A Novel Antibiotic-Producing *Actinomadura*, *Actinomadura kijaniata* sp. nov.

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A new species of *Actinomadura*, *Actinomadura kijaniata*, was isolated from African soil. This organism differs from other species of *Actinomadura* in that it forms deep green vegetative mycelia and white, curled aerial mycelia that fragment into long chains of smooth-walled, elliptical spores and produces a complex of novel acid enol antibiotics. Whole cells contain *meso*-diaminopimelic acid and madurose but lack nitrogenous phospholipids. This species can utilize a wide variety of carbohydrates, grow in the presence of numerous antibiotics, and actively hydrolyze many organic compounds. The type strain of *A. kijaniata* is SCC 1256 (= ATCC 31588).

During an examination of antibiotic-producing *Actinomycetales* isolated from soil, we observed that one strain (SCC 1256) produced a pigment that was distinct from the pigments produced by all of the other strains which we had studied. On agar surfaces, white aerial mycelia and deep green vegetative mycelia were formed; in broth, light to dark green mycelia and soluble pigments were formed. Strain SCC 1256 was particularly interesting because it produced a complex of novel acid enol antibiotics, the major component of which was designated kijanimicin (26). In this paper we identify and classify this kijanimicin-producing strain.

MATERIALS AND METHODS

Bacterial strains. Table 1 lists the strains of *Actinomadura* used in this study. Strain SCC 1256 was isolated from a soil sample collected in Kenya, Africa. A culture of this strain has been deposited in the American Type Culture Collection (ATCC), Rockville, Md., where it has been assigned the number ATCC 31588.

Strain maintenance. The source materials used for this study were freeze-dried preparations of pure cultures. The contents of each vial were suspended in 10 ml of liquid medium (ATCC medium 172) (1) in a 25-mm tube stoppered with a Morton closure. Broth suspensions were incubated at 30°C on a rotary shaker (model G-52; New Brunswick Scientific Co.) at 250 rpm for 3 to 4 days; then 5 ml of each culture was transferred into 50 ml of fresh medium in a 250-ml Erlenmeyer flask stoppered with cotton and incubated as described above. The growth was harvested after 3 to 4 days, and 5-ml quantities of the resulting biomass were dispensed aseptically into sterile screw-capped vials (17 by 60 mm) and stored at -18°C.

Preparation of inocula. A 1-ml sample of thawed cell suspension was used to inoculate each broth tube, as described above. After 3 days at 30°C, the cells were harvested by centrifugation, washed twice with sterile distilled water, and suspended in water to three times the packed-cell volume. The resulting washed suspen-

sions were used as inocula for the tests described below.

TABLE 1. Strains of *Actinomadura* used in this study^a

Laboratory no.	Received as:		Source
	Species	Strain	
SCC 1256	Original isolate	13-363	Soil
A 27887	<i>Actinomadura citrea</i>	ATCC 27887 (type)	ATCC
A 29533	<i>Actinomadura flava</i>	ATCC 29533 (type)	ATCC
A 27295	<i>Actinomadura helvata</i>	ATCC 27295 (type)	ATCC
A 31286	" <i>Actinomadura macra</i> " ^b	ATCC 31286 (type)	ATCC
SCC 908	" <i>Nocardia madurae</i> "	IMRU 777(7)	IMRU ^c
A 27888	<i>Actinomadura malachitica</i>	ATCC 27888 (type)	ATCC
SCC 918	" <i>Nocardia pelletieri</i> "	NCTC 4162 (type)	IMRU
A 27296	<i>Actinomadura pusilla</i>	ATCC 27296 (type)	ATCC
A 27297	<i>Actinomadura roseoviolacea</i>	ATCC 27297 (type)	ATCC
A 27031	" <i>Micromonospora rubra</i> "	ATCC 27031 (type)	ATCC
A 27298	<i>Actinomadura spadix</i>	ATCC 27298 (type)	ATCC
A 27299	<i>Actinomadura verrucosospora</i>	ATCC 27299 (type)	ATCC

^a Of the 24 *Actinomadura* species on the Approved Lists of Bacterial Names (23), only the species listed were available from the ATCC.

^b Names in quotation marks are not on the Approved Lists of Bacterial Names (23) and were not validly published as of 1 January 1980.

^c IMRU, Institute of Microbiology, Rutgers University Busch Campus, Piscataway, N.J.

FIG. 1. Aerial mycelium of strain SCC 1256 fragmenting into chains of elliptical spores on water agar. $\times 450$.

Morphological observations. Washed cells were inoculated onto the surfaces of the following media in petri dishes: YD agar (10 g of yeast extract, 10 g of dextrose, and 15 g of agar in 1,000 ml of tap water, pH 7.0); water agar (15 g of agar in 1,000 ml of tap water, pH 7.0); and half-strength starch agar (2.5 g of yeast extract, 5.0 g of potato starch, and 15 g of agar in 1,000 ml of distilled water, pH 7.0). The inoculated plates were incubated at 30°C for 5, 10, 15, and 20 days and examined after each time period directly under a microscope. Representative plates of strain SCC 1256 were flooded with sterile tap water, the surface growth was gently scraped, and samples were examined microscopically for motile spores. Spores were examined with an electron microscope by using the procedures of Luedemann and Casmer (17).

Chemical analysis of whole cells. The presence and form of diaminopimelic acid and the presence of carbohydrates in whole-cell hydrolysates were determined by the methods of Becker et al. (2) and Lechevalier (14). Phospholipids were analyzed by the procedure of Lechevalier et al. (15).

Growth characteristics. Strain SCC 1256 was cultivated on standard media as described by Shirling and Gottlieb (22) and Waksman (27). Media in plates were inoculated and incubated for 14 to 21 days at 30°C. Each color designation assigned to the vegetative mycelium pigments consisted of a color name (25) and a color chip number (3).

Carbohydrate utilization. Carbohydrate utilization was determined by the procedure of Luedemann and Brodsky (16).

Decomposition of organic compounds. The procedures of Gordon et al. (8) and Goodfellow (5) were

used to determine the decomposition of organic compounds. Hydrolysis of deoxyribonucleic acid was measured by the method of Jeffries et al. (10).

Decomposition of urea and allantoin. The decomposition of urea and the decomposition of allantoin were determined by the procedures of Gordon et al. (8) and Kurup and Schmitt (11), respectively.

Production of lactamase. Lactamase was detected by method B of Schwartz and Schwartz (21).

Temperature relationships. We tested the ability of strain SCC 1256 to grow at 28, 35, 40, and 45°C and to survive at 50°C on ATCC medium 172 by using the techniques of Gordon et al. (8).

Antibiotic susceptibility. The amount of each antibiotic that resulted in a concentration (based on activity) of 1,000 $\mu\text{g/ml}$ was dissolved in the appropriate solvent, diluted in sterile distilled water to a concentration of 500 $\mu\text{g/ml}$, and filter sterilized. Each antibiotic was incorporated into YD agar to a final concentration of 50 $\mu\text{g/ml}$ and then dispensed into sterile petri dishes. Inoculated plates were incubated at 30°C and examined for growth after 5, 7, and 14 days.

RESULTS AND DISCUSSION

Morphological observations. On both YD agar and water agar, abundant aerial mycelia which fragment into long chains of elliptical spores (10 or more) approximately 1.0 to 1.5 μm in diameter by 1.5 to 2.0 μm long (Fig. 1) are formed. Electron microscopic observation indicated that the spores are smooth walled. Aerial mycelia occur as long, open spirals. Motile spores do not occur. The vegetative mycelia form abundant branches and also appear to fragment into chains of spores.

Whole-cell analysis. Hydrolyzed whole cells contain the *meso* isomer of diaminopimelic acid, along with a trace of the L-isomer. Madurose is the characteristic sugar component. A type PI phospholipid pattern, characteristic of *Actinomadura*, was found.

Growth characteristics. Table 2 shows the macroscopic appearance of strain SCC 1256 on various descriptive media. This strain grows well on most organic media. The vegetative mycelium colors range from tan to dark green; shades of green are characteristic. Abundant white aerial mycelia are formed on ISP no. 7 agar and starch agar. Gray, brown, and green diffusible pigments are formed on several media.

Carbohydrate utilization and physiological properties. Table 3 shows the carbohydrate utilization and physiological properties of strain SCC 1256. The positive properties include utilization of adonitol, D-arabinose, L-arabinose, fructose, L-fucose, galactose, glucose, glycerol, inositol, maltose, mannose, rhamnose, ribose, sucrose, trehalose, and D-xylose; methyl- β -D-glucopyranoside is utilized and a distinctive green diffusible pigment is formed. Strain SCC 1256 is capable of hydrolyzing all of the com-

TABLE 2. Growth characteristics of strain SCC 1256 on various media

Medium	Growth	Surface characteristics	Aerial mycelium	Diffusible pigments	Color of growth ^a
Bennett agar	+++ , good	Raised, folded	Present (sparse, white)	Absent	g 22 li (pine green)
Czapek sucrose agar	+++ , good	Ribbon-like	Absent	Absent	g 22 n (dark pine green)
Glucose asparagine agar	+++ , good	Raised, folded	Present (sparse white specks)	Absent	g 22 n (dark pine green)
Glycerol asparagine agar	+++ , good	Flat, folded	Absent	Gray, red-brown	Center, g 19 po (dark laurel); periphery, g 6 ie (redwood)
Nutrient agar	+++ , good	Raised, folded	Absent	Faint dull green	g 23 n (evergreen)
Water agar	± , poor	Granular	Absent	Absent	g 24 ge (dusty green)
Yeast extract malt extract agar (ISP no. 2)	+++ , good	Raised, hard, folded	Present (white specks)	Absent	Center, g 21 nl (dark jade green); periphery, g 2 ec (biscuit)
Potato dextrose agar	++ , moderate	Flat to slightly raised	Absent	Absent	g 2 ge (covert tan)
Emerson agar	+ to ++ , fair to moderate	Flat, granular	Absent	Absent	g 22 ig (bayberry)
NZA glucose agar	+++ , good	Soft, compactly folded	Absent	Absent to dark brown	g 15 po (dark slate)
Yeast extract glucose agar	+++ , good	Raised, deeply folded, hard cracking	Present (white specks)	Absent	Center, g 22 ig (almond green); periphery, g 3 ge (camel)
Tomato paste oatmeal agar	+++ , good	Flat, granular	Present (white specks)	Absent	g 1½ li (light olive drab)
Starch agar (Waksman no. 21)	+ , fair	Flat, granular	Absent	Absent	g 24 ig (sage green)
Calcium maleate agar (Waksman no. 7)	+ to ++ , fair to moderate	Plicate, soft	Absent	Absent	g 2 gc (bamboo)
Oatmeal agar (ISP no. 3)	+ to ++ , fair to moderate	Flat, granular	Present (white specks)	Absent	g 22 nl (dark pine green)
Inorganic salts starch agar (ISP no. 4)	+ , fair	Flat, granular	Absent	Faint green	Center, g 23 li (slate green); periphery, g 4 ge (light fawn)
Peptone iron agar	++ , moderate	Raised, granular	Present (white specks)	Absent	g 2 ge (covert tan)
Casein agar	++ , moderate	Flat, granular	Absent	Absent	g 22 ih (moss gray)
Tyrosine agar (ISP no. 7)	+++ , good	Raised, folded	Present (abundant, white)	Absent	g 5 ih (lead gray)
Starch agar (yeast)	+++ , good	Hard, folded	Present (white)	Absent	g 23 li (slate green)
Calcium citrate agar	+ , fair	Flat, granular	Absent	Absent	g 2 li (covert brown)
Gelatin agar (McDade)	++ , moderate	Raised, compactly folded	Absent	Absent	Center, g 1 po (ebony); periphery, g 1 fe (greige)

^a See references 3 and 25.

TABLE 3. Physiological properties of strain SCC 1256

Test	Results	Test	Results
Growth on carbohydrates		Starch	+ (weak)
Adonitol	+++	Deoxyribonucleic acid	+
D-Arabinose	+++	Gelatin	+
L-Arabinose	+++	Elastin	+
Dextrin	+++	Collagen	+
Dulcitol	—	Formation of:	
Erythritol	—	Urease	+
Fructose	+++	Allantoinase	+
L-Fucose	+++	Lactamase	+
Galactose	+++	Growth at:	
Glucose	+++	28°C	+
α -methyl-D-Glucoside	—	35°C	+
Methyl- β -D-glucopyranoside	+++	40°C	+
	(green DFP) ^a	45°C	+
Glycerol	+++	Survival at 50°C for 8 h	+
Inositol	+++	Growth in the presence of:^b	
Inulin	—	Gentamicin	+
Lactose	—	Kanamycin	+
Maltose	+++	Neomycin	+
Mannitol	—	Paromomycin	+
Mannose	+++	Sisomicin	+
Melibiose	—	Streptomycin	+
Melizitose	—	Erythromycin	+
Raffinose	—	Rosaramicin	—
Rhamnose	+++	Clindamycin	+
Ribose	+++	Lincomycin	+
Sucrose	+++	Tetracycline	+
Trehalose	+++	Everninomicin	+
D-Xylose	+++	Rifamycin	+
Hydrolysis of:		Penicillin G	+
Adenine	+ (weak)	Cephalothin	+
Hypoxanthine	+	Cycloserine	+
Tyrosine	+	Production of:	
Xanthine	+	H ₂ S	—
Xylan	+	Melanin	—
Casein	+		

^a DFP, Diffusible pigment.^b All antibiotics were tested at a concentration of 50 µg/ml.

pounds tested; however, the hydrolyses of starch and adenine are weak. Urea and allantoin are degraded, growth occurs at 45°C, the strain survives at 50°C for 8 h, growth occurs in the presence of most of the antibiotics tested, and lactamase is formed. Strain SCC 1256 is unable to utilize dulcitol, erythritol, α -methyl-D-glucoside, inulin, lactose, mannitol, melibiose, melizitose, or raffinose, to grow in the presence of rosaramicin, and to form hydrogen sulfide and melanin.

Identity of strain SCC 1256. The presence of *meso*-diaminopimelic acid and madurose as characteristic whole-cell components, a type PI phospholipid pattern, and the formation of aerial mycelia with long spore chains identify this strain as a member of the genus *Actinomadura* Lechevalier and Lechevalier (13). Table 4 compares the whole-cell data and differentiating

morphological and physiological (6) characteristics of the strains listed in Table 1 with the data obtained for SCC 1256. Of the strains used in this study and the species of *Actinomadura* described in the literature, none shares with SCC 1256 the combination of deep green vegetative mycelia, white to green aerial mycelia, long spore chains with smooth spores, the ability to hydrolyze xanthine, the ability to utilize methyl- β -D-glucoside, and the ability to produce kijanin.

Our data suggest that strain SCC 1256 represents a new, distinct antibiotic-producing species of *Actinomadura*, for which the name *Actinomadura kijaniata* is proposed. The specific epithet is derived from *kijani*, the Swahili word for green. The type strain is SCC 1256 (= ATCC 31588). Since at present there has been only one strain identified as belonging to this species, (the

TABLE 4. Morphological and chemical characteristics of ATCC *Actinomadura* species and strain SCC 1256

Taxon	Reference(s)	Spore chains	Spore surfaces	Aerial mycelium color	Vegetative mycelium color	meso-Diamino-pimelic acid and madurose	Antibiotic activity	Hydrolysis of xanthine	Utilization of:	
									Methyl- β -D-glucoside	Mannitol
SCC 1256		Flexous spirals, long	Smooth	White to pale green	Dark green	+	+	+	+	-
<i>Actinomadura citrea</i> ATCC 27887	12	Straight to hooked, short	Uneven	Yellow-blue	Lemon yellow	+	-	-	-	+
<i>A. flava</i> ATCC 29533	4	Straight, long	Smooth	Rarely formed	Lemon yellow, yellow-brown	+	+	-	-	+
<i>A. helvata</i> ATCC 27295	19, 20	Closed spirals, short	Smooth	White to pink	Yellow-brown	+	-	-	-	+
" <i>A. macra</i> " ATCC 31286 ^a	9	Straight to flexous, short	Smooth	Grayish	Cream to gray	+	+	-	-	-
<i>A. madurae</i> IMRU 777	7	Curls to hooks, short	Smooth	Rarely formed, white-pink	Pink to red	+	+	-	-	+
<i>A. malachitica</i> ATCC 27888	12	Loose spirals, short	Smooth	Rarely formed, pale green	Cream	+	-	-	-	+
<i>A. pelletieri</i> NCTC 4162	7, 13	Curls to hooks, short	Smooth	Rarely formed, white to pink	Pink to red	+	+	-	-	-
<i>A. pusilla</i> ATCC 27296	19, 20	Closed spirals, short	Smooth	White to pink	Brownish gray, blue-black		+	-	+	-
<i>A. roseoviolacea</i> ATCC 27297	19, 20	Hooks to closed spirals, short	Smooth	White to pink	Red to violet	+	-	-	-	+
<i>A. rubra</i> ATCC 27031	18, 24	Hooks to spirals, short	Smooth to uneven	Rarely formed, white to pink	Brick red	+	+	-	-	+
<i>A. spadix</i> ATCC 27298	19, 20	Hooks to closed spirals, short	Smooth	Grayish	Gray-brown	+	-	-	-	+
<i>A. verrucosospora</i> ATCC 27299	19, 20	Hooks to spirals	Uneven	Grayish blue	Pink	+	- ^b	-	-	+

^a Name not on the Approved Lists of Bacterial Names (23) and not validly published as of 1 January 1980.

^b ATCC 31466, a strain identified as *A. verrucosospora* has been reported to produce a new polycyclic antibiotic (U.S. patent 4,195,079).

type strain), the description presented above applies to both the species and the type strain.

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REPRINT REQUESTS

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LITERATURE CITED

1. American Type Culture Collection. 1980. Catalogue of strains I, 14th 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. Contalner Corporation of America. 1958. Color harmony manual, 4th ed. Container Corporation of America, Chicago.
4. Gause, G. F., T. S. Maksimova, O. L. Olkhovatova, M. A. Sveshnikova, G. V. Kochetkova, and G. B. Ilchenko. 1974. Production of madumycin, an antibacterial antibiotic, by *Actinomadura flava* sp. nov. *Antibiotiki (Moscow)* 9:771-775.
5. Goodfellow, M. 1971. Numerical taxonomy of some nocardioform bacteria. *J. Gen. Microbiol.* 69:33-80.
6. Goodfellow, M., G. Alderson, and J. Lacey. 1979. Numerical taxonomy of *Actinomadura* and related actinomycetes. *J. Gen. Microbiol.* 112:95-111.
7. Gordon, R. E. 1966. Some criteria for the recognition of *Nocardia madurae* (Vincent) Blanchard. *J. Gen. Microbiol.* 45:355-364.
8. 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.
9. 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.
10. Jeffries, C. D., D. F. Holtman, and D. G. Guse. 1957. Rapid method for determining the activity of microorganisms on nucleic acids. *J. Bacteriol.* 73:590-591.
11. Kurup, P. V., and J. A. Schmitt. 1973. Numerical taxonomy of *Nocardia*. *Can. J. Microbiol.* 19:1035-1048.
12. Lavrova, N. V., T. P. Preobrazhenskaya, and M. A. Sveshnikova. 1972. Isolation of soil actinomycetes on selective media with rubomycin. *Antibiotiki (Moscow)* 20:404-409.
13. 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.
14. Lechevalier, M. P. 1968. Identification of aerobic actinomycetes of clinical importance. *J. Lab. Clin. Med.* 71:934-944.
15. Lechevalier, M. P., C. DeBievre, and H. A. Lechevalier. 1977. Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem. Syst. Ecol.* 5:249-260.
16. Luedemann, G. M., and B. Brodsky. 1965. *Micromonospora carbonacea* sp. n., an evernimycin-producing organism, p. 47-52. *Antimicrob. Agents Chemother.* 1964.
17. Luedemann, G. M., and C. J. Casmer. 1973. Electron microscope study of whole mounts and thin sections of *Micromonospora chalcone* ATCC 12452. *Int. J. Syst. Bacteriol.* 21:240-247.
18. Meyer, J., and M. Sveshnikova. 1974. *Micromonospora rubra* Sveshnikova et al. = *Actinomadura rubra* comb. nov. *Z. Allg. Mikrobiol.* 14:167-170.
19. Nonomura, H. 1974. Key for the classification and identification of species of rare actinomycetes isolated from soils in Japan. *J. Ferm. Technol.* 52:71-77.
20. Nonomura, H., and Y. Ohara. 1971. Distribution of actinomycetes in soil. XI. Some new species of the genus *Actinomadura* Lechevalier et al. *J. Ferm. Technol.* 49:904-912.
21. Schwartz, J. L., and S. P. Schwartz. 1979. Production of β -lactamase by non-*Streptomyces* *Actinomycetales*. *Antimicrob. Agents Chemother.* 15:123-125.
22. Shirling, E. B., and D. Gottlieb. 1966. Method for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* 16:313-340.
23. 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.
24. Sveshnikova, M. A., T. S. Maksimova, and E. S. Kudrina. 1969. The species of the genus *Micromonospora* Orskov, 1923, and their taxonomy. *Mikrobiologiya* 38:883-893.
25. Taylor, H. D., L. Knoche, and W. C. Granville. 1950. Descriptive color names dictionary. Container Corporation of America, Chicago.
26. Waltz, J. A., A. C. Horan, M. Kalyanpur, B. K. Lee, D. Loebenberg, J. A. Marquez, G. Miller, and M. G. Patel. 1981. Kijanimicin (Sch 25663), a novel antibiotic produced by *Actinomadura kijaniata* SCC 1256. Fermentation, isolation, characterization and biological properties. *J. Antibiot.* 34:1101-1106.
27. Waksman, S. A. 1961. The actinomycetes, vol. 2. The Williams & Wilkins Co., Baltimore.