Saccharothrix texasensis sp. nov. and *Saccharothrix waywayandensis* sp. nov.

D. P. LABEDA* AND A. J. LYONS

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture,
Peoria, Illinois 61604

Numerical taxonomic and deoxyribonucleic acid relatedness data for several strains belonging to the genus *Saccharothrix*, which has been described previously, clearly delineated two new species, each containing two strains. These new species can be physiologically differentiated from previously described species. The name *Saccharothrix texasensis* sp. nov. is proposed for the species represented by strain NRRL B-16134^T (T = type strain), and the name *Saccharothrix waywayandensis* sp. nov. is proposed for the species represented by strain NRRL B-16159^T.

The genus *Saccharothrix* Labeda et al. (8) contains nocardioform actinomycetes that have type III cell walls (*meso* isomer of diaminopimelic acid) (10), rhamnose and galactose as the diagnostic sugars in whole-cell hydrolysates, and phosphatidylethanolamine as the diagnostic nitrogen-containing phospholipid (phospholipid pattern type PII sensu

commercial interest because researchers have discovered at least two novel antibiotics that are produced by *Saccharothrix* species, the LL-BM782 complex (H. D. Tresner et al., U.S. patent 4,234,717, November, 1980) and rebeccamycin (1). As a result of a numerical taxonomic and molecular systematic study of several recent isolates (7), two new

FIG. 1. Photomicrograph of 14-day growth of *S. waywayandensis* NRRL B-16158 on inorganic salts-starch agar. Note the fragmentation of the mycelium. Bar = 10 μ m.

Lechevalier et al. [9]). Two species of this genus have been described previously, *Saccharothrix australiensis* (8) and *Saccharothrix aerocolonigenes* (6), but numerous other strains have been isolated from soil. Strains belonging to the genus *Saccharothrix* seem to be ubiquitous in soil and are of

species of this genus were delineated. These new species, *Saccharothrix texasensis* and *Saccharothrix waywayandensis*, are described in this paper.

MATERIALS AND METHODS

Maintenance of strains. The strains included in this study are listed in Table 1. The strains were maintained on agar

* Corresponding author.

TABLE 1. Strains of *Saccharothrix* species compared in this study

Strain	Source	Guanine-plus-cytosine content of DNA (mol%)
<i>S. australiensis</i> NRRL 11239 ^T	Soil, Australia	75.9
<i>S. aerocolonigenes</i> NRRL B-3298 ^T	Soil, Nagasaki, Japan	70.0
<i>S. texasensis</i> NRRL B-16107	Soil, Flower Mountain, Tex.	72.5
<i>S. texasensis</i> NRRL B-16134 ^T	Soil, Dallas, Tex.	74.9
<i>S. waywayandensis</i> NRRL B-16158	Soil, Lake Waywayanda, N.J.	71.0
<i>S. waywayandensis</i> NRRL B-16159 ^T	Soil, Lake Waywayanda, N.J.	71.2

slants of American Type Culture Collection (ATCC) medium 172 (NZamine-soluble starch-glucose agar) (2) and were stored at 4°C until they were needed. Inocula for the physiological tests and biomass preparations used for extraction of deoxyribonucleic acid (DNA) were grown in ATCC medium 172 broth from which calcium carbonate was omitted.

Physiological tests. The strains were evaluated for 76 physiological characteristics. Production of acid from carbohydrates, utilization of organic acids, and hydrolysis of adenine, guanine, hypoxanthine, tyrosine, xanthine, casein, esculin, urea, and hippurate were all evaluated by using the media of Gordon et al. (3). Allantoin hydrolysis was evaluated in the basal medium suggested by Gordon for evaluation of esculin hydrolysis. Phosphatase activity was evaluated by using the method of Kurup and Schmitt (5), and Tween 80 hydrolysis was evaluated by using the method of Sierra (15). The temperature range for growth was determined on slants of ATCC medium 172 agar. Tolerance to NaCl was measured by supplementing ATCC medium 172 agar with various concentrations of NaCl.

Chemotaxonomy. The chemotaxonomic properties of strains (isomer of diaminopimelic acid, whole-cell sugar content, phospholipid pattern, and menaquinone content) were determined by using previously described methods (7, 9, 10). The biomass preparations for chemotaxonomic studies were produced as 5-day shaken cultures (28°C) in ATCC medium 172 broth lacking calcium carbonate.

Purification of DNA and determination of relatedness. DNA was extracted from cells by using the method of Marmur (11) and was further purified by chromatography on hydroxyapatite columns. The guanine-plus-cytosine content of the DNA was calculated from the thermal denaturation midpoint, which was determined by the method of Marmur and Doty (12). The percentage of DNA relatedness between strains was calculated from $C_{0t_{0.5}}$ determinations in $5 \times$ SSC ($1 \times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) supplemented with 20% dimethyl sulfoxide at 66°C (thermal denaturation midpoint – 25°C) by using the method of Seidler et al. (13, 14) as described by Kurtzman et al. (4).

RESULTS

Morphologically, all strains belonging to the genus *Saccharothrix* are very similar. The substrate mycelium tends to be plicate (raised and ridged) and yellow to brownish yellow. Sparse white to yellowish white aerial mycelium is produced

TABLE 2. Differential physiological properties of *Saccharothrix* species

Characteristic	<i>S. australiensis</i>	<i>S. aerocolonigenes</i>	<i>S. texasensis</i>	<i>S. waywayandensis</i>
Decomposition of:				
Adenine	— ^a	—	+	—
Allantoin	—	—	v	—
Hippurate	—	—	+	+
Hypoxanthine	—	+	+	+
Potato starch	—	+	+	+
Urea	—	v	v	+
Acid produced from:				
Adonitol	—	—	—	+
Arabinose	—	+	+	+
Erythritol	+	—	—	+
Fructose	+	+	v	+
Inositol	—	+	+	+
Lactose	—	+	+	+
Melibiose	—	+	+	+
Raffinose	—	+	—	+
Rhamnose	—	+	+	+
Salicin	—	+	+	+
Sorbitol	+	—	—	—
Sucrose	—	+	+	+
Xylose	—	+	+	+
α-Methyl-D-glucoside	—	v	+	—
β-Methyl-D-xyloside	—	v	v	—
Utilization of:				
Citrate	—	+	—	+
Lactate	v	+	+	+
Oxalate	—	+	—	+
Propionate	+	+	v	+
Tartrate	—	+	—	—
Production of:				
Nitrate reductase	+	+	+	—
Phosphatase	—	+	+	+
Growth in the presence of:				
4% NaCl	+	+	—	+
5% NaCl	—	w	—	+
Growth at:				
42°C	+	v	+	—
45°C	+	—	—	—

^a +, Positive; —, negative; v, variable response; w, weak response.

by most strains on some media, generally inorganic salts-starch agar (ISP 4 medium) or Czapek agar. Substrate and aerial mycelia typically fragment in strains belonging to the genus *Saccharothrix*, as shown in Fig. 1.

The chemotaxonomic composition of the new strains belonging to the genus *Saccharothrix* which we examined is typical of the genus, with cell walls containing the *meso* isomer of diaminopimelic acid, rhamnose and galactose as the characteristic whole-cell sugars, phosphatidylethanolamine as the diagnostic phospholipid (typical of phospholipid pattern PII), and MK-9(H₄) as the predominant menaquinone. The differential physiological properties of *Saccharothrix* species are summarized in Table 2.

The results of DNA hybridizations between strains of *Saccharothrix* species are shown in Table 3. High levels of DNA relatedness were observed between strains NRRL B-16107 and NRRL B-16134^T (T = type strain) (89% homology) and between strains NRRL B-16158 and NRRL B-16159^T (95% homology).

DISCUSSION

The physiological properties of the strains and the results of DNA hybridization studies clearly support the designation

TABLE 3. DNA relatedness among *Saccharothrix* species

Strain	% DNA relatedness to:				
	Strain NRRL 11239 ^{Ta}	Strain NRRL B-3298 ^T	Strain NRRL B-16107	Strain NRRL B-16134 ^T	Strain NRRL B-16158
<i>S. aerocolonigenes</i> NRRL B-3298 ^T	10				
<i>S. texasensis</i> NRRL B-16107	20	0			
<i>S. texasensis</i> NRRL B-16134 ^T	21	1	89		
<i>S. waywayandensis</i> NRRL B-16158	11	34	4	5	
<i>S. waywayandensis</i> NRRL B-16159 ^T	3	44	19	7	95

^a Strain NRRL 11239 is the type strain of *S. australiensis*.

of two new species in the genus *Saccharothrix*, one species containing strains NRRL B-16107 and NRRL B-16134^T and the other species containing strains NRRL B-16158 and NRRL B-16159^T. The strains in each pair exhibit significant levels of DNA relatedness to each other (>89% homology) and are physiologically similar (>92% similarity, as determined by using the Jaccard similarity coefficient [16] and 76 unit characters), while being quite different from the other species in this genus. Thus, the name *Saccharothrix texasensis* sp. nov. is proposed for strains NRRL B-16107 and NRRL B-16134^T, and the name *Saccharothrix waywayandensis* sp. nov. is proposed for strains NRRL B-16158 and NRRL B-16159^T. These new species can be clearly differentiated from each other and from previously described *Saccharothrix* species by physiological properties that are unique for each species (Table 2). The descriptions of the new taxa are given below.

***Saccharothrix texasensis* sp. nov.** *Saccharothrix texasensis* (tex. as. en' sis. L. adj. *texasensis*, referring to the source of the soil samples, the state of Texas, from which the species was first isolated). Substrate mycelium is dark yellow to brownish yellow. Sparse white aerial mycelium is produced, particularly on inorganic salts-starch agar. A brown to reddish brown soluble pigment is produced on ATCC medium 172. Adenine, casein, esculin, gelatin, hippurate, hypoxanthine, starch, Tween 80, and tyrosine are hydrolyzed; cellulose, guanine, and xanthine are not hydrolyzed; hydrolysis of allantoin and hydrolysis of urea are variable. Nitrate is reduced to nitrite. Phosphatase is produced. Acetate, lactate, DL-malate, and succinate are assimilated; benzoate, citrate, mucate, oxalate, and tartrate are not assimilated; propionate is variably assimilated. Acid is produced from L-arabinose, cellobiose, dextrin, D-galactose, D-glucose, glycerol, myo-inositol, lactose, D-mannitol, D-mannose, melibiose, α-methyl-D-glucoside, L-rhamnose, salicin, sucrose, trehalose, and D-xylose; no acid is produced from adonitol, dulcitol, meso-erythritol, raffinose, and D-sorbitol; acid production from D-fructose and β-methyl-D-xyloside is variable. Grows in the presence of 3% NaCl but not in the presence of 4% NaCl. The temperature range for growth is 20 to 42°C. The guanine-plus-cytosine content of the DNA is 74 mol% (as determined by the thermal denaturation midpoint method). Isolated from soil. The type strain of *S. texasensis* is strain NRRL B-16134 (= LL-37U-77).

***Saccharothrix waywayandensis* sp. nov.** *Saccharothrix waywayandensis* (way. way. an. den' sis. L. adj. *waywayandensis*, referring to the source, Lake Waywayanda, N.J., of the soil samples from which the organism was first isolated). Substrate mycelium is pale yellow to dark yellow. Aerial mycelium is white, when it is produced, particularly on inorganic salts-starch agar. No soluble pigments are produced. Casein, esculin, gelatin, hippurate, hypoxanthine, starch, Tween 80, tyrosine, and urea are hydrolyzed; ade-

nine, allantoin, cellulose, guanine, and xanthine are not hydrolyzed. Nitrate is not reduced to nitrite. Phosphatase is produced. Acetate, citrate, lactate, DL-malate, oxalate, propionate, and succinate are assimilated; benzoate, mucate, and tartrate are not assimilated. Acid is produced from adonitol, L-arabinose, cellobiose, dextrin, meso-erythritol, D-fructose, D-galactose, D-glucose, glycerol, myo-inositol, lactose, D-mannitol, D-mannose, melibiose, raffinose, rhamnose, salicin, sucrose, trehalose, and D-xylose; no acid is produced from dulcitol, α-methyl-D-glucoside, β-methyl-D-xyloside, and D-sorbitol. Grows in the presence of 5% NaCl but not in the presence of 7% NaCl. The temperature range for growth is 20 to 37°C. The guanine-plus-cytosine content of the DNA is 71 mol% (as determined by the thermal denaturation midpoint method). Isolated from soil. The type strain of *S. waywayandensis* is strain NRRL B-16159 (= LL-37Z-15).

ACKNOWLEDGMENT

We express appreciation to E. N. Hoekstra for technical assistance in this study.

LITERATURE CITED

1. Bush, J. A., B. H. Long, J. J. Catino, W. T. Bradner, and K. Tomita. 1987. Production and biological activity of rebeccamycin, a novel antitumor agent. *J. Antibiot.* **40**:668-678.
2. Cote, R., P.-M. Daggett, M. J. Gantt, R. Hay, S.-C. Hay, and P. Pienta. 1984. ATCC media handbook, 1st ed. American Type Culture Collection, Rockville, Md.
3. Gordon, R. A., D. A. Barnett, J. E. Handerhan, and C. H. Pang. 1974. *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int. J. Syst. Bacteriol.* **24**:54-63.
4. Kurtzman, C. P., M. J. Smiley, C. J. Johnson, L. J. Wickerham, and G. B. Fuson. 1980. Two new and closely related heterothallic species, *Pichia amylophila* and *Pichia mississippiensis*: characterization by hybridization and deoxyribonucleic acid reassociation. *Int. J. Syst. Bacteriol.* **30**:208-216.
5. Kurup, P. V., and J. A. Schmitt. 1973. Numerical taxonomy of *Nocardia*. *Can. J. Microbiol.* **19**:1035-1048.
6. Labeda, D. P. 1986. Transfer of "*Nocardia aerocolonigenes*" (Shinobu and Kawato 1960) Pridham 1970 into the genus *Saccharothrix* Labeda, Testa, Lechevalier, and Lechevalier 1984 as *Saccharothrix aerocolonigenes* sp. nov. *Int. J. Syst. Bacteriol.* **36**:109-110.
7. Labeda, D. P. 1988. An evaluation of strains of the genus *Saccharothrix* by numerical taxonomic and molecular taxonomic methods, p. 227-232. In Y. Okami, T. Beppu, and H. Ogawara (ed.), *Biology of actinomycetes '88*. Japan Scientific Society Press, Tokyo.
8. Labeda, D. P., R. T. Testa, M. P. Lechevalier, and H. A. Lechevalier. 1984. *Saccharothrix*: a new genus of the Actinomycetales related to *Nocardiopsis*. *Int. J. Syst. Bacteriol.* **34**:426-431.
9. Lechevalier, M. P., C. DeBièvre, and H. A. Lechevalier. 1977. Chemotaxonomy of aerobic actinomycetes: phospholipid com-

- position. *Biochem. Syst. Ecol.* **5**:249–260.
10. **Lechevalier, M. P., and H. A. Lechevalier.** 1980. The chemotaxonomy of actinomycetes, p. 227–291. *In* A. Dietz and D. W. Thayer (ed.), *Actinomycete taxonomy*. Special publication no. 6. Society for Industrial Microbiology, Arlington, Va.
 11. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.* **3**:208–218.
 12. **Marmur, J., and P. Doty.** 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**:109–118.
 13. **Seidler, R. J., M. D. Knittel, and C. Brown.** 1975. Potential pathogens in the environment: cultural reactions and nucleic acid studies on *Klebsiella pneumoniae* from chemical and environmental sources. *Appl. Microbiol.* **29**:819–825.
 14. **Seidler, R. J., and M. Mandel.** 1971. Quantitative aspects of deoxyribonucleic acid renaturation: base composition, state of chromosome replication, and polynucleotide homologies. *J. Bacteriol.* **106**:608–614.
 15. **Sierra, G.** 1957. A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **23**:15–22.
 16. **Sneath, P. H. A.** 1957. Some thoughts on bacterial classification. *J. Gen. Microbiol.* **17**:184–200.