STUDIES ON THE CHARACTERS OF ANTIBIOTIC STREPTOMYCES. IV. ON THE STRAINS WHICH PRODUCE STREPTOTHRICIN-GROUP SUBSTANCES

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Several antibiotic substances produced by various kinds of streptomyces are resembling each others. They are adsorbed by activated carbon from the fermented broth and eluted into acid methanol. They are soluble in water and insoluble in organic solvents. However, they are different in toxicity, antibacterial spectra, and some chemical characters. At first Umezawa *et al.* (1—4) divided them into two groups, that is, streptomycin and streptothricin-group substances. According to the authors (1), substances of streptothricin-group are in the following characters.

- 1) They are obtained from the broth of streptomyces.
- 2) They inhibit widely Gram-positive and Gram-negative bacteria.
- 3) They are adsorbed by activated carbon, eluted into acid methanol, soluble in water and insoluble in organic solvents such as acetone, ether, benzene and petroleum ether.
- 4) The streptomycin-fast *E. coli* is almost as susceptible to them as the normal culture.
- 5) The streptothricin-fast *E. coli* is more resistant to them than the normal culture.

Thereafter Umezawa classified the streptothricin-group substances into two groups, the streptothricin-group A and the streptothricin B. The former, if injected into mice, shows a later appearing toxicity, but not the latter. A strain of *B. anthracis* in Umezawa's laboratory is more resistant to streptothricin-group A than *B. subtilis* (N. R. R. L. B558), but is rather more susceptible to streptothricin B. Streptothricin-group A substances are produced by various kinds of streptomyces, but streptothricin B is obtained from the broth of strains belonging to *S. fradiae*.

In the present paper characters of strains producing streptothricin-group A substances and streptothricin B are described.

DESCRIPTION OF STRAINS

I. Strains 68, 201, U-33, B-3, B-10, B-18, 42-B, A-6, K-4 and E-2, which produce streptothricin-group A

(A) Microscopical observation.

Aerial mycelium is 0.9–1.1 μ width, well branched and coarse. Numerous spirals are observed. Conidia are oval to sphere (1.0–1.2 $\times$ 1.0–1.2 μ).

(B) Characters on various media.

1) Synthetic agar; 27°C.

Growth; thin, spreading, colorless, develops deep into medium. Aerial mycelium; cottony to velvety, at first white, later grayish with lavender tinge, this lavender

color is often lost during successive cultivation. Reverse; colorless or brownish colored. Soluble pigment; none.

2) Glucose bouillon agar; 37°C.

Growth; thick, wrinkled, cream colored, develops deep into medium. Aerial mycelium; cottony, sometimes with lavender tinge. Soluble pigment; none. Reverse; brownish orange colored.

3) Nutrient agar; 37°C.

Growth; thin, wrinkled, cream colored. Aerial mycelium; white scant. Soluble pigment; brownish orange.

4) Loeffler's serum medium; 37°C.

Growth; thin, dark cream colored. Aerial mycelium; cottony. Substrate changes to brownish around the growth. Liquefaction; none.

5) Egg medium; 37°C.

Growth; thin, cream colored. Aerial mycelium; white. Soluble pigment; none.

6) Starch agar; 27°C.

Growth; transparent, colorless. Aerial mycelium; white with lavender tinge. Soluble pigment; none. Enzymatic zone induced by iodine reaction; 2-3 mm in 5 days.

7) Potato plug; 27°C.

Growth; thin, wrinkled, colorless to cream colored. Aerial mycelium; white. Substrate changes slightly to brownish.

Table 1. Comparison of the strain 68, 201, U-33, B-3, B-10, B-18, 42B, A6, K4, and E2 with *S. lavaendulae* (Waksman & Curtis):

	Description in Bergey's Det. Bact. (1948) ()=Waksman's description (1919)	Description of our strains
Synthetic	Hyphae coarse, branching, spirals close, 5-8 μ in diam., conidia oval, 1.0-1.2 $\times$ 1.6-2.0 μ . Thin, spreading, colorless growth. Aerial mycelium cottony, white, becoming vinouslavender. (S.P. none).	Hyphae coarse, branching, 0.9-1.1 μ in width. Spirals close, abundant, conidia oval to sphere 1.0-1.2 $\times$ 1.0-1.2 μ . Thin, spreading, colorless growth. Aerial mycelium cottony, white, becoming grayish with lavender tinge. S.P. none.
Plain agar	Gray, wrinkled growth (S.P. brown, A.M. none)	Creamy-gray, wrinkled A.M. with in patches. or none S.P. slightly brown in patches.
Potato plug	Thin, wrinkled, cream to yellowish growth. (A.M. none, plug turns to brown.)	Wrinkled, creamy growth. Aerial mycel. white or none. Plug turns to brown.
Gelatin stab	Creamy to brownish surface growth. Liq. (S.P. brown or none)	Creamy surface growth. A.M. none. Liq. slow. S.P., brown or none.
Starch agar	Restricted, glistening, transparent growth. (A.M. lavender.)	Glistening, transparent growth. A.M. lavender colored.
Litmus milk	Cream colored ring. No coag. Pept. with strong alkaline reaction.	Cream colored ring on surface. No coag. Pept. with strong alkaline.
Nitrates reduction	+	+
S.P.	Soluble brown pigment.	Soluble brown pigment.
Starch hydrol.	+	+
Opt. temp.	Aerobic 37°C.	Aerobic 35-38°C.

8) Carrot plug; 27°C.

Growth; abundant, wrinkled, brownish. Aerial mycelium; white powdery, with light pink to lavender tinge. Substrate; unchanged or slightly brownish.

9) Gelatin stab; 20°C.

Growth; glossy surface growth and mass on the bottom of liquefied portion. Liquefaction; slow. Soluble pigment; none or slightly brownish.

10) Litmus milk; 37°C.

Growth; cream colored surface ring. Aerial mycelium; none. Coagulation; occurs in 3-5 days and then peptonization. Reaction changes to alkaline.

11) Nitrites are not produced from nitrates.

In Table 1 these characters are compared with the description of *S. lavendulae* shown in Bergey's Determinative Bacteriology (1948). They are almost same each others and there are not important differences. The strains described here should be *S. lavendulae*.

II. Strain 13A which produces streptothricin-group A

(A) Microscopical observation.

Aerial mycelium is straight and branching. Straight chains of conidia are observed on the end of aerial mycelium. Scale of conidia is $1.0-1.5 \mu \times 1.0-1.5 \mu$ with cylindrical shape. Spirals are not observed.

(B) Characters on various media.

1) Synthetic agar; 27°C.

Growth; at first colorless, later reddish orange. Aerial mycelium; abundant, cottony, chrome orange colored. Soluble pigment; none. Reverse; yellowish.

2) Glucose bouillon agar; 37°C.

Growth wrinkled, creamy to orange colored, glossy. Aerial mycelium; none or white patches. Brown soluble pigment is often produced.

3) Nutrient agar; 37°C.

Growth; wrinkled, dark creamy, glossy. Aerial mycelium; none or thin, grayish white. Soluble pigment; slightly brownish.

4) Blood agar; 37°C.

Growth; wrinkled, greenish gray, glossy. Aerial mycelium; none. Hemolysis; none or very slight.

5) Loeffler's serum medium; 37°C.

Growth; cream colored with brownish tinge. Aerial mycelium; none. Liquefaction; none. Soluble pigment; slightly brownish.

6) Egg medium; 37°C.

Growth; wrinkled, orange colored. Aerial mycelium; white with pink tinge. Soluble brown pigment is around the growth.

7) Starch agar; 27°C.

Growth; orange colored. Aerial mycelium; white with pink tinge. Enzymatic zone induced by iodine reaction; 1.5-2.5 mm in 5 days.

- 8) Potato plug; 27°C.
Growth; elevated, greenish. Aerial mycelium; yellowish white with reddish tinge without margin. Substrate changes to black around the growth.
- 9) Carrot plug; 27°C.
Growth; spreading, brownish. Aerial mycelium; white to pink. Substrate changes slightly to brownish.
- 10) Gelatin stab; 20°C.
Growth; cream colored mass. Aerial mycelium; none. Liquefaction; weak. Soluble pigment; brownish.
- 11) Litmus milk; 37°C.
Growth; cream colored ring with pink tinge on surface. Coagulation starts in 3-4 days and later peptonization. Reaction changes to alkaline.
- 12) Nitrites produced from nitrates.

As seen in Table 2, above characters resemble the characters of *S. ruber*. Apparent differences between them are the formation of spirals, the color of the growth on synthetic agar and the growth on glucose bouillon agar. Though *S. ruber* forms spirals, they are very few. The difference of the color of the growth is not important because they are same in the basic color tone. Therefore, 13A strain is similar to *S. ruber* (Krainsky).

Table 2. Comparison of 13 A with *S. ruber* (Krainsky).

	Description in Bergey's Det. Bact. (1948) ()=Waksman's description (1919)	Description of our strain (13 A)
Synthetic	Straight, branching mycel., radiating. A few spirals. Abundant, spreading, red growth. Aerial mycel. abundant, cottony, chrome-orange. (S.P. none.)	Straight, branching mycel. Spirals are not observed. Abundant, spreading colorless to reddish-orange growth. A.M. abundant, cottony, orange. S.P. none. Reverse yellowish.
Plain agar	Restricted, elevated, wrinkled, olive-green growth. (A.M. thin, ash gray. S.P. brown.)	Wrinkled, creamy, glossy, growth. A.M. thin, grayish. S.P. brown.
Glucose agar	Restricted, abundant, entire, coral-red growth. (A.M. white with pink.)	Wrinkled abundant, reddish-orange growth. A.M. none or white patches.
Potato plug	Elevated wrinkled, greenish growth. (A.M. red with yellow margin.)	Elevated greenish, growth. A.M. yellowish white with reddish tinge without margin.
Gelatin stab	Liquefaction, with yellow flakes. (A.M. none. S.P. brown.)	Abundant, orange growth. A.M. pinkish.
Litmus milk	Dark ring with red tinge. Coag. and pept. with alkaline reaction.	Pinkish cream ring. Coag. and pept. with alkaline reaction.
Nitrates reduction	+	+
S.P.	Brown pigment.	Brown pigment.
Starch hydrol.	+	+
Opt. temp.	37°C.	37°C.

III. Strain 91 which produces streptothricin-group A

(A) Microscopical observation.

Aerial mycelium is well branched, spirals are short, conidia are $1.2-1.5 \mu \times 1.6-1.8 \mu$ in diameters with oval shape.

(B) Characters on various media.

- 1) Synthetic medium; 27°C.
Abundant white to grayish white colored growth. Aerial mycelium; abundant white. Reverse; yellowish. Soluble pigment; none.
- 2) Glucose bouillon agar; 37°C.
Growth; thick, wrinkled, edge is myceloid. Aerial mycelium; grayish white, becoming to brownish. Soluble pigment; none.
- 3) Nutrient agar; 37°C.
Growth; glossy, scant, cream colored. Aerial mycelium; white in patches. Soluble pigment; none.
- 4) Blood agar; 37°C.
Growth; glossy, with brownish gray tinge. Aerial mycelium; none. Soluble pigment; none. Hemolysis; none.
- 5) Loeffler's serum medium; 37°C.
Growth; poor, cream colored. Aerial mycelium; none. Soluble pigment; none. Liquefaction; none.
- 6) Egg medium; 37°C.
Growth; poor. Aerial mycelium; none. Soluble pigment; at first none, later reddish yellow pigment (in 7-10 days).
- 7) Starch agar; 27°C.
Growth; thin, transparent with cream colored tinge. Aerial mycelium; none. Soluble pigment; none. Enzymatic zone induced by iodine reaction; 1-3 mm width in 5 days.
- 8) Potato plug; 27°C.
Growth; wrinkled, brownish orange colored. Aerial mycelium; white, abundant. Plug changes to brown.
- 9) Carrot plug, 27°C.
Growth; cream colored. Aerial mycelium; grayish white. Plug unchanged.
- 10) Gelatin stab; 20°C.
Growth; cream colored surface growth. Aerial mycelium; none or in patches. Slow liquefaction and mass of growth in liquefied portion. Soluble pigment; none.
- 11) Milk; 37°C.
Growth; cream colored ring with brownish tinge on surface. Coagulation and peptonization start from upper portion. Soluble pigment; none.
- 12) Nitrites produced from nitrates.
- 13) No tyrosinase.

Among species described in Bergey's Determinative Bacteriology, *S. albus* is most resembling to this strain. However there are some differences. As shown in Table 3,

the strain, 91, produces reddish pigment on the egg medium, changes the potato to brown, not to purple and hydrolyzes starch. This strain may be a new species though it fairly resembles *S. albus*. On this strain we shall again study in future.

Table 3. Comparison of the strain 91 with *S. albus* (Rossi Doria emend Krainsky) Waksman & Henrici.

	Description in Bergey's Det. Bact. (1948) () = Waksman's description (1919)	Description of our strain
Synthetic	Vegetative hyphae; branched, 1μ in diam. A.M. abundant, white hyphae $1.3-1.7\mu$ in diam. with ellipsoidal spores (1μ long) in coiled chains in lateral branch of the aerial hyphae. (S.P. none.)	Vegetative hyphae branched, $0.8-1.0\mu$. A.M. abundant white, $0.9-1.2\mu$ in diam. with oval conidia ($1.2-1.5 \times 1.6-1.8\mu$) in coiled chains (incomplete spiralform). Reverse yellow. S.P. none.
Glucose agar	Gray A.M. becoming brownish. (Thick, wrinkled growth, edge myceloid. S.P. none.)	Thick, wrinkled growth, edge myceloid. A.M. grayish-white becoming brownish. S.P. none.
Bouillon	No aerial mycel. but chalky-white deposit forms on old colonies. A.M. few white patches, glossy cream colored, spreading.	Grossy, scant, cream colored growth. A.M. white in patches. S.P. none.
Potato plug	Colonies and A.M. white. (Abundant much wrinkled, cream colored. Plug changes purplish.)	Brownish-orange wrinkled growth. A.M. white, plug changes to brownish.
Carrot plug	Excellent growth (cream colored growth. A.M. thin, white.)	Cream colored growth. A.M. grayish-white.
Gelatin stab	Liq. Colonies gray, no S.P. (cream colored growth. A.M. white patches. S.P. brown but losts by artificial cultivating.)	Cream colored growth. A.M. none or white patches. Slow liq. S.P. none.
Starch agar	(Spreading, transparent, A.M. none. S.P. none.)	Thin, transparent with grayish tinge. A.M. none or scant. S.P. none.
Milk	(Brownish surface ring. Coag. none.) Pept. after coag. Alkaline react.	Brownish cream colored ring. Pept. after coag. Reaction alkaline.
Starch hydroly.	— (Broad.)	+
Nitrates reduction	+	+

IV. Strain 284 which produces streptothricin-group A

(A) Microscopical observation.

Well branched aerial mycelium bears short spirals. Conidia are ellipsoidal or oval ($1.2-1.6\mu \times 1.1-1.4\mu$).

(B) Characters on various media.

1) Synthetic agar; 27°C .

Growth; cream colored. Aerial mycelium; abundant, white. Reverse; brownish. Soluble pigment; none.

2) Glucose bouillon agar; 37°C .

Growth; thick, wrinkled. Aerial mycelium; white to grayish white. Reverse; slightly brownish. Soluble pigment; none or slightly brownish.

3) Nutrient agar; 37°C .

Growth; cream colored. Aerial mycelium; white. Soluble pigment; none.

- 4) Blood agar; 37°C.
Growth; brownish, wrinkled. Aerial mycelium; white patches. Soluble pigment; none. Hemolytic action; strong.
- 5) Loeffler's medium; 37°C.
Growth; glossy, cream colored with brownish tinge. Aerial mycelium; white. Liquefaction of coagulated serum; rapid.
- 6) Egg medium; 37°C.
Growth; wrinkled, orange colored with brownish tinge. Aerial mycelium; white in patches. Soluble pigment; none.
- 7) Starch agar; 27°C.
Growth; transparent. Aerial mycelium; none. Enzymatic zone induced by iodine reaction in 5 days; 7-10 mm out side of the growth.
- 8) Potato plug; 27°C.
Growth; cream colored. Aerial mycelium; white. Plug unchanged.
- 9) Carrot plug; 27°C.
Growth; at first cream colored, afterwards with grayish tinge, thick. Aerial mycelium; grayish white. Plug unchanged.
- 10) Gelatin stab; 20°C.
Growth; surface growth is cream colored with brownish tinge. Aerial mycelium; white in patches. Liquefaction; rapid, mass of growth in liquified portion. Soluble pigment; none or orange colored pigment.
- 11) Milk; 37°C.
Growth; cream colored surface ring. Aerial mycelium; none. Coagulation; starts in 4-5 days, thereafter peptonization. Soluble pigment; none. Reaction of substrate; alkaline.
- 12) Nitrites produced from nitrates.

At first the author noticed that this strain should be *S. albus*, but there seem to be many unsuitable points in this determination. The classification will be discussed in detail in future.

V. Strain 9 which produces streptothricin-group A

(A) Microscopical observation.

Straight aerial spore-bearing hyphae. Very few spirals. Conidia spherical; $1.0-1.2 \mu \times 1.0-1.2 \mu$.

(B) Characters on various media.

- 1) Synthetic agar; 27°C.
Growth; spreading, colorless. Aerial mycelium; abundant white with dark yellowish green tinge. Reverse; yellowish brown. Soluble pigment; very slight brownish pigment.
- 2) Glucose bouillon agar, 37°C.
Growth; glossy, dark colored. Aerial mycelium; very scant, but occasionally white patch aerial mycelium with yellowish tinge. Soluble pigment; reddish brown.

- 3) Nutrient agar; 37°C.
Growth; glossy dark cream colored. Aerial mycelium; white with yellowish tinge in patches. Soluble pigment; slight brownish.
- 4) Blood agar; 37°C.
Growth; glossy, brownish. Aerial mycelium; none. Hemolysis; observed.
- 5) Loeffler's serum medium; 37°C.
Growth; light brownish colored. Aerial mycelium; scant white. Liquefaction; none.
- 6) Egg medium; 37°C.
Growth; light brownish colored. Aerial mycelium; dark white. Soluble pigment; grayish brown.
- 7) Potato plug; 27°C.
Growth; wrinkled, brownish colored. Aerial mycelium; none. Plug changed slightly to brownish.
- 8) Carrot plug, 27°C.
Growth; dark cream colored. Aerial mycelium; none. Plug unchanged.
- 9) Gelatin stab; 20°C.
Growth; brownish surface growth. Aerial mycelium; none. Deep brown pigment diffuses into unliquefied portion. Liquefaction; weak or medium.
- 10) Milk; 37°C.
Growth; thick brownish ring on surface. Aerial mycelium; gray. No coagulation and clearing at first. Coagulation occurs after 10 days' incubation. Reaction; slightly alkaline.

Table 4. Comparison of the strain 9 with *S. antibioticus* (Waksman & Woodruff).

	Description in Bergey's Det. Bact. (1948)	Description of our strain
	Spore-bearing hyphae produced in the form of straight aerial hyphae. The conidiophores are arranged in clusters. No spirals formed. The conidia are nearly spherical to somewhat elliptical.	Spore-bearing hyphae produced in the form of straight aerial hyphae. Very few spirals. Conidia spherical.
Potato plug	Folded, brown colored growth with a thin black tinge. No aerial mycelium.	Much folded, deep brown growth. A. M. none.
Carrot plug	Cream colored to faint brownish growth. No aerial mycel. No pig.	Dark cream colored growth. A. M. none. Plug unchanged.
Gelatin stab	Dark brown growth on surface with patches of gray aerial mycel. Dark pig. produced, which gradually diffuse into the unliquefied part of the gelatin. Liquefaction at first very slow, later rapid.	Brownish growth on surface. A. M. none. Deep brown pig. diffuse into the unliquefied part. Liq. weak or medium.
Litmus Milk	Thick brownish ring on surface of milk. Mouse-gray aerial mycel. with greenish tinge, growth becomes brown, especially in the portions adhering to glass. No reaction change, no coag, no clearing with whitish sediment at bottom of tube. Old cultures heavy growth ring on surface of milk, heavy precipitation on bottom and liquid brownish to black in upper portion.	Thick, brownish ring on surface. Gray aerial mycel. slightly alkaline. No coagulation. No clearing at first. Coagulation after 10 days.

11) Starch plate; 27°C.

Growth; dark transparent growth. Aerial mycelium; white with dark yellow tinge. Soluble pigment; none. Enzymatic zone; 3-4 mm in 5 days.

12) Nitrites produced from nitrates.

Comparing this description with the description in Bergey's Determinative Bacteriology (1948), this strain seems to resemble *S. antibioticus* (Waksman & Woodruff) at the points as seen in Table 4. But we find an important difference between them, that is, arranging in clusters of conidiophores.

VI. Strains 117, 260, 250, and 251 which produce streptothricin B

(A) Microscopical observation.

Straight branching filaments and hyphae, no spirals. Conidia are rod to oval $1.0 \times 1.0 \mu$. Spiral is formed on glycerine synthetic agar.

(B) Characters on various media.

1) Synthetic agar; 27°C.

Growth; spreading, colorless to dark yellowish. Aerial mycelium; thick, cottony characteristic sea-shell pink to salmon pink colored. Soluble pigment; none.

2) Glucose bouillon agar; 37°C.

Growth; glossy, cream colored. Aerial mycelium; none or characteristic colored mycelium in patches. Soluble pigment; none.

3) Nutrient agar; 37°C.

Growth; brownish yellow. Aerial mycelium; none or white patches. Soluble pigment; none.

4) Blood agar; 37°C.

Growth; dark with greenish tinge, glossy. Aerial mycelium; none. Hemolytic action; poor.

5) Loeffler's medium; 37°C.

Growth; orange colored. Aerial mycelium; none. Soluble pigment; none. Liquefaction; weak.

6) Egg medium; 37°C.

Growth; thick, wrinkled, orange colored. Aerial mycelium; white. Soluble pigment; none.

7) Starch plate; 27°C.

Growth; slightly brownish or colorless. Aerial mycelium; characteristic color. Soluble pigment; none. Enzymatic zone; 4-6 mm in 5th day.

8) Potato plug; 27°C.

Growth; wrinkled, orange colored. Aerial mycelium; cream colored. Plug unchanged.

9) Carrot plug; 27°C.

Growth; poor, cream colored. Aerial mycelium; none. Plug unchanged.

10) Gelatin stab; 20°C.

Growth; cream colored growth on the surface and bottom of the liquefied portion. Aerial mycelium; white or none. Soluble pigment; none. Liquefaction; rapid.

11) Milk; 37°C.

Growth; cream colored surface ring, Aerial mycelium; none or white patches.

Coagulation occurs within 10 days then clear up. Reaction; slightly alkaline.

12) Nitrites is produced from nitrates.

These strains are very resembling to *S. fradiae*. The characteristic color of the aerial mycelium seems to justify that they should be *S. fradiae* though they are different in the reducing effect of nitrate.

Table 5. Comparison of the strains, 117, 260, 250 and 251 with
S. fradiae (Waksman & Curtis).

	Description in Bergey's Det. Bact. (1948) ()=Waksman's description (1919)	Description of our strain
	Straight, branching filaments and hyphae. No spirals. Conidia red to oval, $0.5-0.7 \times 1.25 \mu$ (Spiral forms on glycerin synthetic agar.)	Same as the left side. Conidia $1.0 \times 1.2 \mu$.
Synthetic	Smooth, spreading, colorless growth. A.M. thick, cottony mass covering surface, sea-shell pink. (S.P. none.)	Spreading, colorless to dark yellowish growth. A.M. thick, cottony, sea-shell pink to salmon pink. S.P. none.
Glucose	Growth; restricted, glossy, buff colored, lichenoid margin. (A.M. sea-shell pink.) (S.P. none.)	Brownish yellow growth, A.M. none or white patches. S.P. none.
Potato plug	Restricted, orange colored growth. (A.M. cream colored patches.)	Wrinkled, orange colored growth. A.M. cream colored.
Gelatin stab	Cream colored to brownish, dense growth on liquid medium. (A.M. white or none. S.P. none.) Liq. +	Cream colored mass on surface and bottom of the liquefied part. A.M. white or none S.P. none. Liq. +.
Milk	Faint, cream colored ring, coagulated; peptonized, becoming alkaline.	Cream colored ring on surface. Coagulation starts in about 4-6 days. Peptonized, becoming alkaline.
Nitrates reduction	— (good, often only trace.)	+
Starch agar	Spreading colorless growth. (A.M. sea shell pink.)	Slightly brownish colorless growth. A.M. characteristic. S.P. none.
Starch hydrol.	+	+
Opt. temp.	25°C.	24°-27°C.

DISCUSSIONS

Among strains of streptomyces which produced the streptothricin-group substances, four strains, 260, 117, 250 and 251 resembled *S. fradiae* and besides produced the streptothricin B which is different from the antibiotics of other strains. These four strains, if their characters are compared with the description of *S. fradiae*, are different from the latter in their liquefaction of coagulated serum and their reduction of nitrates. However, they are similar to the latter in all other points. According to the description in Bergey's book, *S. fradiae* does not reduce nitrate to nitrite, however, in the description by Waksman in 1919 it reduces nitrates. The reduction of the nitrates does not seem to be an important difference. Also the liquefaction of the coagulated serum seems to be not so important to distinguish these strains from *S. fradiae*. It can be concluded that these strains belong to *S. fradiae*. Afterwards, two strains, O-153 and B2 were isolated and found to produce

streptothricin B. They also belong to *S. fradiae*. It is very interesting that the antibiotic of *S. fradiae* is different from the antibiotics of other species.

Among 13 strains which produced streptothricin-group A substances, 9 strains were very similar to *S. lavendulae*. Any important difference of these strains from *S. lavendulae* can not be observed. In an experiment testing the utilization of carbohydrates, as shown in Table 6, the results are almost same as those of Pridham who has shown the utilization of sugars of *S. lavendulae*. However the utilization of sugars suggests that there are some kinds in *S. lavendulae*. From strains of *S. lavendulae* more than one kind of the antibiotics such as streptothricin, lavendulin, streptothricin IV have been obtained. These antibiotics are very resembling each others and seem to belong to our streptothricin-group A. So it can be concluded that streptothricin-group A is most frequently obtained from *S. lavendulae*.

Table 6. The utilization of carbohydrates by strains belonging to *S. lavendulae*.

	201	U-33	B-3	B-10	B-18	42-B	A-6	K-4	68
Rhamnose	+	+	+	+	+	+	+	+	+
Raffinose	—	—	+	—	+	—	+	—	—
Xylose	+	+	—	+	—	—	—	+	—

The strain, 91, is similar to *S. albus* described in Bergey's book, however, if it is compared with the description of Waksman (1919), then this strain is different from the latter in producing reddish brown pigment on egg medium, changing potato to brown, weak hydrolysis of starch and the weak proteolytic action in milk. Therefore this strain may be different from *A. albus* described by Waksman in 1919. The reddish brown growth on potato has been observed in the case of *A. albus* var. *ochroleucus*. The strain 91 is most similar to *A. albus* among the species described in Bergey's book. The strain, 284, seems to be also most resembling to *A. albus*, though several characters do not conform to the latter. It is noteworthy that this strain is more resistant to streptomycin than other strains and it grows on the nutrient agar added with streptomycin at 0.05 mg/cc.

The strain, 13 A, is most similar to *S. ruber*. In the case of *S. ruber* spirals have been observed, but they are very few. So though spirals are not observed in the case of the strain 13 A, it should not be an important difference.

The strain, 9, is found to be most resembling to *S. antibioticus*. However it seems to be different from the latter in the form of spore-bearing hyphae. Besides the production of actinomycin is a specific character of this species. The strain, 9, may be a new species, though it belongs to chromogenic type and resembles *S. antibioticus*.

SUMMARY

(1) Among 13 strains which produced streptothricin-group A substances, 9 strains belonged to *S. lavendulae*, the one to *A. albus* and the one to *S. ruber*. The other two were resembling to *A. albus* and *S. antibioticus*, though they are different in some important points.

(2) Four strains which produced streptothricin B were *S. fradiae*.

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