Saccharopolyspora flava sp. nov. and Saccharopolyspora thermophila sp. nov., novel actinomycetes from soil

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The generic position of two aerobic, Gram-positive, non-acid–alcohol-fast actinomycetes was established following the isolation of their PCR-amplified 16S rRNA genes and alignment of the resultant sequences with the corresponding sequences from representatives of the families Actinosynnemataceae and Pseudonocardiaceae. The assignment of the organisms to the genus Saccharopolyspora was strongly supported by chemotaxonomic and morphological data. The strains were distinguished both from one another and from representatives of validly described Saccharopolyspora species on the basis of a number of phenotypic properties. It is proposed that the organisms, strains 07T (= AS4.1520T = IFO 16345 = JCM 10665) and 216T (= AS4.1511T = IFO 16346 = JCM 10664), be classified in the genus Saccharopolyspora as Saccharopolyspora flava sp. nov. and Saccharopolyspora thermophila sp. nov., respectively.

Keywords: Saccharopolyspora flava sp. nov., Saccharopolyspora thermophila sp. nov., polyphasic taxonomy

INTRODUCTION

The application of the polyphasic taxonomic approach to actinomycete systematics led to the circumscription of the family Pseudonocardiaceae Embley et al. 1988 for mycolateless actinomycetes characterized by the presence of meso-diaminopimelic acid (meso-A₂pm), arabinose and galactose in their peptidoglycan (wall chemotype IV sensu Lechevalier & Lechevalier, 1970). Members of this family are currently assigned to the genera Actinobispora, Amycolatopsis, Kibdelosporangium, Prauserella, Pseudonocardia (including Amycolata), Saccharomonospora, Saccharopolyspora and Thermocribis (Kim & Goodfellow, 1999; Xu et al., 1999; Labeda & Kroppenstedt, 2000). The family Pseudonocardiaceae recognized by Stackebrandt et al. (1997) also included the genera Actinosynema, Saccharothrix and Streptoalloteichus; these taxa were excluded from the family by Warwick et al. (1994), who considered that they might form a ‘sister’ group to the Pseudonocardiaceae clade. This now seems to be the case, as Labeda & Kroppenstedt (2000) have proposed that members of the genera Actinokineospora, Actinosynema, Lentzea and Saccharothrix be classified in a new taxon, i.e. the family Actinosynemataceae. Members of both clades can be distinguished from one another using chemical and morphological markers (Kim & Goodfellow, 1999; Labeda & Kroppenstedt, 2000).

The genus Saccharopolyspora contains eight validly described species: Saccharopolyspora erythraea Labeda 1987; Saccharopolyspora gregorii Goodfellow et al. 1989; Saccharopolyspora hordei Goodfellow et al. 1989; Saccharopolyspora rectivirgula (Krasil’nikov and Age 1964) Korn-Wendisch et al. 1989; Saccharopolyspora spinosa Mertz and Yao 1990; Saccharopolyspora spinosporotrichia Zhou et al. 1998; Saccharopolyspora taberi (Labeda 1987) Korn-Wendisch et al. 1989; and Saccharopolyspora 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 *Pseudonocardiaecae* and can be distinguished using a combination of biochemical, morphological and physiological properties (Zhou et al., 1998). Genus-specific primers are available for the PCR identification of *Pseudonocardia* and *Saccharopolyspora* strains (Morón et al., 1999).

Two actinomycetes isolated from soil samples collected in China were provisionally assigned to the genus *Saccharopolyspora*, using chemical and morphological properties. In the present investigation, these organisms, namely strains 07 and 216, were examined for a range of genotypic and phenotypic properties and found to form distinct new centres of taxonomic variation within the genus *Saccharopolyspora*; the names *Saccharopolyspora flavata* and *Saccharopolyspora thermophila* are proposed for these strains, respectively.

**METHODS**

**Organisms and cultural conditions.** Strain 07 was isolated on an oatmeal agar plate (Shirling & Gottlieb, 1966) seeded with a soil suspension; the latter was prepared from a garden soil collected from Xishan Mountain, Beijing, and incubated at 28 °C for 5 d. Similarly, strain 216 was isolated on selective medium 2 (Lu et al., 2000) inoculated with a soil suspension prepared from a grassland soil sample taken from Nanning, Guangxi Province, Southern China, and incubated at 45 °C for 7 d; the selective medium consisted of Stevenson's basal medium (Stevenson, 1967) supplemented with melezitose (1%, w/v) and neomycin (4 μg ml⁻¹). The strains were maintained on glucose yeast-extract agar (GYEA) (Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20%, v/v) at −20 °C. Biomass for the chemotaxonomic and molecular systematic studies was prepared by growing the strains in shake flasks of modified Sauton's broth (Mordarska et al., 1972) at either 28 °C (strain 07) or 50 °C (strain 216) for 5 d. At maximum growth, the broth cultures were checked for purity, killed with formaldehyde (1%, v/v), washed three times with distilled water and freeze-dried.

**Cultural and morphological properties.** The undisturbed arrangement of hyphae, notably aerial hyphae and spore-chain morphology, was observed on modified Sauton's agar (Mordarska et al., 1972) and ISP medium 2 (Shirling & Gottlieb, 1966) after 7 d, using the coverslip technique of Kawato & Shinobu (1959). Growth on the coverslips was fixed and examined according to the procedure described by Zhou et al. (1998). Additional morphological properties were observed by examining gold-coated, dehydrated specimens of the organisms, using a Hitachi S-570 scanning electron microscope.

**Biochemical and physiological properties.** The strains were examined for a range of phenotypic properties following incubation for 14 d at either 28 °C (strain 07) or 50 °C (strain 216), in most cases according to procedures described in previous studies on *Saccharopolyspora* (Lacey & Goodfellow, 1975; Zhou et al., 1998). To determine lysozyme sensitivity, a 0.05% (w/v) solution of lysozyme was sterilized by membrane filtration and added to sterilized Sauton's broth (McCarthy & Cross, 1984) to give a final concentration of 0.0025% (w/v). Test tubes of the basal medium with and without lysozyme were inoculated with fresh cultures and examined for growth after 10 d at either 28 °C or 50 °C. The antibiotic-sensitivity tests were carried out by placing impregnated filter-paper discs (Goodfellow & Orchard, 1974) over Sauton's agar and incubating the samples for 7–10 d at the incubation temperatures cited above. The two organisms were also examined for their ability to grow at either 20, 25, 37 and 45 °C (strain 07) or at 40, 45, 50, 55 and 60 °C (strain 216).

**Chemotaxonomy.** Established procedures were used to determine the diagnostic isomers of A₄ pm, the predominant whole-organism sugars and the major polar lipids (Leechvalier & Lechevalier, 1980; Hasegawa et al., 1983). Isoprenoid quinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu et al., 1989), with *Streptomyces griseus* as the control. The acid methanolysis procedure was used to screen for mycolic acids (Minnikin et al., 1980). The DNA base ratios of the two strains were determined using the thermal denaturation method of Marmur & Doty (1962), with *Escherichia coli* AS1.365 as the control.

**16S rDNA sequencing.** Genomic DNA was isolated from the strains by following the procedure described by Kim et al. (1995). PCR amplification of the 16S rDNA preparations was carried out as described previously (Kim et al., 1996). The resultant PCR products were purified by using the Wizard PCR purification system (Promega) according to the procedure provided by the manufacturer. The purified products were directly sequenced using a *Tag DyeDeoxy Terminator Cycle Sequencing kit* (Applied Biosystems) and the universal primers 27f (5'-GAGAGTTTGATCCTGGCTCAG-3'), 519f (5'-GTACGGTGAACTTGCCAGCAAG-3') and 765r (5'-CTGTTCCGCTCCACGGTTTC-3') and 1495r (5'-CTAGCGCTACCTTGTTACGA-3'). Sequence gel electrophoresis was carried out and nucleotide sequences 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 of the strains were aligned manually using the **clustal x** program (version 1.6, Thompson et al., 1997) against corresponding nucleotide sequences of representatives of the families *Actinosynmemaetaceae* (Labad & Kroppenstedt, 2000) and *Pseudonocardiaeae* (Kim & Goodfellow, 1999) retrieved from the DDBJ (Thompson et al., 1997) and GenBank (Benson et al., 1997) databases. Evolutionary trees were inferred using the neighbour-joining (Saitou & Nei, 1987) and least-squares (Fitch & Margoliash, 1967) treeing algorithms from the **phylip** package (Felsenstein, 1993). Evolutionary distance matrices were generated as described by Kimura (1980). Tree topologies were evaluated by bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset using the **seqboot** and **consense** options from the **phylip** suite of programs (Felsenstein, 1993).

**RESULTS AND DISCUSSION**

Almost complete 16S rRNA sequences were generated for strains 07 (1443 nucleotides) and 216 (1441 nucleotides). Comparison of these nucleotide sequences with those of representatives of the families *Actinosynmetaetaceae*, *Pseudonocardiaeae* and related taxa (Labad & Kroppenstedt, 2000) clearly showed that the organisms belong to the genus *Saccharo-
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Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rDNA sequences, showing relationships between *Saccharopolyspora* strains 07\(^T\) and 216\(^T\) and representatives of the families Actino-
 synnemataceae and Pseudonocardiaceae and related taxa. Asterisks indicate branches that were also recovered using the Fitch–Margoliash treeing algorithm. The numbers at the nodes indicate the levels of bootstrap support, based on a neighbour-joining analysis of 1000 resampled datasets; only values greater than 50% are given. Bar, 0.02 substitutions per nucleotide position.

*polyspora* (Lacey & Goodfellow, 1975) (Fig. 1). The chemical properties of strain 07\(^T\) are also consistent with its assignment to this taxon. The organism contains *meso*-A\(^{\pm}\)pm as the wall diamino acid, arabinose and galactose as the major wall sugars, a phospholipid pattern containing phosphatidylcholine and tetrahydrogenated menaquinones with nine isoprene units as the predominant isoprenoid quinone, but lacks mycolic acids. This chemical profile distinguishes strain 07\(^T\) from members of wall-chemotype-IV taxa, apart from those classified in the genus *Saccharopolyspora* (Zhou et al., 1998; Kim & Goodfellow, 1999). Strain 216\(^T\) also showed a range of chemical markers typical of saccharopolysporae, though it was unusual in having hexa- and deca-
hydrogenated menaquinones with nine isoprene units as the predominant menaquinones. This apparently anomalous result may reflect the time at which the culture was sampled, as there is evidence that the menaquinone composition of *Amycolatopsis* and *Streptomyces* strains can be age-dependent (Saddler et al., 1986; Yassin et al., 1991). In addition, strains 07\(^T\) and 216\(^T\) were found to have G+C-rich DNA (73.1 and 67.0 mol%, respectively).

The phenotypic properties of the strains are also typical of saccharopolysporae. The organisms are aerobic, non-motile, Gram-positive, non-acid–

alcohol-fast actinomycetes which form extensively branched substrate mycelia. The latter fragment into rod-shaped elements which carry aerial hyphae that differentiate into short chains of smooth-surfaced spores (Fig. 2). Strain 07\(^T\) bears aerial hyphae which differentiate into chains of three to five spores (Fig. 2, right); the aerial hyphae of strain 216\(^T\) differentiate into chains of four to six spores separated by 'empty' hyphae (Fig. 2, left).

The close relationship found between *Saccharo-
polyspora* strain 07\(^T\) and the type strain of *S. spinosa* is supported both by treeing algorithms and by a high bootstrap value (Fig. 1). The two strains share a 16S rDNA nucleotide similarity value of 96.2%, which corresponds to 53 nucleotide differences. In contrast, *Saccharopolyspora* strain 216\(^T\) is not particularly
closely related to any of the type strains of *Saccharopolyspora* species, or to *Saccharopolyspora* strain 07T. Its closest relationship is with the type strain of *S. spinosporotrichia*. The 16S rDNA similarity between these two strains is 93·0%, a value that is equivalent to that of *S. erythraea* and *S. spinosporotrichia*, share a 16S rDNA similarity of 97·5%, a value that corresponds to 31 nucleotide differences. The two most closely related type strains of *Saccharopolyspora* species, namely, those of *S. erythraea* and *S. spinosporotrichia*, share a 16S rDNA similarity of 97·5%, a value that corresponds to 31 nucleotide differences. It is evident, therefore, that *Saccharopolyspora* strains 07T and 216T represent two new centres of taxonomic variation within the genus *Saccharopolyspora*. The two organisms can also be distinguished from one another, and from the type strains of the validly described species of *Saccharopolyspora*, by using a combination of phenotypic properties (Table 1).

The chemotaxonomic, molecular systematic and phenotypic data show that strains 07T and 216T should each be given species status within the genus *Saccharopolyspora*. It is proposed, therefore, that strain 07T be classified as *Saccharopolyspora flava* sp. nov. and strain 216T as *Saccharopolyspora thermophila* sp. nov.

It is also evident from the 16S rDNA tree that the genera *Actinobispora* Jiang et al. 1991 and *Pseudonocardia* Henssen 1957 emend. Reichert et al. 1998 are closely related. Comparative taxonomic studies on representative strains are needed to determine the detailed relationships that exist between members of these genera.

**Description of *Saccharopolyspora flava* Lu et al.**

*Saccharopolyspora flava* (fla’va. L. adj. flaveus yellow, referring to the colour of the substrate mycelium).

Aerobic, Gram-positive, non-acid–alcohol-fast, non-motile actinomycete which forms an extensively branched, yellow substrate mycelium which fragments into rod-shaped elements after 3–4 d at 28 °C. Aerial hyphae are produced upon prolonged cultivation on oatmeal agar. The organism also grows well on glucose-asparagine, ISP medium 2 and modified Sauton’s agars. The aerial mycelium carries abundant chains of 3–5 smooth-surfaced spores (0·4 × 0·5–0·6 μm). Diffusible pigments are not produced. Adenine, cellulose, hypoxanthine, starch, xanthine and xylan are degraded, but casein, chitin, elastin, guanine and tyrosine are not degraded. Aesculin and urea are hydrolysed and nitrate is reduced. Uses adonitol, cellobiose, erythritol, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, methyl α-D-glucoside, raffinose, salicin, sorbitol, sucrose, trehalose, xylitol, acetate, adipate, benzoate, butyrate, citrate, fumarate, H-malate, propionate, pyruvate and succinate as sole carbon sources for energy and growth but does not use L-arabinose, melizitose, sebacic acid or tartrate. Weak growth occurs in the presence of 7% (w/v) NaCl. Growth is inhibited by lysozyme, gentamicin sulphate (discs soaked in 100 μg antibiotic ml⁻¹), streptomycin sulphate (100 μg ml⁻¹) and rifampicin (50 μg ml⁻¹). The temperature growth range is between 28 and 37 °C. The G+C content of the DNA is 67 mol%. Isolated from garden soil collected from Xishan Mountain, Beijing, China. The type strain is strain 07T [= AS4.1520T (Chinese Culture Collection of Microorganisms) = IFO 16345T = JCM 10665T].

**Description of *Saccharopolyspora thermophila* Lu et al.**

*Saccharopolyspora thermophila* (ther.mo’phi.la. Gr. n. thermé heat; Gr. adj. philus loving; M.L. adj thermophila heat-loving organism).

Aerobic, Gram-positive, non-acid–alcohol-fast, non-motile actinomycete which forms an extensively branched colourless to buff substrate mycelium which fragments into rod-shaped elements after 4–5 d at 45 °C. Good growth occurs on ISP medium 2 and modified Sauton’s and oatmeal agars. An abundant aerial mycelium carries long hooked to flexuous chains of 4–6 smooth-surfaced, vesicular spores (0·7–1·1 × 0·85–1·5 μm). Diffusible pigments are not produced. Adenine, guanine, starch, tyrosine and xylan are degraded, but casein, cellulose, chitin, elastin, hypoxanthine and xanthine are not degraded. Aesculin and urea are hydrolysed but nitrate is not reduced.

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**Fig. 2.** Scanning electron micrographs showing (left) vesicular chains of smooth-surfaced spores of a 7-d-old culture of strain 216T grown on ISP medium 2 at 45 °C and (right) short chains of smooth-surfaced spores of a 7-d-old culture of strain 07T grown on oatmeal agar at 28 °C. Bars, 3 μm.
Table 1 Comparison of properties of strains 07\(^T\) and 216\(^T\) with those of the type strains of validly described Saccharopolyspora species

| Characteristic | 07\(^T\) | 216\(^T\) | S. erythraea DSM 4017\(^T\) | S. gregorii DSM 44328\(^T\) | S. hirsuta DSM 43463\(^T\) | S. horderi DSM 44065 | S. rectivirgula DSM 43747\(^T\) | S. spinosa DSM 44228\(^T\) | S. spinopolvetricchia DSM 44350 | S. taberi DSM 43850
|---------------|---------|---------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Spore arrangement | Straight | Hooks or flexuous | Open spirals | Hooks or flexuous | Spiny | Straight to loose spirals | Hairy | Smooth | Smooth or irregularly rough | Spirals | N/A
| Spore ornamentation | Smooth | Smooth | Spiny | Smooth | Spiny | Spiny | Spiny | N/A
| Substrate mycelium | Fragments | Fragments | Branched | Fragments | Fragments | Branched | Fragments in lipid media | W–P | W–G | N/A
| Colour of soluble pigment | None | None | Y–POLr | None | R–O–Y | None | None | C–Br | Br | O–R
| Spires on substrate mycelium | — | — | — | — | — | — | — | — | — | —
| Degradation of: | | | | | | | | | |
| Adenine | + | + | + | + | + | + | + | + | + | +
| Casein | — | — | — | + | + | + | — | — | + | +
| Chitin | — | — | — | — | — | — | — | — | — | —
| Elastin | — | — | — | + | + | + | — | — | + | +
| Starch | + | + | + | + | + | + | — | — | + | +
| Tyrosine | — | + | + | + | + | + | + | — | — | +
| Xanthine | + | + | + | + | + | + | + | + | — | —
| Reduction of nitrate | + | + | + | — | — | — | — | — | — | +
| NaCl tolerance (w/v) | 7 | 7 | < 5 | 13 | < 7 | < 13 | < 10 | < 11 | 2–3 | 7
| Utilization of carbohydrates as sole carbon source | | | | | | | | | |
| L-Arabinose | — | — | + | + | + | + | — | — | — | —
| D-Galactose | + | + | + | + | + | + | — | — | — | —
| D-Lactose | + | + | — | — | — | — | — | — | — | —
| D-Maltose | + | + | + | + | + | + | — | — | — | +
| D-Raffinose | + | + | + | + | + | + | — | — | — | +
| T-Rhamnose | + | + | + | + | + | + | — | — | — | +
| Sucrose | + | + | + | + | + | + | — | — | — | +
| D-Xylose | + | — | + | + | + | + | — | — | — | +

Data for organisms other than the tested strains are taken from Korn-Wendisch et al. (1989) and Zhou et al. (1998).

Uses adonitol, cellobiose, erythritol, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannotol, melezitose, methyl ß-d-glucoside, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, acetate, adipate, benzoate, butyrate, citrate, fumarate, Hmalate, propionate, pyruvate, sebacic acid and succinate as sole carbon sources for energy and growth but does not use L-arabinose or tartrate. Growth occurs in the presence of 7% (w/v) NaCl but is inhibited by lysozyme, gentamicin sulphate, streptomycin sulphate and rifampicin. The temperature growth range is 45–55 °C. The predominant menaquinones are MK-9(H\(_2\)) and MK-9(H\(_3\)). The G+C content of the DNA is 73.1 mol%. Isolated from grassland soil collected from Nanning, Guangxi Province, China. The type strain is strain 216\(^T\) [= AS4.1511\(^T\) (China Culture Collection of Microorganisms) = IFO 16346\(^T\) = JCM 10664\(^T\)].

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