Saccharopolyspora spinosporotrichia sp. nov., a novel actinomycete from soil

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The generic position of an aerobic, Gram-positive, non-acid-alcohol-fast actinomycete was determined following isolation of the PCR-amplified 16S rRNA genes and alignment of the resultant sequence with corresponding sequences from representatives of the family *Pseudonocardiaceae*. The assignment of the organism to the genus *Saccharopolyspora* was strongly supported by chemotaxonomic and morphological data. The strain was distinguished from representatives of validly described *Saccharopolyspora* species by a number of phenotypic properties. It is proposed that the organism, strain AS4.198^T, be classified in the genus *Saccharopolyspora* as *Saccharopolyspora spinosporotrichia* sp. nov.

Keywords: *Saccharopolyspora spinosporotrichia* sp. nov., 16S rDNA sequences

INTRODUCTION

The genus *Saccharopolyspora* was proposed by Lacey & Goodfellow (20) for actinomycetes isolated from spontaneously heated, sugar-cane bagasse. The genus currently encompasses aerobic, non-acid-fast organisms which form extensively branched substrate hyphae that fragment into rod-shaped, non-motile elements, and aerial hyphae which differentiate into bead-like chains of spores. *Saccharopolysporae* are also characterized by the presence of *meso*-diaminopimelic acid, arabinose and galactose in their wall peptidoglycan [wall chemotype IV *sensu* Lechevalier and Lechevalier (21)], have major amounts of phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylmethylethanolamine [phospholipid pattern III *sensu* Lechevalier *et al.* (23)], are rich in iso- and anteiso- branched-chain fatty acids (5, 6, 10), have predominant amounts of tetra-hydrogenated menaquinones with nine isoprene units (5, 10), but lack mycolic acids (5, 10). The DNA base composition of members of the genus falls within the range 66–77 mol% G + C (10).

The genus *Saccharopolyspora* contains seven validly described species, *Saccharopolyspora erythraea* Labeda (19), *Saccharopolyspora gregorii* Goodfellow *et al.* (10), *Saccharopolyspora hordei* Goodfellow *et al.*

(10), *Saccharopolyspora rectivirgula* Korn-Wendisch *et al.* (18), *Saccharopolyspora spinosa* Mertz and Yao (26), *Saccharopolyspora taberi* Korn-Wendisch *et al.* (18), and *Saccharopolyspora hirsuta* Lacey and Goodfellow (20), the type species. These taxa form a distinct phyletic branch within the evolutionary radiation encompassed by the family *Pseudonocardiaceae* (7, 33). Members of the genus *Saccharopolyspora* are a potentially rich source of natural products but only erythromycin, produced by *Saccharopolyspora erythraea*, is currently commercially important (4).

An actinomycete, strain AS4.198^T, isolated during an ecological survey of soil actinomycetes in China, was found to have morphological properties consistent with its assignment to the genus *Saccharopolyspora*. In the present investigation, this organism was examined for an array of genotypic and phenotypic properties and was shown to form a new centre of taxonomic variation within the genus *Saccharopolyspora*. The name *Saccharopolyspora spinosporotrichia* is proposed for this organism.

METHODS

Organisms and culture conditions. Strain AS4.198^T, which was donated by the Institute of Antibiotics of the Chinese Academy of Medical Sciences, was isolated from soil in Jiangxi province, China. The organism was maintained on glucose yeast extract agar (GYEA) (12) at room temperature and as glycerol suspensions (20%, v/v) at –20 °C. Biomass for the chemotaxonomic studies was prepared by growing the strains in shake flasks of nutrient broth (Oxoid) at 28 °C

The GenBank/EMBL accession numbers for the nucleotide sequences described in this paper are 709571 (strain AS4.198), AF002818 (*S. spinosa*) and AF002819 (*S. taberi*).

Fig. 1. Neighbour-joining tree (15) based on nearly complete 16S rRNA gene sequences showing relationships between *Saccharopolyspora spinosporotrichia* AS4.198^T and representative strains of the family Pseudonocardiaceae and related taxa. Asterisks indicate branches that were recovered using the Fitch-Margoliash method. The numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled data sets; only values greater than 50% are given. The scale bar indicates 0.01 substitutions per nucleotide position. The arrow indicates the estimated position of the root organisms.

for 5 d. At maximum growth, the broth cultures were checked for purity, killed with formaldehyde (1%, v/v), harvested by centrifugation, washed three times with distilled water and freeze dried. The microbiological tests were incubated at 28 °C for 2 weeks unless otherwise stated.

Cultural and morphological properties. The undisturbed arrangement of hyphae, especially aerial hyphae and spore chains, was observed on the seven basal media described by Shirling & Gottlieb (30) using the coverslip technique (16). Growth on coverslips was fixed in 5% aqueous tannic acid for 10–15 min, washed in distilled water and stained in aqueous crystal violet (0.2%, w/v) for 3 min then examined at high magnification using a light microscope (Carl Zeiss Universal Microscope). Additional morphological data were obtained by examining dehydrated specimens of the organism, coated with gold, using a Hitachi S-570 scanning electron microscope.

Biochemical and physiological properties. The degradation of casein, gelatin, starch and tyrosine, and the reduction of nitrate were determined using established procedures (11, 13). The production of hydrogen sulphide from peptone iron

agar was detected using lead acetate strips and the ability to grow at various salt concentrations (1–5%, w/v) on oatmeal agar. The organism was also tested for its ability to grow on a range of sole carbon sources using the basal medium of Stevenson (31) and at 10, 25, 30, 37, 45 and 50 °C on oatmeal agar. The ability to degrade cellulose was detected using filter-paper strips immersed in a minimal broth (MgSO₄, 0.5 g; NaCl, 0.5 g; K₂HPO₄, 0.5 g; KNO₃, 1.0 g; distilled water, 1 l), and incubated at 28 °C for 30 d. The organism was examined for its ability to coagulate and peptonize defatted milk after incubation at 28 °C for up to 30 d; the milk was defatted by adding 0.02% (w/v) CaCO₃ and centrifuging at 4000 r.p.m. for 30 min.

Chemotaxonomy. Diagnostic isomers of diaminopimelic acid, sugars and polar lipids were detected using established procedures (22). Isoprenoid quinones were extracted and purified according to Collins (3) and analysed by using HPLC (34). The presence of mycolic acids was tested using the acid methanolysis procedure described by Minnikin *et al.* (27).

DNA base composition. The G + C content of the DNA of the test strain was determined using the thermal de-

naturation method of Marmur & Doty (25) with *Escherichia coli* AS1.365 as the control.

DNA extraction. Biomass was prepared by centrifuging 7-d-old cultures of the test strain, *S. spinosa* DSM 44228^T and *S. taberi* DSM 43856^T grown in shake flasks of tryptic soy broth (Difco) at 30 °C. Genomic DNA was isolated from the test strains by using a procedure (2) slightly modified from that of Pitcher *et al.* (28).

Sequencing of 16S rDNA. PCR amplification of 16S rDNA was performed as described by Kim *et al.* (17). The PCR products were purified using 2-Spin Plus Centrifugal Filters (Gelman Sciences) according to the procedure provided by the manufacturer. The purified products were directly sequenced by using a *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) and previously described oligonucleotide primers (2). Sequence gel electrophoresis was carried out and nucleotide sequences were automatically obtained by using an Applied Biosystems DNA sequencer (model 373A) and software provided by the manufacturer.

Analysis of sequence data. The 16S rDNA sequences were aligned manually with sequences of *Saccharopolyspora* and related strains obtained from Ribosomal Database Project (24), EMBL (14) and GenBank databases by using the AL16S program (1). Evolutionary trees were inferred by using the neighbour-joining (29) and least squares (9) tree-making algorithms. Evolutionary distance matrices for these methods were generated as described by Jukes & Cantor (15). The PHYLIP package (8) was used for making the trees. The resulting unrooted tree topologies were evaluated by carrying out bootstrap analyses of the neighbour-joining data based on 1000 resamplings using the SEQBOOT and CONSENSE programs from the PHYLIP package (8). The root position of the unrooted tree based on the neighbour-joining method was estimated by using *Bacillus subtilis* (GenBank accession no. K00637) and *Bifidobacterium bifidum* (M38018) as the outgroup organisms, as described by Swofford & Olsen (32).

Nucleotide sequence accession numbers. The 16S rRNA gene sequences of the test strains have the following EMBL/GenBank accession numbers: strain AS4.198^T (Y09571), *Saccharopolyspora spinosa* (AF002818) and *Saccharopolyspora taberi* (AF002819). The accession numbers of the reference strains are as follows: *Actinopolyspora halophila* (X54287), *Amycolatopsis azurea* (X53199), *Amycolatopsis mediterranei* (X76957), *Amycolatopsis orientalis* subsp. *orientalis* (X76958), *Kibdelosporangium aridum* subsp. *aridum* (X53191), *Pseudonocardia autotrophica* (X54288), *Pseudonocardia hydrocarbonoxydans* (X76955), *Pseudonocardia petroleophila* (X55608), *Pseudonocardia saturnea* (X76956), '*Saccharomonospora caesia*' (X76960), *Saccharomonospora viridis* (X54286), *Saccharomonospora* sp. (X76961), *Saccharopolyspora erythraea* (X53198), *Saccharopolyspora gregorii* (X76962), *Saccharopolyspora hirsuta* subsp. *hirsuta* (X53196), *Saccharopolyspora hordei* (X53197), *Saccharopolyspora rectivirgula* (X53194), *Saccharopolyspora* sp. A215 (X76967), *Saccharothrix australiensis* (X53193), *Saccharothrix coerulescens* (X76963), *Saccharothrix longispora* (X76964).

RESULTS AND DISCUSSION

Almost complete 16S rDNA sequences (1269 nucleotides) were obtained for the three test strains. Comparison of these nucleotide sequences with those of

Fig. 2. Scanning electron micrograph showing spinous sporotrichites of *Saccharopolyspora spinosporotrichia* strain AS4.198^T. Bar, 3.8 µm

representative strains of the family *Pseudonocardaceae* shows that the strain AS4.198^T belongs to the genus *Saccharopolyspora* (Fig. 1). The nucleotide sequence of this strain shows substantial differences from the corresponding sequences of its nearest neighbours, namely *S. erythraea* (97.3% similarity), *S. hirsuta* (95.7%) and *S. hordei* (95.4%). It also shows 34 nucleotide differences with the 16S rRNA sequence of *S. erythraea*.

The assignment of the test strain to the genus *Saccharopolyspora* Lacey and Goodfellow (20) is also supported by the chemosystematic data. The organism contains meso-diaminopimelic acid as the wall diamino acid; arabinose and galactose as major wall sugars; has a phospholipid pattern containing phosphatidylcholine, and tetra-hydrogenated menaquinones with nine isoprene units as the predominant isoprenoid quinone, but it does not contain mycolic acids. This chemical profile serves to distinguish strain AS4.198^T from members of all other wall chemotype IV taxa, apart from those classified in the genus *Saccharopolyspora* (6, 10, 20, 23).

The phenotypic properties of strain AS4.198^T also fit with those expected of *Saccharopolyspora* strains. The organism is aerobic, non-motile, Gram-positive, non-acid-alcohol-fast and produces an extensively branched substrate mycelium which fragments into rod-shaped elements, and aerial hyphae which carry long chains of spores in a spiral arrangement. The surfaces of the spores are warty (Fig. 2). The organism can be distinguished from members of validly de-

Table 1. Comparison of properties of *Saccharopolyspora spinosporotrichia* and other validly described species in the genus *Saccharopolyspora*

Data for organisms other than *S. spinosporotrichia* are taken from Lacey & Goodfellow (20), Goodfellow *et al.* (10), Labeda (19), Korn-Wendisch *et al.* (18) and Mertz & Yao (26). Abbreviations: Bf, buff; Br, brown; C, colourless; G, grey; O, orange; P, pink; R, red; W, white; Y, yellow; +, positive; –, negative; ND, not determined.

Characteristic	<i>S. spinosporotrichia</i>	<i>S. erythraea</i>	<i>S. gregorii</i>	<i>S. hirsuta</i>	<i>S. hordei</i>	<i>S. rectivirgula</i>	<i>S. spinosa</i>
Spore arrangement	Spirals	Open spirals	Hooks or flexuous	Straight to loose spirals	Hooks and spirals	Straight	Hooks and open loops
Colour of aerial mycelia	W–G	P–BrG–W	W–Y	W	W–Y	W–light P	W–P
Substrate mycelia	Fragments	Branched	Fragments	Fragments	Fragments	Branched	Fragments in lipid media
Spores on substrate mycelia	–	–	–	–	–	+	–
Colour of substrate mycelia	Br–R	OY–RBr	C–Bf	C–Bf	C–Bf	Y–O	G–OY–Br
Colour of soluble pigment	Br	Y–POBr	C	R–O–Y	C	ND	C–Br
Spore ornamentation	Spiny	Spiny	Smooth	Hairy	Smooth	Smooth or irregularly rough	Spiny
Degradation of tyrosine	–	+	–	+	+	+	–
Hydrolysis of starch	–	+	–	+	+	–	–
Reduction of nitrate	–	+	–	–	–	ND	+
NaCl tolerance (% w/v)	2–3	< 5	< 13	< 7	< 13	< 10	< 11
Temperature range (°C)	28–37	20–42	10–35	25–50	20–60	37–63	15–37
Utilization of carbohydrates as sole carbon source							
L-Arabinose	–	+	+	–	+	–	+
D-Fructose	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	ND	–
Glycerol	+	+	+	+	+	ND	ND
D-Lactose	–	–	–	+	+	+	–
D-Maltose	+	+	+	+	+	ND	–
D-Mannitol	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	ND	+
D-Raffinose	+	+	ND	+	ND	+	–
L-Rhamnose	+	+	+	+	+	+	–
Sucrose	+	+	+	+	+	+	–
D-Xylose	+	+	+	+	+	+	–

scribed *Saccharopolyspora* species using a battery of phenotypic properties (Table 1).

It is evident from the chemical, molecular systematic and phenotypic data that strain AS4.198^T should be given species status in the genus *Saccharopolyspora*. It is, therefore, proposed that the organism be classified in the genus *Saccharopolyspora* as *Saccharopolyspora spinosporotrichia* sp. nov.

Description of *Saccharopolyspora spinosporotrichia* sp. nov. Zhou, Liu, Qian, Kim and Goodfellow

Saccharopolyspora spinosporotrichia (spi.no.spo.ro.tri.chi.a. L. adj. *spinosus* thorny; M.L. n. *spora* a spore; M.L. n. *trichia* trichite; *spinosporotrichia* spores bearing needle-like spines).

Aerobic, Gram-positive, non-acid–alcohol-fast actinomycete which forms an extensively branched, reddish-

brown substrate mycelium which fragments into rod-shaped elements; abundant white to grey aerial hyphae are produced. Long spiral spore chains are borne on the aerial mycelium. The spores are spherical with warty surfaces. Good growth occurs on Bennett's, Czapek, Gause's synthetic, inorganic salts-starch, oatmeal and sucrose yeast extract agars. Aerial mycelium production is poor on yeast-maltose extract agar. A brown diffusible pigment is formed on many standard media. Casein and gelatin are degraded but not cellulose, starch or tyrosine. Milk is coagulated and peptonized. H_2S is not produced nor is nitrate reduced. The organism can use D-fructose, D-galactose, D-glucose, glycerol, meso-inositol, D-maltose, D-mannitol, D-mannose, D-melibiose, D-ribose, D-sorbitol, D-sucrose and D-xylose as sole carbon sources for energy and growth, but not L-arabinose, D-lactose and D-sorbose. Grows between 25 and 37 °C and in the presence of 2–3 % (w/v) sodium chloride. The G + C content of the DNA is 70.4 mol %. The remaining chemical properties of the strain were given earlier. The organism was isolated from soil. The type strain is *Saccharopolyspora spinosporotrichia* AS4.198 (Chinese Culture Collection of Microorganisms).

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