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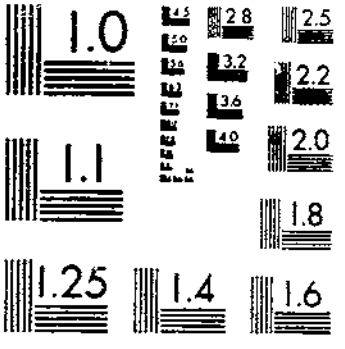
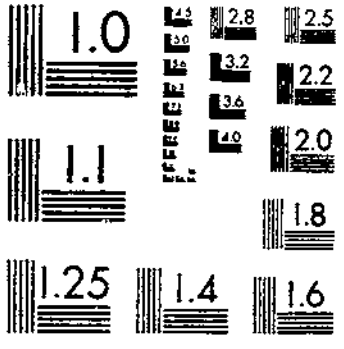


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

STREPTOMYCES GRISEUS (KRAINSKY) WAKSMAN AND HENRICI

A Taxonomic Study of Some Strains

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To clarify the nature and relationships of the important antibiotic-producing Actinomycetes, taxonomic studies were made on a group of 53 streptomycete strains exhibiting the following basic characteristics: Spore chains that are straight to flexuous; sporulating aerial mycelium colored in tints and shades of yellowish gray (Olive-Buff, Ridgway); inability to darken peptone-iron agar or to form brown or black diffusible pigments; and ability to utilize D-xylose but not L-arabinose or L-rhamnose in a chemically defined agar medium. Strains studied include some with the epithet labels, "*brasiliensis*," "*griseus*," "*streptomycini*," and "*vinaceus*" and some that reputedly produce keratinase, vitamin B_{12}, or one or more of the following antibiotics: actinomycin complex, cycloheximide, rhodomycetin, streptocin, and streptomycin. The basic characteristics of each strain were verified and the following additional characteristics determined: optimal temperature range; proteolytic activity, by six different methods; diastatic activity, by two methods; abilities to reduce nitrate; spore-wall ornamentation, by electron micrography; abilities to utilize six additional carbon compounds; sensitivity to lysozyme, by two methods; abilities to decompose L-tyrosine, xanthine, and hypoxanthine; and antibiotic activities. The results suggest that these strains comprise several subspecies of *Streptomyces griseus* (Krainsky) Waksman and Henrici.

This taxonomic study (classification and naming) was made as part of the investigations being conducted at the Northern Regional Research Laboratory (NRRL), Peoria, Ill., on industrial utilization of cereal grains. The Northern Laboratory is headquarters for the Northern Utilization Research and Development Division. The ARS Culture Collection, maintained there, is one of the world's largest and most complete collections of industrially important bacteria, molds, actinomycetes, and yeasts. The Collection serves as a source of authentic micro-organisms for the fermentative production of organic acids, vitamins, antibiotics, enzymes, feeds, beverages, and foods.

Acknowledgment is made to H. D. Tresner and M. C. Davies, Microbiology Department, Biochemical Research Section, Lederle Laboratories, Pearl River, N.Y., for supplying most of the electron microscope data; and to donors of some of the strains used: Bristol Laboratories, Syracuse N.Y., for cultures of streptomycin-resistant bacteria, and Upjohn Co., Kalamazoo, Mich., for a sample of rhodomycetin. This work was supported in part by a grant from the Subcommittee on Taxonomy of the Actinomycetes of the Committee on Taxonomy of the American Society for Microbiology.

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STREPTOMYCES GRISEUS (KRAINSKY) WAKSMAN AND HENRICI

A Taxonomic Study of Some Strains

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INTRODUCTION

A large number of streptomycetes in the ARS Culture Collection have been divided into groups based on morphology; color of aerial mycelium; melaninlike chromogenicity (ability to form brown, deep brown, or black diffusible pigments); and ability to utilize D-xylose, L-arabinose, and L-rhamnose. Particular groups have the additional characteristic that they contain strains which reputedly produce different antibiotics. One group was selected for further taxonomic study in an effort to characterize the strains more precisely and to determine the relationship between the different antibiotic-producing strains. The group selected contains strains that have flexuous spore chains; that form pale-yellow to grayish-yellow [ISCC-NBS Nos. 89 and 90 (*9*) [1] Olive-Buff (*22*)] aerial mycelium; that are nonchromogenic; and that have the ability to utilize D-xylose but not L-arabinose or L-rhamnose.

All the strains of *Streptomyces griseus* (Krainsky) Waksman and Henrici (*2, 10*) in the ARS Collection that have been reported to produce streptomycin fell into the group selected. The group also includes a few strains of *S. griseus* reported to produce cycloheximide, rhodomycetin, and streptocin and strains with the specific epithets "*brasiliensis*," "*streptomycini*," and "*vinaceus*," as well as some strains isolated at the Northern Division.

This work further demonstrates the close relationships of these strains. Classical taxonomic procedures allow the differentiation of these strains and the 1915 culture of *Streptomyces griseus* Waksman and Henrici. The strains studied were separated into several categories (subspecies).

MATERIALS AND METHODS

The taxonomic procedures used with the strains studied (as listed below) are either cited or described. The stock cultures and inocula for the various strains were prepared as outlined by Lyons and Pridham (*14*). The designations, specific epithets, and histories of the streptomycete strains selected from the ARS Culture Collection at the Northern Regional Research Laboratory (NRRL) are given in table 1.

[1] Italic numbers in parentheses refer to Literature Cited, p. 29.

TABLE 1.—*Designations, specific epithets, and histories of selected streptomycete strains from the ARS Culture Collection*

Designation used	Specific epithet	Antibiotic(s) or other metabolic activity	History of strains received by NRRL
Baldacci	*brasiliensis*	(1)	Received 1954 from E. Baldacci, Instituto Patologia Vegetale, Universita di Milano, Italy (IPV). Received by Baldacci from Instituto Superiore di Santa, Rome, Italy, as an isolate from human disease.
ACTU 601	*griseus*	streptomycin	Received 1955 from H. Sakai, Sakaguchi Laboratories, Department of Agricultural Chemistry, Faculty of Agriculture, Tokyo University (ACTU) as No. 601.
B–1281	*griseus*	(1)	Isolated 1950 at NRRL, from Japanese soil.
IMRU 3475	*griseus*	keratinase	Received 1961 from R. Gordon, Institute of Applied Microbiology, Rutgers University (IMRU) as 3475 from U.S. Patent 2,988,488 (*23*) 6/13/61.
IPV 423x	*griseus*	(1)	Received 1959 from E. Baldacci IPV, as strain 17 for International Common Experiment (*12*), ex IPV 423x; ex ATCC (American Type Culture Collection. Strain No. unknown.)
NCIB 8225	*griseus*	(1)	Received 1958 from National Collection of Industrial Bacteria (NCIB) as NCIB 8225, British Drug House Ltd., 1950.
NCIB 9001	*griseus*	streptomycin phage resistant	Received 1958 from NCIB as NCIB 9001, Pamela M. Boyd, MRC Antibiotics Research Station, Clevedon (No. R25), 1952. Actinophage resistant and streptomycin producer.

See footnote at end of table.

TABLE 1.—*Designations, specific epithets, and histories of selected streptomycete strains from the ARS Culture Collection*—Continued

Designation used	Specific epithet	Antibiotic(s) or other metabolic activity	History of strains received by NRRL
NCIB 9004	*griseus*	B_{12}	Received 1958 from NCIB as NCIB 9004, M. Lumb, Boots Pure Drug Co., Ltd. (No. F.D. 196), 1952. Production of vitamin B_{12}.
S-1471	*griseus*	(1)	Isolated 1950 at NRRL from Liberian soil.
SAW 4-1	*griseus*	streptomycin cycloheximide	Received 1957 from ATCC as ATCC 10137. ATCC received it from S. A. Waksman (SAW) as strain 4, derived from strain 3463, the original streptomycin-producing strain with original strain No. 18-16.
SAW 4-2	*griseus*	streptomycin streptocin	Received 1961 from R. Gordon as SAW 3496, same as Waksman 4, a colony isolate of SAW 3463 (18-16).
SAW 4-3	*griseus*	streptomycin	Received 1958 from Torry Research Station, Aberdeen, as NCIB 8506; Waksman 4; ATCC 10137.
SAW 4-4	*griseus*	streptomycin	Received 1950 from J. C. Sylvester, Abbott Laboratories, as Abbott 2 2k-13, derived from *S. griseus*, Waksman 4.
SAW 4-5	*griseus*	streptomycin	Received 1950 from J. C. Sylvester, as Abbott 3 sl-18 as derived from *S. griseus*, Waksman 4.
SAW 4-6	*griseus*	streptomycin	Received 1950 from J. C. Sylvester as Abbott 5 m-365 as derived from *S. griseus*, Waksman 4.
SAW 4-7	*griseus*	(1)	Received 1958 from NCIB as NCIB 8232 (mutant from Waksman's strain 4). M. Lumb, Boots Pure Drug Co., Ltd. (No. F.D. 40), 1951.
SAW 9-1	*griseus*	streptomycin	Received 1957 from ATCC as ATCC 11429 as SAW 9 (3463), Rutgers University, 1953.

See footnote at end of table.

TABLE 1.—*Designations, specific epithets, and histories of selected streptomycete strains from the ARS Culture Collection*—Continued

Designation used	Specific epithet	Antibiotic(s) or other metabolic activity	History of strains received by NRRL
SAW 9–2	*griseus*	streptomycin cycloheximide	Received 1951 from D. Gottlieb, as Gottlieb A–2 from Waksman 9.
SAW 9–3	*griseus*	(1)	Received 1959 from NCIB as NCIB 8237 (mutant from Waksman's strain 9). M. Lumb, Boots Pure Drug Co., Ltd. (No. F.D. 124), 1951.
SAW 3464	*griseus*	streptomycin	Received 1950 from S. A. Waksman as SAW 3464; D–1 isolated in 1944 from the throat of a chicken as original streptomycin producer.
SAW 3481	*griseus*	streptomycin	Received 1950 from S. A. Waksman as SAW 3481, a freshly isolated streptomycin-producing culture.
SAW 3495	*griseus* pink variant	rhodomycetin	Received 1950 from S. A. Waksman as SAW 3495, a natural variant of *S. griseus*.
SL 842	*griseus*	streptomycin	Received 1954 from F. Carvajal, Schenley Laboratories, as SL 842, capable of producing 200 to 500 micrograms of streptomycin per milliliter.
SL 2060	*griseus*	(1)	Received 1946 from G. W. Ward, Schenley Laboratories, as SL 2060.
NIHJ SM–1	*griseus* var. *rhodochrous*	anti-Gram-positive antibiotic produced (not streptomycin)	Received 1962 from Y. Okami, National Institute of Health, Tokyo, Japan (NIHJ), as SM–1.
NIHJ SM–2	*griseus* var. *rhodochrous*	anti-Gram-positive antibiotic (not streptomycin)	Received 1962 from Y. Okami, NIHJ, as SM–2.
NIHJ SN–J–1	*griseus* var. *rhodochrous*	streptomycin	Received 1962 from Y. Okami, NIHJ, as SN–1–J.
NIHJ SN–J–2	*griseus* var. *rhodochrous*	streptomycin	Received 1955 from T. Yamaguchi, University of Tokyo, Japan, who received it from Y. Okami, NIHJ, as SN–1–J.

See footnote at end of table.

TABLE 1.—*Designations, specific epithets, and histories of selected streptomycete strains from the ARS Culture Collection*—Continued

Designation used	Specific epithet	Antibiotic(s) or other metabolic activity	History of strains received by NRRL
NIHJ SN-2 (2)	*griseus* var. *rhodochrous*	streptomycin	Received 1962 from Y. Okami, NIHJ, as SN-2 (2).
NIHJ SN-14-1	*griseus* var. *rhodochrous*	streptomycin	Received 1962 from Y. Okami, NIHJ, as SN-14.
NIHJ SN-14-2	*griseus* var. *rhodochrous*	streptomycin	Received 1955 from K. Saito, Institute of Fermentation (IFO), Osaka, Japan, as IFO 3358, who received it from Y. Okami, NIHJ, as SN-14.
Lilly 1	sp	streptomycin	Received 1950 from J. M. McGuire, Eli Lilly & Co., as Lilly soil isolate, not necessarily *S. griseus* but believed to produce streptomycin.
Lilly 3	sp	streptomycin	Do.
Lilly 5	sp	streptomycin	Do.
Lilly 7	sp	streptomycin	Do.
SAW 3479	sp	actinomycin complex	Received 1955 from S. A. Waksman as SAW 3479, isolated in the Waksman Laboratory in 1948 and belonging to the *S. coelicolor* group (*35*). Also listed as 34-1 (antagonist) (personal communication to T. G. Pridham, 2/6/62).
ARI 1780	*streptomycini*	(1)	Received 1962 from V. D. Kuznetzov, Antibiotics Research Institute (ARI), USSR, Moscow, as strain 1780, a Krasil'nikov culture.
NI 9003	*vinaceus*	(1)	Received 1953 from Nagoa Institute (NI), Japan, as NI 9003, *Actinomyces* S-20 of K. Saito.
Carpenter	----------	(1)	Received 1959 from C. C. Carpenter, Syracuse University Research Foundation, labeled as "coiled verticilate."
E	----------	(1)	Received 1960 from K. Crook, Bristol Laboratories, Syracuse, N.Y., as strain E.

See footnote at end of table.

TABLE 1.—*Designations, specific epithets, and histories of selected streptomycete strains from the ARS Culture Collection*—Continued

Designation used	Specific epithet	Antibiotic(s) or other metabolic activity	History of strains received by NRRL
S–62		(1)	Isolated 1953 at NRRL from San Salvador soil sample.
S–901		(1)	Isolated 1953 at NRRL from Illinois soil sample.
S–1757		(1)	Isolated about 1957 at NRRL as B–6–6.
S–1758		(1)	Isolated about 1957 at NRRL as B–27–1.
S–1759		(1)	Isolated about 1957 at NRRL from Australian soil sample.
S–1760		(1)	Isolated about 1957 at NRRL from South African soil sample.
S–1761		(1)	Isolated about 1957 at NRRL as F–3.
S–1762		(1)	Isolated 1958 at NRRL from California soil sample.
S–1763		(1)	Isolated 1959 at NRRL from Alaska soil sample.
S–1764		(1)	Isolated 1959 at NRRL from Illinois soil sample.
S–1765		(1)	Do.
S–1766		(1)	Do.
S–1767		(1)	Do.

[1] To date there has been nothing reported about antibiotic activity or other metabolic activity that might or might not be present.

Proteolytic Activity

The proteolytic activity of each strain was determined by six methods: (1) cultivation in 15 percent plain gelatin (Difco) in tubes in which the inoculum (0.2 ml. of a 48-hour tryptone-yeast extract (TYE) broth culture) was placed on the surface of the substratum. No stab was made. Cultures were incubated at 28° to 30° C. for 14 days. On the 14th day, each culture was refrigerated at 3° to 5° for 1 hour and then examined for liquefaction and color of diffusible pigment; (2) cultivation in 15 percent plain gelatin and 1 percent soluble starch (Difco) with inoculation, incubation, and reading as in the first method; (3) the Waksman 15 percent plain gelatin-dish method, with incubation at 18° to 20° (*32*); (4) the Waksman 15 percent plain gelatin and 1 percent starch-dish method, with incubation at 18° to 20° (*32*); (5) the Gordon and Smith casein-dish method, with incubation at 28° to 30° (*6*); and (6) the Gordon and Mihm gelatin-hydrolysis method, with incubation at 28° to 30° (*4*). Suitable uninoculated control media were used for comparisons.

All cultures were observed at 14 days; and the degree of liquefaction or hydrolysis of gelatin, degree of clearing of casein agar, and nature of diffusible pigments were recorded.

Diastatic Activity

Two methods were used to detect diastatic activity of each strain: (1) the method outlined in Lyons and Pridham (*14*); and (2) the Gordon and Mihm starch-hydrolysis method modified by use of soluble starch (Difco) and substitution of Lugol's iodine solution for 95-percent ethanol (*4*).

Reduction of Nitrates

The methods are based on procedures outlined by the Subcommittee on the Taxonomy of the Actinomycetes (*26*) and by the Committee on Bacteriological Technic (*3*). Cultures were tested and observed for reduction of nitrate after 14 days' growth at 28° to 30° C.

Spore Morphology and Nature of Spore Surface

Electron micrographs of most of the strains were obtained from Tresner and others. Electron micrographs of the rest of the strains were obtained at the Northern Division, also by the procedure of Tresner and others (*29*). Formvar-coated grids were impressed on the aerial mycelia of 14-day inorganic salts-starch (ST) agar dish cultures.

Morphology of Sporophores and Spore Chains

Morphological studies were made as outlined in reports by Hesseltine and others (*7*), Lyons and Pridham (*14*), and Pridham and others (*19*), except that nutrient agar was not used.

Color

Methods for determination of colors of sporulating aerial mycelium, reverses of cultures, and diffusible pigments are cited and explained in Lyons and Pridham (*14*). In addition to these, observations were noted with potato slants and slices. Potato slants were prepared and inoculated as outlined previously (*14*). Also, peeled white potatoes were cut into slices and soaked in distilled water at 3° to 5° C. for 24 hours. The slices were drained, placed in petri dishes, and sterilized for 15 to 20 minutes at 121°. After sterilization, a small amount of sterilized distilled water was added to each dish to prevent the potato slice from drying out. Each slant and slice were inoculated as outlined (*14*). After 14 days' incubation at 28° to 30°, each preparation was examined and colors of aerial mycelium, vegetative growth, and potato were recorded.

Colors were initially keyed out according to Ridgway (*22*). Later the Ridgway color tabs were matched to their nearest equivalent in the "Color Harmony Manual," 4th edition (*27*). Colors of aerial mycelia were keyed out to the appropriate color series in the system of Pridham and others (*19*) and in the one proposed by Tresner (*28*). Finally, all trivial names of color tabs selected were converted to their ISCC-NBS equivalents (*9*).

Melaninlike Chromogenicity

The ability of each strain to produce brown, deep brown, or black diffusible pigments was determined by observation of TYE broth cultures, gelatin cultures, and potato cultures after appropriate incubation periods.

Darkening of Peptone-Iron Agar

The method for this determination is cited and outlined by Lyons and Pridham (*14*).

Utilization of Carbon Sources for Growth

The method for determining carbon utilization patterns of each of the strains is also outlined by Lyons and Pridham (*14*). Carbon sources used were D-xylose, L-arabinose, L-rhamnose, D-glucose, D-fructose, D-galactose, raffinose, D-mannitol, *i*-inositol, and salicin (all Difco products). Ability to utilize sucrose was determined by cultivating each strain on Czapek's solution (CZ) agar for 14 days at 28° to 30° C.

Sensitivity to Lysozyme

Two methods were used to determine the sensitivity of the strains to lysozyme: (1) the method described by Gordon and Mihm (*5*) and (2) a method based on that of Smolelis and Hartsell (*25*). In the second method 10 ml. of TYE broth was inoculated with 2 loopfuls of spores from 14-day-old tomato paste-oatmeal (TPO) agar or yeast extract (YE) agar slant cultures and incubated for 48 hours at 28° to 30° C. on a rotary shaker. Each culture was centrifuged, the supernatant was discarded, and the cells were adjusted to give a reading of about 10 percent light transmission with a Lumetron colorimeter (red filter) by addition of glycerol broth (*5*). Lysozyme (Nutritional Biochemicals Co., Cleveland, Ohio) solution was prepared according to directions given in Gordon and Mihm (*5*), except that 10 ml. was added to 90 ml. of glycerol broth. Five milliliters of this mixture was added to 5 ml. of the adjusted cell suspension and changes in light transmission were noted at 20-minute intervals for 2 hours. The lysozyme-cell suspensions were incubated at 28° to 30° during the tests. A few additional trials were made at incubation temperatures of 37° and 45°. Cells of *Micrococcus lysodeikticus* Fleming NRRL B–287 were used as a control.

Decomposition of L-Tyrosine

The method outlined by Gordon and Smith (*6*) was used to determine whether any of the strains could decompose L-tyrosine. Cultures were observed after 14 days' incubation at 28° to 30° C. for clearing of the substrate as an indication of decomposition.

Decomposition of Xanthine

The method outlined by Gordon and Mihm (*4*) was used to determine whether any of the strains could decompose xanthine. Cultures were

observed after 14 days' incubation at 28° to 30° C. for clearing of the substrate as an indication of decomposition.

Decomposition of Hypoxanthine

The method outlined by Gordon and Mihm (*5*) was used to determine whether any of the strains could decompose hypoxanthine. Cultures were observed after 14 days' incubation at 28° to 30° C. for clearing of the substrate as an indication of decomposition.

Production of Antibiotic Factors

The antibiotic-producing capacity of each strain was determined by the Waksman agar streak method, by cross-antagonism tests, and by paper-disk assays and paper-strip chromatography of shaken-flask and static fermentation filtrates and mycelial extracts.

The medium used for the Waksman agar streak and cross-antagonism tests was a medium (SCG) based on medium A–4h of Warren and others (*38*) as outlined in Lyons and Pridham (*14*) with 1.5 percent agar added. The primary streaks were incubated for 7 days at 28° to 30° C., and at that time the bacteria, yeast, molds, and streptomycetes were streaked at right angles to the growth. The dishes were held an additional 3 to 5 days, and the zones of inhibition were recorded. Test strains used for agar streak determinations were *Bacillus subtilis* Cohn *emend.* Prasmowski NRRL B–765; *Escherichia coli* (Migula) Castellani and Chalmers NRRL B–766, *E. coli* NRRL B–2748 (streptomycin resistant), *E. coli* NRRL B–2422 (streptomycin resistant), *E. coli* NRRL B–1079 (streptomycin dependent); *Staphylococcus aureus* Rosenbach NRRL B–313, *S. aureus* NRRL B–2747 (streptomycin resistant); *Mycobacterium* sp. NRRL B–692; *Candida albicans* Robin (Berkh.) NRRL Y–477; *Mucor ramannianus* Moel. NRRL 1839; and a strain of *S. aureus* NRRL B–313, whose resistance against rhodomycetin was developed for use in this study.

Shaken-flask fermentations were run in five media for each strain. These media were (1) the A–4h (SCG) broth of Warren and others (*38*) for 4 days; (2) the Pridham and Gottlieb chemically defined basal broth (*18*) containing 1 percent D-glucose and 0.1 percent yeast extract (Difco) for 4 days; (3) the cycloheximide production medium No. 26 of Whiffen (*39*) for 4 days; (4) the rhodomycetin production medium of Shockman and Waksman (*24*) for 6 days; and (5) the streptomycin production medium No. 25 of Whiffen (*39*) for 6 days. The rhodomycetin production medium also was used in a static fermentation in order to detect streptocin production. This medium was arbitrarily selected because the medium used for the production of streptocin was not clearly defined in the two papers by Waksman and associates (*36*) and Kupferberg and coworkers (*11*).

Shaken-flask media were inoculated with 5 percent (v/v) of 48-hour TYE broth cultures of the streptomycetes. Seeded flasks were incubated on a Gump rotary shaker (except those for streptocin production which were held under static conditions) operation at 200 r.p.m. at 28° to 30° C. On the fourth and sixth days, the mycelium was separated from the liquor by filtration and centrifugation. The mycelium was washed with distilled water and separated into two portions. Methanol (10 ml.

of an 80-percent methanol solution) was added to one portion, and the mixture was ground in a tissue grinder and filtered through Whatman No. 1 filter paper. This material comprised the methanol extract. Ethyl ether (10 ml. of anhydrous ether) was added to the second portion, and the mixture was shaken for 30 seconds. The ether was decanted. Five milliliters of distilled water was added to the mycelium and the mixture was ground in a tissue grinder. Two more ether extractions (10 ml. each with 30-second shaking) were made of the mycelium and the three ether extracts were combined. After the ether was allowed to evaporate, the residue was taken up in 10 ml. of 95-percent ethanol to provide the ether-ethanol extracts.

The culture filtrates, methanol extracts, and ether-ethanol extracts were assayed against six test organisms by the paper-disk assay method. The test organisms for the paper-disk assays were *B. subtilis* NRRL B–765, *E. coli* NRRL B–766, *Mycobacterium* sp. NRRL B–692, *Sarcina lutea* NRRL B–1018, *C. albicans* NRRL Y–477, and *M. ramannianus* NRRL 1839. These organisms were grown in M–7 agar (*20*) except for *M. ramannianus* which was grown in Mucor synthetic agar (MSA), a chemically defined medium (*20*).

Paper-strip chromatography studies were carried out on all filtrates, methanol extracts, and ether-ethanol extracts with three solvent systems. These were water-saturated butanol, butanol-saturated water, and 10 percent NH_4Cl. All paper strips were spotted with 150μl. of sample and sealed in a glass chromatography jar. After equilibration for 1 hour, solvents were added to the jars and the strips developed by descending chromatography. The strips were removed from the jars when the solvent fronts approached 1 inch from the bottom of the strips. The fronts were marked, and the strips were inverted and allowed to air-dry for 1 hour. At that time the strips were laid on seeded agar trays for 1 hour in order to permit diffusion of the active factors into the agar. Test organisms used were *B. subtilis* NRRL B–765 and *Saccharomyces pastorianus* Hansen NRRL Y–139. Trays were then incubated at 28° to 30° C. until growth was sufficient to locate zones of antibiotic activity. Control strips were used in all jars.

RESULTS

General Characteristics

All the strains studied exhibited the general characteristics of the genus *Streptomyces*. All grew well aerobically, were of the same size (mycelium about 0.5μ to 1.0μ in diameter), and formed spores in chains with more than three spores per chain.

Optimal Temperature Range

All the strains were able to grow well at temperatures of 18°, 25°, and 28° C. At 37° growth was limited and a few strains were unable to grow. None of the strains grew at 45° or 55°. Strain "Baldacci," which was received with the specific epithet *"brasiliensis"* and reportedly isolated from human disease, required the same temperatures for growth as the rest of the strains that were isolated from soil.

Proteolytic Activity

All the strains studied were proteolytic when tested by the six methods used. Based on these and other experiments, the methods most suitable were the Waksman 15 percent gelatin-dish method and the Gordon and Smith casein-dish method. The Waksman 15 percent gelatin-dish gives well-defined zones of liquefaction, and there is no need to refrigerate cultures before they are read. With the cultures studied, the zone diameters ranged from 18 to 41 mm. The average zone diameter was 33 mm. The Gordon and Smith casein-dish method gives rapid, well-defined clearing, and the medium is simple to prepare. Also, the casein-dish method far excels methods based on whole milk and litmus milk as liquid culture media for determination of action on casein. Zone widths with the casein-dish method were 23 mm. with some of the strains. Some cultures completely cleared the medium. The average width of cleared zones was 30 mm.

Diastatic Activity

All the strains were diastatic. The ST agar cultures, when flooded with Lugol's iodine solution, gave no indication that any starch was left in the agar, according to the method used (*14*).

Results were difficult to interpret when the Gordon and Mihm starch-agar cultures were flooded with 95-percent ethanol according to directions. With Difco soluble starch and 95-percent ethanol, no detectable zone limits could be seen. Control dishes of the starch agar became only faintly cloudy when flooded with 95-percent ethanol. Duplicate cultures, when flooded with Lugol's iodine, gave readily discernible zone limits and control dishes of the agar became deep blue. These results may represent differences in reaction to 95-percent ethanol between the potato starch used by Gordon and Mihm and the Difco soluble starch. With Lugol's iodine reagent, the widths of the decolorized zones on the Gordon and Mihm media cultures ranged from 20 mm. or wider. Some strains gave activities that represented complete hydrolysis of the starch.

Nitrate Reduction

Table 2 shows that neither the chemically defined broth nor the organic-based broth gave uniform results. Strains from the same origin (e.g., SAW 4–1 and SAW 4–2) gave different results. In a number of instances the same strain allowed detection of nitrite in one tube of a pair but not in the other (e.g., SAW 3495 in the organic-based broth). Obviously nitrate reduction tests as used with streptomycetes require modification and improvement.

Nature of Spore Surface

Electron micrographs of each of the strains listed show that the organisms all have smooth-walled spores. The morphology of the spore surfaces was quite uniform. The spores are ellipsoidal and measure approximately $0.5\mu \times 1.0\mu$ (fig. 1).

TABLE 2.—*Reduction of nitrate by strains of streptomycetes* [1]

Designation used	Chemically defined broth		Organic-based broth	
	Tube 1	Tube 2	Tube 1	Tube 2
Baldacci	+	+	−	(+)
ACTU 601	(+)	(+)	+	+
B–1281	(+)	(+)	(+)	(+)
IMRU 3475	(+)	(+)	+	+
IPV 423x	−	−	−	−
NCIB 8225	(−)	−	(+)	(+)
NCIB 9001	−	−	(+)	(+)
NCIB 9004	−	−	−	(+)
S–1471	(+)	(−)	+	+
SAW 4–1	(+)	(−)	(+)	(+)
SAW 4–2	−	−	(−)	−
SAW 4–3	(+)	(+)	(+)	(+)
SAW 4–4	−	−	(−)	(−)
SAW 4–5	−	−	(+)	(+)
SAW 4–6	(−)	(−)	(−)	(−)
SAW 4–7	(−)	(−)	(−)	−
SAW 9–1	−	−	−	−
SAW 9–2	(+)	(+)	+	+
SAW 9–3	−	−	(+)	(−)
SAW 3464	(+)	(+)	+	+
SAW 3481	−	−	−	(+)
SAW 3495	−	−	(+)	−
SL 842	(−)	(−)	+	+
SL 2060	(+)	(+)	+	+
NIHJ SM–1	−	(−)	−	−
NIHJ SM–2	−	−	−	(−)
NIHJ SN–J–1	−	−	(−)	(−)
NIHJ SN–J–2	−	−	−	−
NIHJ SN–2 (2)	−	−	−	−
NIHJ SN–14–1	−	−	(−)	(−)
NIHJ SN–14–2	(−)	(−)	(−)	−
Lilly 1	−	−	(+)	(+)
Lilly 3	(−)	(−)	(+)	+
Lilly 5	−	−	−	−
Lilly 7	−	−	(+)	(+)
SAW 3479	−	−	−	−
ARI 1780	−	−	−	−
NI 9003	(+)	(+)	(+)	(+)
Carpenter	(+)	(+)	+	+
E	(−)	(−)	+	+
S–62	−	−	(+)	(+)
S–901	(+)	(−)	(+)	(+)
S–1757	(+)	(−)	+	+
S–1758	(−)	−	(+)	(+)
S–1759	(+)	(+)	−	−
S–1760	(−)	(−)	(+)	(+)
S–1761	−	−	(−)	(−)
S–1762	(+)	(+)	+	+
S–1763	(−)	(−)	+	+
S–1764	−	(−)	(+)	(+)
S–1765	−	−	(+)	(+)
S–1766	(+)	(+)	+	+
S–1767	+	+	+	+

(See footnote on facing page)

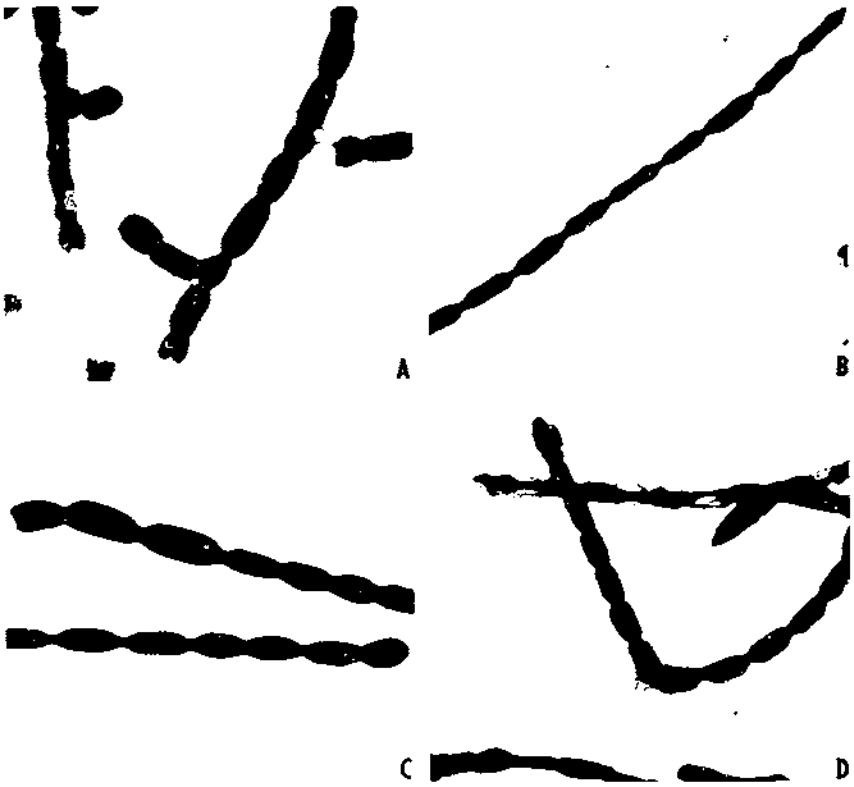

FIGURE 1. Morphology of spores and nature of surfaces of some strains of *Streptomyces griseus*: *A*, Original 1915 culture (IMRU 3326); *B*, SAW 9–1 (IMRU 3463), original streptomycin-producing culture; *C*, SAW 3495, rhodomycetin-producing culture; *D*, ARI 1780 *Actinomyces streptomycini*. Electron microscopy of figures *B* and *C* by H. D. Tresner and M. C. Davies. Magnification, about 6,050× to 8,000×.

Morphology of Spore Chains

All strains listed were relegated to section Rectus-Flexibilis (RF) (*19*) after in situ observations of petri-dish cultures. At low magnification, the chains of spores appear as tufts with relatively little branching. The spore chains are generally flexuous and rarely straight, as shown in figure 2.

Colors of Aerial and Vegetative Mycelium

All the cultures had aerial mycelium colored in tints and shades of yellow (table 3).

(Footnote to table 2)

[1] Symbols used: −, nitrates not reduced; (−), slight pink color produced on addition of reagents; (+), pale red color produced on addition of reagents; +, deep red color produced on addition of reagents. Cultivated on organic-based and chemically defined media (14 days at 28° to 30° C., α-dimethylnaphylamine, sulfanilic acid, and Zn dust reagents).

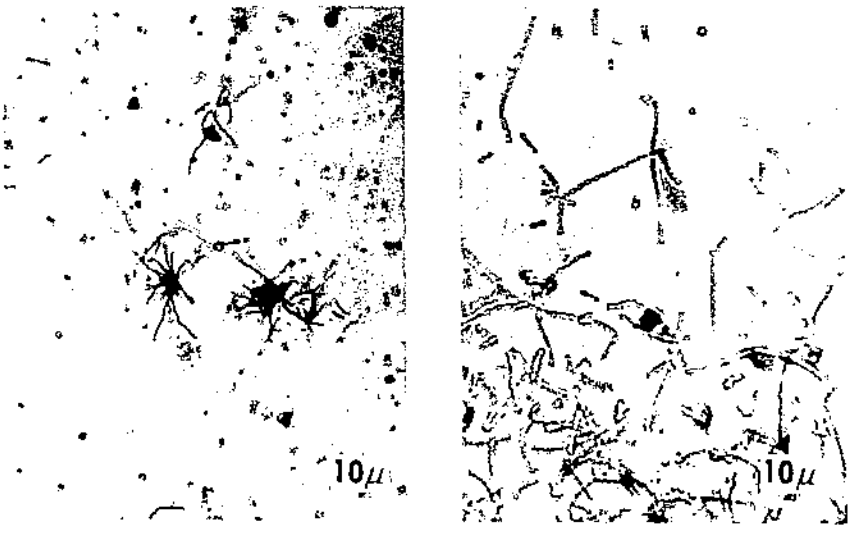

FIGURE 2.—Micromorphology of a representative strain (NI 9003) of *Streptomyces griseus*. Culture grown for 14 days at 28° to 30° C. on inorganic salts-starch agar.

Tints and shades of yellowish pink and orange yellow are often associated with the characteristic yellow colors exhibited by the aerial mycelia of the cultures studied. This characteristic appears true not only for those cultures whose vegetative mycelium is yellow to yellowish brown but also for those cultures whose vegetative mycelium is red to purple. The yellowish pink and orange yellow are more pronounced with those cultures with red to purple vegetative mycelium. This dual color phenomenon has been noted with especial frequency when strains are cultured on TPO agar. The phenomenon suggests that the cultures can be subdivided into two color categories based on the color of their aerial mycelium. On the one hand, when the cultures were compared side by side, the color differences were not sufficient to allow objective separation into two categories. On the other hand, when the cultures were compared on the basis of the color of their vegetative mycelium, it was relatively simple to organize strains into two categories (yellow to yellowish brown and red to purple), as shown in table 4.

Melaninlike Chromogenicity

None of the strains produced brown, deep brown, or black diffusible pigments with TYE broth, gelatin tubes, gelatin dishes, potato slants, or potato slices. Strains with red to purple reverses, however, produced diffusible pigments in pink or violet. Because no other diffusible pigments of importance were noted, it is concluded that all strains studied are nonchromogenic within the limits of our definition.

TABLE 3.—*Colors of aerial mycelium of strains grown for 14 days at 28° to 30° C.*[1]

Ridgway name and plate [2]	Ridgway color group	CHM name and designation [3]	Tresner name and designation [4]	Tresner color wheel series (*28*)	Pridham color series (*19*)	ISCC-NBS color names of Ridgway tabs [5]	ISCC-NBS color names of CHM tabs [5]
Olive-Buff, XL	O-YY	Biscuit 2ec	Parchment 1½db	Yellow	Olive-buff	Pale Yellow 89	Grayish Yellow 90
Pale Olive-Buff, XL	O-YY	Ivory 2db	Ivory 2db	Yellow	Olive-buff	Pale Yellow 89	Pale Yellow 89 Grayish Yellow 90
Deep Olive-Buff, XL	O-YY	Bamboo 2gc	None	Yellow	Olive-buff	Grayish Yellow 90	Grayish Yellow 90
Tilleul-Buff, XL	O-Y	Sand 3cb	Bisque 3ec	Red	Red or olive-buff	Pale Yellowish Pink 31	(No name)
Pale Pinkish Buff, XXIX	O-Y	Pearl 3ba	Pearl pink 3ca	Red	Red or olive-buff	Pale Orange Yellow 73	Yellowish White 92

[1] The color of the aerial mycelium of each of the strains studied is represented by one or more of the color names listed. Colors determined from examination of ST agar dish cultures.

[2] Ridgway color designations representing the closest approximation that could be made.

[3] Color name and designation from Color Harmony Manual (CHM) (*27*), representing the closest approximation that could be made for the Ridgway tabs.

[4] The closest CHM color name and designation that could be made by using the color tabs suggested by H. D. Tresner (*28*) at the workshop on streptomycetes held during the Eighth International Congress of Microbiology at Montreal, Canada, August 18, 1962 (*17*).

[5] The Inter-Society Color Council-National Bureau of Standards (ISCC-NBS) Circular 553 (*9*).

Darkening of Peptone-Iron Agar

None of the strains exhibited a bluish-black coloration of the substratum when grown on peptone-iron agar. There has been some question whether this test actually detects hydrogen sulfide for which it was devised (*13*); however, the results appear to correlate with those for the usual tests for melaninlike chromogenicity.

Utilization of Carbon Compounds

All the strains exhibited a similar carbon utilization pattern (table 5) except for their activities on salicin. No direct correlation could be made between other characteristics and salicin activities.

TABLE 4.—*Colors of vegetative mycelium of strains grown for 14 days at 28° to 30° C.*[1]

Yellow to yellow-brown reverses

Strains: ATCU 601; B-1281; IMRU 3475; IVP 423x; NCIB 8225; NCIB 9001; NCIB 9004; SAW 4-1; SAW 4-2; SAW 4-3; SAW 4-4; SAW 4-5; SAW 4-6; SAW 4-7; SAW 9-1; SAW 9-2; SAW 9-3; SAW 3464; SAW 3481; SL 842; SL 2060; Lilly 1; Lilly 3; Lilly 5; Lilly 7; ARI 1780; Carpenter; E; S-62; S-901; S-1757; S-1758; S-1760; S-1762; S-1763; S-1764; S-1765; S-1766; and S-1767

Ridgway name and plate [2]	CHM name and designation [3]	ISCC-NBS color names of Ridgway tabs [4]	ISCC-NBS color names of CHM tabs [4]
Chamois, XXX	Honey Gold 2ic	Moderate Yellow 87	Moderate Yellow 87
Cinnamon-Buff, XXIX	Lt. Amber 3ic	Moderate Yellow 87 Moderate Orange Yellow 71 Light Yellowish Brown 76	Moderate Orange Yellow 91 Dark Orange Yellow 72
Colonial Buff, XXX	Pastel Yellow 1½fb	Light Yellow 86	Moderate Yellow 87
Cream-Buff, XXX	Bamboo 2fb	Moderate Yellow 87 Light Yellow 86	Moderate Yellow 87 Pale Yellow 89
Deep Chrome, III	Brite Yellow 3na	Strong Orange Yellow 68	Strong Orange Yellow 68
Honey Yellow, XXX	Mustard Gold 2ne	Moderate Yellow 87 Dark Yellow 88	Dark Yellow 88
Isabella Color, XXX	Mustard Gold 2pe	Dark Grayish Yellow 41	Deep Yellow 85
Mustard Yellow, XVI	Maize 2hb	Strong Yellow 84	Moderate Yellow 87
Naples Yellow, XVI	Lt. Wheat 2ea	Light Yellow 86	Light Yellow 86
Ochraceous-Buff, XV	Amber 3lc	Light Yellowish Pink 28 Moderate Yellowish Pink 29	(No name)
Olive-Brown, XL	Clove Brown 3pl	Grayish Yellowish Brown 80	Dark Yellowish Brown 78
Tawny-Olive, XXIX	Topaz 3ne	Moderate Yellowish Brown 77 Light Olive Brown 94	(No name)

(See footnotes on facing page)

Sensitivity to Lysozyme

We were unable to obtain reliable data on sensitivity to lysozyme when the Gordon and Mihm (*5*) method was used. The results in table 6 suggest the same difficulties are experienced as with the nitrate-reduction tests. Uniform results were obtained when a method based on that of Smolelis and Hartsell (*25*) was applied. All strains were sensitive to lysozyme under these test conditions. Selected results are presented in table 7. Some strains were lysed rapidly; others less so. Also, duplicate preparations gave similar results.

Tyrosine Decomposition

All strains used in this study decomposed L-tyrosine.

TABLE 4.—*Colors of vegetative mycelium of strains grown for 14 days at 28° to 30° C.*[1]—Continued

Red to purple reverses

Strains: Baldacci; S–1471; SAW 3495; NIHJ SM–1; NIHJ SM–2; NIHJ SN–J–1; NIHJ SN–J–2; NIHJ SN–2(2); NIHJ SN–14–1; NIHJ SN–14–2; SAW 3479; NI 9003; S–1759; S–1761

Ridgway name and plate [2]	CHM name and designation [3]	ISCC-NBS color names of Ridgway tabs [4]	ISCC-NBS color names of CHM tabs [4]
Brownish Vinaceous, XXXIX	Dusty Coral 6½gc	Light Grayish Red 18	Dark Pink 6 Dark Yellowish Pink 30
Dahlia Carmine, XXVI	Raspberry 9pe	Dark Purplish Red 259	(No name)
Dark Maroon-Purple, XXVI	Raspberry Wine 9pg	Dark Reddish Purple 242	Dark Purplish Red 259 Very Dark Purplish Red 260
Dark Vinaceous, XXVII	Cedar 6½le	Grayish Red 19	Grayish Red 19
Deep Corinthian Red, XXVII	Antique Rose 7le	Grayish Red 19	Grayish Red 19
Deep Livid Brown, XXXIX	Old Wine 7½ng	Grayish Red 19	(No name)
Indian Lake, XXVI	Raspberry 9pc	Moderate Purplish Red 258 Grayish Purplish Red 262	Moderate Purplish Red 258 Dark Purplish Red 259
Purplish Vinaceous, XXXIX	Dusty Rose 7½gc	Light Grayish Red 18	Dark Pink 6
Vinaceous-Purple, XXXVIII	Raspberry 9ne	Moderate Purplish Red 258 Grayish Purplish Red 262	(No name)

[1] The color of the vegetative mycelium (reverse of cultures) of each of the strains studied is represented in one of the color names listed. Colors determined from examination of International glycerol-asparagine agar dish cultures.

[2] Ridgway color designations representing the closest approximation that could be made.

[3] Color name and designation from Color Harmony Manual (CHM) (*27*) representing the closest approximation that could be made for the Ridgway tabs.

[4] The Inter-Society Color Council-National Bureau of Standards (ISCC-NBS) Circular 553 (*9*).

Xanthine Decomposition

All the strains that had yellow to yellowish-brown vegatative mycelium decomposed xanthine. Differences were noted with the strains that have red to purple vegetative mycelium (table 8).

Hypoxanthine Decomposition

All the strains decomposed hypoxanthine.

TABLE 5.—*Utilization of carbon compounds by strains of streptomycetes* [1]

Designation used	Negative control; no carbon	D-Glucose	D-Xylose	D-Arabinose	L-Rhamnose	D-Fructose	D-Galactose	Raffinose	D-Mannitol	i-Inositol	Salicin
Baldacci	−	+	+	(−)	−	+	+	−	+	−	(+)
ATCU 601	−	+	+	−	−	+	+	−	+	−	(+)
B-1281	−	+	+	(−)	−	+	+	−	+	−	(+)
IMRU 3475	−	+	+	(−)	−	+	+	−	+	−	(+)
IVP 423x	−	+	+	−	−	+	+	−	+	−	(+)
NCIB 8225	−	+	+	(−)	−	+	+	−	+	−	(+)
NCIB 9001	−	+	+	(−)	−	+	+	−	+	−	(+)
NCIB 9004	−	+	+	−	−	+	+	−	+	−	(+)
S-1471	−	+	+	(−)	−	+	+	−	+	−	(+)
SAW 4-1	−	+	+	−	−	+	+	−	+	−	(+)
SAW 4-2	−	+	+	−	−	+	+	−	+	−	(+)
SAW 4-3	−	+	+	(−)	−	+	+	−	+	−	(+)
SAW 4-4	−	+	+	−	−	+	+	−	+	−	(+)
SAW 4-5	−	+	+	(−)	−	+	+	−	+	−	(+)
SAW 4-6	−	+	+	(−)	−	+	+	−	+	−	(+)
SAW 4-7	−	+	+	−	−	+	+	−	+	−	(+)
SAW 9-1	−	+	+	−	−	+	+	−	+	−	(+)
SAW 9-2	−	+	+	(−)	−	+	+	−	+	−	(+)
SAW 9-3	−	+	+	−	−	+	+	−	+	−	(+)
SAW 3464	−	+	+	−	−	+	+	−	+	−	(+)
SAW 3481	−	+	+	(−)	−	+	+	−	+	−	(+)
SAW 3495	−	+	+	−	−	+	+	−	+	−	(+)
SL 842	−	+	+	(−)	−	+	+	−	+	−	(+)
SL 2060	−	+	+	−	−	+	+	−	+	−	(+)
NIHJ SM-1	−	+	(−)	(−)	−	(−)	+	−	+	−	(+)
NIHJ SM-2	−	+	+	(−)	−	+	+	−	+	−	(+)
NIHJ SN-J-1	−	+	+	(−)	−	(+)	+	−	+	−	(+)
NIHJ SN-J-2	−	+	+	(−)	−	+	+	−	+	−	(+)
NIHJ SN-2(2)	−	+	(+)	(−)	−	(+)	+	−	+	−	(+)
NIHJ SN-14-1	−	+	(+)	(−)	−	+	+	−	+	−	(+)
NIHJ SN-14-2	−	+	(+)	(−)	−	+	+	−	+	−	(+)
Lilly 1	−	+	+	−	−	+	+	−	+	−	(+)
Lilly 3	−	+	+	(−)	−	+	+	−	+	−	(+)
Lilly 5	−	+	+	(−)	−	+	+	−	+	−	(+)
Lilly 7	−	+	+	(−)	−	+	+	−	+	−	(+)
SAW 3479	−	+	+	−	−	+	+	−	+	−	(+)
ARI 1780	−	+	+	−	−	+	+	−	+	−	(+)
NI 9003	−	+	+	(−)	−	+	+	−	+	−	(+)
Carpenter	−	+	+	−	−	+	+	−	+	−	(+)
E	−	+	+	−	−	+	+	−	+	−	(+)
S-62	−	+	+	(−)	−	+	+	−	+	−	(+)
S-901	−	+	+	(−)	−	+	+	−	+	−	(+)
S-1757	−	+	+	−	−	+	+	−	+	−	(+)
S-1758	−	+	+	(−)	−	+	+	−	+	−	(+)
S-1759	−	+	+	−	−	+	+	−	+	−	(+)
S-1760	−	+	(+)	−	−	+	+	−	+	−	(+)
S-1761	−	+	(+)	−	−	(+)	+	−	+	−	(+)
S-1762	−	+	+	−	−	+	+	−	+	−	(+)
S-1763	−	+	+	−	−	+	+	−	+	−	(+)
S-1764	−	+	+	(−)	−	+	+	−	+	−	(+)
S-1765	−	+	+	−	−	+	+	−	+	−	(+)
S-1766	−	+	+	−	−	+	+	−	+	−	(+)
S-1767	−	+	+	−	−	(+)	+	−	(+)	−	(−)

(See footnote on facing page)

TABLE 6.—*Sensitivity to lysozyme of strains of streptomycetes as determined by the Gordon and Mihm (5) method* [1]

Designation used	Growth in control	Growth in lysozyme broth	
		Tube 1	Tube 2
Baldacci	Poor	(−)	(+)
SAW 4–1	Good	−	−
SAW 9–1	Good	(−)	(+)
SAW 3495	Good	+	+
NIHJ SN–J–1	Good	+	+
ARI 1780	Good	−	−
NI 9003	Good	+	+

[1] Symbols used: −, good growth (not sensitive to lysozyme); (−), less growth; (+), growth present but poor; +, no growth detected (sensitive to lysozyme).

TABLE 7.—*Sensitivity to lysozyme of strains of streptomycetes as determined by a method based on that of Smolelis and Hartsell (25)* [1]

Designation used	Light transmission through culture after addition of lysozyme at 28° to 30° C. for—						
	0 minutes	20 minutes	40 minutes	60 minutes	80 minutes	100 minutes	120 minutes
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Baldacci	22	50	66	73	75	76	76
SAW 4–1	22	72	81	82	83	88	85
SAW 9–1	18	34	51	66	76	81	82
SAW 3495	22	39	61	73	76	80	81
NIHJ SN–J–1	23	36	62	76	75	76	81
ARI 1780	19	30	49	67	77	84	87
NI 9003	20	57	64	70	73	77	80
Micrococcus lysodeikticus NRRL B–287 [2]	20 ca.	95	96	96	96	96	96

[1] Readings made with a Lumetron colorimeter (red filter).
[2] Used as a control.

Antibiotic Activity

It was possible to identify those strains that produce streptomycin through use of the Waksman agar streak method, paper-disk assays of culture filtrates, and cross-antagonism tests. Streptomycin-dependent

(Footnote to table 5)

[1] Pridham and Gottlieb basal agar, 10 days at 28° to 30° C. Symbols used: −, no growth; (−), faint growth, probably no utilization; (+), poor to fair growth; +, good growth and positive utilization.

TABLE 8.—*Decomposition of xanthine by strains of streptomycetes with red to purple vegetative mycelium* [1]

Designation used	Clearing of xanthine medium [2]
Baldacci	+
S-1471	+
SAW 3495	+
NIHJ SM-1	+
NIHJ SM-2	−
NIHJ SN-J-1	−
NIHJ SN-J-2	−
NIHJ SN-2(2)	+
NIHJ SN-14-1	−
NIHJ SN-14-2	−
SAW 3479	+
NI 9003	+
S-1759	+
S-1761	+

[1] Cordon and Mihm (*4*) method, 14 days at 28° to 30° C. Symbols used: +, medium cleared and xanthine decomposed; −, medium not cleared and xanthine not decomposed.

[2] Results from duplicate tests.

and resistant bacteria and grisein- and rhodomycetin-resistant bacteria were especially helpful as test organisms in this regard. In table 9 are presented the results of agar streak tests obtained with strains representing various subcategories finally established in the work. All the known streptomycin-producing strains and 15 other strains allowed the streptomycin-dependent strain of *Escherichia coli* to grow. The rest of the strains under study did not. The 1915 isolate of *Actinomyces griseus* IMRU 3326 (*34*) was included in these tests to determine whether it produced streptomycin, although it does not exhibit the basic characteristics of the group of strains under study. There are several reports in the literature that also suggest the inability of this strain to produce streptomycin (*1, 21, 37*). Only slight antifungal activity was detected with the 1915 strain.

Cycloheximide was presumptively identified by activity of the strains against a yeast and a mold in the agar streak tests and paper-disk assays. Presumptive confirmation of identity was made by paper chromatography and knowledge of the histories of the strains.

In an attempt to determine whether any of the strains could produce streptocin, culture mycelia were extracted with ether. A simplified procedure based on that described by Waksman and others (*36*) and on the solubility of streptocin in ethyl ether and ethanol was used with each strain. Because streptocin is reported to have activity against Gram-positive rods and cocci, acid-fast bacteria, yeasts, and molds, activity of such fractions against these organisms was used to presumptively detect streptocin.

Under the test conditions, mycelial extracts of the original streptocin-producing culture (IMRU 3533) gave activity against four of the five different types of organisms when the culture was grown in a streptomycin-production medium, but not in the streptocin-production medium

TABLE 9.—*Antibiotic activity of representative strains as determined by the Waksman agar streak method with SCG medium incubated at 28° to 30° C.*[1]

Designation used	*Escherichia coli* NRRL B-1070 streptomycin dependent[2]	*Escherichia coli* NRRL B-2422 streptomycin resistant	*Escherichia coli* NRRL B-2748 streptomycin resistant	*Staphylococcus aureus* NRRL B-2747 streptomycin resistant	*Staphylococcus aureus* NRRL B-313 normal strain	*Escherichia coli* NRRL B-766 normal strain	*Bacillus subtilis* NRRL B-765	*Mycobacterium* sp. NRRL B-692	*Candida albicans* NRRL Y-477	*Mucor ramannianus* NRRL 1839
S. griseus 1915 culture	0	0	0	0	0	0	0	0	7	8
SAW 4-1 streptomycin cycloheximide	17	0	0	0	8	2	14	21	0	20
ARI 1780	30	0	0	0	25	15	(3)	30	0	20
SAW 3495	0	7	3	0/12	9	0/5	8	22	0	3
Baldacci	0	0	0	0/7	4/7	0	5	0	0	0
SAW 4-2	32	0	0	0	24	14	28	32	0	20
NI 9003	0	0	0	0	0	0	5	15	0	0
NIHJ SM1-2	0	3	0	3	3	3	4	7	0	0
NIHJ SN-J-1	0	0	0	0	0	0	0	0	0	0
S-1761	0	13	8	10/30	11/25	6	12	25/30	0	0
S-1762	0	0	0	4	5	0	5	5/15	0	0

[1] Figures indicate length of inhibition zones in millimeters except for those for the streptomycin-dependent strain.

[2] Figures indicate millimeters of growth (not inhibition) extending out from the primary streak.

[3] Complete inhibition of bacteria under test conditions.

or any of the other media used. Trace activity, suggestive of streptocin, also was obtained when the strain was grown in the cycloheximide-production medium. The results with those strains whose ether-ethanol extracts showed antibiotic activity are presented in table 10. The original streptocin producer is included, as well as two other strains reported to produce this antibiotic. Preliminary paper chromatographic study of the mycelial extracts from the streptomycin-production medium suggests that the antifungal activity detected is polyenic in nature and is not cycloheximide as originally reported. In view of Yamaguchi and Saburi's (*40*) work showing that many different kinds of streptomycetes exert activity against trichomonads, there is some question as to the precise identity of the antitrichomonal factors reportedly produced by strains other than IMRU 3533. So far, strain IMRU 3533 has been reported to produce streptocin, cycloheximide, a second antitrichomonal antibiotic, and a factor active against Gram-positive cocci. Our preliminary

results suggest that the antifungal activity is not cycloheximide. Strain SAW 3496, a known streptomycin producer, also has been reported to produce streptocin on the basis of antitrichomonal activity of a sublimable fraction (*11*). In our hands, only questionable evidence for the production of streptocin, based on activity of extracts against Gram-positive rods and cocci, was obtained. No activity was detected against the acid-fast organism used. Results of a similar nature were obtained with strain IMRU 3463 reported by Yamaguchi and Saburi (*40*) to exert antitrichomonal activity.

The presumptive detection of rhodomycetin was based on activity of this antibiotic against Gram-positive rods and cocci, acid-fast bacteria, and a rhodomycetin-resistant strain of *Staphylococcus aureus*.

The antibiotic activity of representative strains detected in samples from shaken-flask fermentations with SCG medium is shown in table 11. The results given are only illustrative. In some instances, other media gave better activity. In table 12, results are presented for one strain (SAW 4–1) when cultivated in all of the media studied. Fair, antifungal activity was noted with many strains in the agar streak test. However,

TABLE 10.—*Strains showing antibiotic activity in the ether-ethanol extracts of the mycelium when grown in the streptocin-production medium as determined by the paper-disk method* [1]

Designation used	*Bacillus subtilis* NRRL B–765	*Sarcina lutea* NRRL B–1018	*Mycobacterium* sp. NRRL B–602	*Candida albicans* NRRL Y–477	*Mucor ramannianus* NRRL 1839
SAW 4–1	+	+	−	−	−
SAW 4–2[2]	+	+	−	−	−
SAW 4–4	+	+	+	−	−
SAW 4–7	+	−	−	−	−
SAW 9–1[2]	+	+	−	−	−
SAW 9–3	+	+	−	−	−
SAW 3481	+	+	−	−	−
NCIB 9004	+	+	−	−	−
Lilly 7	+	−	−	−	−
ACTU 601	+	+	−	−	−
E	+	+	−	−	−
Carpenter	+	−	−	−	−
S–62	+	+	−	−	+
S–1761	+	−	−	−	−
S–1760	−	+	−	−	+
S–1764	+	−	−	−	−
S–1765	+	−	−	−	−
S–1766	+	−	−	−	−
NIHJ SN–2(2)	+	−	−	−	−
IMRU 3533[3]	−	−	−	−	−

[1] +, antibiotic activity detected; −, antibiotic activity not detected.
[2] Strains reported to produce streptocin.
[3] Original streptocin-producing strain.

this activity did not appear in assays of samples from the shaken-flask and static fermentations. No single medium gave the best activity for all strains although SCG seemed to be a superior one.

Cross-antagonism studies of all the strains gave results as illustrated in table 13. The 1915 culture of *Streptomyces griseus* was included in these studies and found to be sensitive to streptomycin and to rhodomycetin. These results further suggest that this strain does not belong to the streptomycin-producing group. Although the rhodomycetin-producing strain did not inhibit streptomycin-producing strains, it was inhibited by them.

Strain ARI 1780 inhibited strain SAW 9–1; however, none of the other streptomycin-producing strains inhibited SAW 9–1. No explanation as to the cause of this inhibition can be advanced except that strain ARI 1780 was extremely active on all agar streak tests.

Paper-strip chromatography was run with all of the fermentation samples and extracts from each of the strains used in this study. Representative results are shown in figures 3 and 4. All the strains gave patterns similar to those illustrated if antibiotic activity were present. Standard samples of streptomycin and cycloheximide were run at the same time and compared with the activities shown here. From these studies it was concluded that the antibiotic activities produced by many of the strains are streptomycin and cycloheximide. Another factor (designated factor "X" in fig. 3) was found to be present in the mycelial

TABLE 11.—*Antibiotic activity of representative streptomycete strains in shaken-flask fermentations as determined by paper-disk method* [1]

Strain designation and fermentation products	*Escherichia coli* NRRL B-766	*Bacillus subtilis* NRRL B-765	*Mycobacterium* sp. NRRL B-602	*Sarcina lutea* NRRL B-1018	*Candida albicans* NRRL Y-477	*Mucor ramannianus* NRRL 1839
Baldacci:						
SCG filtrate	0	14	0	0	0	0
SCG methanol extract	0	15	0	14	0	0
SCG ethanol-ether extract	0	0	0	0	0	0
SAW 4–1:						
SCG filtrate	0	21	32	15	0	0
SCG methanol extract	0	0	0	14	0	0
SCG ethanol-ether extract	0	0	0	0	0	0
NIHJ SM–2:						
SCG filtrate	0	15	0	14	0	0
SCG methanol extract	0	0	0	(2)	0	0
SCG ethanol-ether extract	0	15	0	(2)	0	0
SAW 3495:						
SCG filtrate	0	0	15	0	0	0
SCG methanol extract	0	0	0	0	0	0
SCG ethanol-ether extract	0	0	0	0	0	0

[1] Figures indicate diameter of inhibition zones in millimeters.
[2] Trace.

TABLE 12.—*Antibiotic activity of strain SAW 4–1 (reported to produce streptomycin and cycloheximide) in shaken-flask and static fermentations as determined by the paper-disk method* [1]

Media and fractions tested	*Escherichia coli* NRRL B-766	*Bacillus subtilis* NRRL B-765	*Mycobacterium* sp. NRRL B-692	*Sarcina lutea* NRRL B-1018	*Candida albicans* NRRL Y-477	*Mucor ramannianus* NRRL 1839
SCG medium: [2]						
Filtrate	0	21	32	15	0	0
Methanol extract	0	0	0	1	0	0
Ethanol-ether extract	0	0	0	0	0	0
Pridham and Gottlieb: [2]						
Filtrate	0	18	26	(3)	0	0
Methanol extract	0	0	0	0	0	0
Ethanol-ether extract	0	0	0	0	0	0
Cycloheximide production: [2]						
Filtrate	0	20	32	16	0	0
Methanol extract	0	0	0	14	0	0
Ethanol-ether extract	0	0	0	0	0	0
Rhodomycetin production: [4]						
Filtrate	0	22	35	19	0	0
Methanol extract	0	0	0	0	0	0
Ethanol-ether extract	0	0	0	0	0	(3)
Streptomycin production: [4]						
Filtrate	0	0	0	0	0	0
Methanol extract	0	18	0	0	0	0
Ethanol-ether extract	0	18	0	0	0	0
Streptocin production: [5]						
Filtrate	0	20	30	17	0	0
Methanol extract	0	15	0	(3)	0	0
Ethanol-ether extract	0	14	0	(3)	0	0

[1] Figures indicate diameter of inhibition zones in millimeters.
[2] Incubation: 4 days at 28° to 30° C. on rotary shaker.
[3] Trace.
[4] Incubation: 6 days at 28° to 30° C. on rotary shaker.
[5] Incubation: 6 days at 28° to 30° C. in static culture.

extracts of many of the streptomycin producers. This factor might be streptocin. The chromatographic pattern as shown in figure 3 is similar to the pattern obtained with the oily residue isolated from fermentations of strain IMRU 3533, according to procedures of Waksman and others (*36*). Because no standard antibiotic sample of streptocin was available, we cannot conclusively identify the activity as streptocin.

All the strains with red to purple vegetative mycelium gave patterns similar to those of the rhodomycetin-producing culture and to the strains labeled *S. griseus* var. *rhodochrous*, with one exception. The patterns are recorded in figure 4.

Seven strains did not produce streptomycin, were sensitive to a streptomycin-producing culture, and also had yellow to yellow-brown vegetative mycelium. Two had antiyeast activity other than cycloheximide as

TABLE 13.—*Results of cross-antagonism study with representative strains* [1]

[Waksman agar streak method with SCG medium at 28° to 30° C.]

Primary streak designation used	1915 Culture	SAW 9–1	SAW 4–2	ARI 1780	S–1762	SAW 3495	NIHJ SM–2	NIHJ SN–J–1	Baldacci	NI 9003	S–1761
1915 Culture	0	0	----	0	----	0	0	0	0	----	----
SAW 9–1	25	0	0	0	11	9	3	4	10	6	5
SAW 4–2	30	0	0	0	8	7	5	6	8	4	3
ARI 1780	32	10	8	0	20	18	12	13	17	17	11
S–1762	----	4	5	5	0	0	0	0	0	0	2
SAW 3495	20	0	0	0	0	0	0	0	0	0	0
NIHJ SM–2	15	5	0	0	0	0	0	0	0	0	0
NIHJ SN–J–1	15	0	0	0	0	0	0	0	0	0	0
Baldacci	0	0	0	0	0	0	0	0	0	0	0
NI 9003	----	0	0	0	0	0	0	0	0	0	0
S–1761	----	5	7	7	0	3	0	0	3	4	0

[1] Figures indicate length of inhibition zones in millimeters.

shown in figure 3. One strain produced antibacterial activity that gave chromatographic patterns somewhat similar to streptomycin. The sensitivity of the strain to streptomycin, along with its chromatographic pattern (fig. 3), suggests that the activity was not due to streptomycin.

The four other strains in this group did not produce any antibacterial activity. However, three of these strains gave activities similar to cycloheximide when filtrates and extracts were chromatographed. One strain was inactive.

Filtrates and extracts made with the original 1915 strain of *S. griseus* were chromatographed (fig. 4). This strain showed no antibacterial activity but gave an antifungal pattern. Ultraviolet absorption curves indicated this activity was due to a mixture of hexaene and heptaene antifungal agents.

We were unable to confirm the reports of Umezawa and others (*30, 31*) and Okami (*15*) that some of the strains with red to purple reverses produce streptomycin. None of the criteria we used suggested this possibility.

DISCUSSION

Our study confirms, in general, reports on the taxonomy of *Streptomyces griseus* from other laboratories (*16, 33, 37*). Further, these and related studies now in progress suggest that *S. griseus* can be so subdivided into taxa by classical taxonomic methods that certain relationships between the taxonomic characteristics and the qualitative nature of the antibiotics produced by given strains become evident. Identification of individual strains with individual antibiotics reported within each group, however, cannot be accomplished by classical procedures with much confidence. Carbon utilization patterns have been especially helpful. For example,

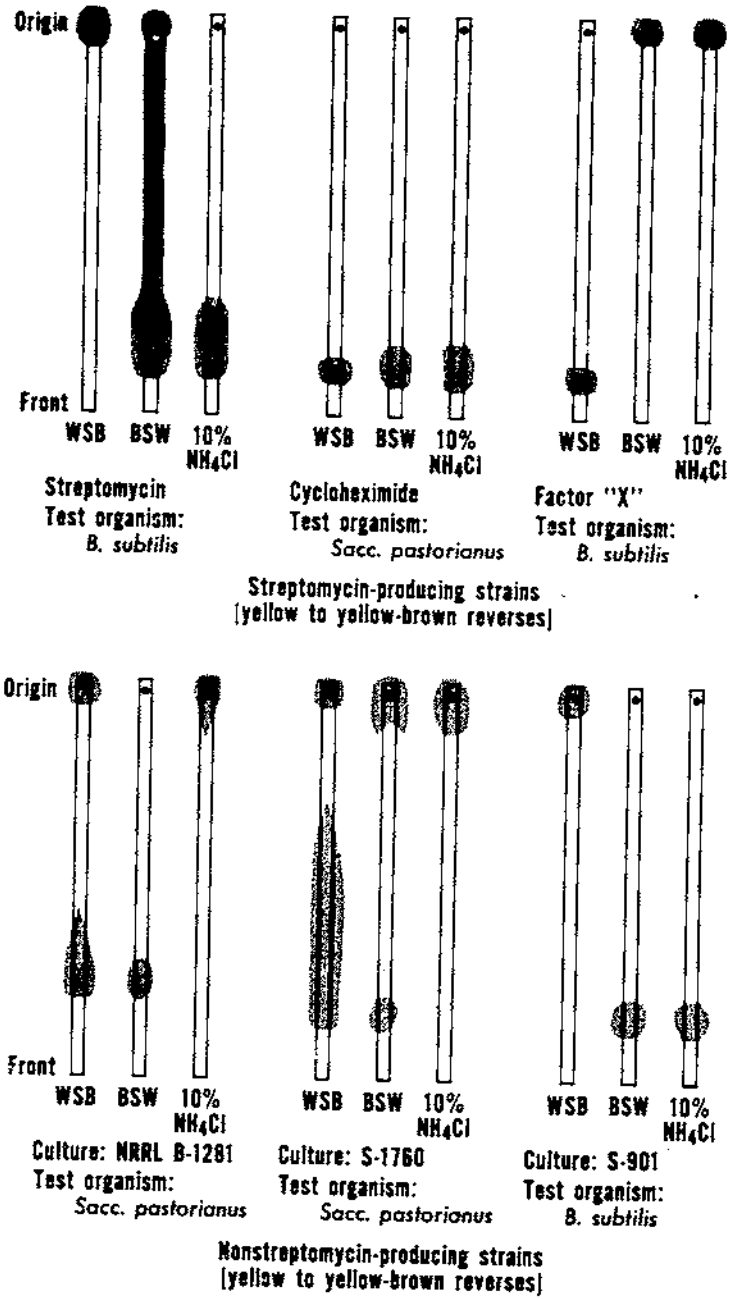

FIGURE 3.—Chromatographic pattern categories obtained with filtrates and mycelial extracts of strains of *Streptomyces griseus*. Solvent systems: WSB, water-saturated butanol; BSW, butanol-saturated water; and 10 percent ammonium chloride.

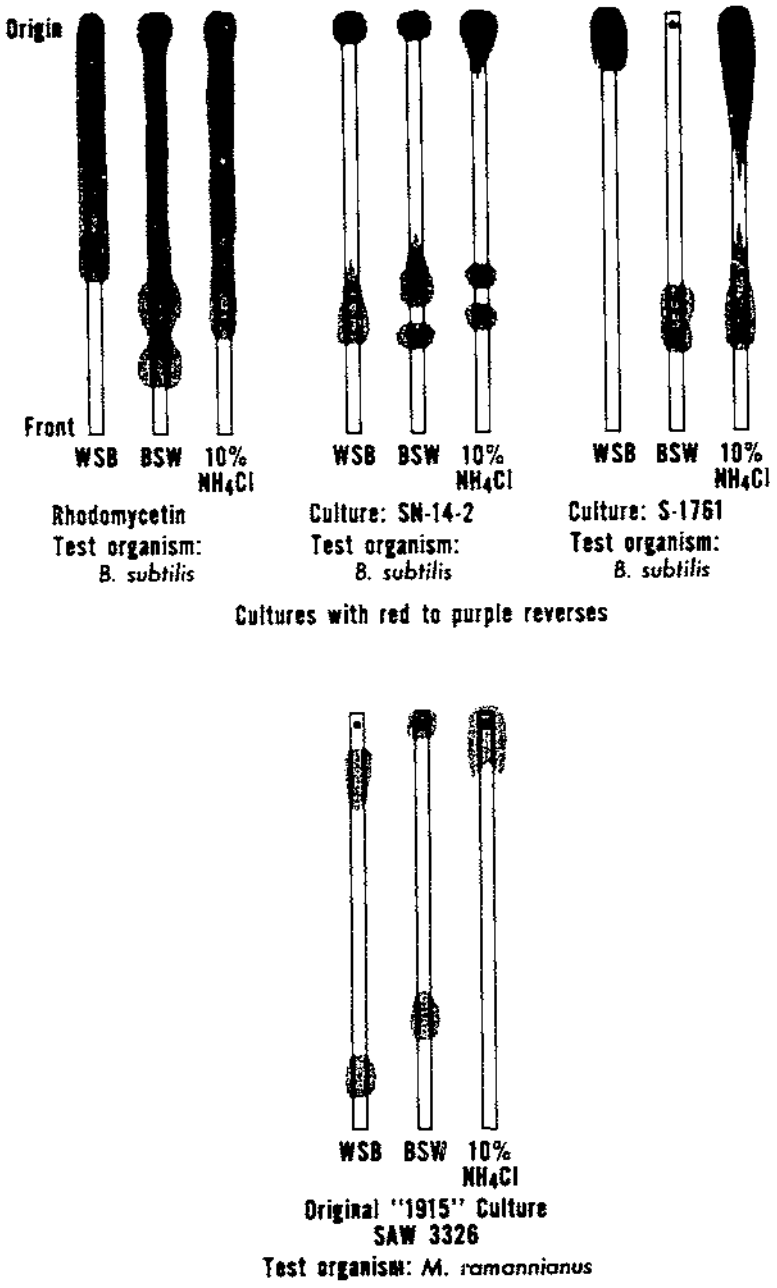

FIGURE 4.—Chromatographic pattern categories obtained with filtrates and mycelial extracts of strains of *Streptomyces griseus*.

all those strains of *S. griseus* in our collection reported to produce streptomycin were placed in the collection of strains at present under study. However, specific information on the total qualitative antibiotic-producing potential of strains is needed to make further separations with confidence.

The results of the present study, based only on criteria in the classical sense, i.e., excluding criteria concerned with production of antibiotics, suggest there are only two distinguishable taxa within the collection of strains examined. These are (1) those strains with yellow to yellowish-brown vegetative mycelia and reverses and (2) those strains with red to purple vegetative mycelium and reverses. These could be designated as two different subspecies of *S. griseus*. In accordance with the International Code of Nomenclature of Bacteria and Viruses (*8*) the taxon, *Streptomyces griseus* (Krainsky) Waksman and Henrici subspecies *griseus* (Krainsky) Waksman and Henrici would automatically be created. As it turns out, these three taxa can be neatly differentiated by classical taxonomic methods; e.g., colors of vegetative mycelium or reverses and carbon utilization patterns. However, the broad taxonomic studies conducted at the Northern Division indicate that, beyond these relatively simple differentiations, collections of strains identified in this manner can be further subdivided, although not with confidence, unless one resorts to more highly sophisticated procedures; e.g., either objective determination of the qualitative nature of the antibiotics or possibly the pigments produced by given strains. It appears that the best way to handle the problem of classification and nomenclature of streptomycetes is to utilize the species-subspecies concept and to take into account that antibiotic production represents an important objective characteristic of the subspecies. Our work thus far suggests that each of the three possible subspecies defined along classical lines does, in fact, contain strains which produce different antibiotics and, hence, can be further subdivided. At the present stage of our work, the following treatment of the strains studied can be made.

Streptomyces griseus (Krainsky) Waksman and Henrici subspecies *griseus* (Krainsky) Waksman and Henrici: strains with flexuous chains of spores; spores smooth-walled; yellow to yellowish-gray aerial mycelium, occasionally tinged with pink; yellow to yellowish-brown vegetative mycelium; nonchromogenic; utilizes D-glucose, D-xylose, L-arabinose, D-fructose, D-galactose, raffinose, D-mannitol, *i*-inositol, and salacin, but not L-rhamnose, when incorporated in the Pridham and Gottlieb chemically defined basal agar (*18*); produces hexaene and heptaene antifungal antibiotics and no, as yet detected, antibacterial antibiotic(s). Proposed neotype strain: IMRU 3326, the 1915 isolate of Waksman and Curtis (*34*), which also is proposed as the neotype strain of *Actinomyces griseus* Krainsky.

Streptomyces griseus (Krainsky) Waksman and Henrici subspecies *streptomycini* (Krasil'nikov) Lyons and Pridham: strains with flexuous chains of spores; spores smooth-walled; yellow to yellowish-gray aerial mycelium, occasionally tinged with pink; yellow to yellowish-brown vegetative mycelium; nonchromogenic; utilizes D-glucose, D-xylose, D-fructose, D-galactose, D-mannitol, and possibly salicin, but not L-arabinose, L-rhamnose, raffinose, or *i*-inositol, when incorporated in the Pridham and Gottlieb chemically defined basal agar; produces the

antibiotics streptomycin, cycloheximide, and an antibacterial antibiotic contained in the mycelium. Holotype strain: SAW 9 (3463) = ATCC 11429, one of the original streptomycin-producing strains isolated at Rutgers University.

The status of the cultures studied that have red to purple vegetative growth is not clear at present. There is little objective information on the identity of the antibiotics produced by these strains. Until such information is obtained little can be said of their subspecific status. Rhodomycetin and viomycins A, B, and C would seem to be antibiotics that should be considered as possible products of these strains.

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