Actinomadura yumaensis sp. nov.

D. P. LABEDA,^{1†} R. T. TESTA,^{1*} M. P. LECHEVALIER,² AND H. A. LECHEVALIER²

Medical Research Division, American Cyanamid Company, Pearl River, New York 19065¹ and Waksman Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey 08903²

A new species of *Actinomadura*, for which we propose the name *Actinomadura yumaensis*, was isolated from a soil sample from Yuma County, Ariz. Whole-cell hydrolysates contain the *meso* isomer of diaminopimelic acid and the sugar madurose; thus, the cell wall composition type is type III. No nitrogenous phospholipids are present (type PI). Spores are formed in short spiral chains on branched, almost verticillate sporophores. The spores are ovoid and have smooth surfaces. This microorganism produces the polyether ionophore antibiotic X-14868A and is characterized by grayish yellow to dark yellowish brown substrate mycelium, white aerial hyphae (when produced) with light gray spores, and yellowish to greenish soluble pigments. Glucose, maltose, sucrose, and trehalose are the only sugars that support good growth on ISP medium 9. The type strain of *A. yumaensis* is strain LL-C23024 (= NRRL 12515).

During the course of screening actinomycetes that were isolated from soil for antibiotic activity, a culture was obtained which produced a greenish brown soluble pigment and an extremely potent polyether ionophore antibiotic which was later found to be identical in structure to antibiotic X-14868, which is produced by *Nocardia* sp. strain ATCC 31585 (C. M. Liu et al., U.S. patent 4,278,663, July 1981). Subsequent studies determined that this organism, designated strain LL-C23024^T (T = type strain), is a new species of the genus *Actinomadura*. This strain is the subject of a United States patent (D. P. Labeda, J. H. Martin, and J. J. Goodman, U.S. patent 4,407,946, October 1983), in which it was incorrectly named "*Actinomadura yumaense*." In this paper, we describe the type strain of the new species *Actinomadura yumaensis*.

MATERIALS AND METHODS

Bacterial strains. Strain LL-C23024^T was isolated from a soil sample that was collected in Yuma County, Ariz. This strain was routinely maintained on ATCC medium 172 slants (1) and stored at 4°C. Inocula for physiological and morphological studies were prepared from 5-day shaken cultures in either ATCC medium 172 or Bennett broth (18); cells were collected by centrifugation at 4°C, washed twice, and then suspended in the same volume of sterile distilled water.

Media. The following media were used in gross morphological studies: ATCC medium 172 (1), Benedict agar (18), Bennett agar (18), Czapek agar (18), Gauze no. 1 agar (4), Gauze no. 2 agar (4), glycerol-asparagine agar (19), Hickey-Tresner agar (18), inorganic salts-starch agar (18, 19), oatmeal agar (19), tomato paste-oatmeal agar (18), and yeast extract-malt extract agar (19). Inoculated media were incubated at 28°C for 2 weeks before observation.

Microscopy. Light microscopic observations of colonial growth were made by using plates containing either ATCC medium 172 or 1.5% agar (Sigma Chemical Co., St. Louis, Mo.) in tap water. Scanning electron microscopy was performed by using either a Hitachi model S-500 or an ISI model SS130 scanning electron microscope. Samples consisting of 14 days of colonial growth on ATCC medium 172 were prepared for scanning electron microscopy by cutting agar

blocks from the growth medium, fixing them overnight in osmium tetroxide vapor, dehydrating them through a graded acetone series, critical point drying them from liquid carbon dioxide, and then mounting them on aluminum stubs and sputter-coating them with approximately 10 nm of gold-palladium.

Cell chemistry. Characteristic whole-cell and cell wall chemical compositions were analyzed by using the methods of Becker et al. (2, 3) and Lechevalier and co-workers

FIG. 1. Scanning electron micrograph of a 14-day culture of *A. yumaensis* grown on tap water agar. Note the branched, almost verticillate sporophores. Bar = 2 μ m.

* Corresponding author.

† Present address: Northern Regional Research Center, U.S. Department of Agriculture, Peoria, IL 61604.

FIG. 2. Scanning electron micrograph of a 14-day culture of *A. yumaensis* grown on ATCC medium 172. Note the spiral chains of smooth spores. Bar = 2 μ m.

(11–13); phospholipid composition was determined by using the method of Lechevalier et al. (14).

Physiological tests. The media and tests used to evaluate the physiological properties of strain LL-C23024^T were those of Gordon et al. (6); the carbohydrate utilization test medium (ISP medium 9) of Shirling and Gottlieb (19) was also used.

RESULTS

Micromorphology. Strain LL-C23024^T forms short, loosely coiled (usually one turn) chains of smooth-surfaced spores (Fig. 1). These chains are usually borne on branched, almost verticillate aerial sporophores (Fig. 2) and average 20 spores in length. The spores are ovoid and measure 0.6 to 0.8 by 1.0 to 1.4 μ m.

Cell chemistry. The cell wall composition type of strain LL-C23024^T is type III sensu Lechevalier and Lechevalier (11); i.e., hydrolysates contain *meso*-diaminopimelic acid, glutamic acid, alanine, muramic acid, and glucosamine. The whole-cell sugar pattern type is type B (madurose is the characteristic sugar), and the phospholipid pattern type is type PI (no nitrogenous phospholipids are present). All of these properties support the assignment of strain LL-C23024^T to the genus *Actinomadura*.

Cultural characteristics. Table 1 shows the macroscopic appearance of strain LL-C23024^T on various culture media. The color descriptions were taken from the Centroid Color Charts (9). The organism grows well on most complex organic media, but does poorly on media containing inorganic nitrogen sources. The vegetative mycelial colors range from pale yellow to dark yellowish brown, and yellowish, orange, or greenish soluble pigments are produced on many media. When produced, aerial mycelia are white to yellowish white, becoming gray as spores are formed.

TABLE 1. Cultural characteristics of *A. yumaensis* LL-C23024^T

Medium	Amt of growth	Aerial mycelium, vegetative mycelium ^a	Soluble pigment	Reverse color ^a
Benedict agar	Poor to moderate	Flat, powdery colonies; aerial mycelium white to light gray (264)	Yellowish	Pale yellow (89)
Bennett agar	Good	No aerial mycelium; convoluted vegetative growth yellowish gray (93) to grayish yellowish brown (80)	Orange	Dark grayish yellowish brown (81)
Czapek sucrose agar	Poor to moderate	Flat growth; moderate white aerial mycelium; vegetative growth grayish yellow (90)	None	Pale yellow (89)
Gauze no. 1 agar	Poor	Colorless flat growth; sparse white aerial mycelium	None	Colorless
Gauze no. 2 agar	Good	Raised convoluted colonies; vegetative mycelium yellowish gray (93); moderate aerial mycelium yellowish white (92)	None	Dark orange yellow (72)
Glycerol-asparagine agar	Poor to moderate	Flat, powdery colonies; white aerial mycelium	Yellow	Pale yellow (89)
Hickey-Tresner agar	Moderate	Flat waxy colonies grayish yellow (90); sparse aerial mycelium; white to light gray (264)	None	Colorless
Inorganic salts-starch agar	Poor	Flat, colorless, powdery colonies; white aerial mycelium	None	Colorless
NZ-amine-starch-glucose agar (ATCC medium 172)	Good	Heavy convoluted growth grayish yellowish brown (61) to brownish black (65); moderate aerial mycelium white to light gray (264)	Greenish brown	Dark yellowish brown (78)
Oatmeal agar	Moderate	Flat, waxy growth grayish yellow (90); moderate white aerial mycelium	None	Grayish yellow (90)
Tomato Paste-oatmeal agar	Good	Flat, waxy growth dark grayish yellow (91); trace of white aerial mycelium	None	
Yeast extract-malt extract agar	Good	Raised, waxy, convoluted colonies yellowish gray (93) to grayish yellowish brown (80); no aerial mycelium	Orange	Dark yellowish brown (78)

^a Color names and numbers taken from reference 9.

Physiological properties. The results of various physiological tests for strain LL-C23024^T are shown in Tables 2 and 3.

DISCUSSION

A comparison of strain LL-C23024^T with *Actinomadura* species described previously (5, 7, 8, 10, 15–17) indicated that it could be related to *Actinomadura macra* or *Actinomadura pelletieri* (unpublished data) based on its carbohydrate utilization pattern on ISP basal medium 9 (19). The micromorphology and gross colonial macromorphology of strain LL-C23024^T are quite different than those of *A. macra*, and, moreover, several physiological differences, such as utilization of calcium malate, hydrolysis of starch and casein, and growth at 45°C were also observed thus eliminating *A. macra* from consideration as the appropriate species. *A. pelletieri* strains tend to lack distinguishing morphological characteristics, but strain LL-C23024^T can be distinguished from strains of this species on the basis of physiological characteristics. The other *Actinomadura* species described previously can be eliminated from consideration based on physiological characteristics or morphological characteristics or both. Therefore, we regard strain LL-C23024^T as the type strain of a new species of the genus *Actinomadura*, for which we propose the name *Actinomadura yumaensis*. This strain has been deposited with the Agricultural Research Service Culture Collection, Northern Regional Research Center, Peoria, Ill., as strain NRRL 12515^T.

***Actinomadura yumaensis* sp. nov.** *Actinomadura yumaensis* (yú ma.en.sis. Lat. derivative of Yuma County, Ariz., the source of the soil sample from which the type strain was isolated) forms branching substrate hyphae and, on some media, produces short, loosely coiled (usually one turn) chains of smooth-surfaced spores. The spore chains are borne on branched, almost verticillate sporophores and average 20 spores in length. The spores are ovoid and measure 0.6 to 0.8 by 1.0 to 1.4 µm.

Substrate mycelium is generally of yellow shades, ranging from pale yellow to dark yellowish brown. Yellow to dark

TABLE 3. Physiological characteristics of *A. yumaensis* LL-C23024^T determined by using the Gordon test series^a

Test	Result
Degradation of:	
Adenine	—
Casein	+
Esculin	+
Gelatin	+
Guanine	—
Hypoxanthine	V ^b
Starch	+
Tyrosine	+
Urea	V
Xanthine	+
Growth in the presence of:	
Lysozyme	+
5% NaCl	—
Salicylate	—
Production of:	
Nitrate reductase	+
Phosphatase	+
Assimilation of:	
Acetate	+
Benzoate	—
Citrate	—
Lactate	—
DL-Malate	+
Mucate	—
Oxalate	—
Propionate	+
Pyruvate	+
Succinate	+
Tartrate	+
Acid produced from:	
Adonitol	—
L-Arabinose	—
Cellobiose	+
Dextrin	—
Dulcitol	—
Erythritol	—
D-Fructose	—
D-Galactose	+
D-Glucose	+
Glycerol	+
Inositol	+
Lactose	—
Maltose	+
D-Mannitol	—
Mannose	+
Melibiose	—
α-Methyl-D-glucoside	—
β-Methyl-D-xyloside	—
Raffinose	—
L-Rhamnose	—
Salicin	—
D-Sorbitol	—
Sucrose	+
Trehalose	+
D-Xylose	—
Growth at:	
10°C	±
37°C	+
45°C	+
55°C	—

^a See reference 6.

^b V, Variable.

TABLE 2. Carbon assimilation by *A. yumaensis* LL-C23024^T on ISP basal medium 9^a

Carbon source	Growth
Adonitol	—
L-Arabinose	—
Dulcitol	—
D-Fructose	+
D-Galactose	+
D-Glucose	+++
Glycerol	+++
myo-Inositol	+
Lactose	—
Maltose	++
D-Mannose	+
D-Mannitol	—
Melibiose	—
Melezitose	+
Raffinose	—
L-Rhamnose	—
Salicin	—
D-Sorbitol	—
Sucrose	++
Trehalose	+++
D-Xylose	—

^a See reference 19.

green soluble pigments are produced on many media. The aerial mycelium is white to yellowish white, becoming gray as spores are formed.

The physiological properties of this species are summarized in Tables 2 and 3.

The optimum temperature for growth is 37°C.

Isolated from soil.

The type strain is strain NRRL 12515 (= LL-C23024).

LITERATURE CITED

1. American Type Culture Collection. 1982. Catalogue of strains I, 15th ed. American Type Culture Collection, Rockville, Md.
2. Becker, B., M. P. Lechevalier, R. E. Gordon, and H. A. Lechevalier. 1964. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. *Appl. Microbiol.* **12**:421-423.
3. Becker, B., M. P. Lechevalier, and H. A. Lechevalier. 1965. Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. *Appl. Microbiol.* **13**:236-243.
4. Gauze, G. F., J. P. Preobrazhenskaya, E. E. Kudrina, N. O. Blinov, I. D. Ryabova, and M. A. Sveshnikova. 1957. Problems in the classification of antagonistic actinomycetes. State Publishing House for Medical Literature, Medgiz, Moscow.
5. Goodfellow, M., G. Alderson, and J. Lacey. 1979. Numerical taxonomy of *Actinomadura* and related actinomycetes. *J. Gen. Microbiol.* **112**:95-111.
6. Gordon, R. E., 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.
7. Horan, A. C., and B. C. Brodsky. 1982. A novel antibiotic-producing *Actinomadura*, *Actinomadura kijaniata* sp. nov. *Int. J. Syst. Bacteriol.* **32**:195-200.
8. Huang, L. H. 1980. *Actinomadura macra* sp. nov., the producer of antibiotics CP-47,433 and CP-47,434. *Int. J. Syst. Bacteriol.* **30**:565-568.
9. Kelley, K. L., and D. B. Judd. 1976. Color. Universal language and dictionary of names. National Bureau of Standards special publication 440. U.S. Department of Commerce, Washington, D.C.
10. Lacey, J., M. Goodfellow, and G. Alderson. 1978. The genus *Actinomadura* Lechevalier and Lechevalier, p. 107-117. In M. Modarski, W. Kurylowicz, and J. Jeljaszewicz (ed.), *Nocardia and streptomyces*. G. Fischer Verlag, New York.
11. Lechevalier, H. A., and M. P. Lechevalier. 1970. A critical evaluation of the genera of aerobic actinomycetes, p. 393-405. In H. Prauser (ed.), *The Actinomycetales*. Gustav Fischer Verlag, Jena.
12. Lechevalier, H. A., M. P. Lechevalier, and N. N. Gerber. 1971. Chemical composition as a criterion in the classification of actinomycetes. *Adv. Appl. Microbiol.* **14**:47-72.
13. Lechevalier, M. P. 1968. Identification of aerobic actinomycetes of clinical importance. *J. Lab. Clin. Med.* **71**:934-944.
14. Lechevalier, M. P., C. DeBievre, and H. A. Lechevalier. 1977. Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem. Syst. Ecol.* **5**:249-260.
15. Meyer, J. 1979. New species of the genus *Actinomadura*. *Z. Allg. Mikrobiol.* **19**:37-44.
16. Nonomura, H., and Y. Ohara. 1971. Distribution of actinomycetes in soil. XI. Some new species of the genus *Actinomadura* Lechevalier et al. *J. Ferment. Technol.* **49**:904-912.
17. Preobrazhenskaya, T. P., M. A. Sveshnikova, and L. P. Terekhova. 1977. Key for identification of the species of the genus *Actinomadura*. *Actinomycetes Rel. Org.* **12**:30-38.
18. Pridham, T. G., P. Anderson, C. Foley, L. A. Lindenfelser, C. W. Hesseltine, and R. G. Benedict. 1957. A selection of media for maintenance and taxonomic study of *Streptomyces*. *Antibiot. Annu.*, p. 947-953.
19. Shirling, E. B., and D. Gottlieb. 1966. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* **16**:313-340.