Saccharothrix: a New Genus of the *Actinomycetales* Related to *Nocardioopsis*

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A species of the new genus *Saccharothrix* is described under the name *Saccharothrix australiensis* sp. nov. This aerobic genus is characterized by fragmentation of both the vegetative and aerial mycelia into ovoid elements and the following novel cell chemistry: type III cell wall (*meso*-diaminopimelic acid present); galactose and rhamnose present as characteristic whole-cell sugars; absence of nocardomycolic acids; and type PII phospholipid pattern. The guanine-plus-cytosine content of the deoxyribonucleic acid is 73 mol%. The type strain of *Saccharothrix australiensis* is strain LL-BM782Ce82 (= ATCC 31947 = NRRL 11239).

Nocardioform microorganisms, as described by Prauser (19), are bacteria included in the *Actinomycetales* which reproduce by fragmentation of their hyphae into irregular to rodlike coccoid elements. In recent years several new nocardioform taxa have been described, such as *Nocardiodens* Prauser (19) and *Nocardioopsis* Meyer (17). In this paper we describe the type strain of a new nocardioform genus. This strain was isolated from a soil sample collected in Australia and was found to produce a complex of novel beta-lysine-containing aminoglycoside antibiotics, including BM782 alpha 1, BM782 alpha 2, and BM782 alpha 1a, which are the subject of a United States patent (Tresner et al., U.S. patent 4,234,717, Nov. 1980) assigned to American Cyanamid Co., Pearl River, N.Y. The cell chemistry of this microorganism differs significantly from the cell chemistry of previously described genera of actinomycetes. We propose to include this strain in the new genus *Saccharothrix*, the type species of which is *Saccharothrix australiensis*. A culture of the type strain, strain LL-BM782Ce82, has been deposited with the American Type Culture Collection (ATCC), Rockville, Md., as strain ATCC 31947 and with the Agricultural Research Service Culture Collection at the U.S. Department of Agriculture Northern Regional Research Center, Peoria, Ill., as strain NRRL 11239.

MATERIALS AND METHODS

Bacterial strain and culture conditions. Strain LL-BM782Ce82^T (T = type strain) was a strain isolated from a soil sample collected in Australia. Stock cultures were grown on yeast extract-malt extract agar (ISP-2 medium) or ATCC medium 172 (1). The other media used in this study were those recommended for use in the International *Streptomyces* Project (23) and included oatmeal agar, inorganic salts-starch agar, glucose-asparagine agar, and yeast extract-malt extract agar.

Physiological tests. The media and tests used to evaluate the physiological properties were the media and tests described by Gordon et al. (9).

Cell chemistry. Characteristic whole-cell chemistry was analyzed by using the methods of Becker et al. (2, 3) and Lechevalier et al. and Lechevalier (12, 13), and phospholipid composition was determined by the method of Lechevalier et al. (14, 15). Menaquinones were extracted, separated, and

purified by using the procedures of Collins et al. (5, 6); they were identified from the masses of their molecular ions by electron impact mass spectroscopy with a Varian MAT model CH-7 unit, using direct insertion, an ionizing voltage of 70 eV, and an inlet temperature of 250°C.

DNA isolation and characterization. Deoxyribonucleic acid (DNA) was extracted and purified by the method of Marmur (16). The guanine-plus-cytosine content of the purified DNA was estimated by the buoyant density method (20). The buoyant density of the DNA was measured by CsCl density gradient centrifugation in a Beckman model E ultracentrifuge. The reference DNA used was from *Tilletia caries* NRRL Y-12807; this DNA had a buoyant density in CsCl of 1.71567 g/cm³, as determined by comparison with *Micrococcus luteus* DNA (Sigma Chemical Co., St. Louis, Mo.).

Electron microscopy. Samples for scanning electron microscopy were prepared from cut agar blocks from colonial growth, which were fixed overnight with osmium tetroxide vapors. The blocks were trimmed and postfixed for 1 h by immersion in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.3). The blocks were washed in cacodylate buffer, dehydrated through a graded acetone series, and then critical-point dried from liquid carbon dioxide. The dried colonies were then mounted on aluminum stubs with colloidal silver paste and sputter coated with approximately 12.5 nm of gold-palladium. The samples were observed by using a Hitachi model S-500 scanning electron microscope operating at 20 keV.

RESULTS

Micromorphology. Both the vegetative and aerial mycelia of *S. australiensis* were composed of hyphae that fragmented into coccoid elements approximately 0.5 to 0.7 by 0.6 to 1.5 µm (Fig. 1 and 2). Figure 1 shows a 28-day culture on NZ-amine-starch-glucose agar (ATCC medium 172), in which all of the vegetative mycelium has fragmented. Figure 2 shows a 14-day culture on the same medium, in which the aerial hyphae are just beginning to fragment. The morphological relationship between this culture and *Nocardioopsis* is evident by comparing Fig. 3, which shows a 14-day culture of *Nocardioopsis dassonvillei* on ATCC medium 172, with Fig. 2. The "zig-zag" morphology of *Nocardioopsis* hyphae observed just before septation of spores is not generally observed in *S. australiensis*.

Gross colonial morphology. A description of the colonial morphology of *S. australiensis* is given in Table 1. The vegetative mycelium on most media is brownish to grayish

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FIG. 1. Scanning electron micrograph of a 28-day culture of *S. australiensis* on ATCC medium 172. Note that the vegetative mycelium has totally fragmented into coccoid elements.

FIG. 2. Scanning electron micrograph of a 14-day culture of *S. australiensis* on ATCC medium 172. Note the fragmentation of the aerial mycelium into spores similar to those of *Nocardioopsis dassonvillei* (see Fig. 3). Bar indicates 2 μ m.

yellow. The aerial mycelium, when produced in significant quantity, is yellowish white to yellowish gray.

Cell chemistry. The cell chemistry of *S. australiensis* is summarized in Table 2. The presence of galactose and rhamnose as whole-cell sugars has taxonomic significance. *S. australiensis* contained phospholipids of the type PII pattern; that is, it contained phosphatidylethanolamine as the characteristic phospholipid. Nocardomycolic acids were not present. The predominant menaquinones present in cells of *S. australiensis* were MK-9(H4) and MK-10(H4), with traces of MK-9(H6), MK-9(H8), and MK-10(H8). The guanine-plus-cytosine content of DNA extracted from *S. australiensis* was 73 mol%.

Physiology. Apparently due to its inability to grow on ammonium as a sole nitrogen source, *S. australiensis* did not utilize any of the carbohydrates tested with ISP-9 basal carbohydrate test medium. Using the physiological tests of Gordon et al. (9), we observed the following: casein, gelatin, and starch were hydrolyzed; nitrate was reduced; growth took place in lysozyme broth; acetate, DL-malate, propionate, pyruvate, and succinate were utilized as sole carbon sources; lactate results were variable. Acid was produced from cellobiose, dextrin, erythritol, fructose, glucose, glycerol, maltose, mannose, sorbitol, and trehalose. The temperature range for growth was 10 to 45°C, and no growth was observed at 53°C. The optimum temperature for growth was

TABLE 1. Morphological characteristics of *S. australiensis* LL-BM782Ce82^T on ISP media^a

Medium	Aerial mycelium ^b	Vegetative mycelium ^b	Soluble pigment
Oatmeal	Sparse, white	Colorless, flat colonies	Slight, brownish gray
Inorganic salts-starch	Sparse, white	Colorless, flat colonies	None
Glucose-asparagine	Fair amount of off-white growth with a slight greenish yellow cast (ISCC-NBS 92, yellowish white)	Light greenish beige to olive tan, slightly raised colonies (ISCC-NBS 91, dark grayish yellow)	Faint grayish brown
Yeast extract-malt extract	Good amount of light brownish gray to off-white growth (ISCC-NBS 93, yellowish gray)	Dark brown, slightly raised colonies (ISCC-NBS 78, dark yellowish brown)	Brownish

^a See reference 23.

^b Inter-Society Color Council-National Bureau of Standards (ISCC-NBS) color designations were determined by using reference 10a.

FIG. 3. Scanning electron micrograph of a 14-day culture of *Nocardioopsis dassonvillei* NRRL-B16089 on ATCC medium 172. Bar indicates 2 μ m.

30°C. A physiological comparison of *S. australiensis* with other actinomycetes having type III cell walls is shown in Table 3.

DISCUSSION

The recognized genera of the *Actinomycetales* that form chains of conidia and are known to have a type III cell wall composition include *Actinomadura*, *Actinosynnema*, *Nocardioopsis*, *Streptoalloteichus*, and *Streptosporangium*. *Actinosynnema* and *Streptoalloteichus* can be excluded from consideration as the appropriate genus even though they have similar whole-cell sugar patterns (i.e., galactose and rhamnose), since members of these genera form motile spores (10, 24), which were not observed in *Saccharothrix*. *Streptosporangium* is likewise excluded from consideration on morphological grounds since all of its species form sporangia (4), whereas *S. australiensis* does not. Morphologically, *S. australiensis* is much more closely related to the genera *Nocardioopsis* and *Actinomadura* (7–9, 11, 17, 21), and, in fact, the closest taxon from a physiological point of view is *Actinomadura pelletieri*. The genus *Actinomadura*, as currently described, contains strains that form relatively short chains of spores and have madurose as the characteristic whole-cell sugar (type B). Since strain LL-BM782Ce82^T has very long chains of spores and its whole cell hydrolysates lack madurose, it cannot be considered for inclusion in this genus. Although *S. australiensis* resembles *Nocardioopsis* species morphologically, it is different from all described *Nocardioopsis* species physiologically, especially regarding its inability

to grow on ISP-9 medium with any carbon source tested, as well as in cell chemical composition. The presence of galactose and rhamnose as a whole-cell sugar pattern has taxonomic significance, since a number of nocardioform actinomycetes have this cell chemistry, including "*Nocardia aerocolonigenes*" (22) and *Nocardioopsis mutabilis* Shearer (21). Strains having this whole-cell sugar pattern have been observed to have either a PII or PIV phospholipid pattern. At this time it appears that only the PII phospholipid pattern should be included in the definition of the new genus *Saccharothrix*, since there are insufficient data at this time to warrant inclusion of strains having a PIV phospholipid pattern in the new genus. Further studies are in progress to clarify this situation.

Descriptions of the genus *Saccharothrix* and the type species *Saccharothrix australiensis* are given below.

***Saccharothrix* gen. nov.** *Saccharothrix* (Sac.' cha. ro. thrix. Gr. neut. noun *sacchar* sugar; Gr. fem. noun *thrix* hair; *Saccharothrix* sugar-containing hair). Branching vegetative mycelium (diameter, approximately 0.5 μ m) and, on some media, aerial mycelium are produced. Both vegetative and aerial hyphae fragment into ovoid elements. Gram positive. Lysozyme resistant. Catalase positive and aerobic. The cell wall is type III (*meso*-diaminopimelic acid) and has a whole-cell sugar pattern consisting of major amounts of galactose and rhamnose. Type PII phospholipid pattern. No nocardomycolic acids are present in the cells. The guanine-plus-cytosine content of the DNA is 73 mol%. The type species is *Saccharothrix australiensis*.

TABLE 2. Chemical composition of *S. australiensis* LL-BM782Ce82^{Ta}

Class	Type
Isomer of diaminopimelic acid	Meso
Whole-cell sugars	Galactose, mannose, rhamnose
Phospholipid pattern	PII (phosphatidyl-ethanolamine)
Menaquinones	MK-9(H4), MK-10(H4)

^a In addition, the guanine-plus-cytosine content of the DNA is 73.0 ± 0.1 mol%.

***Saccharothrix australiensis* sp. nov.** *Saccharothrix australiensis* (aus. tral. i. en' sis. L. adj. *australiensis* referring to the location of the soil sample from which the organism was first isolated). Brownish to grayish yellow substrate mycelium with yellowish white to yellowish gray aerial mycelium (particularly on yeast extract-malt extract agar). Brownish soluble pigment produced on several media. Casein, tyrosine, gelatin, and esculin hydrolyzed. Nitrate reduced to nitrite. Acid produced from cellobiose, dextrin, erythritol, fructose, glucose, glycerol, maltose, mannose, sorbitol, and trehalose. Nitrate not used as a sole nitrogen source. Acetate, DL-malate, propionate, pyruvate, and succinate used as sole carbon sources. Temperature range for growth, 10 to

TABLE 3. Physiological comparison of *S. australiensis* with other actinomycetes having type III cell walls^a

Characteristic	<i>Saccharothrix australiensis</i>	<i>Actinomadura madurae</i> ^b	<i>Actinomadura pelletieri</i> ^b	<i>Nocardia aerocolonigenes</i> ^b	<i>Nocardiopsis dassonvillei</i> ^b	<i>Nocardiopsis mutabilis</i> ^c
Dissimilation of:						
Xanthine	— ^d	—	—	—	+	—
Hypoxanthine	—	+	V	+	+	+
Adenine	—	—	—	—	+	—
Potato starch	—	+	—	+	+	+
Urea	—	—	—	+	V	—
Esculin	+	+	—	+	V	+
Production of:						
Phosphatase	—	ND	ND	+	ND	ND
Nitrate reductase	+	+	+	V	+	+
Growth on lysozyme broth	+	—	—	+	—	+
Alkaline reaction on:						
Citrate	—	V	—	+	+	+
Lactate	V	ND	ND	+	+	+
Malate	+	+	V	+	+	+
Oxalate	—	ND	ND	+	—	—
Succinate	+	V	—	+	+	+
Acid from:						
Adonitol	—	+	—	V	—	—
Arabinose	—	+	—	+	V	+
Cellobiose	+	+	—	+	ND	+
Dextrin	+	ND	ND	+	ND	+
Erythritol	+	—	—	—	—	—
Fructose	+	+	+	+	+	+
Inositol	—	V	—	+	—	+
Lactose	—	V	—	+	—	+
Maltose	+	V	—	+	+	+
Mannitol	—	+	—	V	+	—
Mannose	+	+	—	+	+	+
Melibiose	—	—	—	+	—	+
α-Methyl-D-glucoside	—	—	—	—	—	+
Raffinose	—	—	—	V	—	+
Rhamnose	—	+	—	V	V	—
Salicin	—	ND	ND	+	ND	+
Sorbitol	+	—	—	—	—	—
Sucrose	—	ND	ND	+	ND	ND
Trehalose	+	+	+	+	V	+
Xylose	—	+	—	+	+	+
β-Methyl-D-xyloside	—	ND	ND	—	ND	ND
Growth at:						
10°C	+	—	—	+	—	—
45°C	+	V	V	—	—	+

^a The tests described by Gordon et al. (9) were used.

^b Data from reference 18.

^c Data from reference 21.

^d +, Positive; —, negative; V, variable; ND, no data; w, weak.

45°C. Isolated from soil. The type strain of *S. australiensis* is strain LL-BM782Ce82 (= ATCC 31947 = NRRL 11239).

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