Saccharopolyspora jiangxiensis sp. nov., isolated from grass-field soil

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An actinomycete isolated from soil from a field of grass was analysed using a polyphasic taxonomic approach. The organism, designated strain W12^T, was found to have a range of chemical and morphological properties consistent with its classification in the genus *Saccharopolyspora*. An almost-complete 16S rRNA gene sequence determined for the strain was aligned with corresponding sequences for representatives of the genus *Saccharopolyspora*, using two tree-making algorithms. The novel organism formed a monophyletic clade with the type strain of *Saccharopolyspora antimicrobica*, but the DNA–DNA relatedness value was low. Strain W12^T also had a phenotypic profile that readily distinguished it from recognized representatives of the genus *Saccharopolyspora*. It is evident from the combined genotypic and phenotypic data that the new organism should be classified as a novel species of the genus *Saccharopolyspora*. The name proposed for this new taxon is *Saccharopolyspora jiangxiensis* sp. nov., with strain W12^T (=CGMCC 4.3529^T=JCM 14613^T) as the type strain.

The genus *Saccharopolyspora* was established by Lacey & Goodfellow (1975) and was assigned to the family *Pseudonocardiaceae* (Embley *et al.*, 1988; Warwick *et al.*, 1994). At the time of writing, the genus encompasses 14 species with validly published names: *Saccharopolyspora antimicrobica* Yuan *et al.* 2008; *S. cebuensis* Pimentel-Elardo *et al.* 2008; *S. erythraea* (Waksman 1923) Labeda 1987; *S. flava* Lu *et al.* 2001; *S. gregorii* Goodfellow *et al.* 1989; *S. halophila* Tang *et al.* 2009; *S. hirsuta* subsp. *kobensis* (ex Iwasake and Mori 1979) Lacey 1989; *S. hordei* Goodfellow *et al.* 1989; *S. rectivirgula* (Krasil'nikov and Agre 1964) Korn-Wendisch *et al.* 1989; *S. shandongensis* Zhang *et al.* 2008; *S. spinosa* Mertz and Yao 1990; *S. spinosporotrichia* Zhou *et al.* 1998; *S. taberi* (Labeda 1987) Korn-Wendisch *et al.* 1989; *S. thermophila* Lu *et al.* 2001; and *S. hirsuta* Lacey and Goodfellow 1975 (the type species). Representatives of these species form a distinct phyletic line within the evolutionary radiation encompassed by the family *Pseudonocardiaceae* and can be distinguished using a combination of biochemical, morphological and physiological properties (Lu *et al.*, 2001). Members of the genus *Saccharopolyspora* are potentially rich sources of natural products, but only erythromycin (produced by *S. erythraea*) is currently of commercial importance (Embley, 1992).

An actinomycete, strain W12^T, isolated during an ecological survey of soil actinomycetes in China, was found to

have morphological and chemical properties consistent with its assignment to the genus *Saccharopolyspora*. In the present investigation, a polyphasic approach was used to determine the taxonomic position of this soil isolate. Genotypic and phenotypic data indicated that this strain should be classified as a novel species of the genus *Saccharopolyspora*.

Strain W12^T was isolated on a glucose-yeast extract-malt extract medium (GYM) agar plate, which had been seeded with a soil suspension and incubated at 28 °C for 14 days. The soil sample was collected from a field of grass in Jiangxi Province, China. Strain W12^T was maintained on GYM agar at 4 °C and as suspensions of mycelial fragments in 20 % (v/v) glycerol at –20 °C. Biomass for the chemotaxonomic and molecular systematic studies was prepared as described by Zhang *et al.* (2002) with the modification that the strain was grown in shake flasks containing GYM broth.

Micromorphological and colonial properties were examined on GYM agar, modified Sauton's agar (Mordarska *et al.*, 1972) and standard media used in the International *Streptomyces* Project (Shirling & Gottlieb, 1966), for 7–14 days at 28 °C. The micromorphology was examined using the cover-slip technique of Kawato & Shinobu (1959), following the procedure described by Zhou *et al.* (1998), using a Zeiss Axioskop 20 light microscope. The spore-chain morphology was also investigated by examining gold-coated, dehydrated specimens of the organism, using a Hitachi S-570 scanning electron microscope.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain W12^T is EF143926.

The novel strain was examined for a range of phenotypic properties by using previously described procedures (Lacey & Goodfellow, 1975; Lu *et al.*, 2001; Zhang *et al.*, 2003). Acid production from carbohydrates was assessed using methods described by Gordon *et al.* (1974), while the utilization of sole carbon sources was investigated as described by Gordon & Mihm (1957). Tolerance of pH, temperature and NaCl was investigated using GYM agar plates incubated for up to 14 days. Resistance to lysozyme was determined by using the method of Gordon *et al.* (1974). Resistance to antibiotics was examined by placing antibiotic-impregnated filter-paper discs (Goodfellow & Orchard, 1974) on GYM agar and assessing the results after 14 days incubation at 28 °C.

Genomic DNA preparation, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product from strain W12^T were carried out as described by Rainey *et al.* (1996) and the PCR product was sequenced directly using the method of Lu *et al.* (2001). Sequence gel electrophoresis was performed and the nucleotide sequences were obtained automatically by using an Applied Biosystems DNA sequencer (model 377) and software provided by the manufacturer. The resultant 16S rRNA gene sequence was aligned manually against corresponding sequences from members of the genus *Saccharopolyspora* and from representatives of genera in the family *Pseudonocardiaceae*, available from the GenBank/EMBL/DDBJ databases, using CLUSTAL_X, version 1.8 (Thompson *et al.*, 1997). Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) treeing algorithms. Evolutionary distance matrices were generated as described by Kimura (1980). The resultant unrooted tree topologies were evaluated by means of bootstrap analysis (Felsenstein, 1985) of the neighbour-joining dataset (based on 1000 resamplings), using the SEQBOOT and CONSENSE options. The PHYLIP package (Felsenstein, 1993) was used for all phylogenetic analyses.

Chemotaxonomic studies were performed with strain W12^T to determine whether its chemical profile was typical of members of the genus *Saccharopolyspora* (Lacey & Goodfellow, 1975). Isomers of diaminopimelic acid and whole-organism sugars were analysed according to the procedures developed by Hasegawa *et al.* (1983) and Lechevalier & Lechevalier (1980). Mycolic acids were checked by using the acid methanolysis method of Minnikin *et al.* (1975). Polar lipids were examined using two-dimensional TLC and were identified according to the method of Minnikin *et al.* (1984). Isoprenoid quinones were extracted and purified as described by Collins *et al.* (1987); purified menaquinones were determined using reversed-phase HPLC (Wu *et al.*, 1989). The fatty acids were extracted, purified, methylated and quantified by means of GC using the standard Microbial Identification System (MIDI; Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

The DNA–DNA relatedness between the isolate and representatives of phylogenetically close *Saccharopolyspora* species (*S. antimicrobica* KCTC 19303^T, *S. hirsuta* subsp. *hirsuta* ATCC 27875^T, *S. shandongensis* CGMCC 4.3530^T and *S. spinosa* DSM 44228^T) was determined using a thermal denaturation procedure (De Ley *et al.*, 1970; Huß *et al.*, 1983) with a UV-1206 spectrophotometer (Shimadzu) fitted with a TB-85 thermobath and standard software (Jahnke, 1992); the results were expressed as means of two determinations. The G + C content of the DNA was determined using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* AS 1.365 as the standard.

An almost-complete 16S rRNA gene sequence (1419 nt) was obtained for strain W12^T. Phylogenetic analysis in which this sequence was compared with corresponding sequences for representatives of the genera classified in the suborder *Pseudonocardineae* showed that it contains all of the signature nucleotides expected for members of this taxon (Stackebrandt *et al.*, 1997) and, more specifically, those that are characteristic of members of the family *Pseudonocardiaceae* (Embley *et al.*, 1988; Warwick *et al.*, 1994) and the genus *Saccharopolyspora* (Lacey & Goodfellow, 1975). The high levels of 16S rRNA gene sequence similarity found between the novel organism and representatives of known species of the genus *Saccharopolyspora*, i.e. 98.7% (*S. antimicrobica*) to 93.2% (*S. thermophila*), provide further evidence supporting the assignment of strain W12^T to this taxon.

The classification of strain W12^T within the genus *Saccharopolyspora* is supported by morphological and phenotypic characteristics. The novel organism is an aerobic, Gram-positive actinomycete and it forms an extensively branched substrate mycelium that fragments into irregular, non-motile, coccoid- and rod-shaped elements and carries sparse to moderate, white to buff aerial hyphae that differentiate into long chains of spores (with surfaces that were smooth or irregularly rough) (Fig. 1). The

Fig. 1. Scanning electron micrograph showing long chains of smooth or irregularly rough-surfaced spores of a 14-day-old culture of strain W12^T grown on GYM agar at 28 °C. Bar, 5.0 µm.

novel strain contained *meso*-diaminopimelic acid as the diagnostic diamino acid of the cell wall, whole-organism hydrolysates contained arabinose and galactose as diagnostic sugars (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970), major amounts of phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine were present (phospholipid type III *sensu* Lechevalier *et al.*, 1977), tetrahydrogenated menaquinones with nine isoprene units were the predominant isoprenologues and dihydrogenated menaquinones with 10 isoprene units were minor isoprenologues. The non-hydroxylated fatty acid profile consisted of saturated, monounsaturated iso- and anteiso- components. The fatty acid profile was as follows: iso-C_{15:0} (25.8 %), iso-C_{16:0} (20.3 %), iso-C_{17:0} (15.1 %), anteiso-C_{17:0} (10.7 %), C_{17:0} (6.0 %), C_{17:1ω8c} (3.9 %), 10-methyl C_{17:0} (3.8 %), anteiso-C_{15:0} (3.5 %), iso-C_{14:0} (2.9 %), 10-methyl C_{16:0} (2.2 %), C_{15:0} (1.6 %), C_{16:0} (1.4 %) and C_{18:0} (1.0 %). The novel organism did not contain mycolic acids. In addition, the DNA of strain W12^T was rich in guanine and cytosine (G+C content, 70.3 mol%). All of these properties are consistent with classification of the novel isolate within the genus *Saccharopolyspora*.

As shown in Fig. 2, strain W12^T forms a monophyletic clade with the type strain of *S. antimicrobica*; the two organisms share 98.7 % 16S rRNA gene similarity. Strain W12^T is also closely related to the type strains of *S. hirsuta* subsp. *hirsuta* (97.7 % sequence similarity), *S. shandongensis* (97.5 %) and *S. spinosa* (97.4 %). DNA–DNA relatedness studies provide a reliable way of distinguishing between representatives of species that share high levels of 16S rRNA gene similarity (Goodfellow *et al.*, 1998). In the present study, strain W12^T showed low levels of DNA–DNA relatedness with the type strains of *S. antimicrobica*

(56.3 %), *S. hirsuta* subsp. *hirsuta* (47.8 %), *S. shandongensis* (25.5 %) and *S. spinosa* (21.7 %); all of these values are well below the 70 % cut-off point recommended for the assignment of organisms to the same genomic species (Wayne *et al.*, 1987). A range of phenotypic properties serve to distinguish strain W12^T from recognized members of the genus *Saccharopolyspora* (Table 1).

The genotypic and phenotypic data show that strain W12^T merits recognition as a novel species of the genus *Saccharopolyspora*. The name *Saccharopolyspora jiangxiensis* sp. nov. is proposed for this new taxon.

Description of *Saccharopolyspora jiangxiensis* sp. nov.

Saccharopolyspora jiangxiensis (ji.ang.xi.en'sis. N.L. fem. adj. *jiangxiensis* referring to Jiangxi Province, China, the source of the grass-field soil from which the organism was isolated).

Gram-positive, aerobic, catalase-positive, non-acid–alcohol-fast, non-motile actinomycete. Produces extensively branched (0.4–0.7 µm diameter), colourless to buff substrate mycelium, which fragments *in situ* into coccoid- and rod-shaped elements after 3–4 days at 28 °C. White to buff aerial hyphae, 0.6–1.0 µm in diameter, are produced upon prolonged cultivation on GYM agar. The organism also grows well on ISP3, ISP4 and modified Sauton's agar. The aerial mycelium carries long straight to flexuous chains comprising 6–10 spores (spore size 0.8–1.1 × 1.0–1.3 µm). The spore surface is smooth or irregularly rough. Diffusible pigments are not formed. Colony elevation is convex to irregular and colony margins are filamentous. Grows at 15–45 °C and pH 5.5–9.5. Degrades adenine, elastin, starch,

Fig. 2. Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing the relationships between strain W12^T and the type strains of recognized species of the genus *Saccharopolyspora*. Asterisks indicate branches that were also recovered using the maximum-likelihood tree-making algorithm. Numbers at nodes indicate bootstrap percentages (based on a neighbour-joining analysis of 1000 resampled datasets); only values above 50 % are shown. Bar, 0.02 substitutions per nucleotide position.

Table 1. Comparison of properties of strain W12^T with those of recognized species of the genus *Saccharopolyspora*

Strains: 1, W12^T; 2, *S. antimicrobica* KCTC 19303^T; 3, *S. erythraea* NRRL 2338^T; 4, *S. flava* AS 4.1520^T; 5, *S. gregorii* NCIMB 12823^T; 6, *S. hirsuta* subsp. *hirsuta* ATCC 27875^T; 7, *S. hordei* NCIMB 12824^T; 8, *S. rectivirgula* ATCC 33515^T; 9, *S. shandongensis* CGMCC 4.3530^T; 10, *S. spinosa* DSM 44228^T; 11, *S. spinosporotrichia* AS 4.198^T; 12, *S. taberi* DSM 43856^T; 13, *S. thermophila* AS 4.1511^T. Data were taken from this and previous studies (Korn-Wendisch *et al.*, 1989; Yuan *et al.*, 2008; Zhou *et al.*, 1998; Lu *et al.*, 2001; Zhang *et al.*, 2008). +, Positive; –, negative; w, weak; NA, not applicable (aerial mycelium was not observed); ND, not determined. All strains gave positive results for degradation of aesculin and utilization of D-fructose, D-glucose, glycerol, D-mannitol and D-mannose as sole carbon sources.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Spore arrangement	Straight to flexuous hyphae	Straight	Open spirals	Straight	Hooks or flexuous hyphae	Straight to loose spirals	Hooks and spirals	Straight	Spiral	Hooks and open loops	Spirals	NA	Hooks or flexuous hyphae
Spore ornamentation	Smooth or irregularly rough	Rough	Spiny	Smooth	Smooth	Hairy	Smooth	Smooth or irregularly rough	Spiny	Spiny	Spiny	NA	Smooth
Spores on substrate mycelium	–	–	–	–	–	–	–	+	–	–	–	–	–
Substrate mycelium	Fragments	Branched	Branched	Fragments	Fragments	Fragments	Fragments	Branched	Fragments	Fragments in lipid media	Fragments	Fragments	Fragments
Colour of:													
Substrate mycelium	C-Bf	W-Bf-P	OY-RBr	Y	C-Bf	C-Bf	C-Bf	Y-O	C-Bf	G-OY-Br	Br-R	C-Y	C-Bf
Aerial mycelium	W-Bf	W	P-BrG-W	W	W-Y	W	W-Y	W-light P	W	W-P	W-G	NA	W
Soluble pigment	None	Bf-P-Br	Y-POBr	None	None	R-O-Y	None	None	Br	C-Br	Br	O-R	None
Degradation of:													
Adenine	+	+	+	+	–	+	+	–	+	–	–	+	+
Casein	–	+	–	–	+	+	+	–	+	+	+	+	–
Chitin	–	–	+	–	–	–	+	–	–	–	–	+	–
Elastin	+	–	+	–	+	+	+	–	+	–	+	+	–
Hypoxanthine	–	+	+	+	+	+	–	+	+	–	ND	+	–
Starch	+	+	+	+	+	+	+	–	+	–	–	+	+
Tyrosine	+	+	+	–	+	+	+	+	+	+	–	+	+
Xanthine	+	–	+	+	+	+	+	+	+	–	+	+	–
Urea	+	+	+	+	–	+	–	+	+	+	ND	+	+
Reduction of nitrate	+	+	+	+	–	–	–	+	+	+	–	+	–
NaCl tolerance (% w/v)	<11	≤7	<5	<7	13	<7	<13	<10	<7	<11	2–3	7	<7
Temperature range (°C)	15–45	20–45	20–42	28–37	10–35	25–50	20–60	37–63	15–38	15–37	28–37	20–45	45–55

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Growth on sole carbon sources:													
L-Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+

*Bf, buff; Br, brown; C, colourless; G, grey; O, orange; P, pink; R, red; W, white; Y, yellow.

tyrosine and xanthine, but not casein, cellulose, chitin, guanine, hypoxanthine or xylan. Nitrate is reduced and aesculin, arbutin and urea are hydrolysed. Acid is formed from adonitol, (+)-D-arabinose, dextrin, (+)-D-fructose, (+)-D-galactose, (+)-D-glucose, glycerol, inulin, (+)-maltose, (+)-D-mannitol, (+)-D-mannose, (–)-D-ribose, (+)-sucrose and (+)-trehalose but not from (+)-L-arabinose, (+)-cellobiose, ethanol, *meso*-erythritol, glycogen, *myo*-inositol, (+)-D-lactose, (+)-melezitose, (+)-melibiose, methyl α -D-glucoside, (+)-raffinose, α -L-rhamnose, (+)-D-salicin, (+)-D-sorbitol or (+)-D-xylose. Adonitol, (+)-D-arabinose, *meso*-erythritol, methyl α -D-glucoside, *myo*-inositol, (+)-melezitose, (+)-D-sorbitol, (+)-trehalose, acetate, benzoate, citrate, fumarate, malate, succinate and tartrate are used as sole carbon and energy sources, but (+)-D-salicin, malonate and oxalate are not. Weak growth occurs in the presence of NaCl at 11 %, but not at 12 % (w/v). Resistance is shown to the following antibiotics (μ g per disc, unless indicated otherwise): lysozyme (0.005 %, w/v); amoxicillin plus clavulanic acid (10); ampicillin (10), aztreonam (30); clindamycin hydrochloride (2); erythromycin (15); kanamycin sulphate (30); penicillin G (10 U); and tobramycin sulphate (10). Susceptible to (μ g per disc) amikacin (30), cefotaxime (30), chloramphenicol (30), ciprofloxacin (5), gentamicin sulphate (10), mezlocillin (75), ofloxacin (5), rifampicin (5), streptomycin sulphate (10) and tetracycline hydrochloride (30). Additional phenotypic properties are shown in Table 1. Chemotaxonomic properties are typical of the genus *Saccharopolyspora*. The fatty acid profile is as follows: iso-C_{15:0}, iso-C_{16:0}, iso-C_{17:0}, anteiso-C_{17:0}, C_{17:0}, C_{17:1} ω 8c, 10-methyl C_{17:0}, anteiso-C_{15:0}, iso-C_{14:0}, 10-methyl C_{16:0}, C_{15:0}, C_{16:0} and C_{18:0}. The DNA G+C content of the type strain is 70.3 mol%.

The type strain, W12^T (=CGMCC 4.3529^T=JCM 14613^T), was isolated from a soil sample from a field of grass in Jiangxi Province, China.

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