Studies on Actinomycetes from Soils of Baffin Island[1]

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ABSTRACT. Investigations were made of the abundance and relative incidence of different types of actinomycetes in soil samples from Baffin Island, Northwest Territories. Numbers were relatively low in all samples examined, and no specific group predominated. Cultures grown at 26°C. showed greater metabolic activity than when grown at 10°C., and isolates from different soils varied in degree of activity. Ammonium nitrogen proved to be the best nitrogen source. A comparatively wide range of carbon compounds was utilized; glucose, fructose, mannitol, raffinose and sucrose were the best sources. Moderate growth of a large number of isolates was also obtained with acetate, fumarate, pyruvate and succinate. With one exception the incidence of actinomycetes antagonistic toward bacteria, yeasts and fungi was remarkably high in the soils studied. The samples did, however, differ in proportion of antagonistic types.

RÉSUMÉ. *Études sur les actinomycètes des sols de l'île de Baffin.* On a mené des enquêtes sur l'abondance et l'incidence relative des différents types d'actinomycètes dans des échantillons de sols de l'île de Baffin, Territoires du Nord-Ouest. Tous les échantillons examinés ont donné des comptages relativement bas et aucun groupe spécifique ne dominait. Les cultures venues à 26°C montraient une plus grande activité métabolique que celles venues à 10°C, et des isolats de sols différents avaient des degrés d'activité variables. L'azote de l'ammonium s'est révélé être la meilleure source de cet élément. On a utilisé une gamme étendue de composés carbonés : glucose, fructose, mannitol, raffinose et sucrose étaient les meilleures sources de carbone. On a aussi obtenu avec l'acétate, le fumarate, le pyruvate et le succinate une croissance modérée d'un grand nombre d'isolats. A une exception près, l'incidence des actinomycètes antagonistes des bactéries, levures et champignons était remarquablement élevée. Cependant, ces échantillons différaient entre eux dans leurs proportions de types antagonistes.

РЕЗЮМЕ. *Исследование актиномицетов в почвенных пробах с Баффиновой Земли.* Были произведены исследования по количеству и относительной встречаемости различных типов актиномицетов в почвенных пробах с Баффиновой Земли (Северо-Западные Территории). Во всех случаях оказалось сравнительно мало актиномицетов, и преобладание какой-либо определенной группы установлено не было. В культурах, выращенных при 26°С, наблюдалась более интенсивная метаболическая активность, чем в культурах, выращенных при 10°С. Актиномицеты, изолированные из различных почв, отличались по степени активности. Наилучшим источником азота оказался аммоний. Применялось сравнительно большое количество соединений углерода; наилучшими источниками были глюкоза, фруктоза, маннит, рафиноза и сахароза. За одним исключением, встречаемость актиномицетов, антагонистических по отношению к бактериям, дрожжам и грибам, была очень высокой. В то же время почвенные пробы отличались по содержанию антагонистических форм.

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Actinomycetes form a large and important segment of the microflora of most natural environments. Soils, freshwater, lake and river bottoms, manures and compost contain an abundance of these organisms. They are of universal occurrence in nature, living and multiplying in both cold and tropical zones, and have been reported to occur even under the most extreme conditions of the desert. The temperate zones are, however, generally most favourable for their development.

Actinomycetes participate in many important biochemical processes in the soil. In addition, many species have the capacity to elaborate potent antimicrobial substances. It has been demonstrated that such substances are formed in the soil in association with fragments of organic matter (Wright 1956a, Wright 1956b), possibly affecting other microorganisms in the immediate vicinity (Stevenson 1956). Thus, the actinomycetes are capable of affecting the microbial equilibrium, not only by competitive metabolic activity, but also through the excretion of antimicrobial substances. Most investigations of actinomycetes, and especially of their antibiotic activity, have dealt with isolates from soils of the temperate zones (Nakhimovskaia 1937; Schatz and Hazen 1948; Waksman *et al.* 1941; Strzelczyk and Strzelczyk 1958) and the tropics (Meredith 1944; Johnstone 1947). The antibiotic activity of actinomycetes derived from soils of northern Canada has been studied by Landerkin *et al.* (1950) and Peterson (1954). However, actinomycetes of remote arctic soils have in general received less attention than bacteria (Boyd 1958; Boyd and Boyd 1964; McBee and McBee 1956; Brockman and Boyd 1963) or fungi (Cooke and Lawrence 1959; Cooke and Fournelle 1960; DiMenna 1966).

The aim of this work was to obtain detailed data on the abundance, physiological activity and antimicrobial properties of actinomycetes isolated from soils of Baffin Island, Northwest Territories.

MATERIALS AND METHODS

Data on the 10 soil samples collected for microbiological studies are presented in Table 1. The surface layer of the soil was removed, and samples were taken to a depth of 5 inches. The soil was air dried at the sampling site and shipped by air to the laboratory in plastic specimen bottles where they were stored at 4°C. until examined. The time between sampling and plating did not exceed 2 months. Numbers of bacteria and actinomycetes were determined for all samples on soil extract agar (Lochhead and Chase 1943), one series of plates being held at 10°C. and a second at 26°C. for 14 days. Subsequently, from the series incubated at 26°C., all colonies from one representative plate or a sector thereof, for 5 of the samples, were transferred to slopes of penassay base agar (Difco) and a synthetic medium (B) of the following composition: glucose, 5.0 g.; KNO_3, 1.0 g.; K_2HPO_4, 0.5 g.; $MgSO_4$, 0.2 g.; $FeSO_4$, 0.01 g.; agar 15.0 g.; distilled water, 1000 ml., adjusted to pH 6.8 with 0.1N NaOH. These served as stock cultures. Nitrate reduction, starch hydrolysis and gelatin liquefaction were determined according to the Manual of Methods for Pure Culture Study of Bacteria (Society of American Bacteriologists, 1951).

TABLE 1. Soil samples from Baffin Island, Northwest Territories, 1967.

Sample number	*Location*	*Reference*	*Habitat*	*Dominant vegetation*	pH*
1	Inugsuin Fiord base camp	69° 37′N, 70° 02′W	Adjacent runnel to Koodloo Lake.	*Cassiope tetragona, Vaccinium uliginosum, Epilobium latifolium*	4.8
2	As above	As above	Dry sandy soil at base camp.	*Hierchloe alpina*	4.6
3	As above	As above	Wet sandy soil near small tarn, 1 mile west of base camp.	*Polygonum viviparum, Salix, Poa*	4.5
4	As above	As above	Dried pool bottom 2 miles southwest of base camp.	*Gramineae, Salix Saxifraga cernua*	5.0
5	Dewar Lakes	69° 37′N, 71° 07′W	Sandy flat area near airstrip.	*Calamagrostis*	4.8
6	Longstaff Bluff	68° 56′N, 75° 18′W	Edge of gravelly fellfield plant association 3.5 miles west of site.	*Salix reticulata*	6.3
7	As above	As above	As above	*Oxytropis maydelliana*	6.2
8	As above	As above	As above	*Dryas integrifolia*	6.2
9	As above	As above	As above	*Ledum decumbens*	3.4
10	Cape Dyer	66° 35′N, 61° 37′W	Wet soil on rock ledges.	*Saxifraga nivularis*	4.6

*pH determinations using 0.01M $CaCl_2$ in distilled water (Beckman Zeromatic meter)

Nitrogen and carbon utilization

Nitrogen utilization was tested on medium B with 0.1 per cent KNO_3 and $NH_4H_2PO_4$ (with the N-equivalent of KNO_3) as nitrogen sources and on penassay base broth (Difco) containing 1.5 per cent agar. Carbon utilization was determined using the following carbon compounds: glucose, L-arabinose, D-fructose, I-inositol, D-mannitol, raffinose, L-rhamnose, sucrose, D-xylose, acetate, citrate, fumarate, pyruvate and succinate. These substrates were sterilized by filtration and supplied at a concentration of 0.5 per cent in medium B. Growth of the actinomycetes on the above media was recorded following an incubation period of 7 days at 26°C.

Antimicrobial activity

The capacity of actinomycetes to inhibit growth of other microorganisms was tested by the cross-streak method. For this purpose, both penassay base broth with 1.5 per cent agar and medium B were used. The following test organisms were employed: *Arthrobacter globiformis, Bacillus subtilis, Escherichia coli, Fusarium oxysporum* f. *lini* and *Rhodotorula mucilaginosa*. The actinomycete was streaked near the periphery of a plate and incubated at 26°C. for 7 days, whereupon the test organism was streaked at right angles to, but not in contact with it. After an additional 1 to 2 days of incubation, zones of inhibition were recorded.

RESULTS

Numbers of bacteria and actinomycetes

Data on numbers of bacteria and actinomycetes are presented in Table 2. The data for sample 9 are omitted because fungal overgrowth, probably related to the acid nature of this soil, made an accurate count impossible. However, 20 antifungal actinomycetes were recovered from this sample by transfer from areas where fungal growth was inhibited. All of the soils studied contained relatively few organisms, with greater numbers being recorded on plates incubated at 26°C.

TABLE 2. Numbers of bacteria and actinomycetes in Baffin Island soils*.

Soil number	Incubation temperature 26° Bacteria	26° Actinomycetes	26° B:A	10° Bacteria	10° Actinomycetes	10° B:A
1	76.3	16.5	0.4	36.7	17.8	2.0
2	72.6	6.6	9.5	8.8	4.8	1.8
3	61.3	2.3	22.2	54.5	2.1	26.0
4	25.0	6.3	2.4	8.0	0.4	20.0
5	434.7	4.5	93.4	158.8	—	—
6	38.8	12.7	3.0	10.0	1.0	10.0
7	72.7	21.0	2.9	25.0	11.7	2.1
8	47.7	13.6	3.5	11.3	2.4	4.7
10	31.4	3.0	10.4	11.2	0.8	14.0

*Expressed as thousands per gram of oven dry soil

$$B:A = \frac{\text{Bacteria}}{\text{Actinomycetes}}$$

than on those incubated at 10°C. Nevertheless, the numbers are extremely low as compared with those usually recorded from the soils of temperate zones. The greatest bacterial count recorded (430,000) was from sample 5 on plates incubated at 26°C., but numbers of actinomycetes for this sample were quite low, being approximately one one-hundredth of the bacterial population. In the remaining soils bacteria ranged from 25,000 to 76,000 and actinomycetes from 2,300 to 21,000. The ratios of bacteria: actinomycetes (B:A) ranged from 2.4 to 93.4 for plates incubated at 26°C. and probably reflect marked differences in organic matter content and reaction of the samples studied. Ratios for plates incubated at 10°C. were similar, although somewhat lower, ranging from 1.8 to 26.0.

TABLE 3. Incidence of actinomycete groups from Baffin Island soils.

Group	*Sample number* 1	2	7	8	9
Streptomyces albus	24	14	11	32	0
S. antibioticus	10	24	5	16	20
S. flavus	18	3	7	0	0
S. fradiae	14	6	25	12	0
S. griseus	8	29	18	12	0
S. lavendulae	0	4	30	24	0
S. ruber	0	4	30	0	0
Non-sporing	18	20	2	4	0
Total cultures studied	92	104	128	100	20

Groups of actinomycetes

The isolates were grouped (Table 3) according to morphology and pigment production as outlined by Waksman and Lechevalier (1953). Apart from various non-sporing forms, 7 distinct groups belonging to the genus *Streptomyces* were recognized. No one group predominated in any of the soils studied, but some appeared to be more suitable than others for specific groups of actinomycetes. For example, the *S. albus* group was most numerous in samples 1 and 8, whereas the *S. antibioticus* and *S. griseus* groups predominated in sample 2. The *S. ruber* group was most prevalent in sample 7, but was poorly represented in all other samples. Non-sporing forms were most abundant in samples 1 and 2; however, their incidence was relatively low in samples 7 and 8, and they were not detected in sample 9.

Physiological properties of actinomycetes

Some physiological properties of the actinomycete isolates from four of the samples are shown in Table 4. These data show that the organisms were metabolically more active when grown at 26°C. than at 10°C. Generally, lower activity was associated with poorer growth of the microorganisms; however, an exception was noted where similar growth of a number of cultures occurred at both temperatures, yet the physiological activity was reduced in those cultures grown at the lower temperature. There appeared to be no particular stimulation of specific physiological groups in the soils studied; however, isolates originating from different soils varied in degree of activity. More active proteolytic types were found in samples 1 and 2 than in samples 7 and 8. No relationship was evident between

TABLE 4. Some physiological properties of actinomycetes from Baffin Island soils at two different incubation temperatures.*

Sample	*Gelatin liquefying* 26°				10°				*Starch hydrolysing* 26°				10°				*Nitrate reducing* 26°
number	N	W	M	S	N	W	M	S	N	W	M	S	N	W	M	S	
1	34	18	48	0	88	12	0	0	48	24	14	14	86	14	0	0	40
2	30	28	42	0					70	24	6	0					18
7	38	46	16	0	82	18	0	0	8	78	8	6	40	52	8	0	54
8	68	32	0	0					36	44	20	0					44

*Expressed as percentage of total number of cultures.

N — no activity; W — weak activity (1-5 mm); M — moderate activity (6-10 mm); S — strong activity (>10 mm)

the incidence of starch hydrolysing organisms and type of soil. Nitrate reduction was only evident when the isolates were incubated at 26°C.

Growth response of actinomycetes to different nitrogen and carbon compounds

Variation in growth response due to nitrogen source for actinomycete cultures from 5 of the 10 soil samples studied is shown in Table 5. In general, inorganic nitrogen was a better source than organic, with ammonium nitrogen somewhat better than nitrate. Cultures from samples 7 and 9, however, produced maximum growth on either ammonium or organic nitrogen. Nitrate nitrogen proved to be unsuitable for organisms isolated from sample 9.

A wide range of carbon compounds was utilized by the actinomycetes isolated from these soils; however some compounds were evidently more suitable than others. The best carbon sources were found to be glucose, fructose, mannitol, raffinose and sucrose. Depending on the soil sample, 58 to 90 per cent of the isolates produced moderate or good growth, and 13 to 40 per cent produced weak growth with these compounds. The proportions of cultures failing to grow in the presence of these carbon sources ranged from 0 to 10 per cent. With organic acid salts, acetate, fumarate, pyruvate and succinate, good growth was obtained with 60 to 85 per cent and weak growth with 11 to 30 per cent of the isolates compared

TABLE 5. Growth response of actinomycetes in relation to nitrogen source.*

Sample Number	*Source of nitrogen* Organic			Ammonium			Nitrate		
	A	B	C	A	B	C	A	B	C
1	52	36	12	94	6	0	86	14	0
2	36	52	12	96	4	0	42	58	0
7	100	0	0	100	0	0	96	4	0
8	86	0	14	100	0	0	100	0	0
9	100	0	0	100	0	0	0	0	100

*Expressed as percentage of total cultures studied.

A. Moderate to good growth; B. Weak growth; C. No growth.

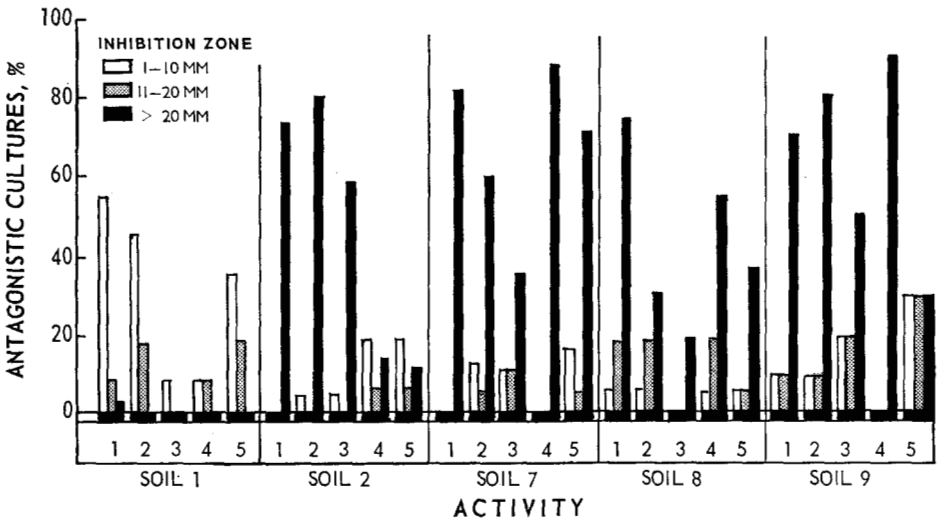

FIG. 1. Antimicrobial activity of antagonistic actinomycetes from Arctic soils. Test organisms. 1. *Arthrobacter globiformis*, 2. *Bacillus subtilis*, 3. *Escherichia coli*, 4. *Rhodotorula mucilaginosa*, 5. *Fusarium oxysporum* f. *lini*.

with glucose as the source of carbon. Arabinose, inositol, rhamnose and xylose appeared to be less favourable for growth of many of the cultures. With these as sources of carbon, 10 to 50 per cent of the cultures produced moderate to good growth, but 37 to 90 per cent produced only weak growth.

Antimicrobial activity

The antagonistic action of 420 actinomycetes against 5 test organisms based on degree of growth inhibition is summarized in Fig. 1. Potent antagonists of both bacteria and fungi were found in 4 of the 5 samples examined. It is clear that cultures arising from sample 1 were the weakest antagonists. As expected, greater antagonism was shown, not only in numbers but also in degree of activity against *Bacillus subtilis* (Gram-positive) and *Arthrobacter globiformis* (Gram-variable) than against *Escherichia coli* (Gram-negative). With the exception of sample 1, all soils harboured greater numbers of actinomycetes antagonistic toward the test fungus and the yeast, with somewhat more showing activity against *Rhodotorula mucilaginosa* than against *Fusarium oxysporum* f. *lini*.

TABLE 6. Antimicrobial activity of actinomycetes from Baffin Island soils.*

Test organism	Sample number 1	2	7	8	9
Arthrobacter globiformis	16	60	28	64	90
Bacillus subtilis	14	74	27	40	100
Escherichia coli	2	52	20	12	90
Rhodotorula mucilaginosa	4	34	34	52	90
Fusarium oxysporum f. *lini*	16	32	32	32	90

*Expressed as percentage of the total number of antagonistic cultures.

TABLE 7. Nature of activity of actinomycetes from Baffin Island soils.*

Type of action	*Sample number* 1	2	7	8	9
Active against bacteria alone	8	46	6	8	10
Active against fungi alone	6	2	4	4	0
Active against both bacteria and fungi	8	34	34	52	90
Inactive	78	18	56	36	0

*Expressed as percentage of the total cultures isolated.

The distribution of active cultures of actinomycetes isolated from Baffin Island soils is given in Table 6. The results indicate that arctic soils differ considerably in occurrence of antagonistic types. Sample 1 yielded the lowest proportion of antagonistic forms, whereas samples 9, 2 and 8 showed the highest. Sample 2 harboured the greatest number (46 per cent) of cultures antagonistic against bacteria alone, (Table 7), with 52 per cent of the actinomycetes originating from that soil inhibiting the growth of *E. coli*. It should be mentioned, however, that elaboration of antibiotic substances depended on the growth medium. A larger number of actinomycetes produced antimicrobial substances and greater activity was observed with medium B containing ammonium nitrogen than with penassay base broth agar.

DISCUSSION

Since the occurrence of actinomycetes in nature is not necessarily limited by climate, the presence of these organisms in soils from the arctic regions is not unexpected. Landerkin *et al.* (1950) indicated that actinomycetes exhibit a high degree of resistance to low temperatures, a property which doubtless accounts for their occurrence in soils that are exposed to extreme and prolonged cold. In a study of microorganisms from frozen soils of southern Canada, Lochhead (1924) noted that numbers of actinomycetes persisted without diminution throughout the period of frost and that subsequent to spring thawing of the soil, no pronounced increase similar to that observed for bacteria occurred.

Although actinomycetes may be found in both cultivated and virgin soils, they are especially abundant under alkaline conditions and in soils of high organic matter content (Burges and Raw 1967). All samples studied during the course of this work were acidic, which may account, at least in part, for the relatively small population of these organisms in the Baffin Island soils. Ivarson (1965) also noted rather low numbers of actinomycetes in northern acidic soils. In addition, the predominant vegetation may also influence actinomycete populations. It has been demonstrated that growing plants exert both a quantitative and qualitative effect on this group of organisms (Landerkin *et al.* 1950, Rouatt *et al.* 1951, Strzelczyk and Strzelczyk 1958, Abraham and Herr 1964).

The data on metabolic activity and growth response to different carbon sources indicate that the actinomycetes are largely non-specific in their carbon requirements. Growth response of the isolates to nitrogen source varied in the different samples, but usually ammonium and organic nitrogen was superior to nitrate nitrogen.

Although relatively few actinomycetes were found in these soils they did contain a high percentage incidence of antagonistic types, as high as, or higher than in soils from other regions. This phenomenon was previously observed by Landerkin *et al.* (1950). It is assumed that the presence of easily available organic matter in such soils favours the development of antagonistic forms (Lochhead 1952, Krassilnikov *et al.* 1953, Brian 1957) and the effect of nutrients on antibiotic production by actinomycetes is well known (Waksman 1947, Duda and Kaszubiak 1957, Strzelczyk and Strzelczyk 1961). The nature of the vegetation occurring at the sampling sites probably influences the actinomycete populations through root exudates and sloughed off root material and might well account for the presence of different groups in the different soils studied.

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REFERENCES

ABRAHAM, T. A. and L. J. HERR. 1964. Activity of actinomycetes from rhizosphere and non-rhizosphere soils from corn and soybean in four physiological tests. *Canadian Journal of Microbiology*, 10: 281-85.

BOYD, W. L. 1958. Microbiological studies of Arctic soils. *Ecology*, 39: 332-36.

BOYD, W. L. and J. W. BOYD. 1964. The presence of bacteria in permafrost of the Alaskan Arctic. *Canadian Journal of Microbiology*, 10: 917-19.

BRIAN, P. W. 1957. The ecological significance of antibiotic production. *Microbial Ecology, 7th Symposium of the Society of General Microbiology*, Cambridge University Press. pp. 168-88.

BROCKMAN, E. R. and W. L. BOYD. 1963. Myxobacteria from soils of the Alaskan and Canadian Arctic. *Journal of Bacteriology*, 86: 605-06.

BURGES, A. and F. RAW. 1967. *Soil biology*. New York: Academic Press, 532 pp.

COOKE, W. B. and D. B. LAWRENCE. 1959. Soil mold fungi isolated from recently glaciated soils in southeastern Alaska. *Journal of Ecology*, 47: 529-49.

COOKE, W. B. and H. T. FOURNELLE. 1960. Some soil fungi from an Alaskan tundra area. *Arctic*, 13: 266-70.

DIMENNA, M. E. 1966. Yeasts in antarctic soils. *Antonie v. Leeuwenhoek*, 32: 29-38.

DUDA, J. and M. KASZUBIAK. 1957. Studies on the effect of the medium on antibiotic activity of soil actinomycetes. *Acta Microbiologica Polonica*, 6: 253-59.

IVARSON, K. C. 1965. The microbiology of some permafrost soils in the MacKenzie Valley, N.W.T. *Arctic*, 18: 256-60.

JOHNSTONE, D. B. 1947. Soil actinomycetes of Bikini Atoll with special reference to their antagonistic properties. *Soil Science*, 64: 453-58.

KRASSILNIKOV, N. A., A. I. KORENIAKO and O. I. ARTAMONOVA. 1953. Distribution of antagonistic actinomycetes in soils. *Microbiology* (U.S.S.R.), 22: 3.

LANDERKIN, G. B., J. R. G. SMITH and A. G. LOCHHEAD. 1950. A study of the antibiotic activity of actinomycetes from soil of Northern Canada. *Canadian Journal of Research*, C, 28: 690-98.

LOCHHEAD, A. G. 1924. Microbiological studies of frozen soil. *Transactions of the Royal Society of Canada,* Ser. 3, 18: 75-96.

LOCHHEAD, A. G. and F. E. CHASE. 1943. Qualitative studies of soil microorganisms: V. Nutritional requirements of the predominant bacterial flora. *Soil Science,* 55: 185-95.

————. 1952. Soil microbiology. *Annual Review of Microbiology,* 6: 185.

MCBEE, R. H. and V. H. MCBEE. 1956. The incidence of thermophilic bacteria in Arctic soils and waters. *Journal of Bacteriology,* 71: 182-85.

MEREDITH, C. H. 1944. The antagonisms of soil organisms to *Fusarium oxysporum cubense. Phytopathology,* 34: 426-29.

NAKHIMOVSKAIA, M. I. 1937. The antagonism between actinomycetes and soil bacteria. *Microbiology* (U.S.S.R.), 6: 131-57.

PETERSON, E. A. 1954. A study of cross antagonisms among some actinomycetes active against *Streptomyces scabies,* and *Helminthosporium sativum. Antibiotics and Chemotherapy,* 4: 145-49.

ROUATT, J. W., M. LECHEVALIER and S. A. WAKSMAN. 1951. Distribution of antagonistic properties among actinomycetes isolated from different soils. *Antibiotics and Chemotherapy,* 1: 185-92.

SCHATZ, A. and E. L. HAZEN. 1948. The distribution of soil microorganisms antagonistic to fungi pathogenic for man. *Mycologia,* 40: 461-77.

SOCIETY OF AMERICAN BACTERIOLOGISTS. 1951. *Manual of methods for pure culture study of bacteria.* 12-Ed., Leaflet V. Geneva, N.Y. pp. 2-24.

STEVENSON, I. L. 1956. Antibiotic activity of actinomycetes in soil as demonstrated by direct observation technique. *Journal of General Microbiology,* 15: 372-78.

STRZELCZYK, A. and E. STRZELCZYK. 1958. Influence of antagonistic actinomycetes on some soil bacteria. *Acta Microbiologica Polonica,* 7: 283-97.

————. 1961. Effect of different carbon and nitrogen sources on the antagonistic activity against *Arthrobacter* and *Rhizobium* in various strains of soil streptomycetes. *Acta Microbiologica Polonica,* 10: 101-07.

WAKSMAN, S. A. 1947. *Microbial antagonisms and antibiotic substances,* Ed. 2, New York: The Commonwealth Fund. 415 pp.

WAKSMAN, S. A., E. S. HORNING, M. WELSCH and H. G. WOODRUFF. 1941. The distribution of antagonistic actinomycetes in nature. *Soil Science,* 54: 281-296.

WAKSMAN, S. A. and H. LECHEVALIER. 1953. *Guide to the classification and identification of the Actinomycetes and their Antibiotics.* Baltimore: The Williams and Wilkins Company, 246 pp.

WRIGHT, J. M. 1956a. The production of antibiotics in soil. III. Production of gliotoxin in wheatstraw buried in soil. *Annals of Applied Biology,* 44: 461-66.

————. 1956b. The production of antibiotics in soil. IV. Production of antibiotics in coats of seeds sown in soil. *Annals of Applied Biology,* 44: 561-66.