STUDIES ON STREPTOMYCES LAVENDULAE[1]

SELMAN A. WAKSMAN, DALE HARRIS, AND M. LECHEVALIER

Rutgers University, New Brunswick, New Jersey

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Since the isolation of streptothricin by Waksman and Woodruff in 1942 from a strain of *Streptomyces lavendulae*, numerous reports have appeared concerning the ability of different actinomycetes to produce this antibiotic or closely related compounds. In most of the antibiotic screening programs, streptothricin and streptothricinlike substances are among the first to be commonly encountered as products of cultures of actinomycetes grown on different media. In most cases these cultures are found to belong to the *S. lavendulae* group. Since only little attention has been paid to the morphology and cultural characteristics of various members of this group of organisms, first isolated and described by Waksman and Curtis in 1916, a detailed examination of its properties was undertaken.

The identity of *S. lavendulae* is established easily by the characteristic lavender coloration of its aerial mycelium, although occasionally white mutants are obtained, and by the production of soluble brown to black pigments in organic media. Antibiotic-producing strains of this organism give rise to a variety of mutants, which differ morphologically, culturally, and physiologically from the parent culture; variants free from aerial mycelium are unable to produce streptothricin (Waksman and Schatz, 1945). Some of the strains of *S. lavendulae* possess marked capacity to produce a number of closely related antibiotics (Hutchison, Swart, and Waksman, 1949); others may form mixtures of antibiotics, such as streptomycin and streptothricin (Trussell, Fulton, and Grant, 1947).

HISTORICAL REVIEW

The original culture of *S.* (*Actinomyces*) *lavendulae* was isolated from soil by Waksman and Curtis (1916) and described as follows:

Czapek's agar, colorless growth; aerial mycelium deep vinaceous lavender (Rdg. xliv-65,,,-d); close spirals 5 to 8 μ in diameter, with abundant, oval conidia, 1.6 to 2 by 1 to 1.2 μ. Gelatin is slowly liquefied, with the production of a brown pigment. On potato plug, growth is brown, color of plug turning black.

In further studies on the distribution of actinomycetes in different soils, the same or similar organisms were isolated from areas in New Jersey, California, and Oregon (Waksman and Curtis, 1918). In a detailed study of the metabolism of actinomycetes (Waksman, 1919*b*, 1920), the original culture of *S. lavendulae* was found to possess strong diastatic and fairly strong proteolytic properties, and to produce brown to black pigments in organic media. It was one of the few actinomycetes that brought about a change of the reaction of gelatin media toward acid, whereas most of the other cultures changed the reaction to the alkaline. Between

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the years 1915 and 1918, the preceding culture was observed to undergo a gradual change in its physiology, as could be determined by the gradual reduction in the rate of gelatin liquefaction. A detailed report of the morphology and the physiology of this organism was presented in 1919 (Waksman, 1919*a*).

In recent studies on the production of antibiotics by actinomycetes, the following procedure has been used as a routine in various laboratories. Samples of soil, manure, or compost material are plated on a simple medium containing egg-albumin agar or glucose-asparagine agar, or on more complex media, such as nutrient or yeast glucose agar. The actinomyces colonies developing on the plate are transferred to agar slants and tested for their ability to inhibit the growth of several bacteria, usually *Bacillus subtilis*, *Bacillus mycoides*, *Staphylococcus aureus*, and *Escherichia coli*. Cultures that show better than average antibiotic activity or a special type of activity, as against gram-negative bacteria, are selected for further study. Such cultures may be reinoculated into sterile soil, to which a small amount of $CaCO_3$ or dried blood has been added, then reisolated. Then they are available for more detailed investigation.

In one of the first surveys on the production of antibiotics by actinomycetes which was done in our laboratory (Waksman, Horning, Welsch, and Woodruff, 1942; Waksman and Woodruff, 1942), a culture of *S. lavendulae* was found to possess marked activity upon gram-negative bacteria. It produced a rose-lavender colored aerial mycelium when grown on synthetic media and a brown pigment on protein-containing media. It possessed marked antibiotic properties against both gram-positive and gram-negative bacteria, as well as fungi. It was found to be effective against mycobacteria (Woodruff and Foster, 1944). Further study of the antibiotic produced by this culture (Waksman and Woodruff, 1942; Waksman, 1943) showed special media to be particularly favorable. The active substance was isolated from the culture filtrate by adsorption on norite and elution with acid-alcohol. Further purification yielded a basic, water-soluble compound, which was designated as *streptothricin*.

The isolation of this antimicrobial agent initiated a new era of antibiotic research since most of the previously known antibiotics (pyocyanase, gramicidin, penicillin, actinomycin) were soluble in organic solvents and not in water, and were active largely against gram-positive bacteria. Here was a relatively simple, water-soluble compound which possessed a remarkable antibiotic spectrum, especially in its activity against gram-negative bacteria. New media had to be developed for its production by the organism, new methods for its removal from the medium, for its concentration into a highly active product, and for measuring its antimicrobial properties.

The 1916 culture of *S. lavendulae*, kept all these years in the culture collection on artificial, mostly synthetic, media, was tested for its antibiotic properties and was found to possess only weak activity, largely against *B. subtilis* (Welsch, 1942). During the more than a quarter of a century that this culture was kept on artificial media, the general appearance and cultural properties were changed greatly. When grown in shake flasks, it produced only a very weak antibiotic substance which was active only against certain gram-positive bacteria. Here we may have

another illustration of a loss in a certain biochemical property by an organism upon long cultivation upon artificial media, a situation that appears to have arisen in connection with streptomycin production by the original culture of *Streptomyces griseus* (Waksman and Harris, 1949).

It can easily be demonstrated that cultures of *S. lavendulae* undergo marked variation and mutation when grown on artificial media (Waksman and Schatz, 1945). They vary in the pigmentation of the aerial mycelium, from lavender to rose to white, in the curvature of the aerial hyphae, some forming spirals and others not, and in the quantitative and qualitative production of an antibiotic substance. Some variants may lose the capacity to produce an aerial mycelium and then to form an antibiotic. The change in pigmentation of the aerial mycelium need not be accompanied by a change in antibiotic production. A number of antibiotics belonging to the streptothricin type are now known. They are produced by different cultures grown in different media. It is sufficient to mention streptin, lavendulin, streptolin, streptothricin VI, aerothricin, roseomycin, and a number of others. Their major characteristic, aside from solubility, stability, and basicity properties, is their characteristic antibiotic spectra. Some of the antibiotics produced by the *S. lavendulae* group are distinct, however, from streptothricin. Others produce no antibiotics at all. Undoubtedly, we are dealing with a very large group of organisms, many of which are capable of producing a variety of antibiotics. Recently one such culture was found capable of forming an antiviral substance which has been designated as ehrlichin (Groupé *et al.*, 1951).

A large number of cultures belonging to this group have been isolated in our laboratory or have been received from other laboratories. A comparative study of these cultures is fully justified at present. Although emphasis is laid upon the ability of different strains to produce antibiotics, it is hardly possible to state that this property is of diagnostic significance.

EXPERIMENTAL STUDIES

The following cultures were used in the present study:

Strain no. 3330. Original isolate of *Streptomyces* (*Actinomyces*) *lavendulae*, kept since 1915 in the culture collection, largely on Czapek's agar and on other synthetic media.

Strains no. 3440-8 and 3440-14. Colonies of original streptothricin-producing culture, isolated from the soil in 1941.

Strain no. 3445. Streptin-producing culture, isolated by Woodruff and Foster (1946).

Strain no. 3465. Culture isolated in 1943 from a soil enriched with dead cells of *Mycobacterium tuberculosis*.

Strain no. 3483. Culture isolated from a garden soil, resistant to the action of streptothricin.

Strain no. 3516. Culture isolated from soil and found to produce streptothricin VI (Hutchison, Swart, and Waksman, 1949).

Strain no. 3516W. A white sporulating mutant of strain no. 3516.

Strains no. 3526 and 3526a. These cultures produce a mixture of streptothricin

and streptomycin and were designated as *Streptomyces* F by Trussell, Fulton, and Grant (1947).

Strains no. 3530, 3531, and 3532. Isolated from a Guatemala soil and found to belong to the *S. lavendulae* group.

Strain 3534. Chloromycetin-producing culture of *Streptomyces venezualae.*

Strains no. 3542 and 3543. Isolated by Dr. W. Kocholaty of the University of Pennsylvania and numbered A-10 and A-105, respectively; received from Dr. H. Norton (*S. lavendulae?*).

Strain no. 3544. Received from The Upjohn Company as their culture 136B.

Strains no. 3555 and 3555a. A culture and a single colony isolate which possess, in addition to antibacterial and antifungal properties, certain antiviral activities (Groupé *et al.*, 1951).

These cultures vary greatly in their ability to produce aerial mycelium when grown in synthetic media; the pigmentation of the mycelium varies from white to various shades of rose and typical lavender. On nutrient agar, all the cultures grow well, producing a soluble brown pigment but no aerial mycelium or only traces of white pigmented mycelium.

The morphological and cultural properties of some of the cultures are shown in table 1. Their capacity to produce an antibiotic agent, as measured by the cross-streak method, is shown in table 2. The differences observed are not only quantitative but also qualitative in nature, as shown by the fact that some cultures produce no activity against gram-negative bacteria. The ability of the different cultures to produce antifungal substances is brought out in table 3. In most cases these correspond with the antibacterial activities of the different cultures. Cultures producing streptothricin are resistant to the effect of this antibiotic (table 4), but remain sensitive to other antibiotics, such as streptomycin. Several of the nonstreptothricin-producing cultures (strains no. 3532, 3555, and 3555a) are sensitive to this antibiotic.

Several cultures were grown in shake flasks in a glucose-peptone-meat extract-NaCl medium. Most of them produced an antibiotic substance, as shown in table 5. The yield and nature of the antibiotic varied greatly. Strain no. 3440-14 gave the highest yield of *E. coli* units, as compared to its activity against the gram-positive bacteria; it had no activity against *B. mycoides.* Strain no. 3531, on the other hand, produced an antibiotic or a mixture of antibiotics which had relatively limited activity against *E. coli*, as compared to its action on gram-positive bacteria. Some cultures produced no soluble antibiotic in the medium, at least on the particular media and under the particular conditions of growth; this was true of strains no. 3530, 3532, 3465, and 3543. Another important difference is found in the relative activity of the different cultures upon *B. mycoides.* It has been shown previously (Waksman and Woodruff, 1942) that typical streptothricin has little activity against this test organism; most of the cultures appeared to produce streptothricin since the antibiotics produced by them had little activity against *B. mycoides;* strain no. 3531 had a higher activity against this organism than against *E. coli*, possibly because of the formation of a mixture of antibiotics.

When studied in further detail, strain no. 3531 was found to produce an acidic

substance in the medium. The ability of this culture to form an antibiotic was definitely favored by the addition of $CaCO_3$ to the medium (table 6).

To determine whether the failure of some of the other cultures, as strains no. 3465 and 3543, to produce antibiotics in the medium is due to the lack of $CaCO_3$, several strains were grown in shake cultures with and without $CaCO_3$. The re-

TABLE 1

Pigmentation and morphology of different strains of S. lavendulae

STRAIN NO.	STRUCTURE AM*	GAA	YEA		POTATO PLUG		GELATIN	
		AM	AM	SP	AM	SP	AM	SP
3330	Close spirals, oval conidia	W-Vn-Lv			W	Bn		Bn
3440-8	Straight, long hyphae	W-Lv	Lv	Bn	Gy-Lv	Bn	W-gy	Dk-Bn
3440-14	Straight, long hyphae	W-Lv	Lv	Bn	W-Lv	Faint Bn	0	Dk-Bn
3445	Straight, long hyphae	W-Lv	0	0	W	Bn	0	Br-Bk
3483	Straight, long hyphae		W	Bn	Gy-W	Bk-Bn	W	Bn
3516	Few, open spirals	Lv	W-Lv	Bn	Gy	Bn	W-Lv	Dk-Bn-Bk
3526	Straight, long hyphae	Lv	Lv	Bn	Gy-W	Bn	0	Gn-Bn
3530	Closed spirals	Lv	W-Gy	Bn	Gy-W	Bn	W-Lv	Dk-Bn
3531	Closed spirals	Lv	Lv	Bn	Trace W	Bn	0	Dk-Bn-Bk
3532	Some spirals	Lv	Lv	Bn	W-Lv	Bn	0	Gn-Bk
3534	Straight, long hyphae	W-Lv	Gn-Vn	Bn	W	Bn	W	Bn
3542	Straight, short hyphae	W-Gy	Vn-Bn	Bn	0	Bn	W	Bn
3555	Long hyphae, spherical to oval conidia	W-Vn	Vn-Bn	Bn	Gy-W	Bn	0	Bn

* GAA = glucose-asparagine agar; YEA = yeast extract glucose agar; AM = aerial mycelium; SP = soluble pigment; Bn = brown; BK = black; GY = gray; Gn = green; Lv = lavender; Vn = vinaceous; W = white.

sults presented in table 7 show that whereas some cultures were favored by the addition of $CaCO_3$, others were not. In the case of strain no. 3542, the addition of $CaCO_3$ was comparable to the prolonged incubation of the cultures; during a short incubation period, the presence of $CaCO_3$ produced a change in pH from 6.2 to 8.2, accompanied by an increase in activity from 0 to 20 *E. coli* units per ml. When this culture was incubated for 7 days, the reaction was alkaline in both

cases and the activity the same. In the case of strain no. 3526, the presence of $CaCO_3$ was favorable and independent of the reaction change upon prolonged incubation. In the case of strain no. 3465, no activity was produced whether or not $CaCO_3$ was present.

Cultural characteristics of different strains of S. lavendulae. Because of their wide occurrence and potential importance as producers of antibiotics, the *S. lavendulae* group deserve careful recognition and detailed consideration. A description of the growth characteristics of some typical cultures is presented here.

TABLE 2

Antibacterial effects of the S. lavendulae group as shown by cross-streak tests

Zone of inhibition on agar plate, measured in millimeters

STRAIN NO.	E. COLI		B. MYCOIDES		M. AUREUS		B. SUBTILIS		MYCOBACTERIUM, 607		S. MARCESCENS	
	N*	YEA	N	YEA	N	YEA	N	YEA	N	YEA	N	YEA
3330	0	0	0	0	0	0	0	0	0	0	0	0
3440-8	28	—	9	—	24	—	32	21	28	—	19	—
3440-14	28	—	9	—	24	—	20	—	29	—	19	—
3445	26	20	19	10	26	20	19	—	28	24	21	19
3465	0	0	0	0	0	0	0	0	0	0	0	0
3483	0	0	0	0	0	0	0	0	0	0	0	0
3516	24	—	11	—	20	—	25	—	22	—	9	—
3516W	19	—	10	—	17	—	22	—	—	—	—	—
3526	20	21	4	4	26	22	24	25	17	27	19	20
3526a	16	—	10	—	15	—	23	—	18	—	18	—
3530	15	—	8	—	16	—	11	—	22	—	18	—
3531	15	—	12	—	14	—	20	—	23	—	19	—
3532	2	—	1	—	8	—	3	—	0	—	0	0
3534	0	0	0	0	0	0	0	0	0	0	0	0
3542	23	17	15	8	22	17	27	21	18	24	17	15
3543	10	13	6	5	14	14	19	17	14	19	8	12
3544	28	14	16	10	20	19	17	21	20	25	16	18
3555	0	29	2	20	7	22	10	20	0	17	0	0
3555a	0	27	0	22	10	23	0	22	0	0	0	0

* N = nutrient agar; YEA = yeast extract glucose agar.

Strain no. 3330. Even after 35 years on synthetic media, this organism produces vigorous growth on a variety of different media with certain few exceptions. On Czapek's agar, it produces a slow starting, lichnoid growth, cream colored to yellowish, which is gradually covered with white powdery aerial mycelium. On glucose-asparagine agar, growth is smooth at first, cream colored to yellowish and somewhat folded; aerial mycelium white later turning vinaceous lavender. On nutrient agar, growth is cream colored, smooth at first, then lichnoid; thin white powdery aerial mycelium covering surface of growth in a somewhat patchy manner. On gelatin, growth is limited, in the form of small flaky colonies throughout the medium; brownish pigmentation; considerable liquefaction. On potato plug, growth is lichnoid, brownish; white powdery mycelium covering growth in

patches, lavender in spots after 7 days; brownish pigmentation of plug. On milk, growth is cream colored, no aerial mycelium; active peptonization of the milk.

Strains no. 3440-8 and 3440-14. On Czapek's agar, growth is lichnoid, cream colored in early stages, later brownish, with greenish tinge in spots; covered with white, turning gray and rose colored aerial mycelium. On glucose-asparagine agar, growth is thin, smooth, cream colored, covered with white to rose to lavender aerial mycelium. On nutrient agar, growth is brownish and in the form of confluent colonies; faint soluble pigment; no aerial mycelium. On gelatin, brownish growth in form of ring on surface; brown soluble pigment; fair liquefaction of

TABLE 3

Antifungal effects of the S. lavendulae group as shown by cross-streak tests

STRAIN NO.	C. ALBICANS		P. NOTATUM		A. NIGER		T. MENTAGROPHYTES	
	N*	YEA	N	YEA	N	YEA	N	YEA
3330	0	0	0	0	0	0	0	0
3440-8	25	13	20	13	14	20	21	14
3440-14	20	13	18	18	17	14	17	15
3445	>45	6	21	15	15	13	16	9
3465	0	0	0	0	0	0	0	0
3483	0	0	0	0	0	0	0	0
3516	18	10	17	15	14	13	16	12
3516W	14	6	12	7	14	9	16	14
3526	18	8	14	12	20	11	18	11
3526a	21	12	20	14	0	20	16	14
3530	16	15	15	19	16	15	17	12
3531	14	5	15	9	25	9	19	8
3532	6	13	6	22	>45	16	22	13
3534	0	0	0	0	0	0	0	0
3542	19	12	16	17	9	10	14	16
3543	21	0	15	0	9	0	0	0
3544	10	13	11	14	18	17	0	15
3555	13	12	21	19	0	16	14	19
3555a	17	13	22	23	0	18	14	16

* N = nutrient agar; YEA = yeast extract glucose agar.

gelatin. On potato plug, brownish lichnoid growth, covered with white to gray aerial mycelium, faint lavender in 7 days; faint brownish pigment. On milk, cream colored surface growth, covered with patches of white aerial mycelium; limited peptonization of milk.

Strain no. 3445. On Czapek's agar, limited cream colored growth; no aerial mycelium. On glucose-asparagine agar, cream colored growth covered by faintly lavender aerial mycelium. On nutrient agar, thin brownish growth, in the form of minute confluent colonies; no aerial mycelium; faint brownish pigment. On gelatin, limited surface brownish growth, in form of ring; soluble brown pigment; limited liquefaction. On potato plug, dark brown folded growth not spreading; trace of white aerial mycelium after 7 days; soluble black pigment. On milk, sur-

face cream colored to brownish growth; good aerial mycelium; white to slightly pigmented; medium peptonization of milk.

TABLE 4

Resistance of different strains of S. lavendulae to streptothricin

A lot of material assaying 70 units per mg was used in these studies.*

STRAIN NO.	AMOUNT OF STREPTOTHRICIN REQUIRED FOR GROWTH INHIBITION	ANTIBIOTIC PRODUCED BY CULTURE
	(μ/ml)	
3330	44	0
3440-8	>56	Streptothricin
3440-14	>56	Streptothricin
3445	>56	Streptin
3465	44	0
3483	>56	‡
3516	>56	Streptothricin VI
3516W	>56	Streptothricin VI
3526	>56	Streptothricin† streptomycin
3526a	>56	Streptothricin† streptomycin
3530	>56	Unknown
3531	>56	Unknown
3532	3	0
3534†	>56	Chloromycetin
3534a†	>56	Chloromycetin
3542	>56	Unknown
3543	44	0
3544	>56	Unknown
3555	<1	0
3555a	<1	0

* Streptomycin was originally standardized against streptothricin; hence one unit of the latter is about equivalent to 1 μg of streptomycin.

† Strains of *Streptomyces venezualae*.

‡ Originally isolated as a streptothricin-resistant culture.

TABLE 5

Production of an antibiotic under submerged conditions by different strains of S. lavendulae

Incubation 4 days at 28 C; dilution units per ml

STRAIN NO.	E. COLI	B. MYCOIDES	M. AUREUS	B. SUBTILIS
3440-14	150	30	100	750
3516	75	7	75	170
3530	100	20	100	500
3531	50	100	300	1,000
3532	<10	<10	<10	<10

Strain no. 3483. On Czapek's agar, slow starting, cream colored, lichnoid growth; patchy white aerial mycelium; no soluble pigment. On glucose-asparagine

TABLE 6

Effect of glucose concentration and $CaCO_3$ on production of antibiotics by S. lavendulae, strain no. 3531

Activity is expressed in units per ml

GLUCOSE, PER CENT	$CaCO_3$, PER CENT	INITIAL pH	2 DAYS		4 DAYS		6 DAYS	
			pH	μ/ml	pH	μ/ml	pH	μ/ml
1	0	6.6	4.4	<3	4.8	<3	5.1	<3
2	0	6.4	4.6	<3	4.8	<3	4.7	<3
4	0	6.2	4.5	<3	4.5	<3	4.4	<3
1	0.75	6.9	7.7	13	8.3	52	8.6	34
2	0.75	6.9	6.0	<3	7.7	53	7.9	64
4	0.75	6.8	7.2	48	5.9	53	7.0	65
1	1.5	7.0	7.6	8	8.2	81	8.4	50
2	1.5	6.9	6.5	<3	7.8	108	7.9	117
4	1.5	6.8	6.7	<3	5.9	<3	5.9	<3

TABLE 7

Effect of $CaCO_3$ upon the antibacterial activity of different strains of S. lavendulae

Medium contains 1 per cent glucose; activity in dilution units

STRAIN NO.	NO $CaCO_3$			$CaCO_3$ ADDED		
	pH	*E. coli*	*B. subtilis*	pH	*E. coli*	*B. subtilis*
4 days' incubation						
3330	5.1	0	0	7.9	0	0
3445	7.9	10	100	8.2	30	>300
3465	4.7	0	0	8.0	0	0
3526	4.6	0	0	8.3	10	10
3542	6.2	0	0	8.2	20	250
3543	7.0	0	0	8.0	0	0
3544	7.0	0	100	8.0	10	70
3555	7.7	0	0	7.9	0	0
7 days' incubation						
3330	8.7	0	0	8.6	0	0
3445	8.8	0	20	8.6	10	250
3465	7.8	0	0	8.6	0	0
3526	8.6	0	0	8.6	10	10
3542	8.5	10	70	8.6	10	70
3543	8.4	0	10	8.7	0	10
3544	8.2	0	0	8.5	0	20
3555	8.5	0	0	8.5	0	0

agar, greenish-cream colored growth; no aerial mycelium; no soluble pigment. On nutrient agar, thin, smooth, brownish growth; no aerial mycelium; soluble brown pigment. On gelatin, cream colored surface ring, white aerial mycelium; soluble brown pigment; limited liquefaction. On potato plug, brown-black lichnoid growth; covered with gray-white aerial mycelium; brown-black soluble pigment. On milk, cream to brown surface colonies; no aerial mycelium; active peptonization.

Strain no. 3516. On Czapek's agar, lichnoid abundant brownish growth, with limited white at first, then brownish aerial mycelium; soluble brownish pigment. On glucose-asparagine agar, cream colored growth covered with lavender colored aerial mycelium. On nutrient agar, thin brownish growth consisting of confluent colonies; soluble brown pigment; no aerial mycelium. On gelatin, brownish growth in form of ring on surface; no aerial mycelium; soluble brown to dark brown to almost black pigment; medium liquefaction. On potato plug, brownish lichnoid growth, covered with white to gray aerial mycelium; soluble brown pigment, black after 7 days. On milk, brownish surface pellicle covered with patches of gray and white aerial mycelium; medium peptonization of milk.

Strain no. 3526. On Czapek's agar, brownish yellow, lichnoid, abundant growth, covered with white to gray aerial mycelium; soluble brownish amber pigment. On glucose-asparagine agar, cream colored growth, covered with typical lavender colored aerial mycelium. On nutrient agar, thin brownish growth consisting of confluent colonies; faint soluble brown pigment; no aerial mycelium. On gelatin, brownish ring on surface; no aerial mycelium; soluble brown to greenish-brown pigment; limited liquefaction. On potato plug, brownish lichnoid growth; thin white aerial mycelium, notably in drier parts of growth; faint soluble brown pigment. On milk, brownish surface growth; no aerial mycelium; medium peptonization.

Strain no. 3530. Similar to 3531 with some minor differences (see table 1).

Strain no. 3531. On Czapek's agar, thin, smooth, cream colored slow-growing; no aerial mycelium; no soluble pigment. On glucose-asparagine agar, cream colored smooth growth, covered abundantly with typical lavender aerial mycelium. On nutrient agar, thin brownish growth, consisting of confluent colonies, faint soluble brown pigment; no aerial mycelium. On gelatin, brownish ring on surface; no aerial mycelium; soluble brown to dark brown or almost black pigment; limited liquefaction. On potato plug, abundant, brownish, lichnoid growth; no aerial mycelium; typical black soluble pigment. On milk, good surface pellicle, with patchy white aerial mycelium; medium peptonization.

Strain no. 3532. On Czapek's agar, cream-yellow lichnoid growth, with heavy white aerial mycelium, turning lavender; soluble amber pigment. On glucose-asparagine agar, cream-yellow lichnoid growth with traces of white aerial mycelium; no soluble pigment. On nutrient agar, thin, smooth tan growth; no aerial mycelium; soluble brown pigment. On gelatin, brown surface ring, white aerial mycelium; soluble brown pigment; limited liquefaction. On potato plug, brown lichnoid growth, with white turning lavender aerial mycelium; faint soluble

brown pigment. On milk, cream to brown surface ring; white aerial mycelium; limited peptonization.

Strain no. 3534. Czapek's agar, cream colored lichnoid growth, covered with white aerial mycelium; soluble brownish pigment. On glucose-asparagine agar, cream colored growth, covered with thin, white-lavender aerial mycelium; no soluble pigment. On nutrient agar, thin brownish growth, with thin, white aerial mycelium; soluble dark brown pigment. On gelatin, brownish surface ring, with whitish-gray aerial mycelium. On potato plug, dark brown lichnoid growth, with traces of white aerial mycelium; soluble brown pigment. On milk, cream to brown surface ring, with thin, white aerial mycelium; rapid peptonization of milk.

Strain no. 3452. On Czapek's agar, cream colored smooth growth covered with thin white aerial mycelium in patches; faint brownish pigment. On glucose-asparagine agar, cream colored smooth growth, covered with powdery white to grayish aerial mycelium. On nutrient agar, thin brownish, smooth growth covered in patches with white aerial mycelium; soluble brownish pigment. On gelatin, brownish ring on surface; soluble brown to almost black pigment; limited liquefaction. On potato plug, brownish lichnoid growth, covered in spots with very thin, gray-white aerial mycelium; soluble black pigment. On milk, surface cream colored growth, with thin ring of whitish to lavender aerial mycelium; limited peptonization.

Strain no. 3544. On Czapek's agar, yellowish-brown, slow starting lichnoid growth; abundant, thin, patchy whitish aerial mycelium; limited brownish soluble pigment. On glucose-asparagine agar, yellowish-brown lichnoid growth; thin patchy white aerial mycelium; no soluble pigment. On nutrient agar, thin brownish growth, consisting of confluent colonies; faint soluble brown pigment; no aerial mycelium. On gelatin, surface pellicle brown colored; thin white aerial mycelium; brownish soluble pigment; medium liquefaction. On potato plug, lichnoid, brownish, good growth, with thin patchy white aerial mycelium; soluble brownish pigment. On milk, surface cream colored growth with white aerial mycelium; limited peptonization.

Strain no. 3555. On glucose-asparagine agar, thin spreading colorless growth; cottony-white aerial mycelium, brown vinaceous, later vinaceous-fawn in color. On nutrient agar, restricted colorless growth; no aerial mycelium. On gelatin, cream colored surface growth; soluble brown pigment; rapid liquefaction. On potato plug, lichnoid, yellowish colored growth, later turning to red-brown with greenish margin; sparse aerial mycelium; soluble brown pigment. On milk, gray-brown ring; peptonization without change in reaction.

SUMMARY

Streptomyces lavendulae (Waksman and Curtis) Waksman and Henrici represents a widely distributed and extremely variable group of organisms. It has been isolated from many soils throughout the world.

Streptothricin, the first water-soluble, basic antibiotic that has ever been isolated from the culture filtrate of an organism, was produced by a strain of *S.*

lavendulae typical of the group. Since 1942, when the isolation of streptothricin was reported, this antibiotic or closely related compounds have been isolated in various laboratories throughout the world. It would appear that in any screening program for the production of antibiotics by actinomycetes, streptothricin is among the first compounds always encountered.

Not all the strains, however, of *S. lavendulae* are able to produce antibiotics. Further, not all the antibiotics produced by active strains are of the streptothricin type.

The various cultures of *S. lavendulae* isolated from different soils and in different laboratories vary morphologically and physiologically. Some produce a straight aerial mycelium, which later gives rise to spores, whereas others form spirals, mostly of the closed type. The cultures also differ in the degree of coloration of the aerial mycelium when grown on artificial media; the pigment ranges from white to lavender to vinaceous. All strains of *S. lavendulae* are chromogenic, producing dark pigments on organic media, these pigments varying in shade from brown to dark brown, to almost greenish-black. Physiologic differences can be measured by the degree of acid production and rate of proteolysis.

The antibiotic substances produced by most of the strains of *S. lavendulae* have a relatively high activity against *Escherichia coli*, *Bacillus subtilis*, *Micrococcus pyogenes* var. *aureus* and *Mycobacterium* strain no. 607, and a relatively low activity against *Bacillus mycoides*, a phenomenon characteristic of the antibiotic spectrum of streptothricin. Some strains are active also upon *B. mycoides*, however, pointing to the formation by such strains of another type antibiotic, either alone or admixed with streptothricin.

Not all the strains of *S. lavendulae* that show activity by the cross-streak method are able to produce antibiotics in liquid media, at least not in those that have been used in these experiments and under the particular conditions of culture.

REFERENCES

GROUPÉ, V., FRANKEL, J. W., LECHEVALIER, M. P., AND WAKSMAN, S. A. 1951 Ehrlichin, a new antibiotic with specific antiviral properties. Bact. Proc., Chicago, Ill.

HUTCHISON, D., SWART, E. A., AND WAKSMAN, S. A. 1949 Production, isolation and antimicrobial, notably antituberculosis, properties of streptothricin VI. Arch. Biochem., **22**, 16–30.

TRUSSELL, P. C., FULTON, C. O., AND GRANT, G. A. 1947 Two antibiotics produced by a streptomyces. J. Bact., **53**, 769–780.

WAKSMAN, S. A. 1919*a* Cultural studies of species of actinomyces. Soil Sci., **8**, 71–215.

WAKSMAN, S. A. 1919*b* Studies in the metabolism of actinomycetes. J. Bact., **4**, 189–216, 307–330.

WAKSMAN, S. A. 1920 Studies in the metabolism of actinomycetes. III. Nitrogen metabolism. J. Bact., **5**, 1–30, 31–48.

WAKSMAN, S. A. 1943 Production and activity of streptothricin. J. Bact., **46**, 299–310.

WAKSMAN, S. A., AND CURTIS, R. E. 1916 The actinomyces of the soil. Soil Sci., **1**, 99–134.

WAKSMAN, S. A., AND CURTIS, R. E. 1918 The occurrence of actinomycetes in the soil. Soil Sci., **6**, 309–319.

WAKSMAN, S. A., AND HARRIS, D. A. 1949 Streptomycin-producing capacity of different strains of *Streptomyces griseus*. Proc. Soc. Exptl. Biol. Med., **71**, 232–235.

WAKSMAN, S. A., HORNING, E. S., WELSCH, M., AND WOODRUFF, H. B. 1942 Distribution of antagonistic actinomycetes in nature. Soil Sci., **54**, 281–296.

WAKSMAN, S. A., AND SCHATZ, A. 1945 Strain specificity and production of antibiotic substances. VI. Strain variation and production of streptothricin by *Actinomyces lavendulae*. Proc. Natl. Acad. Sci., **31**, 208–214.

WAKSMAN, S. A., AND WOODRUFF, H. B. 1942 Streptothricin, a new selective bacteriostatic and bactericidal agent, particularly active against gram-negative bacteria. Proc. Soc. Exptl. Biol. Med., **49**, 207–210.

WELSCH, M. 1942 Bacteriostatic and bacteriolytic properties of actinomycetes. J. Bact., **44**, 571–588.

WOODRUFF, H. B., AND FOSTER, J. W. 1944 *In vitro* inhibition of mycobacteria by streptothricin. Proc. Soc. Exptl. Biol. Med., **57**, 88–89.

WOODRUFF, H. B., AND FOSTER, J. W. 1946 Streptin, an antibiotic from a species of *Streptomyces*. J. Bact., **52**, 502.