Saccharopolyspora rosea sp. nov., isolated from a patient with bronchial carcinoma

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The taxonomic status of a bacterium isolated from a patient with bronchial carcinoma was established using a polyphasic taxonomic approach. The strain had morphological and chemotaxonomic characteristics consistent with those of members of the genus *Saccharopolyspora*. The generic assignment was confirmed by comparative analysis of the 16S rRNA gene sequence, which showed that the strain constituted a distinct phyletic line, displaying 93.5–96.9% sequence similarity with respect to members of the genus *Saccharopolyspora*. The isolate was distinguished from the type strains of recognized members of the genus *Saccharopolyspora* by means of various biochemical tests. The genotypic and phenotypic data obtained demonstrated that strain IMMIB L-1070^T represents a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora rosea* sp. nov. is proposed. The type strain is IMMIB L-1070^T (=DSM 45226^T=CCUG 56401^T).

The genus *Saccharopolyspora* was first described by Lacey & Goodfellow (1975) and (at the time of writing) contains 13 species with validly published names: *Saccharopolyspora antimicrobica* (Yuan *et al.*, 2008); *S. cebuensis* (Pimentel-Elardo *et al.*, 2008), *S. 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. shandongensis* (Zhang *et al.*, 2008), *S. spinosa* (Mertz & Yao, 1990), *S. spinosporotrichia* (Zhou *et al.*, 1998), *S. taberi* (Labeda, 1987; Korn-Wendisch *et al.*, 1989) and *S. thermophila* (Lu *et al.*, 2001). 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 (Embley, 1992). During the course of the identification of bacterial isolates encountered in clinical specimens, a hitherto unknown filamentous, thermotolerant bacterium was characterized that did not correspond to any recognized taxon. On the basis of the results of a polyphasic taxonomic study, a novel species of the genus *Saccharopolyspora* is described.

Strain IMMIB L-1070^T was isolated from a bronchial lavage sample from a 45-year-old patient with bronchial carcinoma. After treatment of the bronchial lavage sample with *N*-acetylcysteine, the strain was isolated on Löwenstein–Jensen medium and subsequently cultured on Columbia agar supplemented with 5% sheep blood

agar and brain heart infusion agar. The organism was grown on yeast extract-malt extract agar (ISP2 medium), oatmeal agar (ISP3 medium) and inorganic salts-starch agar (ISP4 medium) as described by Shirling & Gottlieb (1966) and was examined for pigmentation, for production of aerial mycelium and for other morphological characteristics. Air-dried smears from Columbia agar were stained using the standard Gram and Ziehl–Neelsen methods in order to determine the Gram stain and acid-fastness, respectively. The micromorphology of the organism was determined by using a culture grown at 37 °C on ISP2 medium. Growth temperatures were determined on Columbia agar by incubating the organism at 28, 37, 42 and 50 °C. The physiological properties of strain IMMIB L-1070^T were determined by using tests to investigate hydrolysis of complex substrates, as described previously (Gordon, 1966, 1967; Gordon & Mihm, 1957), as well as tests to determine carbon-source utilization (as described by Yassin *et al.*, 1995). Melanoid pigment production was determined using peptone-yeast extract-iron agar (ISP6 medium) and tyrosine agar (ISP7 medium) as described by Shirling & Gottlieb (1966). The isomeric form of the diaminopimelic acid was determined using the methods of Becker *et al.* (1964) and the whole-cell sugars were determined using the method of Lechevalier (1968). Lipids were extracted using acid methanolysis and mycolic acids were detected by means of TLC as described by Minnikin *et al.* (1980). Non-hydroxylated fatty acids were analysed as described recently by Yassin *et al.* (2007). Polar lipids were extracted, purified and identified using two-dimensional TLC as described by Yassin *et al.* (1993). Menaquinones were extracted and purified as described by

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain IMMIB L-1070^T is AM992060.

Collins *et al.* (1977). Mass spectral analyses of the menaquinones were recorded as described by Yassin & Hupfer (2006) in positive-ion mode on a Q-TOF 2 mass spectrometer (Micromass) equipped with a nanospray source.

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene sequence and purification of PCR products were carried out using procedures described previously (Rainey *et al.*, 1996). The purified PCR products were sequenced using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) as described in the manufacturer's protocol. A Genetic Analyzer (model 310; Applied Biosystems) was used for electrophoresis of the sequencing reaction products. The 16S rRNA gene sequences of isolate IMMIB L-1070^T and recognized members of the genus *Saccharopolyspora* (obtained from GenBank) were added to the ARB database (Ludwig *et al.*, 2004) and aligned using the respective tool from the ARB package. The resulting alignment was corrected manually and evolutionary trees were inferred using maximum-parsimony (Fitch, 1971), neighbour-joining (Saitou & Nei,

1987) and maximum-likelihood (Felsenstein, 1981) methods. The evolutionary distance matrix for the neighbour-joining method was calculated using the correction of Jukes & Cantor (1969). The topology of the neighbour-joining tree was evaluated using bootstrap analyses (Felsenstein, 1985) based on 500 resamplings.

Cultures of strain IMMIB L-1070^T produced abundant growth on Columbia agar and ISP2, ISP3 and ISP4 media. On these ISP media, the organism formed yellowish to brownish yellow, extensively branched substrate mycelia that fragmented into rod-shaped elements. The aerial hyphae were yellowish to white and differentiated into long chains of smooth-surfaced spores. A pink-coloured diffusible pigment was weakly produced on ISP2 and ISP3 media, whereas strong production of this pigment was observed on ISP4 medium. The organism was found to be aerobic, and the hyphae stained Gram-positive and were non-acid-alcohol fast. A battery of phenotypic and biochemical tests were performed on strain IMMIB L-1070^T, the results of which are given in the species description and Table 1.

Table 1. Differential physiological properties of strain IMMIB L-1070^T and the type strains of the species of the genus *Saccharopolyspora*

Strains: 1, IMMIB L-1070^T; 2, *S. cebuensis* SPE 10-1^T; 3, *S. gregorii* DSM 44324^T; 4, *S. spinosporotrichia* DSM 44350^T; 5, *S. spinosa* DSM 44228^T; 6, *S. erythraea* DSM 40517^T; 7, *S. hirsuta* subsp. *hirsuta* DSM 43463^T; 8, *S. hordei* DSM 44065^T; 9, *S. rectivirgula* DSM 43747^T; 10, *S. flava* AS4.1520^T; 11, *S. thermophila* AS4.1511^T; 12, *S. taberi* DSM 43856^T; 13, *S. shandongensis* JCM 14614^T; 14, *S. antimicrobica* I05-00074^T. Data for strain IMMIB L-1070^T are from this study; data for all other strains are from Pimentel-Elardo *et al.* (2008) and Lu *et al.* (2001), except for data concerning utilization of hypoxanthine and D-mannitol, which are from Goodfellow *et al.* (1989), Labeda (1987), Lacey & Goodfellow (1975), Mertz & Yao (1990) and Zhou *et al.* (1998). *S. cebuensis* SPE 10-1^T is the only known species of the genus *Saccharopolyspora* with a strict requirement for salt. +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Utilization of:														
L-Arabinose	+	+	+	–	+	+	–	+	–	–	–	–	+	+
D-Galactose	+	+	+	+	–	+	+	+	+	+	+	+	+	+
D-Lactose	–	+	–	–	–	–	+	+	+	+	+	+	–	+
Maltose	+	+	+	+	–	+	+	+	+	+	+	+	+	+
D-Mannitol	+	–	+	+	+	+	+	+	+	+	+	+	ND	ND
Raffinose	–	+	+	+	–	+	+	+	+	+	+	+	+	+
L-Rhamnose	–	+	+	+	–	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	–	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	–	+	+	+	+	+	–	+	+	+
Temp. range for growth (°C)	22–42	15–37	10–35	28–37	15–37	20–42	25–50*	20–60	37–63*	28–37	45–55	20–45	15–38	20–45
Nitrate reduction	–	–	–	–	+	+	–	–	+	+	–	+	+	+
Degradation of:														
Adenine	+	–	–	–	–	+	+	+	–	+	+	+	+	+
Casein	+	–	+	+	–	–	+	+	–	–	–	+	+	+
Chitin	ND	–	–	–	–	+	–	+	–	–	–	+	–	–
Elastin	–	ND	+	+	–	+	+	+	–	–	–	+	+	–
Hypoxanthine	+	–	+	+	+	+	+	+	+	+	–	+	+	+
Tyrosine	+	+	+	–	+	+	+	+	+	–	+	+	+	+

*The temperature data from Lu *et al.* (2001) for the type strains differ slightly from those of Korn-Wendisch *et al.* (1989), who reported temperature ranges of 20–50 °C for *S. hirsuta* and 37–60 °C for *S. rectivirgula*.

Cell-wall analysis revealed that strain IMMIB L-1070^T contained chemical markers that supported its assignment to the genus *Saccharopolyspora*. The cell wall contained *meso*-diaminopimelic acid as well as arabinose and galactose (i.e. wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970). Analysis of the non-hydroxylated cellular fatty acids of strain IMMIB L-1070^T revealed the presence of straight-chain, saturated fatty acids C_{14:0} (0.3 % of total fatty acids), C_{15:0} (0.8 %), C_{16:0} (2.1 %), C_{17:0} (8.4 %) and C_{18:0} (0.3 %) and branched-chain fatty acids iso-C_{15:0} (8.6 %), anteiso-C_{15:0} (3.5 %), iso-C_{16:0} (12.8 %), iso-C_{17:0} (15.5 %), anteiso-C_{17:0} (42.2 %) and iso-C_{18:0} (0.9 %). Polar lipid analysis showed that the organism contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and phosphatidylinositol as the characteristic phospholipids (i.e. phospholipid type PIII *sensu* Lechevalier *et al.*, 1977). Mass spectral analysis of the respiratory quinones showed that a tetrahydrogenated menaquinone with nine isoprene units, MK-9(H₄), was the predominant menaquinone. In addition, a substantial amount of a tetrahydrogenated menaquinone with 10 isoprene units, MK-10(H₄), was detected.

To ascertain the phylogenetic relationships of strain IMMIB L-1070^T, the almost-complete 16S rRNA gene sequence of the novel strain (1492 nt) was determined in this study. Comparative 16S rRNA gene sequence analysis revealed that strain IMMIB L-1070^T displayed highest sequence similarities with respect to species of the family *Pseudonocardiaceae* (data not shown). Strain IMMIB L-1070^T displayed highest sequence similarity (93.5–96.9 %) with species of the genus *Saccharopolyspora*. Phylogenetic analysis confirmed the association of strain

IMMIB L-1070^T with the genus *Saccharopolyspora*, with the strain forming a distinct subline. A phylogenetic tree for strain IMMIB L-1070^T, constructed with the neighbour-joining method, is shown in Fig. 1. It is evident from the tree that strain IMMIB L-1070^T displays a phylogenetic affinity with a subcluster of species consisting of *S. gregorii* and *S. cebuensis*; however, the branching of the strain at the base of this subcluster was not supported by either bootstrap analysis or other treeing methods. Strain IMMIB L-1070^T showed highest sequence similarities with respect to *S. spinosa* DSM 44228^T (96.9 %), *S. shandongensis* 88^T (96.7 %), *S. gregorii* ATCC 51265^T (96.3 %), *S. antimicrobica* I05-00074^T (96.2 %) and *S. cebuensis* DSM 45019^T (96.1 %); other species showed lower levels of sequence similarity. However, it is generally accepted that organisms displaying ≥3 % 16S rRNA gene sequence divergence are not members of the same species (Stackebrandt & Goebel, 1994). Support for the distinctiveness of strain IMMIB L-1070^T is also evident from phenotypic data: the strain can be readily distinguished from all of the recognized members of the genus *Saccharopolyspora* (Table 1). In particular, it differed from *S. spinosa* DSM 44228^T by its ability to utilize D-galactose, maltose, sucrose and D-xylose as carbon sources and by its ability to hydrolyse adenine and casein. Strain IMMIB L-1070^T can also be distinguished biochemically from *S. shandongensis* 88^T by the inability to utilize raffinose and L-rhamnose as carbon sources and the inability to hydrolyse elastin. Similarly, strain IMMIB L-1070^T varies from *S. gregorii* ATCC 51265^T by its ability to hydrolyse adenine and the inability to utilize raffinose and L-rhamnose as carbon sources. Therefore, on the basis of both phenotypic and phylogenetic criteria, strain IMMIB L-1070^T represents

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain IMMIB L-1070^T within the radiation of species of the genus *Saccharopolyspora*. Numbers at nodes indicate bootstrap percentages (based on 500 resampled datasets). Filled circles indicate that the corresponding nodes (groupings) were also recovered in maximum-likelihood and maximum-parsimony trees. Asterisks indicate that the corresponding nodes were recovered only in the neighbour-joining and maximum-likelihood trees. Bar, 5.0 % sequence divergence.

a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora rosea* sp. nov. is proposed.

Description of *Saccharopolyspora rosea* sp. nov.

Saccharopolyspora rosea (ro.se'a. L. fem. adj. *rosea* rose-coloured, pink, referring to the colour of the diffusible pigment produced by the organism).

Cells are aerobic, catalase-positive, oxidase-negative and thermotolerant, growing at 22–42 °C and pH 6.0–8.0. Gram-positive and non-acid-alcohol fast. Forms yellowish to brownish yellow, extensively branched substrate mycelia (0.4–0.5 µm in diameter) that fragment into rod-shaped elements and carry yellowish to white aerial hyphae (0.5–0.7 µm) that differentiate into long chains of smooth-surfaced spores. Pink-coloured diffusible pigment is produced on ISP2, ISP3 and ISP4 media. Melanin pigment is not formed in ISP6 or ISP7 media. Adenine, aesculin, casein, gelatin, hypoxanthine, tyrosine and urea are hydrolysed; elastin, guanine, keratin, testosterone and xanthine are not hydrolysed. Nitrate is not reduced. Assimilates acetate, adipate, adonitol, iso-amyl alcohol, L-arabinose, 2,3-butanediol, citrate, *meso*-erythritol, D-galactose, D-gluconate, D-glucose, *myo*-inositol, L-lactate, maltose, D-mannitol, sucrose, D-sorbitol, trehalose and D-xylose as carbon sources, but not cellobiose, *m*-hydroxybenzoate, *p*-hydroxybenzoate, D-lactose, melezitose, 1,2-propanediol, raffinose or L-rhamnose. Acetamide, L-alanine, arginine, gelatin, ornithine, L-proline and L-serine are utilized as simultaneous sources of carbon and nitrogen.

The type strain, IMMIB L-1070^T (=DSM 45226^T=CCUG 56401^T), was isolated from bronchial lavage from a 45-year-old patient with bronchial carcinoma.

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