

Streptomyces steffisburgensis sp.n.

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Streptomyces steffisburgensis is described as a new species and named to conform with the 1966 International Code of Nomenclature of Bacteria. The description of the organism is accompanied by a color print of it on six agar media and electron micrographs of the spore chains.

This communication describes a new species of *Streptomyces*, named to conform with the International Code of Nomenclature of Bacteria (8). An outstanding property of the culture is its production of U-20,661, an antimicrobial agent described by Bergy and Reusser (1).

MATERIALS AND METHODS

Inoculum. The inoculum for determining the various characteristics was prepared as previously described (2), but the cells were washed according to the procedure of Pridham and Gottlieb (12). The blended inoculum was streaked in a cross-hatch pattern on diagnostic agar media in four-sector petri plates. For seeding broth media and gelatin stabs in test tubes, 0.2 ml of the inoculum was used.

Color characteristics. The color characteristics of the culture were first determined according to the method of Dietz (2) as modified by Dietz and Mathews (3). Reference color determinations were made on three of the media: Bennett's, Czapek's sucrose, and maltose-tryptone using the *Color Harmony Manual* (9) and NBS Circular No. 553 (10).

Microscopic characteristics. The sporophore type was determined by examination by light microscope of the surface of the culture on agar in petri plates. The spore type was determined by examination by electron microscope of spores according to the method of Dietz and Mathews (3).

Media for cultural and biochemical characteristics. The six agar media for Ektachromes (3) were used as well as the following media: calcium malate (7), glucose-asparagine, skim milk (Difco, with 1.5% agar), and tyrosine (5), xanthine (6), yeast extract-malt extract (13), nutrient starch agar, plain gelatin, nutrient gelatin, nutrient nitrate broth, and synthetic nitrate broth (15). Difco litmus milk was used for determining the action of the culture on milk. Carbon utilization was determined according to the method of Pridham and Gottlieb (12) except that the tests were run on four-sector plates rather than on slants.

Incubation. Tubes photographed on Ektachromes were incubated for 7 days at 28 C. All other plates and tubes were incubated for 14 days at 28 C. Temperature studies were made using Bennett's, Czapek's sucrose, and maltose-tryptone agars at 18, 24, 28, 37, and 55 C.

DESCRIPTION

Streptomyces steffisburgensis Dietz, sp. n.

Color characteristics. Gray aerial mycelium. Melanin-positive. Appearance on Ektachromes is given in Fig. 1. Color characteristics on three agar media are given in Table 1. The culture may be placed in the Gray (GY) color series of Tresner and Backus (16).

Microscopic characteristics. Short spiny spores (Fig. 2) borne on short, straight to open spiral to spiral sporophores (RF, RA, S) in the sense of Pridham et al. (14).

Cultural and biochemical characteristics. See Table 2.

Carbon utilization. The ability of the culture to grow on carbon compounds was determined in the synthetic medium of Pridham and Gottlieb (12). Growth was good on D-xylose, L-arabinose, rhamnose, D-fructose, D-galactose, D-glucose, D-mannose, maltose, sucrose, lactose, cellobiose, raffinose, dextrin, inulin, soluble starch, glycerol, D-mannitol, and inositol; moderate on D-sorbitol, salicin, sodium citrate, and sodium succinate; slight on dulcitol, sodium oxalate, and sodium tartrate. There was no growth on phenol, cresol, sodium formate, sodium salicylate, sodium acetate, and the control.

Temperature. The culture did not grow at 18 C. It grew poorly at 24 C. At 55 C there was a slight colorless vegetative growth after 24 hr. Its optimal temperature is between 28 and 37 C.

Antibiotic-producing properties. The culture produces the antibiotic U-20,661, which has antimicrobial properties as described by Bergy and Reusser (1).

Source. Soil.

Type culture. UC-5044, NRRL 3193 (U.S. Patent 3,309,273, 1967).

DISCUSSION

S. steffisburgensis is an actinomycete of the genus *Streptomyces* which was isolated from a

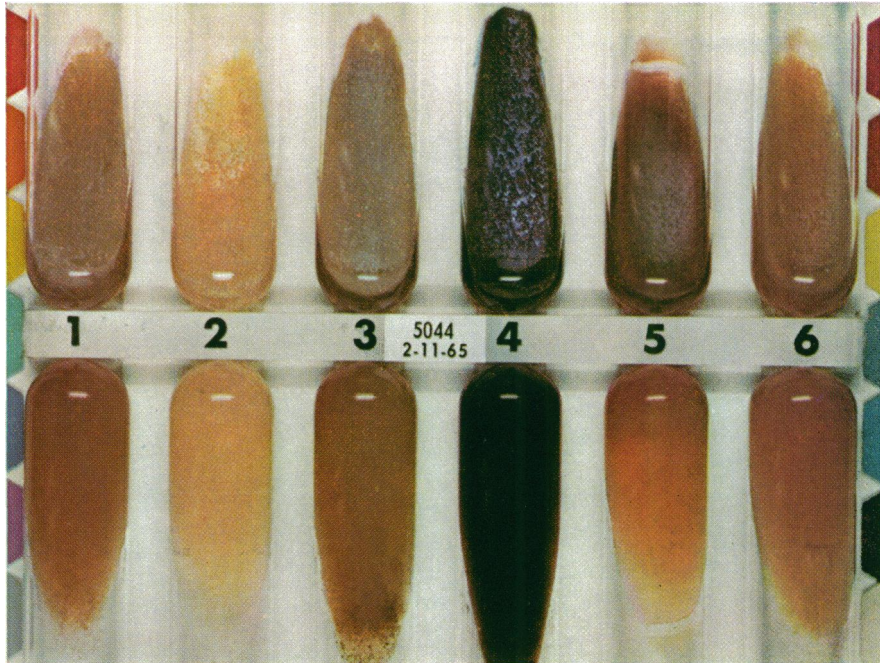


FIG. 1. Ektacolor print of *Streptomyces steffisburgensis* on six agar media, showing obverse and reverse. The agar media are: (1) Bennett's, (2) Czapek's sucrose, (3) maltose-tryptone, (4) peptone-iron, (5) 0.1% tyrosine, (6) casein starch.

TABLE 1. Reference color characteristics of *Streptomyces steffisburgensis*

Medium	<i>Color Harmony Manual</i> , 3rd ed., 1948	ISCC-NBS method of designating color and a dictionary of color names, Circular 553, 1955
<i>Bennett's agar</i>		
Surface	3ig beige brown, mist brown	80m grayish yellowish brown 95g moderate olive brown
Reverse	3lg adobe brown, cinnamon brown, light brown	77gm moderate yellowish brown
Pigment	None	
<i>Czapek's sucrose agar</i>		
Surface	2ie light mustard tan	91gm dark grayish yellow 94g light olive brown 106g light brown
Reverse	2ie light mustard tan	91gm dark grayish yellow 94g light olive brown 106g light olive
Pigment	none	
<i>Maltose Tryptone Agar</i>		
Surface	a white 3ge beige camel	263 gm white 79m light grayish yellowish brown 94m light olive brown
Reverse	3ng yellow maple	77m moderate yellowish brown
Pigment	3ie camel, maple sugar, tan	76m light yellowish brown 77g moderate yellowish brown

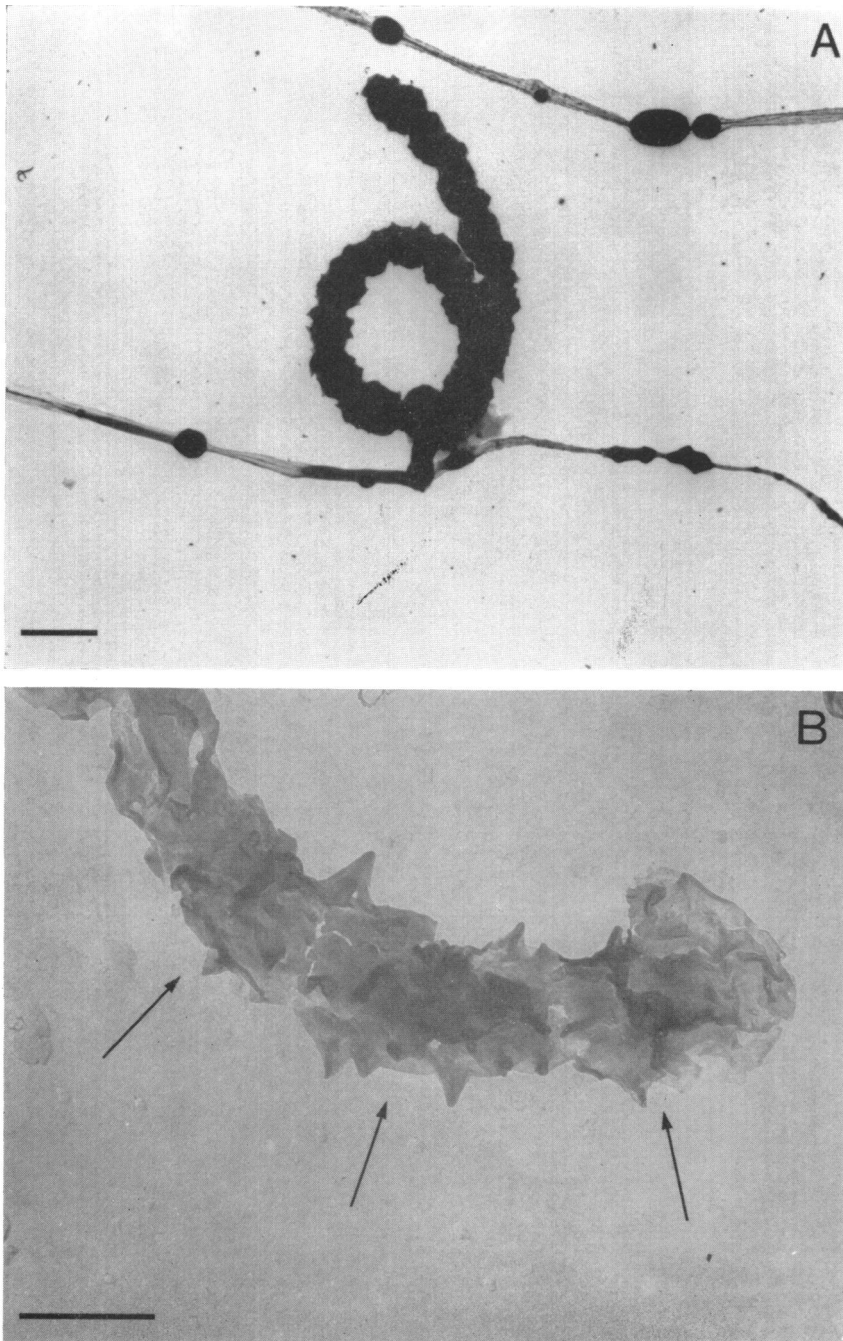


FIG. 2. Electron micrographs of spores of *Streptomyces steffisburgensis*. Each index mark equals 1 μ . (A) Whole spore mount. (B) Carbon repligraph of spore chain. Arrows indicate individual spores.

TABLE 2. Cultural characteristics of *Streptomyces steffisburgensis*

Medium	Surface	Reverse	Other
<i>Agar media</i>			
Peptone-iron	No aerial growth	Brown	Brown pigment Melanin-positive
Calcium-malate	Trace white aerial growth	Colorless	No pigment Malate solubilized (\pm)
Glucose-asparagine	Very slight trace of gray-white aerial growth	Yellow	No pigment
Skim milk	Very slight trace of gray aerial growth	Tan	Tan pigment Casein not solubilized
Tyrosine	Trace gray-white aerial growth	Pink-tan	Pink-tan pigment Tyrosine not solubilized
Xanthine	Trace gray-white aerial growth	Brown	Brown pigment Xanthine solubilized
Nutrient starch	Trace gray-white aerial growth	Pale yellow-tan	No pigment
Yeast extract-malt extract	Fair gray-white aerial growth	Tan	Red-tan pigment
Bennett's	Trace gray aerial growth	Pink-tan	Pink-tan pigment
Czapek's sucrose	None to trace gray aerial growth	Yellow	No pigment
Maltose-tryptone	Gray-pink aerial growth	Pink-tan	Pink-tan pigment
<i>Gelatin media</i>			
Plain			Brown pigment in upper $\frac{1}{4}$ of medium Liquefaction in pigment area
Nutrient			Tan pigment in upper $\frac{1}{4}$ of medium Liquefaction in pigment area
<i>Broth media</i>			
Nutrient nitrate			Colorless surface growth Colorless flocculent growth at base No pigment Nitrate not reduced to nitrate
Synthetic nitrate			Colorless surface growth Colorless flocculent growth at base No pigment to trace tan pigment Nitrate not reduced to nitrite
Litmus milk			Colorless surface ring Partial reduction pH 6.3-6.6

soil sample and found to produce the antibiotic U-20,661. The culture was compared for identity with actinomycete cultures in the Upjohn collection and the literature descriptions in Gauze (4), Krassilnikov (11), and Waksman (17).

S. steffisburgensis showed some similarity to *Streptomyces diastatochromogenes* NRRL B-2518. Both cultures are melanin-positive and

sporulate poorly. Both have similar growth patterns on carbon compounds in the synthetic medium of Pridham and Gottlieb (12). *S. diastatochromogenes* has straight to flexuous sporophores rather than short straight to open spiral to spiral sporophores as seen in *S. steffisburgensis*. The spores as observed by electron microscopy are smooth rather than spiny. *S.*

diastatochromogenes NRRL B-2518 does not produce the antibiotic U-20,661. Furthermore, *S. diastatochromogenes* NRRL B-2518 differs greatly from *S. diastatochromogenes* as described in Waksman (17). Criteria for *S. diastatochromogenes* as described in Waksman (17) are not definitive and a review of it and other *S. diastatochromogenes* strains is in preparation.

S. steffisburgensis was readily distinguished by color, microscopic, macroscopic, and cultural characteristics from named species of *Streptomyces* in the Upjohn culture collection and as far as can be determined from those described in the literature. These data, in conjunction with the fact of production of a distinctly new antimicrobial agent (U-20,661), require the culture to be considered a new species of *Streptomyces* designated *Streptomyces steffisburgensis* sp.n.

It is proposed that the organism described here and deposited as NRRL 3193 be designated the type strain.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. BERGY, M. E., AND F. REUSSER. 1967. A new antibacterial agent (U-20, 661) isolated from a streptomycete strain. *Experientia* **23/4**:241-320.
2. DIETZ, A. 1954. Ektachrome transparencies as aids in actinomycete classification. *Ann. N.Y. Acad. Sci.* **60**:152-154.
3. DIETZ, A., AND J. MATHEWS. 1962. Taxonomy by carbon replications. I. An examination of *Streptomyces hygrosopicus*. *Appl. Microbiol.* **10**:258-263.
4. GAUZE, G. F., T. P. PREOBRAZHENSAYA, E. S. KUDRINA, N. O. BLINOV, I. D. RYABOVA, AND M. A. IVESHNIKOVA. 1957. Problems in the classification of antagonistic actinomycetes. State Publishing House for Medical Literature, Moscow. English edition translated by Fritz Danga; David Gottlieb [ed.]. The American Institute of Biological Sciences, Washington, D.C.
5. GORDON, R. E., AND M. M. SMITH. 1955. Proposed group of characters for the separation of *Streptomyces* and *Nocardia*. *J. Bacteriol.* **69**:147-150.
6. GORDON, R. E., AND J. M. MIHM. 1957. A comparative study of some strains received as nocardiae. *J. Bacteriol.* **73**:15-27.
7. HEINEMAN, B., M. A. KAPLAN, R. D. MUIR, AND I. R. HOOPER. 1953. Amphomycin, a new antibiotic. *Antibiot. Chemotherapy* **3**:1239-1240.
8. INTERNATIONAL CODE OF NOMENCLATURE OF BACTERIA. 1966. Edited by The Editorial Board of the Judicial Commission of the International Committee on Nomenclature of Bacteria. *Intern. J. System. Bacteriol.* **16**:459-490.
9. JACOBSON, E., W. C. GRANVILLE, AND C. E. FOSS. 1948. Color harmony manual, 3rd ed. Container Corporation of America, Chicago, Ill.
10. KELLY, K. L., AND D. B. JUDD. 1955. The ISCC-NBS method of designating colors and a dictionary of color names. U.S. Dept. Comm. Circ. 553.
11. KRASSILNIKOV, N. A. 1949. *Actinomycetes*. In Guide to the identification of bacteria and *Actinomycetes*. Academy of Sciences, U.S.S.R., Moscow. English edition translated by J. B. Routien, Chas. Pfizer & Co., Inc., 1957.
12. PRIDHAM, T. G., AND D. GOTTLIEB. 1948. The utilization of carbon compounds by some Actinomycetales as an aid for species determination. *J. Bacteriol.* **56**:107-114.
13. 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. Ann.* 1956-57, p. 947-953.
14. PRIDHAM, T. G., C. W. HESSELTINE, AND R. G. BENEDICT. 1958. A guide for the classification of streptomycetes according to selected groups. Placement of strains in morphological sections. *Appl. Microbiol.* **6**:52-79.
15. SOCIETY OF AMERICAN BACTERIOLOGISTS, Committee on Bacteriological Technic. 1946. A manual of methods for pure culture study of bacteria. Biotechnical Publications, Geneva, N.Y.
16. TRESNER, H. D., AND E. J. BACKUS. 1963. System of color wheels for streptomycete taxonomy. *Appl. Microbiol.* **11**:335-338.
17. WAKSMAN, S. A. 1961. The actinomycetes, vol. 2. Classification, identification, and descriptions of genera and species. The Williams & Wilkins Co., Baltimore.