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Saccharopolyspora antimicrobica sp. nov., an actinomycete from soil

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Three Gram-positive, aerobic, non-motile, non-acid–alcohol-fast strains, designated I05-00051, I05-00074^T and I03-00808, were isolated from different soil samples in Beijing and Sichuan, China. Phylogenetic analysis based on 16S rRNA gene sequences and DNA–DNA hybridization experiments revealed that these three isolates represented the same genospecies. These three strains showed <97.0 % 16S rRNA gene sequence similarity with the type strains of recognized species of the genus *Saccharopolyspora*, with the exception of *Saccharopolyspora hirsuta* subsp. *hirsuta* DSM 43463^T (98.1 % gene sequence similarity) and *Saccharopolyspora spinosa* DSM 44228^T (98.0 % similarity). Chemotaxonomic data, including *meso*-diaminopimelic acid as the diagnostic diamino acid, arabinose and galactose as predominant sugars, iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$ and anteiso-$C_{17:0}$ as major fatty acids, MK-9(H_4) as predominant menaquinone and polar lipids dominated by diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol, supported the affiliation of these three organisms to the genus *Saccharopolyspora*. The genomic DNA G+C contents of the three isolates were 68.2–69.9 mol%. The results of DNA–DNA hybridization experiments among these three isolates and *S. hirsuta* subsp. *hirsuta* DSM 43463^T and *S. spinosa* DSM 44228^T, in combination with chemotaxonomic and physiological data, demonstrated that the three new isolates represent a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora antimicrobica* sp. nov. is proposed. The type strain is I05-00074^T (=CCM 7463^T=KCTC 19303^T).

The genus *Saccharopolyspora* was proposed by Lacey & Goodfellow (1975) to accommodate actinomycetes from sugar cane bagasse that produced white aerial mycelia with bead-like chains of spores enclosed in a characteristic hairy sheath. The genus encompasses aerobic, non-acid-fast organisms which form extensively branched substrate hyphae that fragment into rod-shaped elements and aerial hyphae that segment into bead-like chains of spores. The chemical properties of the genus *Saccharopolyspora* include a pattern of *meso*-diaminopimelic acid, arabinose and galactose in the cell-wall peptidoglycan that lacks mycolic acids (Embley *et al.*, 1987; Goodfellow *et al.*, 1989), the presence of iso-branched and anteiso-branched-chain fatty acids (Embley *et al.*, 1987, 1988; Goodfellow *et al.*, 1989), major amounts of phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylmethylethanolamine (a type PIII phospholipid pattern) (Lechevalier *et al.*, 1977) and MK-9(H_4) as the predominant menaquinone (Embley *et al.*, 1987; Goodfellow *et al.*, 1989). The DNA G+C contents of members of the genus are in the range 66–77 mol% (Goodfellow *et al.*, 1989). At the time of writing, the genus *Saccharopolyspora* comprises ten recognized species, namely *Saccharopolyspora erythraea* (Labeda, 1987), *S. flava* (Lu *et al.*, 2001), *S. gregorii* (Goodfellow *et al.*, 1989), *S. hirsuta* (Lacey & Goodfellow, 1975), *S. hordei* (Goodfellow *et al.*, 1989), *S. rectivirgula* (Korn-Wendisch *et al.*, 1989), *S. spinosa* (Mertz & Yao, 1990), *S. spinosporotrichia* (Zhou *et al.*, 1998), *S. taberi* (Korn-Wendisch *et al.*, 1989) and *S. thermophila* (Lu *et al.*, 2001).

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain I05-00074^T is EF693956.

Tables giving the cellular fatty acid profiles of strains I05-00074^T, I05-00051 and I03-00808 and levels of DNA–DNA relatedness among these three novel strains and reference strains *Saccharopolyspora hirsuta* subsp. *hirsuta* DSM 43463^T and *Saccharopolyspora spinosa* DSM 44228^T are available with the online version of this paper.

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During the course of a screening programme to identify new antibiotics, strains I05-00051, I05-00074^T and I03-00808 were isolated from soil samples collected from Beijing and Sichuan, China, by using yeast extract-malt extract agar medium (ISP 2; Shirling & Gottlieb, 1966), incubated at 28 °C for 21 days. These three strains were maintained on ISP 2 agar slants at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C. Biomass for molecular systematic and chemotaxonomic studies was obtained after incubation in shake flasks of trypticase soy broth medium (Difco) at 28 °C for 4–7 days.

Aerial spore-mass colour, substrate mycelial pigmentation and the production of diffusible pigments were observed on ISP 2, ISP 3, ISP 4, ISP 5 (Shirling & Gottlieb, 1966), Czapek solution agar (Waksman, 1961), nutrient agar (Difco) and potato agar (Waksman, 1961) media following incubation at 28 °C after 7, 14, 21 and 28 days. Spore-chain morphology was recorded by examining gold-coated dehydrated specimens of 28-day cultures grown on ISP 2 agar with a scanning electron microscope (Quanta; FEI). The coverslip technique (Zhou *et al.*, 1998) was used to observe the hyphae and spore-chain characteristics.

All the following experiments for the three novel strains were carried out with *S. hirsuta* subsp. *hirsuta* DSM 43463^T and *S. spinosa* DSM 44228^T as controls. Growth temperature was tested at 0, 4, 10, 15, 20, 28–37 (at intervals of 0.5 °C), 40, 45 and 55 °C on ISP 2. The ability of the strains to grow at different pH values (5.0–11.0) (at intervals of 0.5 pH units) and NaCl concentrations (0–20 %, w/v) (at intervals of 0.5 %) was examined on ISP 2 as basal medium. Carbon source utilization tests and qualitative enzyme tests were determined by using API 50 CH and API ZYM test kits. Hydrolysis of urea was determined on peptone-glucose agar comprising (per litre distilled water): 1 g peptone, 1 g glucose, 5 g NaCl and 2 g KH_2PO_4, supplemented with 2 % (w/v) urea and 0.001 % (w/v) phenol red. Hydrolysis of starch was determined on peptone-beef extract agar containing 0.2 % (w/v) soluble starch by flooding of the plates with iodine solution. Hydrolysis of casein was tested on casein agar by observation of clear zones around the colonies. The incubation period for hydrolysis of urea, starch and casein was 4 days at 28 °C. Gelatin hydrolysis was determined by incubation for 3 weeks at 28 °C on peptone-gelatin medium (per litre distilled water: 5 g peptone and 120 g gelatin). Milk coagulation and peptonization were determined by using 20 % (w/v) skimmed milk as the medium with incubation for 3 weeks at 28 °C. Susceptibility to lysozyme and antibiotics was determined by using the disc-diffusion plate method. The following antibiotics were tested (concentration per millilitre in parentheses) on tryptone soy agar (TSA; Oxoid) medium: amikacin (30 μg), aureomycin (30 μg), ciprofloxacin (10 μg), chloramphenicol (30 μg), erythromycin (15 and 30 μg), gentamicin sulfate (10 μg), kanamycin (15 μg), netilmicin (10 μg), novobiocin (5 and 30 μg), oleandomycin (10 μg), penicillin G (10 U), polymyxin B (10 and 300 U), streptomycin sulfate (10 and 25 μg), terramycin (2.5 and 30 μg), tetracycline (10 and 30 μg), tobramycin sulfate (10 μg) and vancomycin (10 μg). The antimicrobial activities of strains I05-00051, I05-00074^T and I03-00808 were investigated by using media containing *Staphylococcus aureus*, *Escherichia coli* and *Mycobacterium smegmatis* (all at 10^8 c.f.u. ml^{-1}).

Strains I05-00051, I05-00074^T and I03-00808 developed well on the test media, including ISP 2, ISP 3, ISP 4, ISP 5, potato agar, Czapek's agar and nutrient agar. Vegetative hyphae were long and well developed. Buff, pink to brown diffusible pigments were produced when strains I05-00051, I05-00074^T and I03-00808 were grown on the above media for 7, 14 and 21 days. Aerial mycelia developed well with long spore chains. All spores were non-motile and rough-surfaced (Fig. 1). Good growth occurred at 28–37 °C and at pH 7–7.5 with 0–7 % (w/v) NaCl. Detailed physiological and biochemical characteristics of strain I05-00074^T are given in Table 1 and in the species description below. Strains I05-00051, I05-00074^T and I03-00808 produced a substance that inhibited growth of *Staphylococcus aureus* and *E. coli*.

The whole-cell sugar pattern and the diagnostic isomers of diaminopimelic acid were determined by TLC (Lechevalier & Lechevalier, 1965, 1980). Menaquinones were extracted and analysed by using the method of Collins (1985). Phospholipids were extracted and identified as described by Minnikin *et al.* (1984). The resultant fatty acids were prepared and analysed following the standard Sherlock MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Arabinose and galactose were detected as the major components of sugars in the whole-cell hydrolysates of strains I05-00051, I05-00074^T and I03-00808 with very small amounts of ribose and glucose. *meso*-Diaminopimelic acid was the diagnostic diamino acid in the cell-wall peptidoglycan. The phospholipids comprised

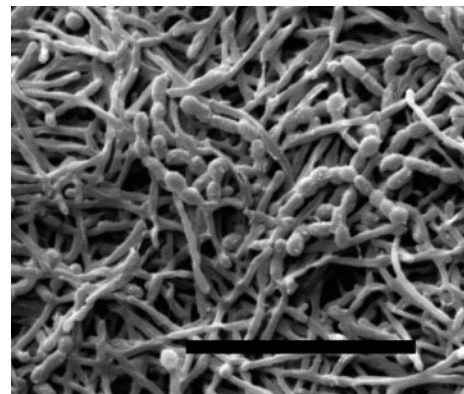

Fig. 1. Scanning electron micrograph showing vesicular chains of rough-surfaced spores of a 28-day-old culture of strain I05-00074^T grown on ISP medium 2 at 28 °C. Bar, 10 μm.

Table 1. Differential phenotypic characteristics between strain I05-00074^T and the type strains of recognized species of the genus *Saccharopolyspora*

Strains: 1, I05-00074^T; 2, *S. hirsuta* subsp. *hirsuta* DSM 43463^T; 3, *S. spinosa* DSM 44228^T; 4, *S. erythraea* DSM 40517^T; 5, *S. gregorii* DSM 44324^T; 6, *S. thermophila* AS4.1511^T; 7, *S. horderi* DSM 44065^T; 8, *S. rectivirgula* DSM 43747^T; 9, *S. flava* AS4.1520^T; 10, *S. spinospirotrichia* DSM 44350^T; 11, *S. taberi* DSM 43856^T. Data for strain I05-00074^T, *S. hirsuta* subsp. *hirsuta* DSM 43463^T and *S. spinosa* DSM 44228^T are from the present study; other data are taken from Korn-Wendisch *et al.* (1989) and Lu *et al.* (2001). BF, Buff; BR, brown; C, colourless; G, grey; O, orange; P, pink; R, red; W, white; Y, yellow; +, positive; −, negative; NA, not applicable (aerial mycelium was not observed); ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Spore arrangement	Straight	Straight to loose spirals	Hooks and open loops	Open spirals	Hooks/flexuous hyphae	Hooks/flexuous hyphae	Hooks and spirals	Straight	Straight	Spirals	NA
Spore ornamentation	Rough	Hairy	Spiny	Spiny	Smooth	Smooth	Smooth	Smooth or irregularly rough	Smooth	Spiny	NA
Substrate mycelium	Branched	Branched	Branched	Branched	Fragments	Fragments	Fragments	Branched	Fragments	Fragments	Fragments
Colour of:											
Aerial mycelium	W	W	W-P	P-BRG-W	W-Y	W	W-Y	W-light P	W	W-G	NA
Substrate mycelium	W-BF-P	C-BF	G-OY-BR	OY-RBR	C-BF	C-BF	C-BF	Y-O	Y	BR-R	C-Y
Soluble pigment	BF-P-BR	R-O-Y	C-BR	Y-POBR	None	None	None	None	None	BR	O-R
Spores on substrate mycelium	−	−	−	−	−	−	−	+	−	−	−
Degradation of:											
Adenine	+	+	−	+	−	+	+	−	+	−	+
Casein	+	+	−	−	+	−	+	−	−	+	+
Chitin	−	−	−	+	−	−	+	−	−	−	+
Elastin	−	+	−	+	+	−	+	−	−	+	+
Starch	+	+	−	+	+	+	+	+	+	+	+
Tyrosine	+	+	+	+	+	+	+	+	−	−	+
Xanthine	−	+	+	+	+	−	+	+	+	+	+
Reduction of nitrate	+	−	+	+	−	−	−	+	+	−	+
NaCl tolerance (w/v)	≤7	<7	<11	<5	13	7	<13	<10	7	2–3	7
Temperature range (°C)	20–45	25–50	15–37	20–42	10–35	45–55	20–60	37–63	28–37	28–37	20–45
Utilization of carbohydrates as sole carbon source											
L-Arabinose	+	−	−	+	+	−	+	−	−	−	−
D-Galactose	+	+	+	+	+	+	+	+	+	+	+
D-Lactose	+	+	+	−	−	+	+	+	+	−	+
Maltose	+	+	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+	+
L-Rhamnose	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	−	+	+	+	+	+	+	+	+

diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol. The predominant menaquinone was MK-9(H_4); MK-9(H_2) and MK-9(H_6) were detected as minor components. The detailed cellular fatty acid profiles of strains I05-00051, I05-00074^T and I03-00808 are given in Supplementary Table S1 in IJSEM Online; iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$ and anteiso-$C_{17:0}$ were the major fatty acids.

Extraction of genomic DNA and amplification of the 16S rRNA gene were performed as described by Li *et al.* (2007). Purified PCR products were sequenced by using an ABI PRISM automatic sequencer (model 3730XL). The 16S rRNA gene sequences were aligned with available nucleotide sequences of members of the genus *Saccharopolyspora* and related genera retrieved from the DDBJ/GenBank/EMBL databases by using the CLUSTAL_X 1.8 program (Thompson *et al.*, 1997) based on the BLAST results in NCBI. Subsequently, a phylogenetic analysis was performed by using MEGA version 2.1 (Kumar *et al.*, 2001). Distances were calculated according to the Kimura two-parameter model (Kimura, 1980, 1983) and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Levels of 16S rRNA gene sequence similarity were recalculated by using a web-based tool in http://www.eztaxon.org as described by Chun *et al.* (2007). Bootstrap analysis (1000 resamplings) was used to evaluate the tree topology of the neighbour-joining data (Felsenstein, 1985).

Levels of DNA–DNA relatedness among the three test strains and reference strains *S. hirsuta* subsp. *hirsuta* DSM 43463^T and *S. spinosa* DSM 44228^T were determined according to the thermal renaturation method (De Ley *et al.*, 1970), by using a UV-1700 spectrophotometer (Shimadzu) equipped with a DCW-2008 thermo bath. The G+C content of the genomic DNA was determined by using the thermal denaturation (T_m) method (Marmur & Doty, 1962) with *E. coli* AS1.365 as a control.

The almost-complete 16S rRNA gene sequences of strains I05-00051, I05-00074^T and I03-00808 were generated. Levels of sequence similarity among these three strains were above 99.0 %. Comparison of their nucleotide sequences with those of representatives of the families *Actinosynnemataceae* and *Pseudonocardiaceae* and related taxa (Labeda & Kroppenstedt, 2000) clearly showed that the three isolates belonged to the genus *Saccharopolyspora*. In the phylogenetic tree constructed based on 16S rRNA gene sequences (Fig. 2), the three organisms were included in the clade comprising members of the genus *Saccharopolyspora*. Strains I05-00051, I05-00074^T and I03-00808 showed less than 97.0 % 16S rRNA gene sequence similarity to the type strains of all recognized species of the genus *Saccharopolyspora*, with the exception of *S. hirsuta* subsp. *hirsuta* DSM 43463^T (98.1 %) and *S. spinosa* DSM 44228^T (98.0 %). Levels of DNA–DNA hybridization among strains I05-00051, I05-00074^T and I03-00808 were all above 70 %, while values between any one of these three strains and either *S. hirsuta* subsp. *hirsuta* DSM 43463^T or *S. spinosa* DSM 44228^T were significantly below 70 % (see Supplementary Table S2 in IJSEM Online), the cut-off point recommended by Wayne *et al.* (1987) for the recognition of genomic species. Therefore, the three novel strains could not be classified as representing any of the recognized species of the genus *Saccharopolyspora*.

Morphological, physiological and chemotaxonomic similarities of the three new isolates combined with high levels of 16S rRNA gene sequence similarity and DNA–DNA relatedness indicated that strains I05-00051, I05-00074^T and I03-00808 represent the same genomic species. The G+C contents of the genomic DNA of strains I05-00051, I05-00074^T and I03-00808 were 68.2, 69.3 and 69.9 mol%, respectively.

Based on the phenotypic differentiation (Table 1) and genotypic data presented above, we consider that strains I05-00051, I05-00074^T and I03-00808 represent a novel species of the genus *Saccharopolyspora*. In view of their antimicrobial activities towards *Staphylococcus aureus* and *E. coli*, we propose the name *Saccharopolyspora antimicrobica* sp. nov. to accommodate these organisms.

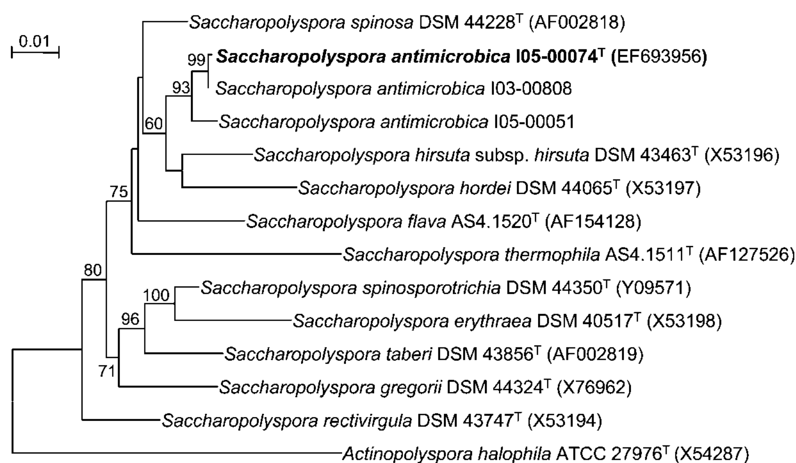

Fig. 2. Neighbour-joining tree showing the phylogenetic relationships among strains I05-00074^T, I05-00051 and I03-00808 and the type strains of related taxa based on 16S rRNA gene sequences. Numbers at branch nodes are bootstrap values (percentages of 1000 replicates). Bar, 1 nt substitution per 100 nt.

Description of *Saccharopolyspora antimicrobica* sp. nov.

Saccharopolyspora antimicrobica (an.ti′mi.cro.bi.ca. Gr. prep. *anti* against; N.L. n. *microbium* microbe; L. adj. suff.-*cus* -*a* -*um* suffix used with various meanings; N.L. fem. adj. *antimicrobica* antimicrobial).

Aerobic, Gram-positive, non-acid–alcohol-fast, non-motile actinomycete. Good growth occurs on ISP 2, ISP 3, ISP 4, ISP 5, Czapek's agar, nutrient agar and potato agar media. Forms extensively branched, white, buff to pink substrate mycelia that later fragment into rod-shaped elements that carry aerial hyphae that differentiate into long chains of rough-surfaced spores. White aerial hyphae are produced upon cultivation on ISP 2 agar. Diffusible pigments, buff, pink to brown, are produced on some agar media. Adenine, hypoxanthine, tyrosine, casein, xylan, starch and gelatin are degraded, but cellulose, chitin, elastin and xanthine are not. Milk is coagulated and peptonized. Aesculin and urea are hydrolysed, nitrate is reduced, but H_2S is not produced. Utilizes adonitol, cellobiose, fructose, galactose, glucose, inositol, lactose, L-arabinose, melezitose, maltose, mannitol, raffinose, salicin, sorbitol, sucrose, xylose, acetate, citrate, malonate, phenylalanine, rhamnose, sorbitol, glyconate, dulcitol, mannose, D-ribose, turanose, melibiose, glucosamine and tartrate as sole carbon sources for energy and growth but does not use trehalose, erythritol or methyl α-D-glucoside. Is able to produce β-glucosaccharase, α-glucosaccharase, β-galactosidase, *N*-acetylglucosaminidase, alkaline phosphatase and acid phosphatase. NaCl tolerance range is 0–7 % (w/v). Temperature and pH ranges for growth are 20–45 °C and pH 6.0–8.5, respectively. Optimal temperature and pH for growth are 28–37 °C and pH 7.0–7.5, respectively. Growth is inhibited by lysozyme. Resistant ($\mu g\ ml^{-1}$) to cefotaxime (30), aztreonam (30), amikacin (30), streptomycin (10), oxacillin (1), tobramycin (10), ceftazidime (30), gentamicin (10), erythromycin (15), chloromycetin (30), ampicillin (10), furadantin (300) and penicillin G (10 U). Shows antimicrobial activities against *Staphylococcus aureus* and *E. coli*. Contains *meso*-diaminopimelic acid and mainly arabinose and galactose in whole-organism hydrolysates. Phospholipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol. The predominant menaquinone is MK-9(H_4). The major fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$ and anteiso-$C_{17:0}$. The G+C content of the genomic DNA is 69.3 mol% for the type strain.

The type strain, I05-00074^T (=CCM 7463^T=KCTC 19303^T), was isolated from soil from the Temple of Heaven, Beijing, China. Strains I05-00051 and I03-00808, isolated from soil samples, are reference strains.

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