass for chemical and molecular analysis was obtained by growth on modified PDA medium at 28 °C for 10 days. Whole cell sugars and cell wall amino acids were detected by HPLC after precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP) (Tang et al. 2009a). Polar lipids were analyzed according to the methods of Minnikin et al. (1984). Menaquinones were isolated according to (Collins 1994) and were analyzed by HPLC (Kroppenstedt 1982). Biomass for fatty acid analysis of strain AFM 10238^T was obtained after growth on Tryptic Soy Agar (Becton–Dickinson and Company USA) at 28 °C for 10 days. Extraction and analysis of fatty acids were performed as described by Sasser (1990) by using the Microbial Identification System (MIDI) (Sherlock version 6.1; MIDIdatabase TSB A6).

Phylogeny

Extraction of chromosomal DNA, PCR amplification of 16S rRNA gene and sequencing of the purified products were carried out as described by Li et al. (2007). The 16S rRNA gene sequence of strain AFM 10238^T was aligned manually with reference strains retrieved from DDBJ/EMBL/GenBank via the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/;

Kim et al. 2012). Multiple alignments with sequences of the closely related *Saccharopolyspora* species were carried out by using the program CLUSTAL X (Thompson et al. 1997). Phylogenetic trees were constructed using MEGA version 5.0 (Tamura et al. 2011) with the neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971) and maximum-likelihood (Felsenstein 1981) algorithms respectively. The topologies of the phylogenetic trees were evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The G + C content of the DNA was determined according to the method of Marmur (1961) and was determined by reversed-phase HPLC of nucleosides according to Mesbah et al. (1989). DNA–DNA hybridization experiments were performed according to the method described by Ezaki et al. (1989) and He et al. (2005).

Results and discussion

Strain AFM 10238^T was found to be an aerobic, Gram-positive filamentous actinomycete. The substrate mycelia were well developed, and fragmented into rod shaped elements. Aerial mycelia developed well with long spore chains (Fig. 1). The spore chains were straight or flexuous. The spores were non-motile, smooth-surfaced, oval or sphere-shaped. The growth of the isolate AFM 10238^T was good on PDA, nutrient agar, ISP 2, ISP 3, and ISP 5, weak on Czapek's agar and ISP 4. The aerial mycelia were light gray on PDA, nutrient agar, ISP 2, ISP 3 and ISP 4, white on czapek's agar and ISP 5. The substrate mycelia were light yellow on PDA, yellow white on nutrient agar and czapek's agar, yellow on ISP 2, white on ISP 3, ISP 4 and ISP 5. No soluble pigment was produced. Temperature and pH ranges for strain AFM 10238^T growth were 15–45 °C and pH 6.0–12.0, with optimal at 28 °C and pH 7.5. The NaCl concentration range for growth was 0–15 %, with optimal growth occurring at 10 %. Other detailed physiological and biochemical characteristics that differentiate the strain AFM 10238^T from recognized *Saccharopolyspora* species are summarized in Table 1 and also given in the species description.

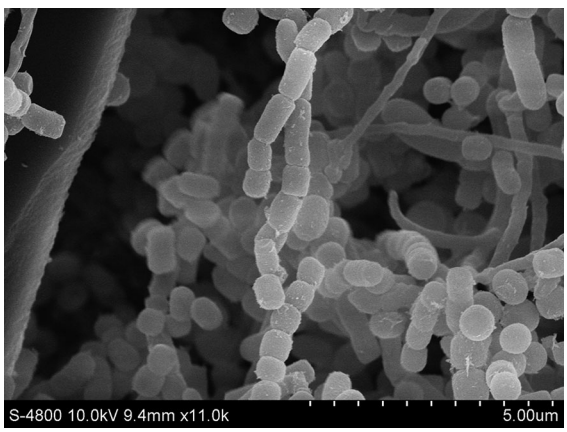

Fig. 1 Scanning electron micrograph of strain AFM 10238^T grown on PDA medium for 28 days at 28 °C

Strain AFM 10238^T contained meso-diaminopimelic acid (meso-DAP) as the cell wall amino acids, with galactose and arabinose as the whole cell sugars. The predominant menaquinone was MK-9(H_4) (75.3 %), and minor amounts of MK-9(H_6) (14.2 %), MK-8(H_4) (5.4 %) and MK-9(H_8) (5.1 %) were detected. The polar lipids were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC) and two unidentified phospholipids (PLs) (Supplementary Fig. S1). Major fatty acids were iso-$C_{16:0}$ (25.5 %), anteiso-$C_{17:0}$ (18.1 %), iso-$C_{17:0}$ (11.4 %), $C_{17:1}\omega 8c$ (11.3 %) and iso-$C_{15:0}$ (10.3 %). The chemotaxonomic date for strain AFM10238^T are consistent with its assignment to the genus *Saccharopolyspore* (Lacey and Goodfellow 1975; Korn-Wendisch et al. 1989).

The almost-complete 16S rRNA gene sequence (1514 bp) of the isolate was determined. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain AFM 10238^T belongs to the genus *Saccharopolyspora*. In the phylogenetic tree based on the neighbour-joining algorithm, strain AFM 10238^T formed a distinct clade with *S. halophila* YIM 90500^T, and the two strains shared a branch with a bootstrap value of 93 % (Fig. 2). Topologies of phylogenetic trees built using the maximum-likelihood and maximum-parsimony algorithms were similar to that of the tree constructed by neighbour-joining analysis. The 16S rRNA gene sequence similarity between strain AFM 10238^T and its close neighbor, *S. halophila* YIM 90500^T, *S. spinosa* DSM 44228^T, *S. dendranthemae* KLBMP 1305^T and *S. cebuensis* DSM 45019^T were 98.24, 97.23, 97.07 and 97.02 %, respectively. The sequence similarity with other *Saccharopolyspora* species was lower than 97 %. It has been suggested

Table 1 Differential phenotypic and chemotaxonomic characteristics between stain AFM 10238^T and its closest neighbours of the genus *Saccharopolyspora*

Characteristic	AFM 10238^T	*S. halophila*YIM 90500^T	*S. cebuensis* DSM 45019^T	*S. spinosa* DSM 44228^T	*S. dendranthemae* KLBMP 1305^T
NaCl range for growth(%,w/v)	0–15	3–20	2.5–12.5	0–11	0–17
Temperature range for growth (°C)	15–45	10–45	15–37	15–37	15–37
Soluble pigment	−	−	+	+	−
Degradation of					
Casein	+	+	−	+	−
Starch	+	−	+	−	+
Reduction of nitrate	−	+	−	+	−
Utilization of					
D-galactose,	+	+	+	−	−
D-lactose,	+	+	+	−	−
D-mannose,	+	+	+	+	−
D-xylose	+	+	+	−	+
D-sorbitol	+	−	−	+	−
Trehalose	+	−	+	+	+
Menaquinones	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)
	MK-9(H_6)	MK-9(H_2)MK-9(H_6)	MK-8(H_4)	MK-9(H_6)	MK-9(H_2)
	MK-9(H_8)		MK-10(H_4)		
	MK-8(H_4)				
Polar lipids	DPG,PC,PG,	PC,DPG,PI	PC,PE,PME,DPG, PG,PI	PC,DPG	DPG,PG,PE, PC
Major fatty acids	iso-$C_{15:0}$	iso-$C_{15:0}$	iso-$C_{15:0}$	iso-$C_{15:0}$	iso-$C_{16:0}$
(>10 %)	iso-$C_{16:0}$	iso-$C_{16:0}$	iso-$C_{16:0}$	iso-$C_{16:0}$	anteiso-$C_{15:0}$
	iso-$C_{17:0}$	anteiso-$C_{17:\ 0}$	iso-$C_{17:0}$	iso-$C_{17:0}$	anteiso-$C_{17:0}$
	anteiso-$C_{17:0}$		anteiso-$C_{17:0}$	anteiso-$C_{17:0}$	$C_{18:1}$ ω8c/ω6c
	$C_{17:1}$ ω8c		$C_{17:1}$ ω8c		

Data are from this study and from Tang et al. (2009b), Pimentel-Elardo et al. (2008), (Mertz and Yao 1990) and Zhang et al. (2013)

+, positive; −, negative

DPG Diphosphatidylglycerol, *PC* phosphatidylcholine, *PI*, phosphatidylinositol, *PE* phosphatidylethanolamine, *PME* phosphatidylmethylethanolamine, *PG* phosphatidylglycerol

that bacterial strains sharing less than 97 % 16S rRNA gene sequence similarity are different genomic species (Stackebrandt and Goebel 1994). DNA–DNA hybridization was performed to differentiate between strain AFM 10238^T and its close neighbours *S. halophila* YIM 90500^T and *S. spinosa* DSM 44228^T. The level of DNA–DNA relatedness between the AFM 10238^T, *S. halophila* YIM 90500^T and *S. spinosa* DSM 44228^T, were 58.6 ± 2.08 % and 57.4 ± 1.45 %, which are below the 70 % cut-off point recommended for the delineation of genomic species (Wayne et al. 1987). DNA–DNA hybridization experiments between strain AFM 10238^T and *S. dendranthemae* KLBMP 1305^T and *S. cebuensis* DSM 45019^T were not carried because of the lower level of 16S rRNA gene sequence similarity. The G + C content of DNA of strain AFM 10238^T was 72.7 mol%.

The morphological and chemical characteristics and the phylogenetic data of strain AFM 10238^T support the conclusion that it is a member of the geuns *Saccharopolyspora*. However, strain AFM 10238^T can be distinguished from its close phylogenetic neighbours by using phenotypic and chemotaxonomic

characteristics (Table 1), such as differences in tolerance to NaCl, in the temperature ranges for growth and production of soluble pigment, in the hydrolysis of casein and starch, in nitrate reduction and in the utilization of sole carbon sources and in the profiles of menaquinones, polarlipids and major fatty acids. In conclusion, based on the phenotypic, chemotaxonomic and phylogenetic data presented, we suggest that strain AFM 10238^T represents a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora griseoalba* sp. nov. is proposed.

Description of *Saccharopolyspora griseoalba* sp. nov.

Saccharopolyspora griseoalba (gri.se.o. al'ba. N.L. adj. *griseus* gray; L. adj *alba* white; N.L. fem. adj. *griseoalba* gray- white.)

Aerobic, Gram-positive, halotolerant, filamentous actinomycete. Aerial mycelia are light gray and well developed with long spore chains. The spore chains are straight or flexuous. The spores are non-motile, smooth-surfaced, oval or sphere-shaped. The substrate mycelia are light yellow to white, well developed, fragment into rod shaped elements. No soluble pigment is produced. Temperature, pH, and NaCl ranges for growth are 15–45 °C, pH 6–12 and 0–15 % (w/v). The optimal growth is at 28 °C, pH 7.5 and NaCl 10 %. Utilizes D-arabinose, cellobiose, dextrin, fructose, D-galactose, D-galactitol, D-glucose, lactose, maltose, D-mannose, L-raffinose, rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, starch, sucrose, D-trehalose and D-xylose, but does not utilize D-mannitol and inositol as sole carbon sources. Utilizes L-alanine, L-arginine, L-aspartic acid, L-cysteine, glycine, L-histidine, L-methionine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tyrosine, ammonium acetate, ammonium citrate, ammonium nitrate, diammonium phosphate, potassium nitrate and sodium acetate, but does not utilize D-glutamic acid, ammonium molybdate and ammonium dihydrogen citrate as sole nitrogen sources. Positive for catalase, milk peptonization, milk coagulation, indole production, and hydrolysis of casein, gelatin, hippurate, starch, Tweens 20, Tweens 80 and urea. Negative for nitrate reduction, methyl red and Voges–Proskauer test, H_2S production, and hydrolysis of cellulose. Cell wall amino acids are *meso*-DAP. Whole cell sugars are

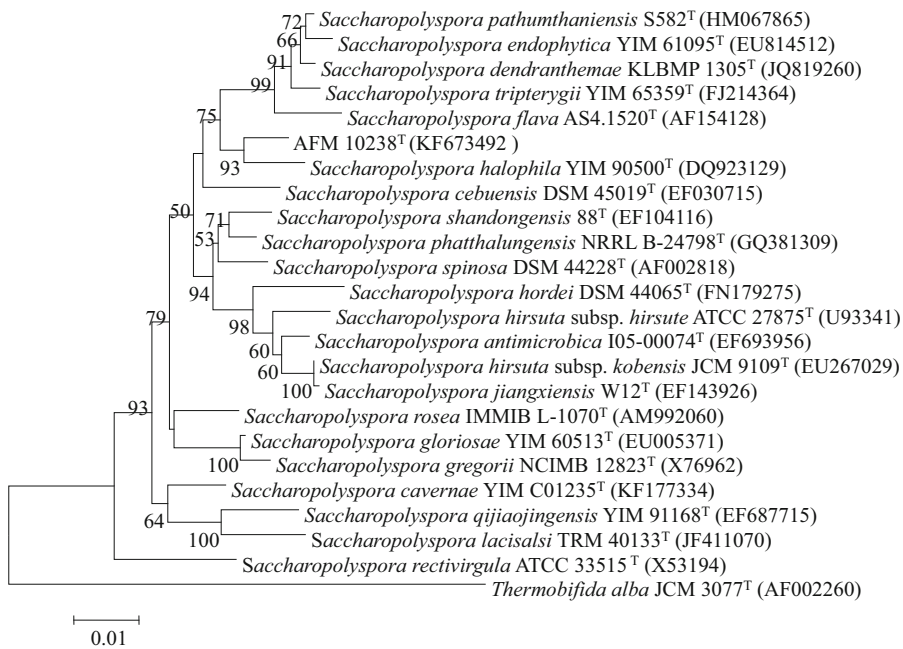

Fig. 2 Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship between strains AFM 10238^T and closely related species of the genus *Saccharopolyspora*. Expressed as percentages of 1000 replications, are given at the branching points. Accession numbers are given in parentheses. *Bar*, 0.01 substitutions per nucleotide position

galactose and arabinose. The major menaquinone is MK-9(H_4). The polar lipids are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC) and two unidentified phospholipids (PLs). Major fatty acids are iso-$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{17:0}$, $C_{17:1}\omega 8c$ and iso-$C_{15:0}$. The DNA G + C content of the type strain is 72.7 mol%.

The type strain, AFM 10238^T (= DSM 46663 = CGMCC 4.7124), was isolated from a sample collected from the Dead Sea, Israel. The GenBank accession number for the 16S rRNA gene sequence of strain AFM 10238^T is KF673492.

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