Saccharopolyspora phatthalungensis sp. nov., isolated from rhizosphere soil of *Hevea brasiliensis*

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The taxonomic position of a rhizosphere soil isolate, designated strain SR8.15^T, was determined by using a polyphasic approach. Phylogenetic analysis based on an almost-complete 16S rRNA gene sequence of the strain showed that it formed a well-separated sub-branch within the radiation encompassing the genus *Saccharopolyspora*. Highest levels of 16S rRNA gene sequence similarity were found between strain SR8.15^T and *Saccharopolyspora shandongensis* CGMCC 4.3530^T (98.9 %) and *Saccharopolyspora spinosa* DSM 44228^T (98.5 %). However, these strains shared low levels of DNA–DNA relatedness (<26 %). Strain SR8.15^T had chemical characteristics consistent with its classification in the genus *Saccharopolyspora*. It contained *meso*-diaminopimelic acid as the diagnostic diamino acid. Whole-cell hydrolysates contained arabinose and galactose. The diagnostic phospholipids were phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol. The main menaquinone was MK-9(H₄). No mycolic acid was detected. The predominant cellular fatty acid was iso-C_{16:0}. The G + C content of the genomic DNA of strain SR8.15^T was 70.3 mol%. Strain SR8.15^T had a phenotypic profile that readily distinguished it from recognized representatives of the genus *Saccharopolyspora*. It is evident from its combined genotypic and phenotypic properties that strain SR8.15^T represents a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora phatthalungensis* sp. nov. is proposed. The type strain is SR8.15^T (=TISTR 1921^T=BCC 35844^T=NRRL B-24798^T).

The genus *Saccharopolyspora* was first erected by Lacey & Goodfellow (1975) with the description of *Saccharopolyspora hirsuta* as the type species. At the time of writing, the genus comprises 16 recognized species, namely *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. halophila* (Tang *et al.*, 2009), *S. hirsuta* (Lacey & Goodfellow, 1975), *S. hordei* (Goodfellow *et al.*, 1989), *S. jiangxiensis* (Zhang *et al.*, 2009), *S. rectivirgula* (Korn-Wendisch *et al.*, 1989), *S. rosea* (Yassin, 2009), *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). Most species of the genus were isolated from soil samples, but *S. gregorii* and *S. hordi* were isolated from fodder (Goodfellow *et al.*, 1989),

S. cebuensis from a marine sponge (Pimentel-Elardo *et al.*, 2008) and *S. halophila* from a saline lake (Tang *et al.*, 2009). The most recently described member, *S. rosea*, was isolated from a patient with bronchial carcinoma (Yassin, 2009). Representatives of the genus *Saccharopolyspora* form a distinct phyletic line within the evolutionary radiation encompassing the family *Pseudonocardiaceae* and can be distinguished based on a combination of biochemical, morphological and physiological properties (Embley, 1992).

During the course of a programme of isolation of actinomycetes from rhizosphere soil of rubber trees (*Hevea brasiliensis*) in Phatthalung Province, Thailand, strain SR8.15^T was isolated by using the dilution plating method on starch casein agar plates (Küster & Williams, 1964) supplemented with ketoconazole and nalidixic acid as antifungal and antibacterial agents, respectively. The plates, which had been seeded with soil suspension, were incubated at 28 °C for 14 days. One colony was isolated

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SR8.15^T is GQ381309.

and purified on glucose yeast extract (GYE) agar [containing 1.0 % (w/v) glucose, 1 % (w/v) yeast extract and 1.5 % (w/v) agar]. The pure culture was maintained as a 20 % glycerol suspension at -20°C or as lyophilized cells for long-term preservation. *S. shandongensis* JCM 14614^T and *S. spinosa* JCM 9375^T were used as reference strains.

The cultural characteristics of strain SR8.15^T were examined on International *Streptomyces* Project (ISP) media 1–7 (Shirling & Gottlieb, 1966) and GYE agar. The colour of mycelium and soluble pigment was determined by comparing them with colour chips from the *Color Harmony Manual* (Jacobson *et al.*, 1958). Morphological characteristics were observed by light microscopy and scanning electron microscopy (JEOL-JSM 5600 LV) using 14-day-old cultures grown on GYE agar and ISP medium 3. Acid production from carbohydrates was determined by using the media and methods described by Gordon *et al.* (1974). Activity profiles of 19 enzymes were tested by using the API ZYM system (bioMérieux). Urease activity was determined based on a colour change on urea agar (Gordon *et al.*, 1974). The temperature range for growth was determined on ISP medium 2 (Shirling & Gottlieb, 1966) by using a temperature gradient incubator (Tokyo Kagaku Sangyo). The production of hydrogen sulfide was detected by using lead acetate strips. Hydrolysis of casein and gelatin and reduction of nitrate were examined according to the methods of Gordon & Mihm (1957). Production of melanoid pigment was determined according to Shirling & Gottlieb (1966). NaCl tolerance was studied on ISP medium 2 containing NaCl at final concentrations of 1–12 % (w/v) (at intervals of 1.0 %).

To analyse chemotaxonomic markers, cell biomass was obtained by growing strain SR8.15^T in GYE broth in shake flasks for 3 days at 28°C . The resultant cells were washed three times in distilled water and freeze-dried. Standard procedures were used to determine the isomers of diaminopimelic acid (Hasegawa *et al.*, 1983). The acyl type of the cell wall was analysed according to the method of Uchida & Aida (1984). Whole-cell sugars were analysed according to the method of Becker *et al.* (1965). Polar lipids were examined by TLC by using the method of Minnikin *et al.* (1977). Menaquinones were extracted and purified by the method of Collins *et al.* (1977), and isoprene units were subsequently analysed by LC/MS (JMS-T100LP; JEOL) with a PEGASIL ODS column ($2\phi \times 50$ mm) by using methanol:2-propanol (7:3). Mycolic acids were detected by TLC according to the method of Tomiyasu (1982). The G+C content of the DNA was determined by HPLC according to the method of Tamaoka & Komagata (1984). Analysis of the fatty acids in cells grown in GYE broth at 28°C for 3 days was performed according to the procedures of the Sherlock Microbial Identification System (Microbial ID).

The total DNA of strain SR8.15^T was extracted and purified following the method of Kieser *et al.* (2000). The 16S rRNA gene was amplified as described by Duangmal *et al.* (2005).

The PCR products were purified and sequenced (Macrogen) by using universal primers (Lane, 1991). The almost-complete 16S rRNA gene sequence of strain SR8.15^T (1448 bp) was aligned with corresponding sequences of representatives of the genus *Saccharopolyspora*, retrieved from the GenBank database, by using the CLUSTAL X (Thompson *et al.*, 1997) and PHYDIT (<http://plaza.snu.ac.kr/~jchun/phydit/>) programs. Phylogenetic trees were inferred by the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) methods within the PHYLIP suite of programs (Felsenstein, 1993). The resultant tree topologies were evaluated by using bootstrap analyses (Felsenstein, 1985) based on 1000 resampled datasets of the SEQBOOT and CONSENSE options from the PHYLIP package. The resultant phylogenetic trees were viewed by using the TreeView program (Page, 1996).

Strain SR8.15^T was a Gram-positive, aerobic, filamentous actinobacterium. Good growth developed on ISP medium 1, ISP medium 2 and GYE agar with white aerial mycelium and yellowish-brown substrate mycelium after 14 days at 28°C . Moderate growth occurred on ISP media 3, 6 and 7. Poor growth was observed on ISP media 4 and 5. Brownish-black soluble pigment was produced on ISP medium 2. Chains of oval spores with spiny surfaces were observed on GYE agar (Fig. 1).

Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain SR8.15^T forms a distinct cluster with members of the genus *Saccharopolyspora* (Fig. 2). High levels of 16S rRNA gene sequence similarity were found between strain SR8.15^T and its nearest neighbours, *S. shandongensis* CGMCC 4.3530^T (98.9 %) corresponding to 16 nt differences over 1417 positions, and *S. spinosa* DSM 44228^T (98.5 %) corresponding to 21 nt differences over 1399 positions. DNA–DNA relatedness studies provide a reliable way of distinguishing between representatives of

Fig. 1. Scanning electron micrograph of cells of strain SR8.15^T grown on GYE agar for 14 days at 28°C . Bar, 1 μm .

0.02 substitutions/site

Fig. 2. Neighbour-joining tree derived from 16S rRNA gene sequences showing the relationship between strain SR8.15^T and members of the genus *Saccharopolyspora*. Asterisks indicate branches of the tree that were also found by using the least-squares (Fitch & Margoliash, 1967) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. Numbers at nodes indicate the percentage bootstrap support from an analysis of 1000 resampled datasets. Bar, 0.02 substitutions per site.

species that share high 16S rRNA gene sequence similarity (Stackebrandt & Ebers, 2006). Mean levels of DNA–DNA relatedness between strain SR8.15^T and *S. shandongensis* JCM 14614^T and *S. spinosa* JCM 9375^T were 45.7 and 37.7%, respectively, values well below the 70% cut-off point recommended by Wayne *et al.* (1987) for the delineation of genomic species.

Further DNA–DNA relatedness studies were not carried out between strain SR8.15^T and the type strains of related species that shared more than 97% 16S rRNA gene sequence similarity, such as those of *S. antimicrobica* (97.5%), *S. hirsuta* (97.2%) and *S. jiangxiensis* (97.3%), as it has previously been demonstrated that some phylogenetically closely related strains sharing high 16S rRNA gene sequence similarity show DNA–DNA relatedness values well below the 70% cut-off point (Wayne *et al.*, 1987). For example, the type strain of *S. jiangxiensis* shared 97.5 and 97.4% 16S rRNA gene sequence similarity with the type strains of *S. shandongensis* and *S. spinosa*, respectively, but had DNA–DNA relatedness levels of 25.5 and 21.7%, respectively (Zhang *et al.*, 2009).

The results of chemical analysis indicated that strain SR8.15^T has chemotaxonomic markers characteristic of the genus *Saccharopolyspora* (Korn-Wendisch *et al.*, 1989). It contained *meso*-diaminopimelic acid in the peptidoglycan and MK-9(H₄) as the main menaquinone. Arabinose and galactose were detected as the major components of sugars in whole-cell hydrolysates. Polar lipid analysis showed that the organism contained phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol. No mycolic acids were detected. The G+C content of the DNA of strain SR8.15^T was 70.3 mol%.

The predominant fatty acids of strain SR8.15^T were iso-C_{16:0} (43.7%), 10-methyl C_{17:0} (11.8%), iso-C_{17:0} (8.3%), iso-C_{16:0} 2-OH (7.9%), iso-C_{15:0} (7.5%) and 9-methyl C_{16:0} (5.9%); other cellular fatty acids detected as

minor components were 10-methyl C_{18:0} (4%), anteiso-C_{17:0} (4%), C_{16:1} 2-OH (2.5%), C_{17:1} *cis* (1.9%), iso-C_{14:0} (1.4%) and C_{17:0} (1.2%).

The assignment of strain SR8.15^T to the genus *Saccharopolyspora* was supported by biochemical, chemotaxonomic, morphological and physiological characteristics. Strain SR8.15^T was positive for catalase, hydrogen sulfide production and urease. The temperature range for growth was determined in a temperature gradient incubator set to between 5 and 50 °C. Observation of cultures over 14 days revealed growth at 18–42 °C, with the optimum temperature for growth being 28–34 °C. No growth was observed at or above 42 °C. The strain was able to grow at pH 5.0–9.0, and in the presence of up to 7% NaCl. Melanin pigment was not produced. Strain SR8.15^T was readily differentiated from its closest relatives, *S. shandongensis* and *S. spinosa*, on the basis of physiological properties (Table 1), in particular the production of allantoinase and nitrate reduction, both of which were negative. These characters were positive in *S. shandongensis* and *S. spinosa*. Strain SR8.15^T produced H₂S and was sensitive to 0.005% lysozyme, whereas *S. shandongensis* gave the opposite results. Strain SR8.15^T was not able to degrade adenine, arbutin, starch or xanthine, in contrast to results for *S. shandongensis*. Strain SR8.15^T produced acid from D(+)–galactose, *myo*-inositol and D(+)–xylose, again contrasting with results for *S. shandongensis* and *S. spinosa*. Spore arrangement of strain SR8.15^T was open loops compared with a spiral arrangement in *S. shandongensis*. Moreover, growth of strain SR8.15^T occurred between 18 and 42 °C, as compared with 15–38 °C for *S. shandongensis* and 15–37 °C for *S. spinosa*.

It was evident on the basis of genotypic and phenotypic data that strain SR8.15^T can be distinguished from its closest neighbours, *S. shandongensis* and *S. spinosa*. We therefore conclude that strain SR8.15^T represents a novel

Table 1. Differential characteristics between strain SR8.15^T and the type strains of closely related *Saccharopolyspora* species

Strains: 1, SR8.15^T; 2, *S. shandongensis* JCM 14614^T; 3, *S. spinosa* JCM 9375^T. Data are from the present study. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3
Spore arrangement	Hooks and open loops	Spiral	Hooks and open loops
Degradation of:			
Adenine	–	+	–
Aesculin	+	+	–
Allantoin	–	+	+
Arbutin	–	+	–
Starch	–	+	–
Xanthine	–	+	–
Acid production from:			
D(+)–Cellobiose	–	+	–
D(+)–Galactose	+	–	–
myo–Inositol	+	–	–
Maltose	–	+	–
D(+)–Xylose	w	–	–
Hydrogen sulfide production	+	–	+
Lysozyme resistance (0.005 %)	No growth	Growth	No growth
Nitrate reduction	–	+	+
NaCl tolerance (% w/v)	<7	<7	<11
Temperature range (°C)	18–42	15–38	15–37

species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora phatthalungensis* sp. nov. is proposed.

Description of *Saccharopolyspora phatthalungensis* sp. nov.

Saccharopolyspora phatthalungensis (phat.tha.lun.gen'sis. N.L. fem. adj. *phatthalungensis* referring to Phatthalung Province, Thailand, the source of the rhizosphere soil from which the type strain was isolated).

Aerobic, Gram-positive, non-acid–alcohol-fast, non-motile actinomycete. Cells are filamentous and are differentiated into substrate and aerial mycelium. The aerial mycelium is white, and the substrate mycelium is yellowish to yellowish-brown on ISP media 1, 2, 5, 6 and 7 and GYE agar. A soluble dark-brown pigment is produced on ISP medium 2 but not on ISP media 1, 3, 4, 5, 6 and 7. Spore chains have a bead-like appearance with a spiny spore surface and have an open loop arrangement. Positive for catalase, H₂S production and urease. Negative for allantoinase production and nitrate reduction. Acid is produced from adonitol, D(–)-fructose, D(+)–galactose, D(+)–glucose, glycerol, myo–inositol, D(–)-mannitol, D(+)–mannose, D(–)-ribose and D(+)–trehalose, and weakly produced from D(+)–xylose. No acid production from L(+)–arabinose, D(+)–cellobiose, fucose, β–lactose, malt-

ose, melibiose, D(–)-rhamnose, raffinose, D(–)-sorbitol, sorbose, sucrose or xylitol. Degrades aesculin, casein, gelatin, hypoxanthine and L-tyrosine, but not adenine, arbutin, cellulose, chitin, guanine, starch or xanthine. Growth occurs at pH 5.0–9.0. The temperature range for growth on ISP medium 2 is 18–42 °C with optimum growth at 28–34 °C. Good growth occurs on ISP medium 2 at 0–5 % NaCl, moderate growth at 6 % NaCl and poor growth at 7 % NaCl. Alkaline phosphatase and leucine aminopeptidase are detected with the API ZYM enzyme assay; negative for acid phosphatase, chymotrypsin, cystine aminopeptidase, esterase (C4), α–fucosidase, α–galactosidase, β–galactosidase, α–glucosidase, β–glucosidase, β–glucuronidase, lipase (C8), lipase (C14), α–mannosidase, N–acetyl–β–glucosaminidase, trypsin phosphoamidase and valine aminopeptidase. The diagnostic diamino acid of the peptidoglycan is meso–diaminopimelic acid. Whole-cell sugars are arabinose and galactose. The glycan moiety of the murein is acetylated. Phospholipids are phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol. The main menaquinone is MK-9(H₄). Mycolic acids are not detected. The predominant fatty acids are iso–C_{16:0}, 10-methyl C_{17:0}, iso–C_{17:0}, iso–C_{16:0} 2-OH, iso–C_{15:0} and 9-methyl C_{16:0}. The G + C content of the DNA of the type strain is 70.3 mol%.

The type strain, SR8.15^T (=TISTR 1921^T=BCC 35844^T=NRRL B-24798^T), was isolated from rhizospheric soil of a rubber tree (*Hevea brasiliensis*), collected from Phatthalung Province, Thailand.

Acknowledgements

We would like to thank Dr J. P. Euzéby for advice on naming the species, Onsiri Duangchui for providing soil samples and Yuki Inahashi for technical support. This study was supported by the Commission on Higher Education and the Thailand Research Fund, Kasetsart University Research and Development Institute, Faculty of Science at Kasetsart University and Kitasato University.

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