PATHOGENICITY AND CULTURAL CHARACTERISTICS

OF

STREPTOMYCES ISOLATES ASSOCIATED WITH SWEET POTATO POX IN MARYLAND

By

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INTRODUCTION AND REVIEW OF LITERATURE

Pox or soil rot of sweet potatoes (Ipomoea batatas Poir.) has been of considerable importance in the commercial sweet potato growing areas of Maryland since the early part of the present century. Severity of this disease has apparently increased during the last few years.

There has been considerable controversy concerning the causal agent of pox since the disease was first reported. The disease was first described by Halstead (5)[1] in New Jersey in 1890. He gave it the name "soil rot" and stated that it was caused by the fungus Acrocystis batatae E. and H. Little work was done on the disease until 1916 when Elliott (4) claimed, after extensive study, that it was produced by a slime mold, Cystospora batata Ell. In 1918 Taubenhaus (17) confirmed Elliott's work and also mentioned the presence of an Actinomycete, which was described as Actinomyces poolensis Taub. He concluded, however, that the latter was a wound parasite following Cystospora batata. Manns (7) in 1924 after restaining Elliott's slides on pox material found an Actinomycete present in each case. Previous work by Manns had indicated that an Actinomycete was the causal agent. Later studies by Manns and Adams (8, 9, 10, 11) proved definitely by artificial inoculation that pox was caused by an Actinomycete, which they named Actinomyces Pox. However, the organism was not described. In 1929 Adams (1) showed conclusively that an Actinomycete, which he designated Actinomyces p., was the causal agent of pox.

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[1]Numbers in parentheses refer to literature cited.

In 1940 Person and Martin (15) described an Actinomycete causing pox in Louisiana, to which they gave the name Actinomyces ipomoea Person and Martin. When the Actinomycetes were reclassified by Waksman (20), A. ipomoea was placed in the genus Streptomyces, and, accordingly, in Bergey's Manual (3) the causative agent of sweet potato pox is given as Streptomyces ipomoea (Person and Martin) Waksman and Henrici.

Control of pox by lowering the soil pH with sulfur and acid phosphate has been reported by Harter (6) and others (15, 17). Application of sulfur for this purpose was investigated in detail by Person (14) in 1946. However, sulfur has given inconsistent results when used for pox control in Maryland and its use has in general been discontinued. This indicates that possibly different organisms or conditions have been encountered in this State than were met by other workers who had success with its use.

It has been noted by the author and other workers in Maryland during the past few years that cracking of storage roots during the growing season has been associated with pox. The actual cause of cracking is not known. According to Harter (6), it is probably connected in some way with soil moisture. Nutrition and other factors in conjunction with injuries have been considered to be important in the cracking problem.

This research was undertaken because of the lack of adequate and recent studies on the causal organism; lack of information on the pox situation in Maryland, and the need for better control measures. The work was carried out with the following aims in view: (1) to isolate the causal organism or orgamisms, (2) to determine some of the pathological, physiological, morphological, and cultural characteristics of the isolated organisms, (3) to determine, if possible, any relation of pox to cracking, and (4) to show in any way why pox control with the use of sulfur has not been successful in Maryland.

MATERIALS AND METHODS

Sweet potato plants used in all experiments were of the Maryland Golden variety[2] and in every case were vine cuttings bearing no adventitious roots at the time of planting.

The four pathogenic organisms used in all experiments were designated SP 12, SL 2, SL 5, and La. SP 12 was isolated from an infected sweet potato. SL 2 and SL 5 were isolated from infested soil. Methods used will be discussed under Isolations. The organism designated La, a culture of *Streptomyces ipomoea* (Person and Martin) Waksman and Henrici, was obtained from Dr. W. J. Martin, Louisiana State University, Baton Rouge, Louisiana, and in all experiments was used in comparison with the other three isolates.

To determine the pathogenicity of isolates obtained, a screening technique devised by Person and Martin (15) was used. Since the results were not always reproducible, a second laboratory method was devised by the author to recheck isolates that gave positive results under the first test. Isolates that were shown to be pathogenic in the laboratory were then tested in the greenhouse. Details of the above methods will be discussed under Pathogenicity of Isolates.

To learn some of the physiological characteristics of the pathogenic isolates, tests were conducted to determine the effects of temperature, hydrogen ion concentration, and carbon source. Cultural characteristics on some common synthetic and artificial media were also observed.

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[2]A strain of Big Stem Jersey variety introduced by T. H. White, University of Maryland, 1932.

In temperature experiments, isolates were cultured on "Difco" potato dextrose agar and incubated in constant temperature units. Temperatures at which studies were made were 50°, 60°, 70°, 80°, 90°, 100°, 110°, and 120°F.

In determining pH requirements, isolates were grown in liquid shake culture using sweet potato extract medium[3] which had been adjusted, with either 0.1 N solution of KOH or HCl, using a Beckman inline pH meter, to the following pH readings: 6.2, 6.0, 5.8, 5.3, 4.9, and 4.5. Cultures were grown at room temperature (about 75°-80°F.) for one week, filtered, dried, and weighed on an analytical balance. After passage through Whatman #1 filter paper, pH of the filtrates was determined.

To determine the effect of carbon source on growth, isolates were grown in Wellman's differential agar[4] in which eight different carbohydrates were substituted. In preparation of the media, samples of the non-reducing carbohydrates were removed after autoclaving and tested with Benedict's solution to show that partial hydrolysis did not occur during the sterilization process. Cultures were incubated at 90°F. and results recorded after one week.

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[3]Sweet potato extract medium was made by autoclaving 200 grams of peeled, diced sweet potatoes for 45 minutes; the extracted juice was filtered and used in the preparation of one liter of medium.

[4]One liter of Wellman's differential agar contained: proteose peptone, 5 grams; dipotassium phosphate, 0.5 gram; magnesium sulfate, 0.5 gram; glucose, 20 grams; ferrous sulfate, 0.03 gram; agar, 20 grams; water, 1 liter. Medium was adjusted to neutrality.

EXPERIMENTS AND RESULTS

Isolations

Isolations were made from "poxed" sweet potatoes and from "pox-infested" soil.

In the first method of isolation diseased sweet potatoes used were obtained from ten different areas of Maryland. The technique employed was as follows: small portions of roots taken from diseased areas were surface sterilized with 2.5 percent sodium hypochlorite (2 - 3 minutes); the pieces were then placed in tubes containing 15 ml. of unsolidified sterile soil extract agar[5], ground up with a sterile glass rod, plated in sterile petri dishes, and incubated for one week at 90°F. Isolates obtained from sweet potatoes were designated SP, and numbered serially from 1 to 15. Of this series only three isolates, SP 3, SP 12, and SP 15, were identified as Streptomyces.

Due to difficulty in isolating Streptomyces from sweet potato tissue, a second method, using soil, was employed which yielded a high percentage of this genus.[6] Soil samples were obtained from six pox-infested fields in the sweet potato growing areas of Maryland. Two grams of soil were taken from each sample and mixed with ten ml. of water. Dilutions of 1/10,000 were made and one ml. plated out in petri dishes containing soil extract agar. Isolates from soil appearing to be Streptomyces were

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[5]Soil extract agar was made by mixing 20 grams of soil with one liter of water. After settling, the supernatent was used in the preparation of two percent agar medium.

[6]This technique was obtained by letter from Mr. C. H. Meredith, Jamaica, B. W. I., who has done considerable work with soil Actinomycetes.

designated SL, and were also numbered serially.

A total of 35 Streptomyces isolates were obtained by the two methods. Origin of these isolates is shown in Table I. All isolates were transferred to and maintained on potato dextrose agar at 90°F.

Table I. Origin of Streptomyces Isolates.

Isolate Number	County of Maryland	City of Maryland [a]
	From infected sweet potatoes	
SP 3	Wicomico	Salisbury
SP 12	Wicomico	Salisbury
SP 15	Prince George's	Beltsville
	From infested soil	
SL 1 - 2	Wicomico	Salisbury
SL 3 - 6	Dorchester	Hurlock
SL 7 - 8	Wicomico	Salisbury
SL 9 - 27	Prince George's	Clinton
SL 28 - 32	Wicomico	Salisbury

[a] Vicinity of city listed.

Pathogenicity of Isolates

To determine pathogenicity of isolates obtained, a screening technique devised by Person and Martin (15) was used. Small sections of sweet potato stem containing one or two nodes were surface sterilized with 2.5 percent sodium hypochlorite and placed in petri dishes containing sterile two percent water agar, made by suspending 20 grams of agar in one liter of distilled water. These were incubated at 90°F. After several days adventitious roots, of one to two inches in length, were produced along the stems. Discs were then cut from petri dish cultures of isolates to be tested and placed on the roots (Figure 1). The plates were then incubated at 90°F. for one week and disease production was then

observed. Positive results were indicated by occurrence of lesions or discoloration. Fifteen of the 35 isolates showed some degree of pathogenicity. These were SP 12, SL 2, SL 3, SL 5, SL 7, SL 9, SL 13, SL 17, SL 21, SL 23, SL 24, SL 27, SL 29, SL 31, and SL 32. A second method was devised to recheck isolates that gave positive results with the first technique.

In this method all isolates were grown in 125-ml. Erlenmeyer flasks, in a culture-shaking apparatus, using liquid sweet potato extract medium. After one week, the resulting growth was centrifuged at approximately 3,000 r.p.m., washed three times with sterile water, and replaced in autoclaved 125-ml. Erlenmeyer flasks with 50 ml. of sterile water. Into each of these flasks was placed a sweet potato cutting, six to eight inches long, with the lower leaves removed. The lower part of the stem had previously been treated with 2.5 percent sodium hypochlorite (2 - 3 minutes). A collar of absorbent cotton was placed at the mouth of the flask to hold the plant secure (Figure 2). These flasks were replaced on the shaking apparatus to insure good aeration of the liquid. All tests were replicated four times and compared with plants growing in La inoculum and check plants growing in tap water. After one week three isolates, SP 12, SL 2, and SL 5, were found to be pathogenic to sweet potatoes under these conditions. Symptoms produced by these isolates are shown in Figures 3, 4, and 5, respectively. Figure 6 shows symptoms produced by S. ipomoea. All other isolates appeared to be non-pathogenic. Figure 7 illustrates growth of sweet potato roots in a water suspension of a non-pathogenic isolate, SL 3. Clean white roots with slightly darkened tips, a natural condition, were produced.

To determine pathogenicity under soil conditions, a greenhouse experiment was set up using SP 12, SL 2, and SL 5 in comparison with

La. Sweet potato vine cuttings were planted in six-inch pots containing sandy soil obtained from an area on the University of Maryland research farm, near Beltsville, which had not been planted in sweet potatoes for at least five years. The plants were allowed to grow for one month, at which time they were carefully removed and the roots washed and dipped into a suspension containing macerated mycelium and spores of the isolate to be tested. Check plants were dipped into tap water. All plants were repotted and randomized in five replications. Each replication contained ten plants of each treatment and six check plants.

At the end of one month, one-half of the plants were removed. The roots were washed and lesions, of macroscopic size, on each plant were counted. As shown in Table II, the plants were divided into four infection groups according to the severity of infection, as determined by the number of lesions evident.

Table II. Pathogenicity of *Streptomyces* isolates to feeding roots of sweet potatoes grown in soil.

Inoculum used in treatment	Total number of plants	Degree of Infection[a]				Percentage infection
		Severe	Moderate	Slight	None	
La	25	1	2	20	2	92
SP 12	24[b]	1	2	20	1	96
SL 2	25	5	15	5	0	100
SL 5	25	6	10	9	0	100
Check (In tap water)	15	0	1	7	7	53

[a]Severe - more than 25 lesions evident
Moderate - 10 to 25 lesions evident
Slight - 1 to 10 lesions evident
None - no lesions evident

[b]One plant died before data were obtained.

A few lesions were found on approximately one-half of the check plants. This can probably be explained by the fact that the soil had not been previously sterilized and may have been naturally infested. The percentage of infection was considerably higher on inoculated plants than on check plants. Plants inoculated with SL 2 and SL 5 appeared to be more severely infected than plants inoculated with the other isolates.

After two months the remainder of the plants were dug and examined. Due to the extensiveness of some of the root systems, lesions were counted only on storage roots. Results (Table III) show the number of lesions found on small storage roots (approximately two centimeters in diameter).

Table III. Pathogenicity of Streptomyces isolates to sweet potatoes grown in soil.

Inoculum used for treatment	Number of storage roots	Total number of lesions	Average number lesions per root	Number of roots showing cracking	Percent cracking
La	30	221	7.4	12	40
SP 12	29	170	5.9	13	45
SL 2	29	192	6.6	14	48
SL 5	28	169	6.0	10	36
Check (Tap water)	15	11	0.7	0	0

Again a few lesions were found on check plants, but the number was extremely small in comparison with the number of lesions on roots that had been inoculated. Symptoms produced by SP 12, SL 2, SL 5, and La are illustrated in Figures 8 through 11. Lesions were, in general, eliptical in shape and were dark brown to black in color (Figure 13). Some lesions

showed healing accompanied by the sloughing off of necrotic tissue which is associated with pox lesions found in the field. Figure 12 illustrates the clean healthy appearance of the check roots. Rows of spots that can be seen on these roots are scars left by branch roots. Forty-two percent of the inoculated roots showed cracking of the type typical of field symptoms, in contrast to no cracking of any check roots. Cracks on many roots were apparently initiated by pox lesions (Figure 14).

Cultural and Physiological Characteristics of Isolates

Isolates were grown on various types of media to determine some of their cultural characteristics. Cultures were grown for two weeks in petri dishes at 90°F., using the following eight different agar media: lima bean[7], yeast dextrose[7], potato dextrose[7], sweet potato[8], corn meal[7], Czapek's[9], Wellman's[4] differential, and tyrosine[10]. Growth characteristics for SP 12, SL 2, SL 5, and La are given in Table IV. These data represent the mean from either three or four different experiments.

In general SL 5 had a more rapid growth rate on most media than other isolates tested. It was the only culture to show any appreciable growth on Czapek's medium. A more abundant production of aerial mycelium on most media was observed for SL 5 than for the other three organisms.

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[7]"Difco" media.

[8]Made from sweet potato extract by the addition of two percent agar.

[9]One liter of Czapek's agar contained: sodium nitrate, 2 grams; dipotassium phosphate, 1 gram; potassium chloride, 0.5 gram; magnesium sulfate, 0.5 gram; ferrous sulfate, 0.01 gram; sucrose, 30 grams; water, 1 liter; agar, 20 grams.

[10]One liter of tyrosine medium contained: glucose, 10 grams; tyrosine, 1 gram; ammonium sulfate, 0.5 gram; dipotassium phosphate, 0.5 gram; agar, 20 grams; water, 1 liter. Medium was adjusted to pH 7 with sodium hydroxide.

Table IV. Cultural characteristics of *Streptomyces* isolates grown on various types of media.

Culture medium	Isolate	Growth[a] type	Radius of colony (mm)	Aerial[b] mycelium growth	Soluble[b] pigment
Yeast Dextrose Agar	SP 12	W	4	O	O
	SL 2	W	5	O	O
	SL 5	W	6	++	++
	La	W	5	O	O
Potato Dextrose Agar	SP 12	S	4	++	O
	SL 2	S	4	+	O
	SL 5	S	8	+++	O
	La	S	9	++	O
Wellman's Differential Agar	SP 12	W	8	O	O
	SL 2	W	5	O	O
	SL 5	W	6	O	O
	La	W	8	T	O
Tyrosine Agar	SP 12	R	1	O	O
	SL 2	R	1	O	O
	SL 5	R	1	O	O
	La	R	1	O	O
Lima Bean Agar	SP 12	F	5	O	O
	SL 2	W	5	O	O
	SL 5	S	8	+	O
	La	S	7	+++	O
Sweet Potato Agar	SP 12	W	4	+	+
	SL 2	W	5	+	+
	SL 5	W	9	+++	O
	La	S	4	O	T
Corn Meal Agar	SP 12	S	4	+	O
	SL 2	S	4	+	O
	SL 5	S	8	+	O
	La	S	8	++	O
Czapek's Agar	SP 12	-	O[c]	O	O
	SL 2	-	O[c]	O	O
	SL 5	S	5	++	O
	La	-	O[c]	O	O

[a]Type of growth: F, Folded; W, Wrinkled; S, Smooth; R, Rough

[b]Key: O, none; T, trace; +, slight; ++, moderate; +++, abundant

[c]No growth at the end of two weeks. Slight growth at the end of one month.

Appearance of cultures grown for two weeks on potato dextrose, lima bean, sweet potato, corn meal, and yeast dextrose agars are shown in Figures 15 through 19.

To determine the effect of temperature on growth, experiments were conducted as previously described. Cultures were grown for two weeks on potato dextrose agar, and measurements were made of colony radius and amount of aerial mycelium. These data are summarized in Table V.

Table V. Relation of temperature to growth of *Streptomyces* isolates in culture.

Temp. °F.	Isolates							
	SP 12		SL 2		SL 5		La	
	Radius of colony (mm)	Aerial mycelium growth	Radius of colony (mm)	Aerial mycelium growth	Radius of colony (mm)	Aerial mycelium growth	Radius of colony (mm)	Aerial mycelium growth
50°	0	0	0	0	0	0	0	0
60°	T	0	T	0	3	++	T	T
70°	1.5	0	1.5	0	4	+++	2	+
80°	3	++	3	T	6	+++	3	++
90°	4	++	4.5	++	8	+++	4	++
100°	5	0	5	T	3	0	9	T
110°	3	0	2.5	0	0	0	5	0
120°	0	0	0	0	0	0	0	0

Key: 0, none; T, trace; +, slight; ++, moderate; +++, abundant

The range of growth for SP 12, SL 2, and La extended from 60° to 110°F. with the optimum approximately 90°F. Person and Martin (15) reported optimum growth of La to be approximately 90°F. SL 5, on the other hand, had a growth range from 60° to 100°F. with the optimum essentially

the same as the other isolates at 90°F. SL 5 grew better than the other isolates at temperatures below optimum although the minimum was essentially the same. In determining the point of optimum growth, two criteria were taken into consideration: (1) maximum colony growth, and (2) maximum production of aerial mycelium. These did not necessarily occur at the same temperature; for example, La produced the largest colony at 100°F. but most aerial mycelium at 80° to 90°F. Comparative growths of SP 12, SL 2, SL 5, and La at the various temperatures used are illustrated in Figures 20 and 21.

Effect of hydrogen ion concentration on growth was determined as previously described. Results (Table VI) show that La, SP 12, and SL 2 have a higher pH requirement than SL 5 and that they, unlike SL 5, do not produce favorable growth at low pH values. Upon statistical analysis of the data, it was found that reduction of growth at the lower pH values was statistically significant for SP 12, SL 2, and La but not for SL 5.

Table VI. Effect of pH on growth of Streptomyces isolates grown in sweet potato extract medium.

pH	La		SP 12		SL 2		SL 5	
	Mean yield in mg.	Final pH of medium	Mean yield in mg.	Final pH of medium	Mean yield in mg.	Final pH of medium	Mean yield in mg.	Final pH of medium
6.2	62.4	8.0	23.5	8.5	48.9	8.6	32.3	4.8
6.0	61.4	8.0	29.6	8.5	53.0	8.6	28.3	4.8
5.8	74.1	7.8	21.8	8.4	50.2	8.5	30.9	4.8
5.3	66.7	7.6	32.1	8.2	38.5	8.4	30.2	4.7
4.9	28.8	5.8	3.9	6.0	20.8	5.4	29.8	4.7
4.5	12.6	4.6	4.3	4.5	17.6	4.5	34.9	4.7

Least difference between means required for significance:

At 5% point	14.6
At 1% point	19.6

Observations made during the period of growth showed that SL 5 grew more slowly at the lower pH values at first, but after one week there was no apparent difference. An examination of the media after growth showed that La, SP 12, and SL 2 raised the pH value in every case where any appreciable growth occurred. SL 5, on the other hand, changed all samples to approximately 4.7, indicating that the metabolic products of growth from SL 5 were acid in contrast to the alkaline products produced by the other isolates.

It was observed that when all four isolates were grown on sweet potato extract more rapid growth occurred on the plain extract than when two percent glucose was added. Plain sweet potato extract medium became more discolored and, upon examination after growth, the medium containing glucose was more acid in every case, as shown in Table VII.

Table VII. Growth of isolates as affected by addition of glucose to sweet potato extract medium.

Isolate	Relative amount of growth[a]		Color of medium after growth		pH of medium after growth	
	With Glucose	Without Glucose	With Glucose	Without Glucose	With Glucose	Without Glucose
SP 12	+	+++	Tan	Greenish Brown	7.3	7.9
SL 2	++	+++	Tan	Greenish Brown	7.8	8.2
SL 5	+++	++++	Tan	Amber	4.0	4.3
La	++	++++	Tan	Amber	4.3	8.1

[a]Key: +, trace; ++, slight; +++, moderate; ++++, abundant

These observations led to the next experiment in which eight different carbohydrates (glucose, levulose, maltose, lactose, raffinose, dextrin, and starch) were substituted in Wellman's differential agar and isolates

cultured thereon as previously described. Effect of carbon source on growth of isolates is shown in Table VIII. Some growth occurred on all substrates. Maltose in general produced best growth, as is illustrated by the fact that aerial mycelium was produced by SP 12 and SL 2 on this medium only. La produced more aerial growth on this substrate than any other. Maximum growth of SL 5 also occurred on maltose. Pigmentation of the medium occurred only with SL 5 on disaccharides, trisaccharides, and polysaccharides, and not on monosaccharides. Aerial mycelium was produced by SL 5 on all media except glucose. Hydrolysis of dextrin and starch occurred with all four isolates but at a greater rate with La and SL 5 (Figure 26). Appearance of cultures at one week is shown in Figures 22 through 25.

Identification of Isolates

Identification and classification of the pathogenic isolates were attempted, but without complete success. All were placed under the genus *Streptomyces*, according to Bergey's Manual (3) and Waksman's classification (20). Specific nomenclature was not attempted. All isolates produced spores eliptical in shape which were borne in spiral chains when grown on potato dextrose agar medium.

Cultures of SP 12, SL 2, and SL 5 were sent to Dr. Selman A. Waksman, of Rutgers University, for identification. After examination, he stated that "Two of these isolates, SL 2 and SP 12 appear to be similar to *S. ipomoea*. SL 5, however, appears to be quite different."

Tyrosinase reaction was negative for all three isolates, thus differentiating them from *Streptomyces scabies* (Thaxt.) Waksman and Henrici, the causative agent of white potato scab. According to Bergey's Manual, the latter is the only other *Streptomyces* species named that is known to be a plant pathogen.

Table VIII. Effect of carbohydrate source on growth of *Streptomyces* isolates.

Carbon source	Radius of colony (mm)	Aerial[a] mycelium	Soluble[a] pigments	Appearance of[b] colony		Hydrolysis in advance of growth (mm)
			SL 2			
Glucose	3.0	0	0	Dark cream	Tr	-
Levulose	1.5	0	0	Dark cream	Tr	-
Maltose	3.0	+	0	Tan	Tr	-
Sucrose	3.0	0	0	Dark cream	Tr	-
Lactose	2.0	0	0	Dark cream	Tr	-
Raffinose	2.0	0	0	Light cream	Tr	-
Dextrin	2.0	0	0	Light cream	Tr	3
Starch	2.0	0	0	Light cream	Tr	2
			SP 12			
Glucose	5.0	0	0	Yellow cream	Tr	-
Levulose	3.0	0	0	Yellow cream	Tr	-
Maltose	3.0	+	0	Yellow cream	Tr	-
Sucrose	3.0	0	0	Bright yellow	Tr	-
Lactose	4.0	0	0	Yellow cream	Tr	-
Raffinose	2.0	0	0	Cream	Tr	-
Dextrin	5.0	0	0	Yellow cream	Tr	3
Starch	2.0	0	0	Yellow cream	Tr	4
			SL 5			
Glucose	4.0	0	0	Tan	Op	-
Levulose	5.0	+++	0	Gray white	Op	-
Maltose	10.0	+++	+++	Silver gray	Op	-
Sucrose	6.0	+	++	Silver gray	Op	-
Lactose	4.0	++	T	Silver white	Op	-
Raffinose	3.0	+	+	Gray white	Tr	-
Dextrin	5.0	+++	++	Silver white	Op	7
Starch	5.0	++	+++	Gray white	Op	11
			La			
Glucose	5.0	T	0	Light cream	Tr	-
Levulose	4.0	T	0	Light cream	Tr	-
Maltose	5.0	+	0	Gray tan	Tr	-
Sucrose	6.0	0	0	Yellow cream	Tr	-
Lactose	4.0	T	0	Light cream	Tr	-
Raffinose	4.0	0	0	Light cream	Tr	-
Dextrin	5.0	0	0	Light cream	Tr	7
Starch	6.0	T	0	Light cream	Tr	14

[a]Key: 0, none; T, trace; +, slight; ++, moderate; +++, abundant

[b]Appearance of colony: Tr, translucent; Op, opaque

DISCUSSION

Results of the work reported in this paper indicate that three different Streptomyces isolated in Maryland will, upon inoculation of healthy sweet potato roots, produce symptoms similar and comparable with those caused by La (S. ipomoea), the reported causative agent of pox.

Information obtained from cultural and physiological experiments and from Dr. Waksman indicates that SL 2 and SP 12 are in some way related to each other and to La. These may have arisen as mutations from S. ipomoea or together with this species may have originated from some common source. Variants, as evidenced through sectoring, occur quite frequently when culturing Streptomyces. These are illustrated in Figure 15, in which several sectors differing macroscopically have arisen from a colony of La. SP 12 and SL 2, while differing slightly from La in appearance, may in fact be strains of the same organism. SL 5 due to its radical differences from the other isolates and from La, with respect to cultural and physiological properties, is not closely related and may represent a separate species. However, more work of a cultural and morphological nature would be required before SL 5 could be described and named as a new species.

Much research has been conducted on a closely related organism, Streptomyces scabies (Actinomyces scabies), the cause of white potato scab. Varying pathological and cultural characteristics have been reported (2, 19). Millard and Burr (12), in 1926, reported different species of Actinomyces causing scab. According to Skinner, Emmons, and Tsuchiya (16), there are several species now known to cause scab; the best known species, S. scabies, is sometimes used as a "catch-all" term for all the potato scab

Actinomycetes. With so many forms and strains reported causing scab, it seems quite possible that S. ipomoea, an organism similar to S. scabies, may also have many different strains or forms which are pathogenic to sweet potatoes.

Cracking of storage roots during growth has long been known under field conditions. In Maryland cracking is frequently found wherever pox is prevalent. Moore (13) in other experiments has shown that there was significant corrolation between pox and cracking in field plots in 1948, 1949, and 1950. It is possible that cracking is induced by pox although it may occur in the absence of this disease. It is the opinion of the author that cracks are a symptom of pox which is expressed under certain environmental conditions, which promote abnormal growth of internal tissues and periderm. In many cases in the field, cracks have been observed to exist between what appeared to be two pox lesions, and in other cases on either side of one apparent pox lesion. Also, cracking commonly appears to originate directly from a pox lesion (Figure 27). Isolate SP 12 was isolated from a root on which pox and cracking were both present. Cracking is a condition not previously described as a symptom of pox or soil rot.

The fact that SL 5 grows well at pH values below 5.3 may be a factor in explaining why control of pox by acidifying the soil with the use of sulfur has not been successful when tried in certain areas of Maryland. Distribution and abundance of SL 5 in the microflora of sweet potato-growing areas of Maryland and elsewhere is not known at this time. However, it was shown that SL 5 will produce symptoms indistinguishable from pox and will grow in culture at pH values below that which will support the growth of the organism reported as the cause of pox.

SUMMARY

Three *Streptomyces* isolates, designated as SP 12, SL 2, and SL 5, were selected from isolates obtained from "poxed" sweet potatoes and from "pox-infested" soil. These isolates were shown to be pathogenic to sweet potatoes in both laboratory and greenhouse tests. Symptoms produced on storage roots in the greenhouse were similar and comparable with those produced by *Streptomyces ipomoea*.

When grown on various common laboratory culture media, SP 12, SL 2, and SL 5 differed in their growth characteristics from each other and *S. ipomoea*. With respect to physiological characteristics, SP 12 and SL 2 were quite similar to each other and to *S. ipomoea*. SL 5, however, differed radically in the following respects: (1) more rapid growth rate on most media, (2) more abundant production of aerial mycelium, (3) more abundant production of soluble pigments, (4) more rapid growth rate at temperatures below the optimum, (5) good growth at pH values of 4.5 to 5 in contrast to little or no growth of the other isolates below pH 5.3.

In greenhouse experiments cracking occurred on 42 percent of the sweet potatoes produced by inoculated plants. No cracking occurred on uninoculated check plants. It is considered that cracking of storage roots is a symptom of pox expressed under certain environmental conditions.

The occurrence of SL 5 in certain soils in Maryland may be a factor in explaining why control of pox by use of sulfur to lower the soil pH has not proved to be satisfactory.

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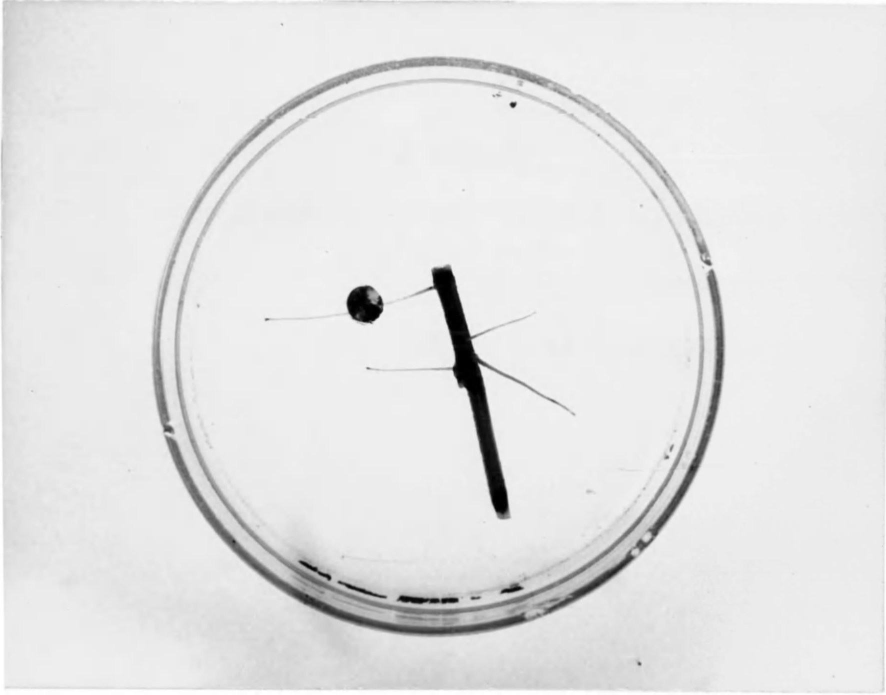
Figure 1

Screening technique, devised by Person and Martin, used to determine pathogenicity of *Streptomyces* isolated. Note disc from culture placed on adventitious root.

Figure 2

Method devised by the author to further test isolates that showed some degree of pathogenicity by technique shown in Figure 1.



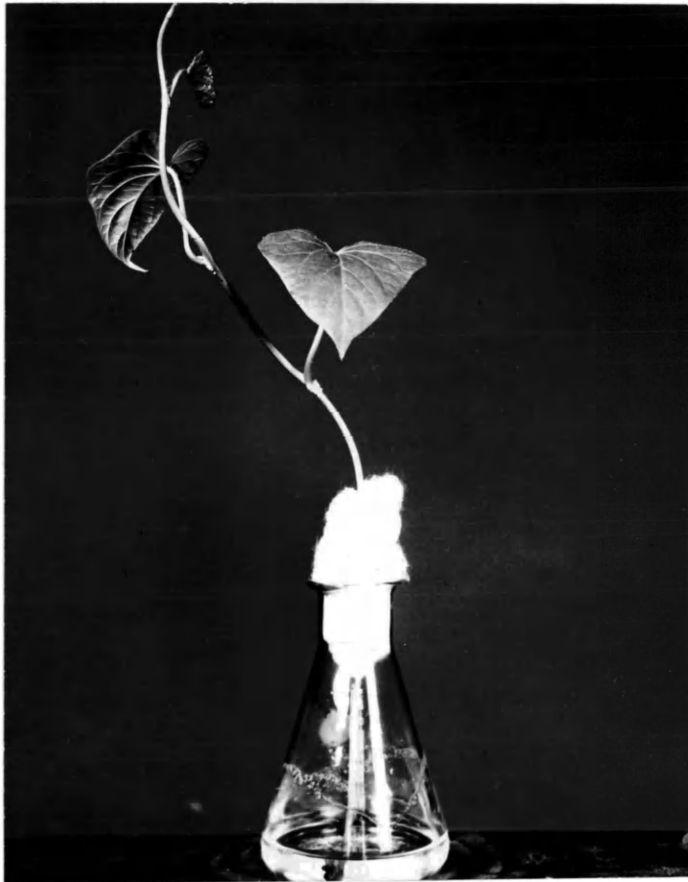
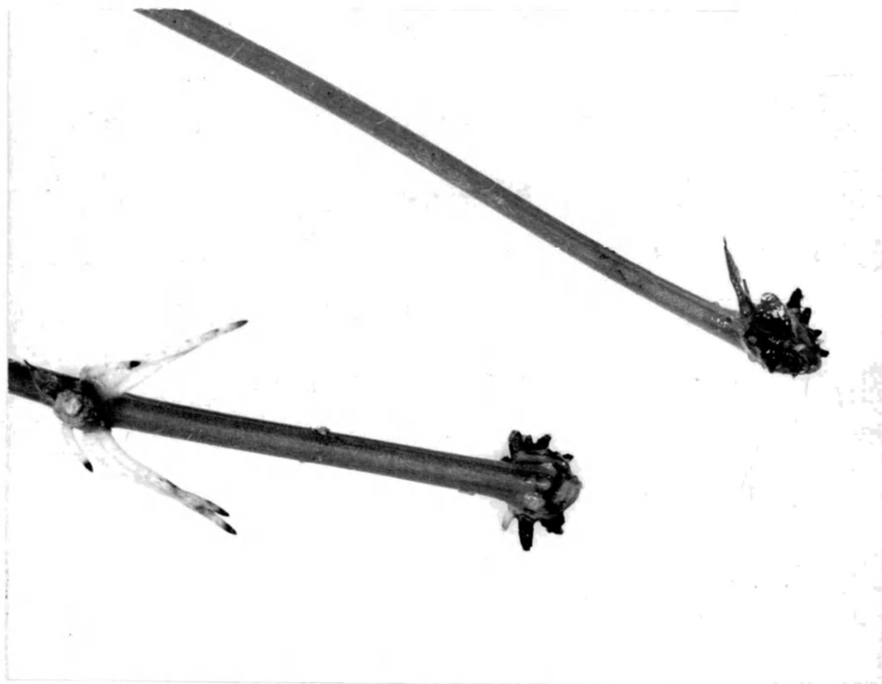


Figure 3

Appearance of adventitious roots produced on sweet potato cuttings immersed in a water suspension of SP 12. Note black, stunted condition of the roots produced.

Figure 4

Appearance of adventitious roots produced on sweet potato cuttings immersed in a water suspension of SL 2. Note black, stunted condition of the roots produced.



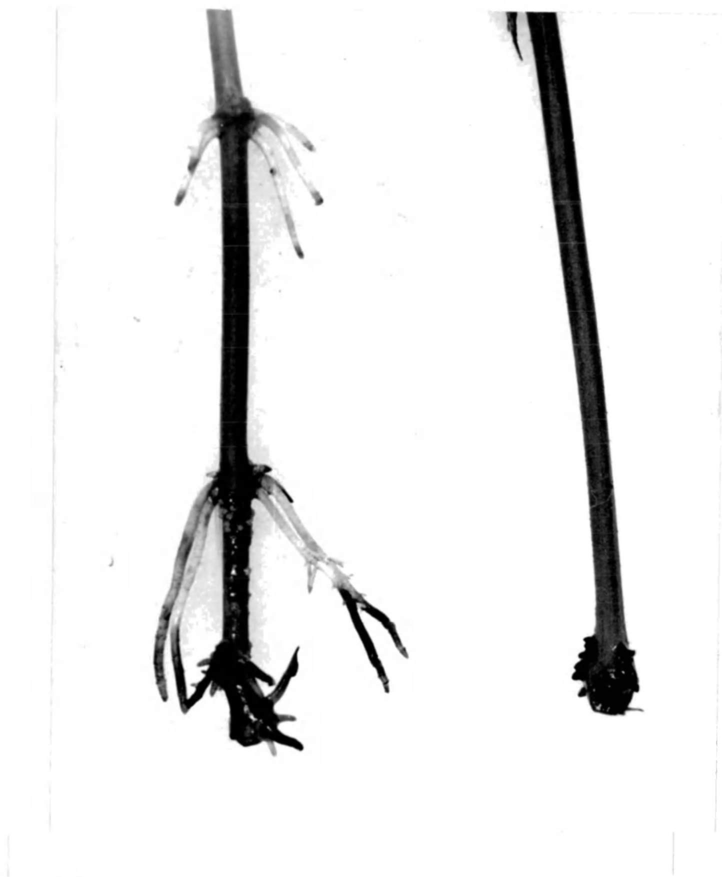


Figure 5

Appearance of adventitious roots produced on sweet potato cuttings immersed in a water suspension of SL 5. Note lesions and darkened areas on the roots produced.

Figure 6

Appearance of adventitious roots produced on sweet potato cuttings immersed in a water suspension of La. Note black, stunted condition of roots produced.

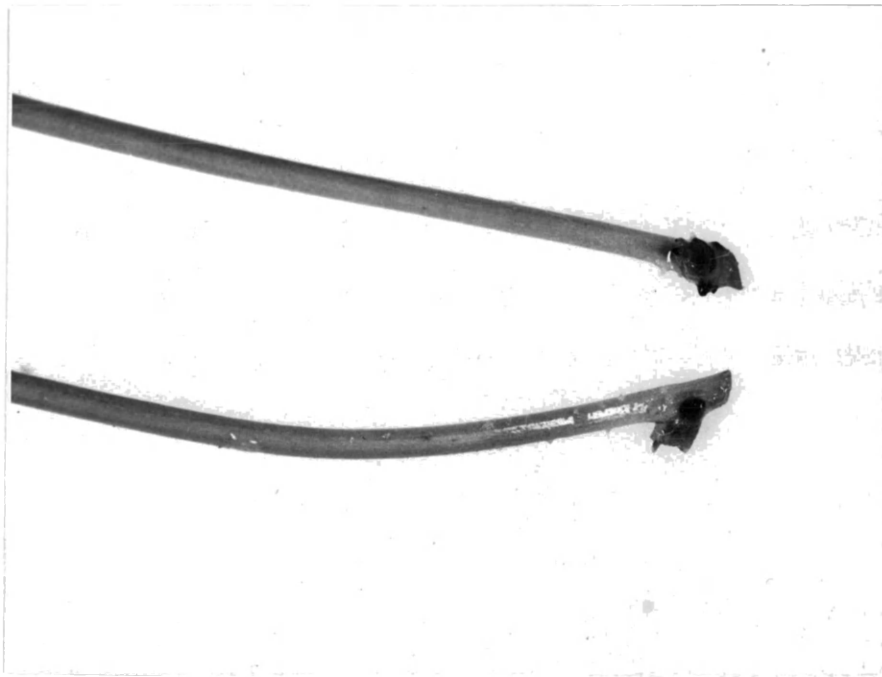


Figure 7

Appearance of adventitious roots produced on sweet potato cuttings immersed in a water suspension of a non-pathogenic isolate, SL 3. Note clean white appearance of the roots. Dark tips are the natural appearance of the root caps of this variety.

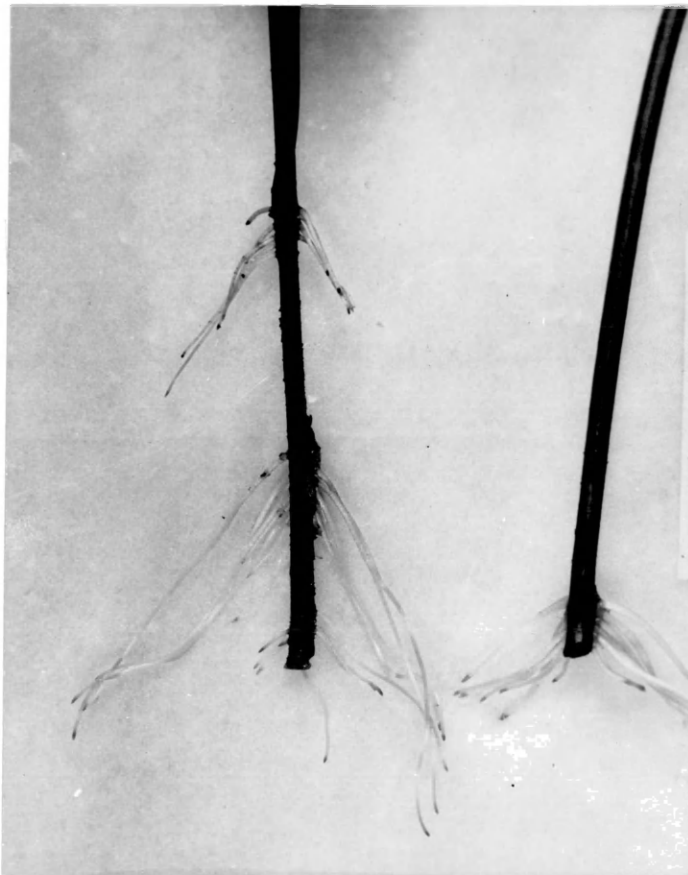


Figure 8

Appearance of storage roots grown in soil for two months after inoculation with a water suspension of SP 12. Note healing of some lesions.

Figure 9

Appearance of storage roots grown in soil for two months after inoculation with a water suspension of SL 2. Note healing of some lesions.

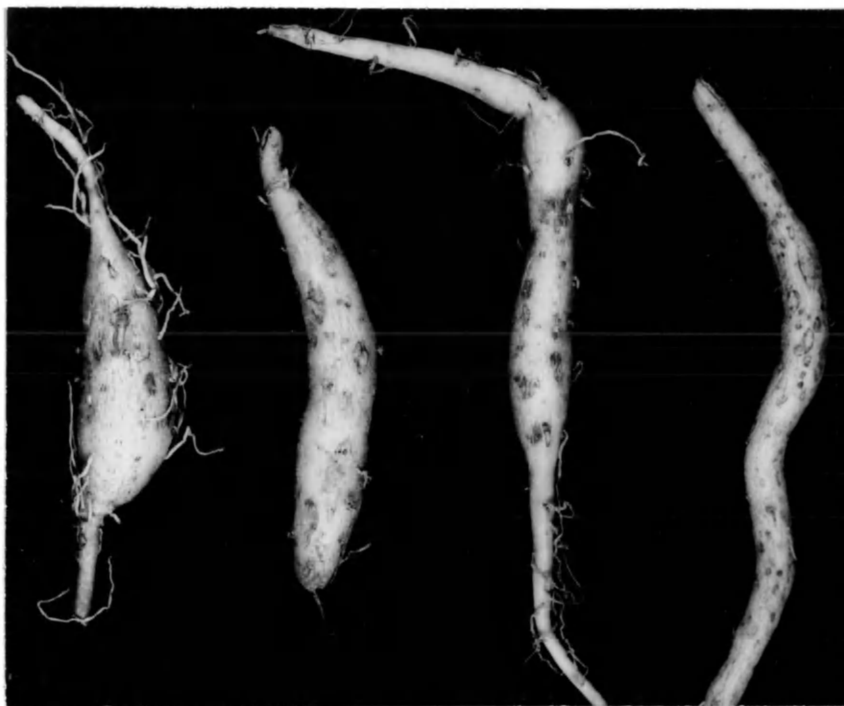


Figure 10

Appearance of storage roots grown in soil for two months after inoculation with a water suspension of SL 5. Note healing of some lesions.

Figure 11

Appearance of storage roots grown in soil for two months after inoculation with a water suspension of La. Note healing of some lesions.

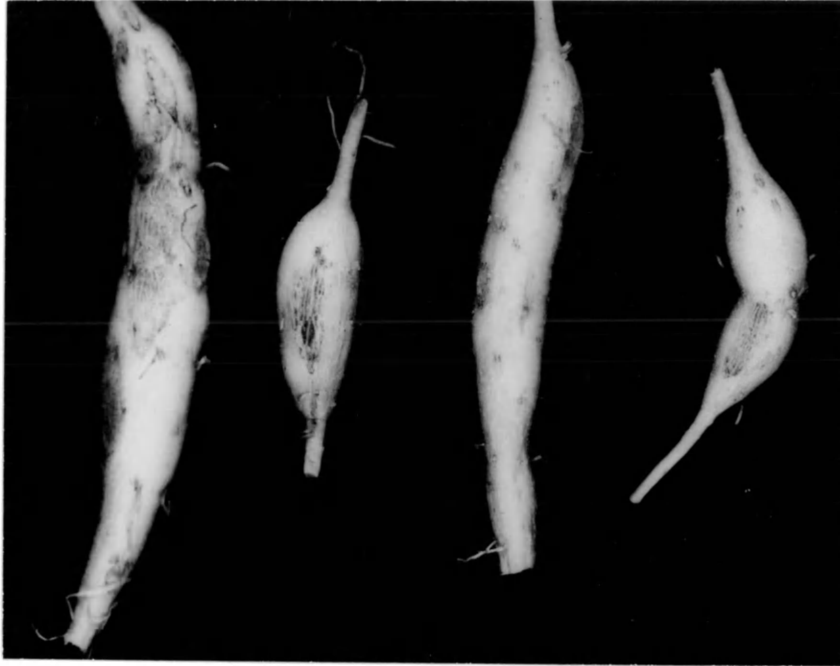


Figure 12

Appearance of uninoculated roots grown for two months in soil. The rows of spots are scars left from branch roots.

Figure 13

Close-up of an infected root showing elliptical shape and depressed condition of pox lesions.

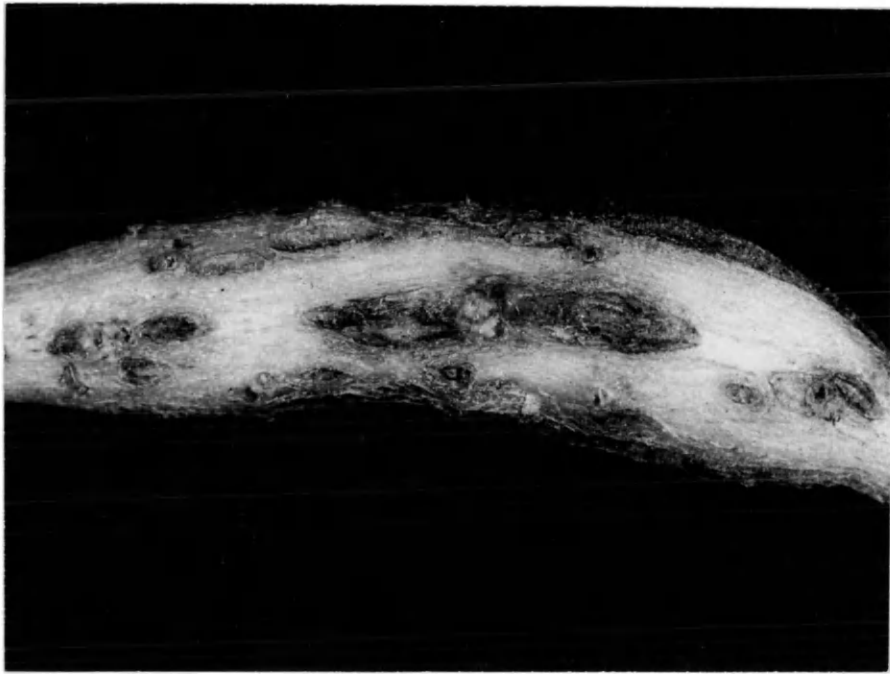


Figure 14

Cracking occurring on artificially inoculated roots. Note various stages of cracking on the different roots. No cracking occurred on the check roots shown at left. The small dark spots are scars left from branch roots.

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Figure 15

Two-week old cultures of SP 12, SL 2, SL 5, and La on potato dextrose agar.

(Cultures appear from left to right in order given.)

Figure 16

Two-week old cultures of SP 12, SL 2, SL 5, and La on lima bean agar.

Figure 17

Two-week old cultures of SP 12, SL 2, SL 5, and La on sweet potato agar.

Figure 18

Two-week old cultures of SP 12, SL 2, SL 5, and La on corn meal agar.

Figure 19

Two-week old cultures of SP 12, SL 2, SL 5, and La on yeast dextrose agar.

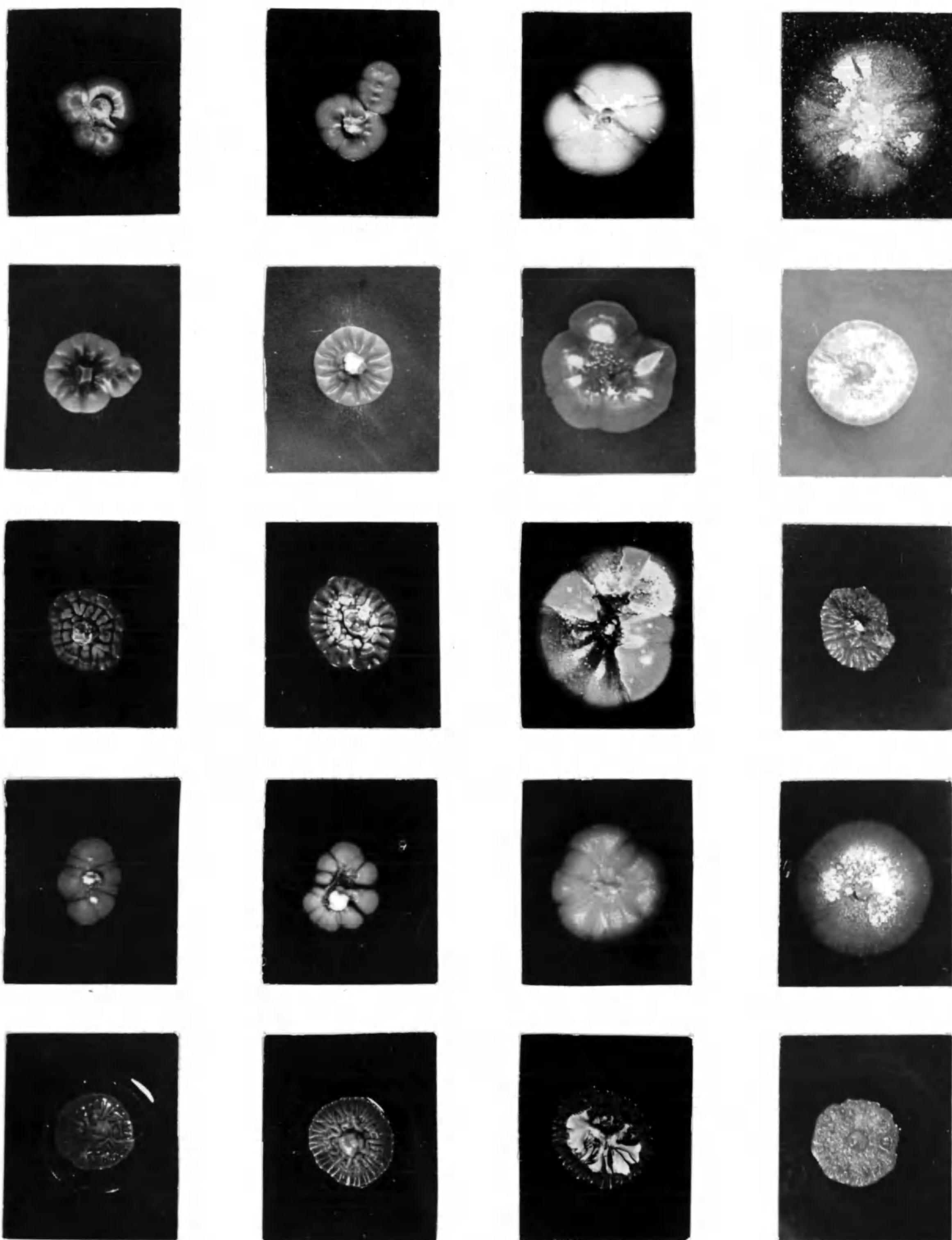
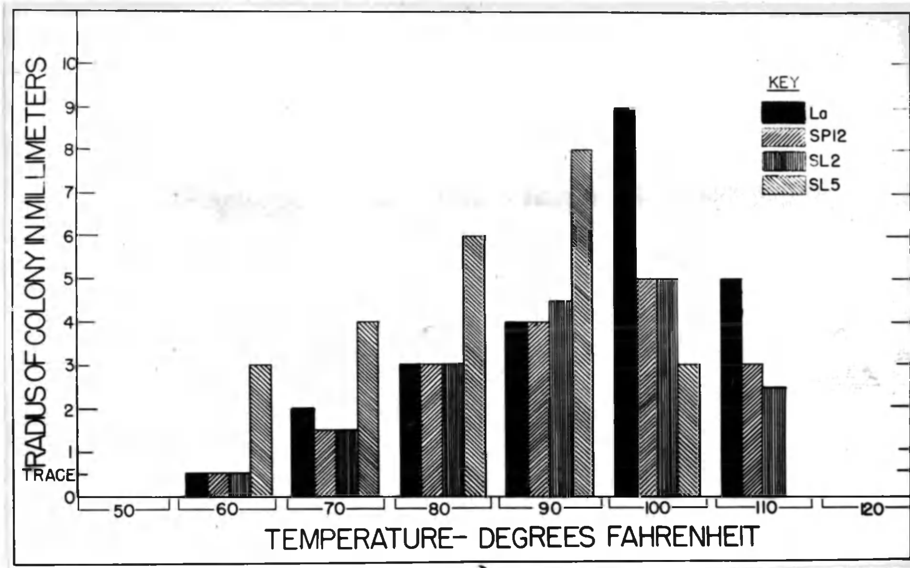


Figure 20

Effect of temperature on colony size of *Streptomyces* isolates SP 12, SL 2, SL 5, and La when grown for two weeks on potato dextrose agar.

Figure 21

Effect of temperature on production of aerial mycelium of *Streptomyces* isolates SP 12, SL 2, SL 5, and La when grown for two weeks on potato dextrose agar.


RADIUS OF COLONY IN MILLIMETERS
10
9
8
7
6
5
4
3
2
1
TRACE
0
KEY
La
SP12
SL2
SL5
50
60
70
80
90
100
110
120
TEMPERATURE- DEGREES FAHRENHEIT

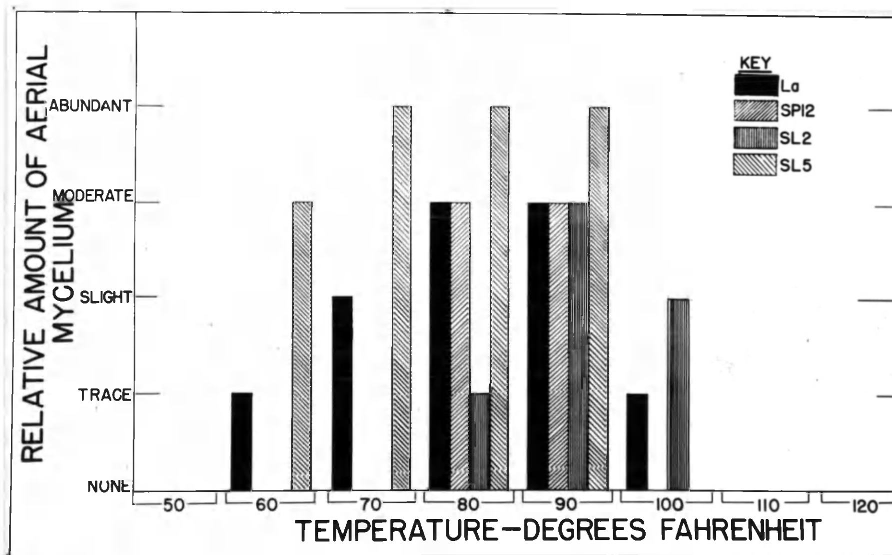

RELATIVE AMOUNT OF AERIAL MYCELIUM
ABUNDANT
MODERATE
SLIGHT
TRACE
NONE
KEY
La
SP12
SL2
SL5
50
60
70
80
90
100
110
120
TEMPERATURE—DEGREES FAHRENHEIT

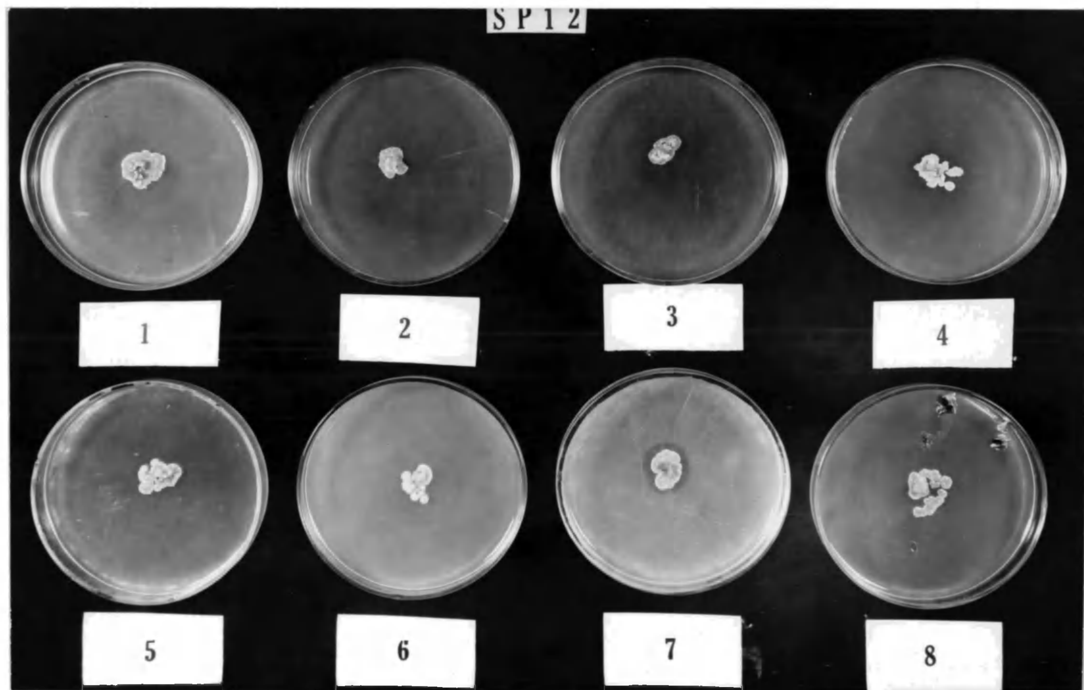
Figure 22

Growth of SP 12 on Wellman's differential agar in which eight different carbohydrates were substituted.

Key:
1 - Glucose
2 - Levulose
3 - Maltose
4 - Sucrose
5 - Lactose
6 - Raffinose
7 - Dextrin
8 - Starch

Figure 23

Growth of SL 2 on Wellman's differential agar in which eight different carbohydrates were substituted. (Irregularity and slightness of growth due to use of spores instead of mycelial discs when transfers were made.)


S P 1 2
1
2
3
4
5
6
7
8

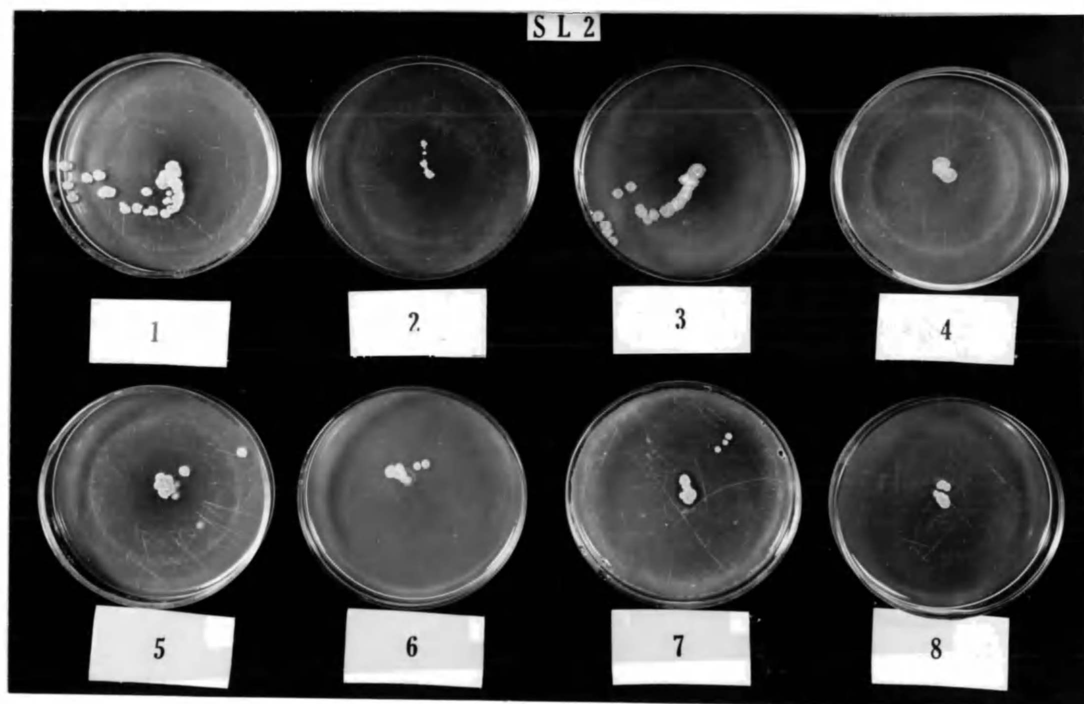

S L 2
1
2
3
4
5
6
7
8

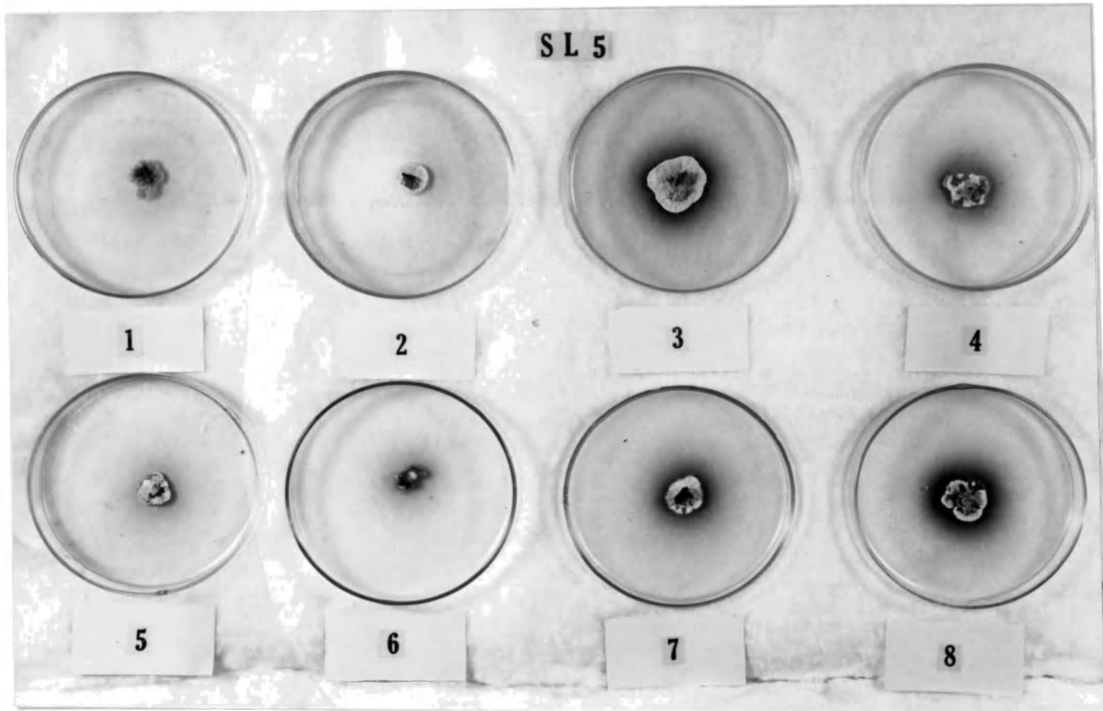
Figure 24

Growth of SL 5 on Wellman's differential agar in which eight different carbohydrates were substituted. Note varying amounts of soluble pigments produced on all but the monosaccharides.

Key: 1 - Glucose
2 - Levulose
3 - Maltose
4 - Sucrose
5 - Lactose
6 - Raffinose
7 - Dextrin
8 - Starch

Figure 25

Growth of La on Wellman's differential agar in which eight different carbohydrates were substituted.


S L 5
1
2
3
4
5
6
7
8

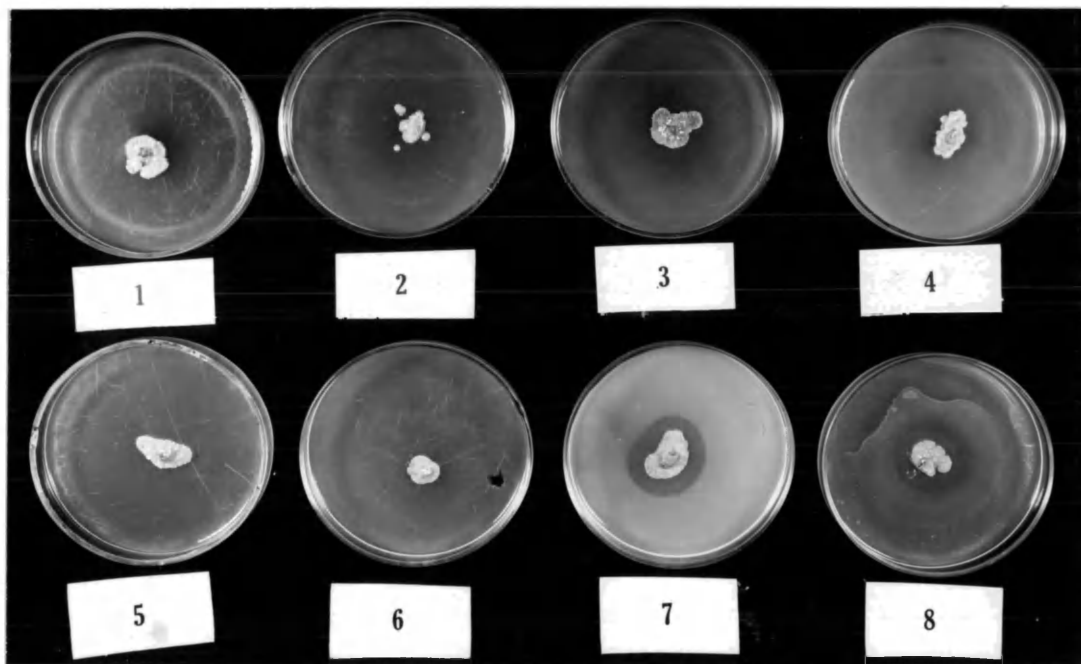
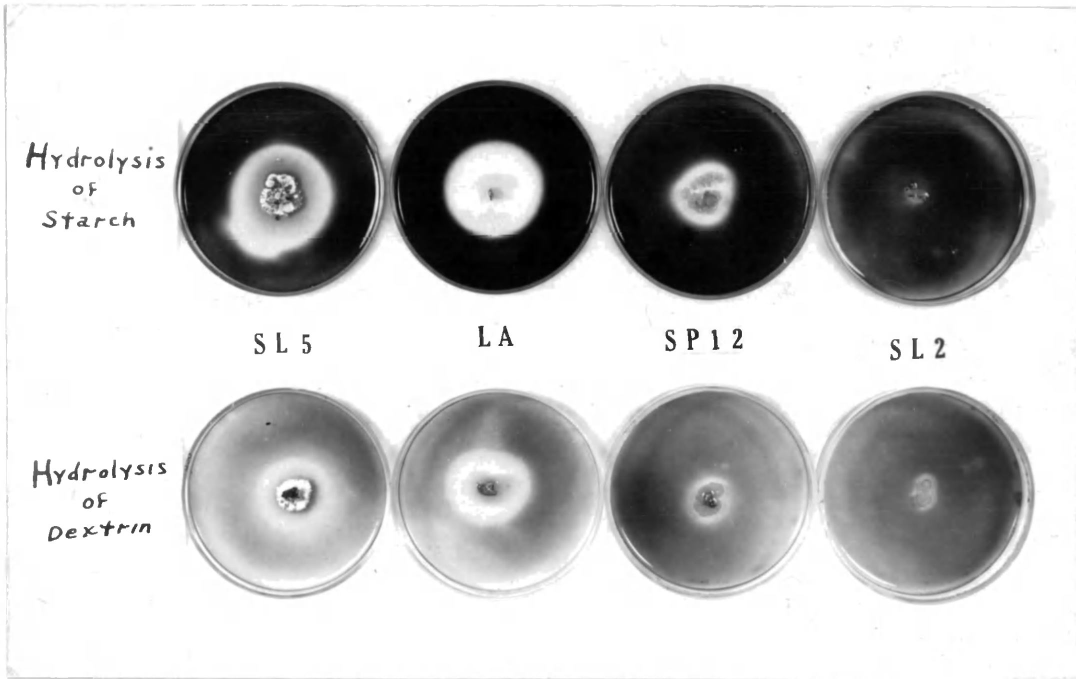

1
2
3
4
5
6
7
8

Figure 26

Hydrolysis of starch and dextrin shown by flooding the plates with iodine after growth of one week by the isolates on Wellman's medium containing starch and dextrin.

Figure 27

Sweet potatoes collected from the field showing cracking apparently initiated by pox lesions.


Hydrolysis
of
Starch
SL 5
LA
SP12
SL 2
Hydrolysis
of
Dextrin