STREPTOMYCES GRISEUS (KRAINSKY) WAKSMAN AND HENRICI[1]

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Since the announcement of the isolation of streptomycin, an antibiotic substance produced by certain strains of *Streptomyces griseus* (Schatz, Bugie, and Waksman, 1944), an extensive literature has accumulated dealing with this antibiotic and with the organism producing it. Although most of the investigations are concerned with the production, isolation, and chemical purification of streptomycin, its antimicrobial properties, and especially its utilization as a chemotherapeutic agent, various reports are also devoted to the isolation of new streptomycin-producing strains of *S. griseus* and of other actinomycetes and to the development of more potent strains. Only a very few of the cultures of *S. griseus* that have been isolated from natural substrates, following the islation of the two original cultures in 1943, were found capable of producing streptomycin. One such culture was reported from this laboratory (Waksman, Reilly, and Johnstone, 1946), and one or two from other laboratories (Carvajal, 1946*a*,*b*). It has been shown more recently (Waksman, Reilly, and Harris, 1947) that by the use of selective methods other streptomycin-producing strains can easily be isolated and identified.

In addition to *S. griseus*, certain other actinomycetes are able to form streptomycin or streptomycinlike substances. One such culture was described by Johnstone and Waksman (1947) as *Streptomyces bikiniensis*, another culture was isolated by Trussell, Fulton, and Grant (1947) and was found capable of producing a mixture of two antibiotics, one of which appeared to be streptomycin and the other streptothricin.

HISTORICAL REVIEW

The first isolation of an organism described as *Actinomyces griseus* was reported by Krainsky (1914), who obtained this culture from a Russian soil. This report appeared at the outbreak of the First World War, and the culture itself was, therefore, not available for comparative studies in this country. When soon afterward a similar culture was isolated from an American soil by Waksman and Curtis (1916), the comparative studies had to be based entirely upon the description of the culture made by Krainsky. Although the cultural characteristics of the new isolate appeared to correspond closely to those given for the original culture, doubt was expressed as to the identity of the two cultures, as indicated by the statement that "the color of the aerial mycelium is somewhat lighter than that described by Krainsky."

The culture isolated by Waksman and Curtis grew readily upon synthetic media, producing an olive-buff vegetative growth. The aerial mycelium ap-

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peared at an early stage in the form of a thick powdery mass of a water-green color. Microscopically, this mycelium was found to consist of long filaments with very little branching; these fragmented readily into rod-shaped conidia, 1 to 1.5 by 0.8 μ. Heavy growth was produced in liquid glucose media. It consisted of round colonies (1 to 3 mm in diameter) floating on the surface of the medium, with a powdery white aerial mycelium covering the growth.

In a further study of the distribution of actinomycetes in various soils, Waksman and Curtis (1918) isolated *S. griseus* from three other American soils obtained from California, Oregon, and Texas. Because the ability of actinomycetes to produce antibiotic substances was not recognized at that time, these cultures were not tested either for their capacity to inhibit the growth of bacteria or of other organisms or to produce substances that had such capacity.

As will be shown later, the streptomycin-producing strains of *S. griseus* give rise to two kinds of variants or mutants, one of which does not produce any antibiotic and the other produces an antibiotic that is distinct from streptomycin. This suggests the possibility that the original New Jersey culture may have had the capacity of producing streptomycin or another antibiotic but that this property was lost upon continued cultivation on artificial media, or that inactive variants or variants possessing a different kind of activity were produced during these three decades of artificial cultivation of this culture.

One of the most important characteristics of *S. griseus* is its marked variability when it is grown on artificial media. This was recognized in the early studies of this organism, as is brought out by the following quotation (Waksman, 1919):

> Even more striking than the morphological are the physiological variations. These depend chiefly on the substratum of the mother culture, temperature and length of incubation, amount and kind of inoculum (vegetative or aerial mycelium or spores). For example, a certain culture (*A. griseus*) may at one time clot the milk at 37° in 2 days and then peptonize it (dissolve the clot) in 5–6 days; at another time, the same culture, under the same conditions, will clot the milk only in 5–6 days, then peptonize it in 12–15 days; while at a third time, some tubes may not show any clot at all, and the milk is hydrolyzed (cleared up without any previous clot).

The variability of *S. griseus* is both quantitative and qualitative in nature. The quantitative differences can be expressed in the ability of different strains isolated from different substrates or of different substrains isolated from the same culture to produce varying amounts of streptomycin; quantitative variations are also observed in the characteristics of the organisms, such as degree of liquefaction of gelatin, shade of pigmentation of aerial mycelium, etc. The qualitative differences are varied in nature, as is shown by the preferential ability of certain strains to grow under submerged conditions of culture and of others to grow under surface conditions, by the nature of the aerial mycelium, by the pigmentation of the vegetative growth, and by the ability of different strains to produce antibiotic substances.

From the point of view of their ability to produce antibiotic substances, certain variations among the different strains of *S. griseus* may be summarized briefly as follows:

(1) Some, if not most, of the strains of *S. griseus* isolated from natural substrates do not produce any antibiotic at all (Waksman, Schatz, and Reynolds, 1946).

(2) Some of the antibiotic-producing strains form streptomycin as the major antibiotic; in addition, they also form other antibiotics, as the antifungal substance actidione and the anti-gram-positive bacterial agent present in the mycelium of the organism.

(3) Some of the antibiotic-producing strains of *S. griseus* form grisein as the major antibiotic.

(4) Some streptomycin-producing strains of *S. griseus* are more active under stationary and others under submerged conditions of growth; they vary greatly in their quantitative production of streptomycin.

(5) Streptomycin-producing strains of *S. griseus* give rise to at least two types of mutants. One mutant has lost the capacity to produce aerial mycelium and streptomycin (Schatz and Waksman, 1945). The other mutant produces a pink to red pigmented vegetative mycelium and yields an antibiotic that is distinct from streptomycin (Waksman, Reilly, and Johnstone, 1946).

(6) Different strains of *S. griseus* vary greatly in their sensitivity to actinophage. The phage active against streptomycin-producing strains of *S. griseus* is not active against any other strains of this organism (Reilly, Harris, and Waksman, 1947).

These variations in strain specificity may help to explain certain differences observed in the production of antibiotics by freshly isolated cultures of *S. griseus* or by closely related species of *Streptomyces*. The following experiments tend to throw further light upon these variations.

EXPERIMENTAL RESULTS

Effect of the addition of streptomycin to the medium upon the selection of active strains. The fact that streptomycin-producing cultures of *S. griseus* give rise to inactive variants and the fact that these variants are sensitive to streptomycin, the active cultures being resistant to this antibiotic (Schatz and Waksman, 1945; Waksman, Reilly, and Johnstone, 1946), suggested the possibility of utilizing the method of enriching plating media with streptomycin in order to eliminate inactive strains and to permit the isolation of more active streptomycin-producing strains.

Four cultures of *S. griseus* capable of forming streptomycin and originally isolated from soil or from other natural substrates were used for this purpose. These cultures were grown on agar slants, and various dilutions of the spore suspension were plated out, two media being used, one an ordinary nutrient agar and another agar enriched with 50 μg per ml of streptomycin. The plates were incubated at 28 C and the total numbers of colonies developing were counted. The results obtained (table 1) show that the cultures of *S. griseus* isolated from natural substrates vary greatly in the number of streptomycin-resistant colonies produced from spores. Two of the cultures contained a much greater percentage of resistant colonies than the other two cultures.

A number of colonies were picked from the plates containing the two agar

media, grown on agar surfaces, and tested for their ability to produce streptomycin. The colonies developing on the streptomycin-enriched agar gave no better streptomycin-producing cultures than the colonies picked from the streptomycin-free nutrient agar plates. Some of the colonies, independent of the medium from which they were isolated, gave more active strains than the parent cultures; others gave less active strains. The differences obtained among the various colonies were quantitative rather than qualitative. One culture (no. 3498) obtained from such a colony produced much less streptomycin under stationary conditions than under submerged conditions of growth, as is brought out in table 2.

Isolation of non-streptomycin-producing variants. The streptomycin-producing cultures of *S. griseus* give rise to two types of variants, one of which is inactive antibiotically and produces no aerial mycelium (Schatz and Waksman, 1945)

TABLE 1

Sensitivity of different streptomycin-producing S. griseus cultures to streptomycin

Colonies developing from similar spore suspensions on different media

CULTURE NO.	STREPTOMYCIN-FREE NUTRIENT AGAR	STREPTOMYCIN-CONTAINING NUTRIENT AGAR	PER CENT OF ORGANISMS RESISTANT TO 50 μg/ML OF STREPTOMYCIN
3463	3,000,000	97,000	3.2
3464	4,600,000	3,000,000	69.1
3480	3,150,000	90,000	2.9
3481	96,500,000	71,000,000	73.6

TABLE 2

Production of streptomycin by no. 3498 grown under submerged and stationary conditions

EXPERIMENT NO.	SUBMERGED GROWTH		STATIONARY GROWTH	
	2 days	6 days	6 days	8-13 days
1	43	65	21	12
2	22	80	<10	<10

and the other produces pink to red vegetative growth and an antibiotic substance that is distinct from streptomycin. The second variant was obtained by plating active cultures of *S. griseus* on agar plates and picking those colonies that form a pink to cherry-red pigmented growth, notably on glucose asparagine agar. The greenish-gray aerial mycelium of these strains does not differ much from that of the typical *S. griseus* cultures. When the pigmented strains are grown on media favorable for streptomycin production, no streptomycin is obtained. Another antibiotic is produced, however, that differs greatly in its bacteriostatic spectrum from that of streptomycin, especially in its lack of activity against *Escherichia coli* and in its very high potency against *Staphylococcus aureus* (up to 10,000 dilution units per 1 ml of metabolite solution). Actinophage active against streptomycin-producing strains of *S. griseus* has no effect upon the pink substrain.

The pink substrain was grown on media commonly used for the production of

streptomycin. Excellent growth, of a deep-red color, was obtained in submerged cultures. The filtrate had a high potency against gram-positive bacteria but nct against *E. coli* or other gram-negative bacteria. The active substance was dissolved in ether and was found to be largely active against gram-positive bacteria.

Antibiotic activity of the 1915 S. griseus culture. The original strain of *S. griseus* isolated from a New Jersey soil in 1915 was available in two cultures: one that was kept for over 30 years in the New Jersey culture collection, where it has been grown continuously in synthetic media; and the other, a transfer of this culture, that was kept in the Centraalbureau voor Schimmelcultures in Baarn, Holland, where it was deposited in 1921 and where it was grown in organic media, such as potato agar. The New Jersey culture was tested recently several times for its ability to inhibit bacterial growth, but it showed no antibiotic

TABLE 3

Production of an antibiotic substance by different colony isolates from the 1915 culture of S. griseus kept at Baarn, S326a

STRAIN NO.	DILUTION UNITS OF ACTIVITY AGAINST DIFFERENT BACTERIA							
	Stationary growth 12 days				Submerged growth 5 days			
	E. coli	*B. subtilis*	*B. mycoides*	*S. aureus*	*E. coli*	*B. subtilis*	*B. mycoides*	*S. aureus*
1	5	60	30	50	50	>1,000	100	60
2	5	150	225	275	<10	<10	<10	<10
3	5	150	300	250	0	0	0	0
4	20	200	800	750	0	0	0	0
5	20	125	400	600	0	0	0	0
6	20	150	500	300	<10	<10	12	15
7	15	125	350	250	<10	8	15	15
8	15	100	400	350	<10	12	20	28
9	15	1,000	250	600	40	500	225	600
10	15	150	350	300	<10	<10	8	15
11	40	200	750	750	<10	<10	<10	<10

activity. The Baarn culture, however, possessed definite antibacterial properties and produced an antibiotic substance in the medium. Our attention was directed to this culture by several European investigators who obtained it from the Baarn collection and, believing that they were working with one of the streptomycin-producing strains, grew the culture on a large scale. Although they found that the culture produced a considerable amount of some antibiotic substance, they were unable to isolate any streptomycin by the methods developed for the isolation of this antibiotic.

When recently received from Baarn, the culture was plated out, and a few individual colonies were picked and transferred to agar slants. The colony isolates were grown in a glucose peptone meat extract medium commonly used for the study of streptomycin production. These isolates differed greatly in their ability to produce an antibiotic substance, as is brought out in table 3. Although all the 11 isolates thus obtained were found to produce an antibiotic

when grown under stationary conditions, only 2 cultures formed such a substance under submerged conditions of growth. The activity of this antibiotic against *E. coli* was rather limited, although it was very active against various gram-positive bacteria.

The antibiotic substance produced by the active isolates from the Baarn culture possessed solubility properties distinctly different from those of streptomycin. Although the antibiotic was readily adsorbed on norite, it could not be removed from it by acidified alcohol, as is usually the case with streptomycin; it was soluble in organic solvents, although only a small part was removed, especially by ethyl acetate and by chloroform. This is brought out in table 4.

TABLE 4

Comparative bacteriostatic spectra of culture filtrates and isolated preparations from the original 1915 strain and the streptomycin-producing strains of S. griseus

PREPARATION	*E. coli*	*B. subtilis*	*B. mycoides*	*S. aureus*
S. griseus: Original 1915 culture, Baarn strain				
Culture filtrate, 1 ml	10	200	400	500
Total units in solution 1,150 ml	11,500	230,000	460,000	575,000
Ether soluble*	100	1,280	480	960
Ethyl acetate soluble	500	2,500	1,500	1,375
Chloroform soluble	180	2,400	600	1,800
Acid alcohol soluble	200	525	210	525
Total units recovered in all fractions	500	6,705	2,790	4,660
S. griseus: Streptomycin-producing culture 3463				
Culture filtrate, 1 ml	75	500	—	30
Total units in solution 1,000 ml	75,000	500,000	—	30,000
Total units recovered Acid alcohol preparation†	40,000	200,000	—	24,000

* Adsorbed on norite, treated with various solvents. Activity reported for total used in this experiment 1,150 ml.

† Adsorbed on norite, removed with acid-alcohol, treated with ether; more than 50 per cent of activity was recovered.

Production of different antibiotics by S. griseus. Different strains of *S. griseus* are thus shown to vary greatly in their capacity to produce antibiotic substances. Some of the strains produce streptomycin, others grisein, still others yield mixtures of these with other antibiotics. This was first pointed out by Carvajal (1946*b*), who emphasized the danger of designating an antibiotic substance as "streptomycin" merely because it is produced by a strain of *S. griseus* or because a strain of the latter causes inhibition of bacterial growth comparable to that of the streptomycin-producing strains of *S. griseus*.

On the basis of their ability to form antibiotic substances, the various strains of *S. griseus* that have so far been tested can be divided into four groups: (1)

streptomycin-producing strains; (2) grisein-producing strains; (3) strains producing antibiotics other than streptomycin or grisein, as in the case of the red-pigmented variant of the streptomycin strain described above or the Baarn variant of the 1915 culture; and (4) non-antibiotic-producing strains.

An attempt was made to establish whether any morphological or cultural differences existed among these four groups of cultures. The cultures were isolated from natural substrates, selected by colony isolates of older cultures, or obtained from other laboratories.

General morphological and cultural characteristics of S. griseus. *S. griseus* is characterized by certain morphological and cultural properties that make possible its identification and its ready separation from other species of *Streptomyces*.

Drechsler (1919) made the first comprehensive study of the morphology of *S. griseus*. He reported that the aerial mycelium of the organism showed proliferations of fertile branches at moderately close intervals along the axial hyphae, but no spirals. This early description and the illustrations correspond well with the production of tufts in the aerial mycelium of *S. griseus* found in recent photomicrographs (Carvajal, 1946*a*, Reynolds and Waksman, 1948). The early description of *S. griseus* (Waksman, 1919) covers the cultural and biochemical properties of the organism, which as a rule characterize rather well both the streptomycin-producing and grisein-producing cultures, with only certain minor differences.

S. griseus produces a typical growth on solid media. On glucose nitrate agar, it forms a thin, spreading growth, developing deep into the medium, at first colorless, then turning olive buff. The pigment of the growth may be lost on successive transfers. The aerial mycelium is abundant, powdery, and of a water-green color. Growth on a potato plug is lichnoid and yellowish or cream-colored; the aerial mycelium is powdery with characteristic greenish pigmentation; the plug remains colorless or tends to become slightly brown. On gelatin, at 18 C, *S. griseus* produces a cream-colored to greenish-yellow growth, with white-gray to greenish aerial mycelium; the gelatin is rapidly liquefied, without the formation of a soluble pigment. Milk is rapidly peptonized with complete clearing, with or without previous coagulation.

The organism is strongly proteolytic and can attack starch, sugars, sugar alcohols, and organic acids. It can obtain its nitrogen from a large variety of compounds, both organic and inorganic.

S. griseus undergoes a typical life cycle. The spores germinate rapidly and produce a typical, monopodially branched mycelium, followed by the formation of a sporulating mycelium. The culture undergoes rapid lysis, especially under submerged conditions of growth, until it is completely disintegrated. The onset of lysis is accompanied by maximum streptomycin production; the completion of lysis is accompanied by a decrease in streptomycin concentration or potency. The glucose-containing media first become slightly acid, then change to alkaline, reaching pH 8.6 to 8.8 during lysis of the culture.

The streptomycin-producing strains of *S. griseus* are subject to destruction by actinophage, which is distinct from that of lysis of the culture, as is brought out

elsewhere (Reilly, Harris, and Waksman, 1947). Phage-resistant strains are produced readily, although these may still carry some of the phage; the streptomycin-producing capacity of such resistant strains does not differ from that of the parent cultures.

Cultures used for comparative studies. Eleven representative cultures of *S. griseus* were taken from the collection for a comparative study of their cultural and biochemical properties. The source and antibiotic-producing capacity of these cultures are shown in table 5. On the basis of their ability to produce antibiotic substances, these cultures can be grouped as follows:

(1) The streptomycin producers included nos. 3463, 3464, 3481, and 3496, the last culture being a colony isolate of the first. These cultures were all sensitive

TABLE 5

Nature and antibiotic activity of cultures studied

COLLECTION NO.	ORIGIN OF CULTURE	ANTIBIOTIC SPECTRUM*	SENSITIVITY BY CROSS-STREAK METHOD† TO		SENSITIVITY TO PHAGE‡
			3463	3478	
3326	1915 culture kept in N. J. collection	0	++++	++++	0
3326a	1915 culture kept in Baarn collection	+	++++	++++	0
3463	Original streptomycin-producing 18-16	±ac	0	+	+
3464	" " " D-1	±ac	+	++	+
3481	Freshly isolated streptomycin-producing culture	±ac	0	+	+
3496	Colony (no. 4) from 3463	±	0	++	+
3478	Grisein-producing, original 25-G	±	++	0	0
3510	Produces a griseinlike substance, 16-F	±	+++	0	0
3527	Produces a griseinlike substance	±	+++	0	0
3495	Pink variant of 3463	+	+++	0	0
3522	Bucherer culture of *S. griseus* obtained from Baarn collection	0	++++	+++	0

* + = active against gram-positive bacteria only; ± = active against both gram-positive and gram-negative bacteria; ±ac = ±, but also active against acid-fast bacteria; 0 = inactive.

† ++++ = very sensitive; +++ and ++ = sensitive; + = slightly sensitive; 0 = not sensitive.

‡ + = sensitive to phage; 0 = resistant to phage.

to actinophage; they were resistant, by the cross-streak method, to the antibiotic effect of the original streptomycin-producing culture 3463 and were also partly sensitive to the effect of the grisein-producing 3478.

(2) The grisein producers included nos. 3478, 3510, and 3527. These were resistant to the action of actinophage; they were sensitive to the antibiotic effect of the streptomycin-producing 3463 and were resistant to the action of the grisein-producing 3478.

(3) Two cultures that produced antibiotics other than streptomycin or grisein were included in this group, namely 3526a, the Baarn culture derived from the original 1915 isolate, and 3495, the pink mutant derived from one of the streptomycin-producing cultures. These two cultures produced antibiotics that had no

effect upon gram-negative bacteria. They were resistant to actinophage. They were sensitive to the antibiotic action of 3463; one was sensitive and the other was resistant to the action of 3478.

(4) The group of inactive cultures included 3326, the original 1915 isolate kept in the New Jersey collection, and 3522, a culture isolated by Bucherer and received from the Baarn collection. The were resistant to actinophage and were very sensitive to both the streptomycin-producing 3463 and the grisein-producing 3478.

Morphologically, none of these cultures could be distinguished from another. They all produced straight aerial mycelium, with a tendency to form tufts, and no spirals. The manner of sporulation, of spore germination, and of growth both in stationary and submerged culture was similar to that reported by Drechsler (1919) and by Carvajal (1946*a*,*b*). There were minor variations among the cultures, as in the rapidity of lysis and in the pigmentation of the aerial mycelium; these variations did not appear to be fundamental in nature and could not be associated with the formation of any particular antibiotic.

The cultures were also similar in their characteristic growth upon different media, the variations being more of degree than of kind. Some of the more significant cultural characteristics are listed here.

Growth on gelatin. All the cultures grew well on the gelatin, and nearly all brought about rapid liquefaction of the gelatin. Cultures 3326, 3326a, and 3522 produced only slow liquefaction; this reduction in proteolytic potency may have been the result of the storage of these cultures in collections for many years. It is to be recalled that 3326 was originally one of the strongest proteolytic actinomycetes (Waksman, 1919).

Only the streptomycin-producing strains formed a brownish pigment in gelatin media; this pigment was faint and quite distinct from the type of pigment usually produced by the chromogenic actinomycetes, as, for example, by *Streptomyces lavendulae* or *Streptomyces scabies*. Of the other cultures, 3495, the pink mutant, was the only one to produce also a slight pigmentation of the gelatin.

Potato plug. All cultures produced a lichnoid, somewhat brownish growth on potato plugs. It rapidly became covered with a white, powdery aerial mycelium. The greenish tinge of this mycelium was more pronounced in the case of the streptomycin-producing cultures; the pigmentation of the mycelium either remained white or tended to become gray in the case of the other cultures. The color of the plug was unchanged, except in the case of the streptomycin-producing strains, which tended to produce a faint brownish pigment around the growth.

Nutrient agar. Growth was similar for all cultures, being cream-colored and lichnoid in appearance. The aerial mycelium was powdery, white to light gray. The only differences observed were in the amount of mycelium covering the growth. None of the cultures produced any soluble pigment.

Glycerol agar. With the exception of the pink variant 3495, the color of which was carmine red, all the cultures produced a thin, cream-colored growth with white to gray aerial mycelium. Only the streptomycin-producing strains and the pink variant produced the typical greenish pigmentation of the mycelium.

Glucose asparagine agar. On this medium as well, all the cultures, with the

exception of 3495, which produced a vinaceous-colored growth, produced a thin, cream-colored growth. The aerial mycelium was light gray, varying in abundance with each culture. The greenish tinge of the streptomycin-producing cultures and the mouse-gray color of 3522 were the exceptions.

Starch agar and Czapek's agar. Cream-colored growth, similar for all cultures except for 3495, in which it was somewhat pinkish to lavender, was produced. Aerial mycelium of most of the non-streptomycin-producing strains was white to cream-colored, varying in abundance and in shade of color; 3522 produced a mouse-gray mycelium; the streptomycin-producing strains, as well as 3495, were covered with a greenish mycelium.

These results show that it is hardly possible to separate any one group from the others in an attempt to create a separate species. It could possibly be done for the streptomycin-producing group or for the pink variant. The streptomycin cultures are characterized by their ability to produce streptomycin, their sensitivity to actinophage, the greenish pigmentation of the aerial mycelium, and the slight brownish pigment on gelatin and certain other complex organic media. The fact, however, that 3326 and 3326a when originally isolated also produced the typical watery greenish pigmentation of the mycelium, and also the fact that the grisein-producing cultures frequently show the greenish coloration of the aerial mycelium, would speak against using this property as a major distinguishing characteristic between constituent groups within the species *S. griseus*. The variations in other properties also are not sufficient to justify, for the time being at least, subdividing this species.

SUMMARY

Streptomyces griseus (Krainsky emend. Waksman and Curtis) Waksman and Henrici represents an extremely variable group of organisms.

Only some of the cultures of *S. griseus* isolated from natural substrates are able to produce antibiotic substances. A few cultures produce streptomycin, and a few form other antibiotics.

On the basis of their ability to produce antibiotic substances, the various cultures of *S. griseus* have been grouped into four categories: (1) those that produce streptomycin; (2) those that produce grisein; (3) those that form an antibiotic that is neither streptomycin nor grisein; and (4) those that do not form any antibiotic.

Streptomycin-producing cultures give rise to a number of variants or mutants, which differ in their capacity to form streptomycin quantitatively under the same conditions or under different conditions of cultivation. Some of the variants do not form any streptomycin; others may form a different type of antibiotic.

A study of the morphological characteristics and cultural properties of different strains of *S. griseus* revealed sufficient similarity between them to justify considering them all, for the time being at least, as members of a single, very variable species.

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