Actinomadura macra sp. nov., the Producer of Antibiotics CP-47,433 and CP-47,434

L. H. HUANG

Central Research, Pfizer Inc., Groton, Connecticut 06340

A new species of *Actinomadura* Lechevalier and Lechevalier is described, for which the name *Actinomadura macra* is proposed. It is characterized by white, pale gray, or grayish aerial mycelia, straight, flexuous, hooked to coiled (once or twice) short spore chains, and spores with a smooth surface. The type strain of *A. macra* is Pfizer FD 25934 (=ATCC 31286).

Inventors W. D. Celmer, W. P. Cullen, C. E. Moppett, J. R. Oscarson, L. H. Huang, R. Shibakawa, and J. Tone described a bacterial strain producing the polycyclic ether antibiotics CP-47,433 and CP-47,434 and gave it the name *Actinomadura macer* (sic) (U.S. patent 4,148,882, April 10, 1979; assignee, Pfizer Inc.). This strain, which was given the designation Pfizer FD 25934, was deposited at the American Type Culture Collection (ATCC) and accessioned there as ATCC 31286. The taxon *A. macra* (not *A. macer* as appeared incorrectly in the patent) has been mentioned previously (Abstr. Joint Int. Congr. Chemother. 11th and Intersci. Conf. Antimicrob. Agents Chemother. 19th, abstr. no. 1030, 1979). This paper presents a characterization of the taxon, for which the name *Actinomadura macra* sp. nov. is proposed.

MATERIALS AND METHODS

Bacterial strains. Strain Pfizer FD 25934 and the type strain (16) of *Actinomadura spadix* (ATCC 27298) were used in this study. The former was isolated from a soil sample collected in Okayama, Japan, and the latter was purchased from the ATCC. The Pfizer strain was inoculated into liquid ATCC medium 172 (1), grown for 4 days at 28°C on a rotary shaker, and then transferred to fresh liquid ATCC medium 172. After 7 days of incubation at 28°C on a shaker, the growth was centrifuged, washed twice with sterile distilled water, and then used as an inoculum for various studies.

Media. Washed cells were inoculated onto the following media: ISP media 2 to 5 (15); mineral medium 1 and organic medium 2 of Gauze et al. (6); Jensen dextrose-casein isolation medium (9) with 0.1% calcium carbonate added; gelatin agar and starch agar (7); casein agar and tyrosine agar (8); Czapek sucrose agar (18); Emerson agar (18); yeast-starch agar (5); nutrient agar (18); calcium malate agar (17); peptone Czapek agar (4); potato carrot agar (11) modified by using only 30 g of potatoes, 2.5 g of carrots, and 20 g of agar; yeast extract-malt extract agar with coconut milk (ISP medium 2 plus 50 ml of coconut milk per liter of medium); potato dextrose agar with coconut milk (potatoes, 100 g; dextrose, 10 g; coconut milk, 50

ml; agar, 20 g; tap water added to 1,000 ml); and 2% tap water agar. Inoculated plates were placed in an incubator at 28°C.

Morphological characterization. The morphologies of the spore chains and the mycelia of the cultures grown on Jensen dextrose-casein isolation medium, Czapek sucrose agar, and tyrosine agar were studied with a light microscope. The surfaces of the spores and the spore chains were studied with a Hitachi HU-12A transmission electron microscope.

Cultural characterization. Readings of the results of the growth on the various media were made at 14 days. The colors of the mycelia were described in common terminology, but the exact colors were determined by comparing the mycelia with color chips from the *Color Harmony Manual* (3).

Physiological tests. The media used for the physiological tests were as follows: ISP media 1, 6, and 7 for melanin production (15); ISP medium 6 with lead acetate strips for H₂S production; gelatin agar and starch agar; casein agar and tyrosine agar; organic nitrate broth (7); dextrose nitrate broth (medium 1 [18] with 3 g of dextrose substituted for 30 g of sucrose and agar omitted); Jensen cellulose broth (9); cellulose broth of Levine and Schoenlein (medium 2511 [12]); and ATCC medium 172 (for determination of the temperature range).

Carbohydrate utilization. Carbohydrate utilization was studied by the procedures described by Shirling and Gottlieb (15) and by Luedemann and Brodsky (13).

Cell wall analysis. Cell walls were analyzed by the procedure of Becker et al. (2), and whole cells were analyzed by the method of Lechevalier (11).

RESULTS AND DISCUSSION

Morphological properties. The vegetative hyphae of strain Pfizer FD 25934 were narrow and branched and measured 0.4 to 0.6 µm in diameter. On tyrosine agar, terminal or intercalary swellings were produced on the substrate mycelium; these swellings were globose, oval, or broadly elliptical to elongated and measured 1.5 to 3.5 µm in diameter or 1.1 to 2.8 by 2.8 to 5.5 µm. Numerous attempts at inducing spore formation on various common laboratory media used for the identification of the *Actinomyce-*

tales failed. However, after 5 weeks of incubation on Jensen isolation medium and Czapek sucrose agar, a culture produced a few short spore chains on the aerial mycelium. The spore chains (Fig. 1) contained 4 to 15 spores and were straight, flexuous, hooked, or coiled (once or twice). The spores were oval to elliptical and measured 0.8 to 1.0 by 1.2 to 2.0 μm . They had a smooth surface, as revealed by transmission electron microscopy (Fig. 2).

Cultural characteristics. Table 1 lists the cultural characteristics of the strain. Generally, growth was poor on the defined media but moderate on most of the organic media. The aerial mycelium, if present, was short and sparse and varied from white to pale grayish to grayish. The color of the substrate mycelium was cream, pale yellow, pale grayish, faint pink, pinkish orange, avellaneous, lavender, grayish, grayish black, or black. Except for the production of a brown soluble pigment on ISP medium 2 and ISP medium 2 plus coconut milk and of a pale yellow soluble pigment on yeast-starch agar, no soluble pigment was produced on the agar media used.

Physiological tests. Positive physiological

properties for the strain included production of hydrogen sulfide, gelatin liquefaction, nitrate reduction, clearing of milk, digestion of tyrosine, and growth at 21, 28, and 37°C. Negative properties included melanin production, starch hydrolysis, growth on cellulose broths, coagulation and peptonization of milk, digestion of calcium malate, digestion of casein, and growth at 45°C.

Carbohydrate utilization. On ISP medium 9 the organism utilized glucose and sucrose but not arabinose, inositol, mannitol, raffinose, or rhamnose. Doubtful utilization was noted with fructose and xylose as the carbon sources. Better growth was obtained on the medium of Luedemann and Brodsky. With this medium, glucose, sucrose, and trehalose were utilized, the utilization of inositol, xylose, galactose, glycerol, ribose, sorbose, and starch was doubtful, and arabinose, fructose, mannitol, raffinose, rhamnose, adonitol, cellobiose, dulcitol, lactose, mannose, melizitose, melibiose, salicin, and sorbitol were not utilized.

Cell wall analysis. The cell walls contained *meso*-diaminopimelic acid in addition to alanine, glutamic acid, muramic acid, and glucosamine. *meso*-Diaminopimelic acid and madurose were present in whole-cell hydrolysates. Thus, the culture can be considered to have a cell wall of type III of Becker et al. (2) and a type B whole-cell sugar pattern (11).

Identity of strain Pfizer FD 25934. The production of short spore chains, the cultural characteristics, and the presence of *meso*-diaminopimelic acid and madurose in the whole-cell hydrolysates place strain Pfizer FD 25934 in the genus *Actinomadura* Lechevalier and Lechevalier (10, 16). When compared with descriptions of known species of *Actinomadura*, this strain most closely resembled *A. spadix* Nonomura and Ohara (14). A culture of the type strain of *A. spadix* (ATCC 27298) was therefore used for comparison in this study. Both strain Pfizer FD 25934 and *A. spadix* ATCC 27298 shared the following properties: production of grayish aerial mycelium, production of spores with a smooth surface, cultural characteristics on organic agar 2 of Gauze et al. (6) and ISP medium 5, no production of melanin, and a positive nitrate reduction test. On ISP medium 2, colonies of strain Pfizer FD 25934 were gray to grayish black and produced a brown soluble pigment, whereas those of *A. spadix* ATCC 27298 were white to pale yellow and did not produce soluble pigment. On ISP medium 3, colonies of the former did not produce soluble pigment, whereas those of the latter produced a pink, soluble pigment. On ISP medium 4 and mineral medium 1 of Gauze et al., colonies of strain Pfizer FD 25934 were pale gray at the end

FIG. 1. Spore-bearing hyphae of strain Pfizer FD 25934 on Czapek sucrose agar. Light microscope. $\times 189$.

FIG. 2. Spores of strain Pfizer FD 25934. Electron microscope. Bar represents 1 μm .

TABLE 1. *Cultural characteristics of A. macra sp. nov. on various media*

Medium	Growth ^a	Aerial mycelium	Color of reverse side of colony	Soluble pigment
ISP medium 2	Good, grayish to grayish black (near gray series 2dc to 2dl), raised, wrinkled	White to grayish	Black	Brown
ISP medium 3	Moderate, cream to faint pink (near gray series 3ba), thin, smooth	None to scant, short	Same	None
ISP medium 4	Very scant, colorless or pale grayish at end of streak (near gray series 2fe), very thin, smooth	None	Colorless	None
ISP medium 5	Poor, cream (near gray series 1ba), thin, smooth	None to scant, short	Same	None
Mineral medium 1 of Gauze et al.	Poor to moderate, off-white or pale grayish (2ec) at end of streak, thin, smooth or occurring as isolated dots	None	Same	None
Organic medium 2 of Gauze et al.	Moderate, lavender (6ie), roughened	None to scant, short	Same	None
Czapek sucrose agar	Poor, pale cream (near gray series 2ba), thin, smooth	None to scant, short	Colorless	None
Emerson agar	Moderate to good, grayish, black (near gray series 2ih), raised, wrinkled	None	Same	None
Nutrient agar	Poor to moderate, cream (2ca), smooth, with a few black dots	None	Same	None
Jensen isolation agar	Poor to moderate, colorless to cream (near gray series 1ba), thin, smooth	Sparse, short	Same	None
Gelatin agar	Moderate, avellaneous (3ec to 3ge), slightly raised, wrinkled or granular in some areas	None	Same	None
Starch agar	Moderate, avellaneous (4ec to 4ge), thin to slightly raised at end of streak, smooth or slightly roughened at end of streak	White, scant	Same	None
Calcium malate agar	Scant to poor, colorless to pale grayish (near gray series 2dc to 2fe), thin, smooth	None	Same	None
Casein agar	None to a few isolated colonies, pale pinkish orange (4ea), raised, wrinkled	None	Same	None
Tyrosine agar	Poor, cream to pale yellowish (1ca to 1ea), thin, smooth	None	Same	None
Peptone Czapek agar	Moderate, pinkish orange (5ea to 5ga), thin, smooth	None	Same	None
Potato carrot agar	Poor to moderate, pale grayish (near gray series 1cb to 1dc), thin, smooth	None	Same	None
Potato dextrose agar	Moderate, grayish (near gray series 1fe to 1ih), thin, smooth to granular	White to pale grayish	Same	None
Yeast-starch agar	Moderate, pinkish orange (5gc), thin, smooth to slightly granular	White, scant	Same	Pale yellow
ISP medium 2 plus coconut milk	Good, pale grayish (near gray series 2dc to 2ih), raised, granular to wrinkled	White to pale grayish	Grayish	Brown
Tap water agar	Scant, colorless, thin, smooth	None to scant, very short	Same	None

^a For color designations, see reference 3.

of the streak, and those of *A. spadix* ATCC 27298 were cream colored. In addition, *A. spadix* ATCC 27298 differed from strain Pfizer FD 25934 in that the former was positive for starch hydrolysis, negative for gelatin liquefaction, and positive for milk peptonization and showed slight growth at 45°C. In carbohydrate utilization, *A. spadix* ATCC 27298 but not strain Pfizer

FD 25934 utilized arabinose, fructose, mannitol, raffinose, and rhamnose. Thus, strain Pfizer FD 25934 is considered to represent a heretofore undescribed species of the genus *Actinomadura*, for which the name *A. macra* sp. nov. is proposed. The specific epithet (L. fem. adj. *macra* lean) refers to the poor, thin growth of this organism on many media used in the identifica-

tion of similar organisms. Strain Pfizer FD 25934 is the type strain of *A. macra*; a culture of this strain has been deposited at the ATCC under the accession number ATCC 31286. Since the description of *A. macra* given above is based on a single strain, the type strain, it also serves as the description of the type strain.

ACKNOWLEDGMENTS

I thank W. D. Celmer for his encouragement and interest in this study and J. Bright for expert technical assistance. Appreciation is also expressed to J. Tone and H. Maeda for isolating the organism and providing photographs.

REPRINT REQUESTS

Address reprint requests to: Dr. L. H. Huang, Central Research, Pfizer Inc., Groton, CT 06340.

LITERATURE CITED

1. American Type Culture Collection. 1978. Catalogue of strains I, 13th ed. American Type Culture Collection, Rockville, Md.
2. Becker, B., M. P. Lechevalier, and H. Lechevalier. 1965. Chemical composition of cell wall preparations from strains of various form genera of aerobic actinomycetes. *Appl. Microbiol.* 13:236-243.
3. Container Corporation of America. 1958. Color harmony manual, 4th ed. Container Corporation of America, Chicago, Ill.
4. Couch, J. N. 1963. Some new genera and species of the *Actinoplanaceae*. *J. Elisha Mitchell Sci. Soc.* 79:53-70.
5. Emerson, R. 1958. Mycological organization. *Mycologia* 50:589-621.
6. Gauze, G. F., J. P. Preobrazhenskaya, E. S. Kudrina, N. O. Blinov, I. D. Ryabova, and M. A. Sveshnikova. 1957. Problems in the classification of antagonistic actinomycetes. *Medgiz, Moscow*.
7. Gordon, R. E., and J. M. Mihm. 1957. A comparative study of some strains received as nocardiae. *J. Bacteriol.* 73:15-27.
8. Gordon, R. E., and M. M. Smith. 1955. Proposed group of characters for the separation of *Streptomyces* and *Nocardia*. *J. Bacteriol.* 69:147-150.
9. Jensen, H. L. 1930. The genus *Micromonospora* Ørskov, a little known group of soil microorganisms. *Proc. Linn. Soc. N.S.W.* 55:231-248.
10. 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.
11. Lechevalier, M. P. 1968. Identification of aerobic actinomycetes of clinical importance. *J. Lab. Clin. Med.* 71:934-944.
12. Levine, M., and H. W. Schoenlein. 1930. A compilation of culture media. The Williams & Wilkins Co., Baltimore.
13. Luedemann, G. M., and B. Brodsky. 1965. *Micromonospora carbonacea* sp. n., an evernimicin-producing organism, p. 47-52. *Antimicrob. Agents Chemother.* 1964.
14. 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.
15. Shirling, E. B., and D. Gottlieb. 1966. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* 16:313-340.
16. Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.). 1980. Approved lists of bacterial names. *Int. J. Syst. Bacteriol.* 30:225-420.
17. Waksman, S. A. 1957. Species concept among the actinomycetes with special reference to the genus *Streptomyces*. *Bacteriol. Rev.* 21:1-19.
18. Waksman, S. A. 1961. The actinomycetes, vol. 2. The Williams & Wilkins Co., Baltimore.