Saccharothrix violaceirubra sp. nov., isolated from soil and plant litter

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Two strains of nocardioform actinomycetes, isolated from soil and plant litter in Yamanashi prefecture, Japan, showed substrate mycelium with purple to dark-red colours. 16S rRNA gene sequence analysis indicated that the organisms belonged to the family *Actinosynnemataceae* and were related closely to *Saccharothrix* strains (96.7–98.0% sequence similarity). The isolates contained MK-9(H₄) as the predominant menaquinone, *meso*-diaminopimelic acid, galactose as the diagnostic whole-cell sugar and phosphatidylethanolamine as the predominant polar lipid. Based on a combination of cultural, physiological and chemotaxonomic properties, in addition to the phylogenetic analysis and DNA–DNA hybridization data, we propose a novel species, *Saccharothrix violaceirubra* sp. nov., for these strains, with the type strain YU 692-1^T (=NBRC 102064^T =KCTC 19326^T).

The genus *Saccharothrix* was first described by Labeda *et al.* (1984) for actinomycete strains that were characterized by nocardioform mycelia and rod-shaped spores, with *meso*-diaminopimelic acid (*meso*-A₂pm) in the cell wall and with galactose and small amounts of mannose in whole-cell hydrolysates. Menaquinone MK-9(H₄) and phosphatidylethanolamine (phospholipid type II) are contained.

In recent years, several *Saccharothrix* strains have been transferred to other new taxa, including the genera *Lentzea* (Yassin *et al.*, 1995), *Lechevalieria* (Labeda *et al.*, 2001), *Goodfellowia* (Labeda & Kroppenstedt, 2006), *Crossiella* (Labeda, 2001) and *Umezawaea* (Labeda & Kroppenstedt, 2007). As a result, the genus *Saccharothrix* currently includes nine species.

During a screening of actinomycetes, strains YU 692-1^T and YU 724-61, which exhibited synnemata and rod-shaped spores, were isolated from soil and plant litter in Japan, respectively. These isolates were subjected to polyphasic taxonomy, including phylogenetic analysis, chemotaxonomy, DNA–DNA relatedness and cultural and physiological properties. On the basis of these characteristics, including their characteristic colony colour,

the strains were accommodated in the genus *Saccharothrix*. Accordingly, we propose the name *Saccharothrix violaceirubra* sp. nov. for strains YU 692-1^T and YU 724-61.

Strains YU 692-1^T and YU 724-61 were isolated from samples of soil and plant litter in Yamanashi prefecture, Japan, using humic acid/vitamin (HV) agar (Hayakawa & Nonomura, 1987) supplemented with cycloheximide (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹). Morphological features of the strains grown on YS medium (2 g yeast extract, 10 g soluble starch and 15 g agar l⁻¹; pH 7.3) or HV agar were observed by light and scanning electron microscopy (SEM) (model JSM-6060; JEOL). Preparation of samples for SEM was done as described previously (Tamura *et al.*, 1994).

Cultural, physiological and biochemical characteristics were examined by using methods described previously (Gordon *et al.*, 1974; Shirling & Gottlieb, 1966; Williams *et al.*, 1983). A₂pm isomers, whole-cell sugar patterns, fatty acid compositions, isoprenoid quinones and polar lipids were analysed as described by Tamura *et al.* (1994). Genomic DNA was obtained by using the method of Saito & Miura (1963). The G+C content of the DNA was determined by HPLC as described by Tamura *et al.* (1994). DNA–DNA hybridization was performed fluorometrically in microdilution wells using photobiotin, as described by Ezaki *et al.* (1989).

The 16S rRNA gene was amplified by PCR and sequenced following the procedures described by Tamura & Hatano (2001), using a model ABI PRISM 3100 Genetic Analyzer

Abbreviations: *meso*-A₂pm, *meso*-diaminopimelic acid; SEM, scanning electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Saccharothrix violaceirubra* YU 692-1^T is AB284261.

A scanning electron micrograph of the synnemata of strain YU 692-1^T is available with the online version of this paper.

according to the manufacturer's protocol. The 16S rRNA gene sequences obtained in the present study were aligned with sequences of species of the family *Actinosynnemataceae* with validly published names that were available from GenBank/EMBL/DDBJ by using the MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 (Kumar *et al.*, 2004) and CLUSTAL_X (Thompson *et al.*, 1997) programs. A phylogenetic tree was inferred by using the neighbour-joining tree algorithm (Saitou & Nei, 1987) and the minimum-evolution and maximum-parsimony methods (Takahashi & Nei, 2000). The topology of the constructed tree was evaluated by bootstrap analysis with 1000 replicates (Felsenstein, 1985).

The isolates, strains YU 692-1^T and YU 724-61, produced synnemata and formed spore chains on their aerial mycelia; however, they did not form sporangia. The aerial hyphae were well-developed on yeast extract/malt extract agar (ISP medium 2). The colour of the substrate mycelium was purple to dark red on ISP medium 2 and vivid yellow on ISP medium 6. White aerial mycelium was observed on ISP media 2 and 4. These hyphae became septate and gave rise to chains of spores. The formation of branched substrate hyphae and fragmentation into rod-shaped or irregular elements were observed by microscopy. The spores had a smooth surface and were non-motile.

Whole-cell hydrolysates of the two strains contained *meso*-A₂pm, galactose, mannose and rhamnose (wall chemotype III *sensu*; Lechevalier & Lechevalier, 1970). The predominant menaquinones of isolate YU 692-1^T were MK-9(H₄) (46 %), MK-9(H₆) (22 %) and MK-9(H₈) (32 %). The isolates also contained phosphatidylethanolamine (phospholipid type PII *sensu*; Lechevalier *et al.*, 1977) as a diagnostic phospholipid. The major isoprenoid quinone and phospholipid patterns of the isolates were consistent with those of *Saccharothrix australiensis* (Labeda *et al.*, 1984). The major cellular fatty acids were iso-C_{16:0} (21–23 %), anteiso-C_{15:0} (17–18 %), anteiso-C_{17:0} (8–9 %) and C_{17:1} (8–10 %) (Table 1). The DNA G+C contents of isolates YU692-1^T and YU 724-61 were 70.8 and 70.1 mol%, respectively.

Phylogenetic analysis of the almost-complete 16S rRNA gene sequences (1432 nt) of isolates YU 692-1^T and YU 724-61 revealed that these novel isolates belonged to a cluster of the genus *Saccharothrix* (Fig. 1). It is clear that strain YU 692-1^T formed a distinct phyletic line with *Saccharothrix texasensis* NRRL B-16134^T. Three tree-making algorithms supported the position of strain YU 692-1^T as being consistently in the same clade as *Saccharothrix algeriensis* NRRL B-24137^T, *S. australiensis* NRRL B-11239^T, *Saccharothrix espanaensis* NRRL 15764^T, *Saccharothrix mutabilis* subsp. *capreolus* DSM 40225^T and *S. texasensis* NRRL B-16134^T. Sequence similarities between strain YU 692-1^T and these most closely related *Saccharothrix* type strains ranged from 98.0 % (with *S. australiensis*) to 96.4 % (with *S. mutabilis* subsp. *capreolus*), whilst strain YU 692-1^T exhibited 100 % sequence similarity

Table 1. Cellular fatty acid composition of *Saccharothrix violaceirubra* strains YU692-1^T and YU 724-61 and the phylogenetically most closely related species, *Saccharothrix australiensis*

Taxa: 1, YU 692-1^T; 2, YU 724-61; 3, *S. australiensis* NBRC 14444^T. Values are percentages of total fatty acids. –, Not detected. Fatty acid identities were determined from the Microbial Identification System software (MIDI Inc.) peak-naming table.

Fatty acid	1	2	3
iso-C _{14:0}	7.1	6.3	4.7
iso-C _{15:0} G	1.3	1.7	2.4
iso-C _{15:0}	10.4	10.6	30.3
anteiso-C _{15:0}	17.9	17.8	4.8
iso-C _{16:1} H	12.7	14.2	2.7
iso-C _{16:0}	23.0	21.2	13.6
anteiso-C _{16:0}	–	0.6	3.3
<i>cis</i> 9-C _{16:1}	–	–	1.2
C _{16:0} 9-methyl	–	–	10.5
iso-C _{17:1} ω9 <i>c</i>	1.6	2.0	–
anteiso-C _{17:1} ω9 <i>c</i>	1.6	2.3	–
anteiso-C _{17:1} C	–	–	1.0
iso-C _{17:0}	0.8	0.7	6.5
anteiso-C _{17:0}	8.9	8.0	11.3
<i>cis</i> 9-C _{17:1}	–	–	1.0
C _{17:1} ω6 <i>c</i>	7.2	7.0	–
C _{16:1} 2-OH	–	–	1.1
anteiso-C _{17:0} 2-OH	–	–	1.3
C _{17:0} 2-OH	0.8	1.0	–

to strain YU 724-61. According to sequence similarity values and phylogenetic trees, the isolate was related most closely to the type strain of *S. australiensis*.

Physiological properties of the two isolates and the type strains of phylogenetically related *Saccharothrix* species are shown in Table 2. The isolates can utilize glucose and sucrose, but dextrin, D-xylitol and sorbitol are not utilized. They cannot grow at 37 °C or in the presence of 4 % (w/v) NaCl. The strains exhibited good growth at 20–30 °C, with an optimum at 28 °C. In contrast, *S. australiensis* NRRL 11239^T, which is physiologically the most closely related species to the isolates, utilizes sorbitol and dextrin, decomposes calcium malate and grows in 4 % NaCl and at 37 °C. The resistance of strain YU 692-1^T to various antibiotics is given in the formal species description below. Some results of antibiotic resistance are different between isolates YU 692-1^T and YU 724-61 (data not shown).

Strain YU 692-1^T exhibited DNA–DNA relatedness values of 97–112 % to YU 724-61. Strain YU 724-61 was accordingly identified as belonging to the same species as strain YU 692-1^T. In contrast, the DNA–DNA relatedness of these isolates to *S. espanaensis* NBRC 15908^T (=NBRC 15066^T), *S. algeriensis* NBRC 101915^T, *S. australiensis* NBRC 14444^T, *S. mutabilis* subsp. *capreolus* NBRC 12847^T and *S. texasensis* NBRC 14971^T was 13.0, 11.5, 8.0, 7.5 and 7.5 %, respectively.

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987), based on nearly complete 16S rRNA gene sequences for members of the genus *Saccharothrix*, showing the position of *Saccharothrix violaceirubra* YU 692-1^T. *Actinosynnema mirum* NBRC 14064^T (GenBank accession no. D85475) was used as an outgroup. Numbers on branches indicate percentage bootstrap values from 1000 replicates (only values >70% are shown). Asterisks indicate that the corresponding branches were also recovered in the minimum-evolution and maximum-parsimony trees (Takahashi & Nei, 2000). Bar, 0.005 K_{nuc} .

Table 2. Differential phenotypic properties of isolates YU 692-1^T, YU 724-61 and the type strains of phylogenetically related *Saccharothrix* species

Taxa: 1, strains YU 692-1^T and YU 724-61; 2, *S. australiensis* NRRL 11239^T; 3, *S. coeruleofusca* DSM 43679^T; 4, *S. espanaensis* NRRL 15764^T; 5, *S. mutabilis* subsp. *capreolus* DSM 40225^T; 6, *S. mutabilis* subsp. *mutabilis* NRRL B-16077^T; 7, *S. syringae* DSM 43886^T; 8, *S. texasensis* NRRL B-16134^T; 9, *S. xinjiangensis* AS 4.1731^T. +, Positive; –, negative; NR, not reported; w, weakly positive; v, variable. Data for reference species were taken from Labeda (2002) and Hu *et al.* (2004).

Characteristic	1	2	3	4	5	6	7	8	9
Decomposition of:									
Hippurate	+	–	–	+	+	+	+	+	NR
Hypoxanthine	–	–	–	+	+	+	–	+	NR
Starch hydrolysis	–	–	+	–	+	+	+	+	+
Tyrosine	+	+	+	–	–	+	+	+	+
Production of nitrate reductase	+	+	–	w	–	+	–	+	–
Assimilation of:									
Citrate	–	–	–	v	+	+	–	–	–
Lactate	–	w	–	+	–	+	–	+	–
Malate	–	+	–	+	+	+	+	+	NR
Utilization of:									
Arabinose	–	–	+	–	+	+	+	+	+
Dextrin	–	+	+	–	+	+	+	+	NR
Inositol	–	–	–	–	+	+	–	+	NR
Lactose	–	–	+	–	–	+	+	+	+
Melibiose	–	–	–	–	+	+	+	+	+
Raffinose	–	–	+	–	–	+	+	–	+
Rhamnose	–	–	+	–	–	–	+	+	+
Salicin	–	–	+	–	+	+	w	+	NR
Sorbitol	–	+	+	–	–	–	–	–	–
Sucrose	+	–	+	+	–	+	+	+	+
D-Xylose	–	–	+	v	+	+	+	+	+
Methyl α -D-glucoside	–	–	+	–	–	+	–	+	+
Growth in/at:									
4% NaCl	–	+	+	+	+	–	+	–	–
5% NaCl	–	–	+	–	+	–	+	–	–
37 °C	–	+	+	+	+	+	+	+	+
45 °C	–	+	+	–	+	+	+	–	+

Based on 16S rRNA gene sequence data and morphological and chemotaxonomic properties, strains YU 692-1^T and YU 724-61 belong to the genus *Saccharothrix*. On the basis of DNA–DNA relatedness and physiological properties, these isolates represent a distinct species in this genus. Accordingly, we propose a novel species, *Saccharothrix violaceirubra* sp. nov., for isolates YU 692-1^T and YU 724-61. The type strain of the species is YU 692-1^T (=NBRC 102064^T =KCTC 19326^T).

Description of *Saccharothrix violaceirubra* sp. nov.

Saccharothrix violaceirubra (vi.o.la.ce.i.ru'bra. L. adj. *violaceus* violet; L. adj. *ruber* -bra -brum red; N.L. fem. adj. *violaceirubra* violet-red.)

Aerobic and Gram-positive. Forms branched vegetative hyphae and white aerial mycelia, which fragment into ovoid elements. Colonies on ISP medium 2 are purple to dark red in colour. Soluble pigment is not produced. Glucose, maltose, mannose, sucrose and trehalose are utilized. D-Xylose, raffinose, rhamnose, D-ribose, D-mannitol and D-arabinose are not utilized. Negative in tests for starch hydrolysis and calcium malate decomposition. Temperature range for growth is 15–30 °C, with an optimum at approximately 28 °C. Growth occurs in the presence of 0.005% lysozyme, 0.1% phenyl ethanol, 0.01% potassium tellurite, kanamycin (40 mg ml⁻¹), neomycin (40 mg ml⁻¹), leucomycin (2 mg ml⁻¹), lincomycin (16 mg ml⁻¹), novobiocin (1 mg ml⁻¹), streptomycin (20 mg ml⁻¹) and vancomycin (3 mg ml⁻¹). No growth occurs at 37 °C or in the presence of 4% NaCl, ampicillin (20 mg ml⁻¹), oxytetracycline (40 mg ml⁻¹), puromycin (40 mg ml⁻¹) or rifampicin (20 mg ml⁻¹). The major cellular fatty acids are iso-C_{16:0}, anteiso-C_{15:0}, anteiso-C_{17:0} and C_{17:1}. The G+C content of the type strain is 70.8 mol%.

The type strain is YU 692-1^T (=NBRC 102064^T =KCTC 19326^T), isolated from a soil sample collected in Yamanashi prefecture, Japan.

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