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Inhibition of the Growth of Fungi by *Streptomyces* spp. in Relation to Nutrient Conditions

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SUMMARY: Several species of soil actinomycetes arrested the growth of fungi by antibiotic secretions on agar media containing 10 g. glucose/l. On media of lower glucose concentration, the fungi continued to grow in the presence of the actinomycetes, but there was evidence that traces of antibiotic substances were still being formed. In sand moistened with liquid medium containing glucose, *S. albidoflavus* limited early growth of *Fusarium culmorum* by antibiotic action and also attacked preformed fungus mycelium directly. The effectiveness of these antagonistic mechanisms was decreased when the glucose concentration was lowered. The fungus and the actinomycete grew together on a variety of natural organic materials, but only when dried grass was used did the actinomycete arrest growth of the fungus at a distance.

Actinomycetes are numerous in soils (Waksman, 1950), and a high proportion of strains isolated can antagonize bacteria and fungi in culture (Waksman, Horning, Welsch & Woodruff, 1942; Schatz & Hazen, 1948). There is no doubt that most examples of antagonism encountered in mixed cultures are due to the production of inhibitory substances by the actinomycetes. These antagonistic effects are often so striking that it is tempting to consider such substances as being factors of considerable importance in influencing the balance of natural soil populations. Nevertheless, though many species of soil actinomycetes can produce their antibiotics in artificial culture when supplied with nutrients of the right kinds in adequate amounts, it is by no means certain that they can form the antibiotics in sufficient quantity to antagonize other micro-organisms in soil where the quality and amounts of nutrients may not permit rapid growth (Robison, 1945; Waksman, 1945). A study has accordingly been made of the ability of some soil actinomycetes to suppress the growth of fungi, particularly by the production of antifungal inhibitors, when growing under conditions unfavourable to luxuriant development.

METHODS

Organisms

Five test fungi (*Fusarium culmorum*, *Rhizoctonia goodyerae-repentis*, *Fusarium* sp., *Stemphylium* sp., and an unidentified sterile mycelial form) were used in preliminary tests for selecting soil actinomycetes which displayed antibiotic activity. Five species of *Streptomyces*, each strongly inhibitory to the growth of all the five test fungi on several different agar media, were isolated from local soils. In most of the experiments one of these actinomycetes, identified

as *Streptomyces albidoflavus* (Rossi Doria) comb.nov. (Skinner, 1951) was used with *Fusarium culmorum*, a strain of which was obtained from Dr W. J. Dowson of the Botany School, Cambridge.

Media

Artificial soil solution media. Solid and liquid media were prepared from an 'artificial soil solution' similar to that described by Erikson (1947). This solution (hereafter 'AS solution') consisted of (g./l. distilled water): CaSO_4 , 0.8; $\text{Ca}(\text{NO}_3)_2$, 0.33; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.7; K_2SO_4 , 0.025; K_2HPO_4 , 0.005; NaHCO_3 , 0.2; FeCl_3 , trace. This solution plus specified amounts of glucose was used to moisten sand cultures; it was also used as a solid medium by the addition of 1.5% (w/v) water-washed agar. These media were sterilized by steaming for 20 min. on 3 successive days. The pH value was adjusted to 7.0–7.2.

Plain buffered agar medium (pH 7.0) was made by dissolving 15 g. agar in 500 ml. buffer solution and sufficient distilled water to make 1 l. (This volume of buffer was made by mixing 195 ml. of M/15 KH_2PO_4 and 305 ml. of M/15 Na_2HPO_4 .) The medium was autoclaved for 15 min. at 15 lb./sq. in.

Glucose asparagine solution. Glucose, 10 g.; asparagine 0.5 g.; K_2HPO_4 , 0.5 g.; distilled water, 1 l. The solution was adjusted to pH 7.0–7.2 and sterilized by steaming for 20 min. on 3 consecutive days. Buffered glucose asparagine solution was made by mixing equal volumes of the phosphate buffer solution used for plain buffered agar medium and double-strength glucose asparagine solution from which the phosphate had been omitted. This solution was also sterilized by intermittent steaming.

Other materials

Leaf mould. This was washed as free as possible from mineral particles in a column of water agitated by a stream of air (Chesters, 1947).

Root fraction. Soil from the top 6 in. of local pasture was washed on a wire-mesh sieve to separate the larger pieces of fresh and partially decomposed organic matter. This material (consisting mainly of fine roots) was washed in the same way as the leaf mould.

Leaf mould, root fraction and other organic materials (dried grass, wheat straw, farmyard manure, horticultural peat) were all dried at 50°, milled and sifted. Fractions passing a 0.5 mm. sieve were used.

RESULTS

Inhibition of fungal growth by actinomycetes on agar media containing different concentrations of glucose

Three media were prepared from the AS solution solidified with 1.5% (w/v) agar. Medium A contained no glucose; medium C, 0.1 g. glucose/l., and medium E, 10 g. glucose/l. Five fungi were tested against five actinomycetes on each medium in Petri dish cultures. Three actinomycete species were tested in each dish, the inocula being placed as spots near the edge of the dish. After 5 days

of incubation at 25°, a small spot inoculum of the test fungus was placed in the centre; control plates with fungi alone were also set up. The cultures were incubated and daily measurements were made of (1) the distance grown by each fungus from the centre towards each actinomycete colony; (2) the distances which separated fungus and actinomycete colonies along lines joining their centres.

Table 1. *Distances grown by fungi towards actinomycete colonies after 9 days of incubation*

		Medium: AS solution. (1) Distances grown (mm.). (2) Distances between fungus and actinomycete colonies					
Fungus	Actino- mycete	No glucose		0.01 % glucose		1 % glucose	
		(1)	(2)	(1)	(2)	(1)	(2)
<i>Fusarium culmorum</i>	A 2	24.5	0	23.0	1.0	16.0	9.0
	A 30	24.5	0	23.0	0	14.5	9.0
	A 40	23.5	0	18.0	4.0	18.0	5.0
	S.a.	20.0	4.0	16.5	7.0	17.5	6.5
	S.l.	23.0	0	18.0	3.0	16.5	5.0
	.	44.0+	.	44.0+	.	44.0+	.
<i>Fusarium</i> sp.	A 2	25.0	0	23.5	1.0	13.5	11.0
	A 30	24.0	0	23.0	0	12.0	11.5
	A 40	22.0	0	22.0	0	18.0	4.0
	S.a.	.	.	18.5	5.0	15.5	7.5
	S.l.	21.5	0	20.0	0	16.0	5.5
	.	35.8	.	37.0	.	32.8	.
<i>Mycelia sterilia</i>	A 2	18.0	6.0	11.0	9.0	9.0	15.0
	A 30	22.0	1.0	19.0	4.0	9.0	14.0
	A 40	23.5	0	14.5	7.0	12.5	9.0
	S.a.	20.5	3.0	19.0	4.5	13.5	9.0
	S.l.	21.0	0	17.5	3.5	14.5	6.0
	.	25.9	.	24.1	.	27.0	.
<i>Stemphylium</i> sp.	A 2	20.0	4.5	15.5	8.5	9.5	15.5
	A 30	23.5	0	20.0	3.0	8.0	15.0
	A 40	23.0	0	18.5	4.5	14.5	6.0
	S.a.	21.0	3.0	19.0	5.0	15.5	7.5
	S.l.	22.0	0	21.0	0	17.0	5.0
	.	40.0	.	34.3	.	39.5	.
<i>Rhizoctonia goodyerae-repentis</i>	A 2	20.0	4.5	.	.	17.0	8.0
	A 30	24.0	0	.	.	8.0	15.0
	A 40	23.0	0	17.0	5.5	16.0	6.0
	S.a.	24.0	0	.	.	14.0	9.0
	S.l.	22.0	0	17.5	2.5	7.0	6.5
	.	40.5	.	44.0+	.	41.0	.

S.a. = *Streptomyces albidoflavus*; S.l. = *S. lavendulae*.

Measurements of fungal growth after 9 days are given in Table 1. The inhibitory effect of the actinomycetes on the fungi in all combinations was directly related to the glucose supply. The fungi reached the actinomycetes within 9 days in 17 out of the 25 combinations on medium A, in only 5 combinations with medium C, and in no case with medium E. After 23 days of incubation

the fungi reached the actinomycetes in all but one combination on media A and C and in only 4 combinations on medium E. Only on medium E did the actinomycetes virtually arrest fungal extension.

Antagonism in sand cultures with artificial soil solution

Various flask culture experiments were made in order to study the behaviour of an actinomycete and a fungus when growing together under conditions approximating more closely to those in soil. *Streptomyces albidoflavus* was used with the fungus *Fusarium culmorum*. Ten g. of coarse, washed and ignited sand were placed in a 50 ml. conical flask which was then plugged and autoclaved for 1 hr. at 15 lb./sq.in. After sterilization, each flask received 1–2 ml. of culture solution containing the inoculum. The contents of each flask were then mixed thoroughly by shaking.

Sampling and estimation of fungal growth. About 1 g. of sand was removed from the flask with a flamed spatula and transferred to a weighed crucible. One ml. of water was added, the sample ground with the end of a glass rod for 5 min. and 0.5 ml. of the resulting suspension mixed with an equal volume of acetic acid-aniline blue solution (Jones & Mollison, 1948). A drop of this mixture was placed on a haemocytometer of 0.1 mm. depth and the number of stained fragments counted; a $\frac{2}{3}$ in. objective was used. The number of hyphal fragments/ml. suspension was estimated from the mean count for 10 random microscope fields on each of 4 replicate slides. This value was corrected for moisture to give the number of fragments/g. of culture.

AS solution E (10 g. glucose/l.) was used to moisten the sand in the culture flasks. Suspensions of spores of both organisms were made in this medium. Three sets of sand flasks (2 flasks/set) received the following additions: *Set A.* 1 ml. fungus spore suspension + 1 ml. medium. *Set B.* 1 ml. fungus spore suspension + 1 ml. actinomycete spore suspension. *Set C.* 1 ml. actinomycete spore suspension + 1 ml. medium. After 5 days of incubation at 25°, each flask in set C received 1 ml. of fungus spore suspension. The flasks were sampled after a further 2 and 7 days of incubation and the number of fragments of fungus mycelium/g. dry wt. culture estimated as described above. Since fungus growth in duplicate flasks was obviously similar, quantitative estimations were made on only one flask of each set (A1, B1 and C1). In flask B1 the fungus grew almost as well as it did in the control flask A1 (Table 2), and was accompanied by only slight development of the actinomycete. There was no evidence of any antibiotic action in flask B1, in which both organisms had been present for the same length of time, so it was discarded after 7 days.

In flask C1 the actinomycete grew only very slowly during the 5 days which preceded inoculation with the fungus. After inoculation the fungus at first grew rapidly (thereby showing that no effective concentration of antibiotic had previously been built up) but later the amount of detectable fungus mycelium in the culture declined. This decline coincided with marked development of the actinomycete. Seven days after fungus inoculation, the actinomycete was growing on all the few remaining recognizable fragments of fungus

mycelium. The nature of this direct attack on the fungus is illustrated in Pl. 1, figs. 1, 2. After 15 days, all trace of fungus growth had disappeared. At this time, both C cultures were tested for glucose (by Fehling's solution), nitrate (by Gries-Ilosvay reagent) and phosphate (by the benzidine-blue spot test; Feigl, 1943). Both glucose and nitrate were present but no positive test was obtained for phosphate.

Table 2. *Growth of Fusarium culmorum with Streptomyces albidoflavus in sand moistened with AS solution containing 10 g. glucose/l.*

	No. fragments fungus mycelium ($\times 10^3$)/g. dry wt. culture.	
	Incubation after inoculation with fungus for (days)	
	2	7
A 1. Control (no actinomycete)	46.7	299.9
B 1. Fungus and actinomycete inoculated simultaneously	39.5	240.5
C 1. Fungus inoculated 5 days after the actinomycete	91.1*	3.5

* This figure is probably high because each C flask received 3 ml. medium (2 ml. initially and 1 ml. with the fungus inoculum), whereas each A and B culture received only 2 ml.

Antagonism in sand cultures with actinomycete and fungus inocula of different strengths

An experiment was set up to determine whether antibiotic effects would become apparent and direct-attack effects modified by changes in the numbers of fungus and actinomycete spores present in the original inocula.

A suspension of spores of *Streptomyces albidoflavus* in sterile 0.75% (w/v) NaCl solution was freed from particles of mycelium by filtration through a sterile no. 1 filter-paper and 0.1 ml. of the filtered suspension was mixed with an equal volume of acetic acid-aniline blue stain and allowed to stand for 10 min. The number of stained spores/ml. was then estimated microscopically with a Thoma haemocytometer and oil-immersion objective. Appropriate dilutions were made from this original suspension. The number of spores of *Fusarium culmorum*/ml. was estimated directly with a haemocytometer and dilutions prepared.

Each of twenty sand culture flasks received 2 ml. of AS solution E. These 2 ml. portions were made up by combining appropriate volumes of standardized suspensions of spores of both organisms prepared with the AS solution. Sterile medium was added to the flasks as required to bring the total volume of liquid added to each flask to 2 ml. A small sample was removed daily from each flask and mixed with a little acetic acid-aniline blue solution. Each resulting suspension was examined microscopically (Table 3).

Similar results were obtained with either 10^3 or 10^4 fungus spores/flask; only those results obtained with 10^3 spores/flask are therefore given. In all the test cultures, the fungus developed rapidly at first and actinomycete growth

was delayed. Nevertheless, by 22 days, the actinomycete had in all cases attacked the fungus directly. Clearly, the faster growing fungus had a great initial advantage over the actinomycete, and this situation was not affected very much by the numbers of actinomycete and fungus spores which comprised the inocula. It was clear from this and the preceding experiment that

Table 3. *Growth of Fusarium culmorum with Streptomyces albidoflavus in sand moistened with ASP solution containing 10 g. glucose/l.*

Actinomycete spores (no./flask)	Actinomycete (amount of growth); days of incubation			Fungus (kind of mycelium); days of incubation		
	7	12	22	7	12	22
0 (Control)	—	—	—	N	N	N
5×10^3	—	+	++	N	N	N
5×10^4	—	++	+++	N	U	O
5×10^5	—	++	+++	N	U	O
5×10^6	—	++	+++	N	U	O

— = no growth; + = slight growth; ++ = moderate growth; +++ = abundant growth; N = normal deeply stainable mycelium; U = largely unstainable mycelium; O = no stainable mycelium.

any demonstration of early antagonistic effects in sand culture (as distinct from the direct attack which always took place when the fungus grew before the actinomycete) depended on the actinomycete being able to grow and produce its antibiotic before the fungus inoculation. When using AS solution E, the actinomycete did not grow sufficiently rapidly to produce an effective concentration of the antibiotic even when inoculated into the culture 5 days before the fungus; it was thought that phosphate deficiency might be responsible for this slow actinomycete growth even though satisfactory growth of this organism and other actinomycetes had taken place on the same medium solidified with agar.

A test was made by growing the actinomycete in small quantities of (a) AS solution E as used above, and (b) the same medium in which the concentration of K_2HPO_4 had been raised to 0.5 g./l. This test showed that the actinomycete was able to grow much faster in the latter medium than in the former. Luxuriant actinomycete development took place in the medium with high phosphate within 5 days, whereas practically no growth occurred in the unmodified medium in this time. In all subsequent experiments, ASP solution refers to AS solution modified with the extra phosphate.

Effect of glucose concentration of the inhibition of Fusarium culmorum by Streptomyces albidoflavus in sand culture with ASP solution

Eight sand culture flasks each received 1 ml. of ASP solution containing 0.1 g. glucose/l.; eight more received 1 ml. ASP solution containing 10 g. glucose/l. Four flasks of each set were inoculated with actinomycete spores and incubated for 5 days at 25°. Each flask was then inoculated with spores of

Fusarium culmorum in 1 ml. of medium. The amount of fungus growth was measured after 1 and 2 days of further incubation (Table 4). At the higher glucose concentration the growth of the fungus was greatly inhibited in the presence of the actinomycete. Two days after the fungus inoculation, glucose was present in abundance, and nitrate and phosphate were also present in these mixed cultures. The antagonism, therefore, may have been due to antibiotic action or to competition for some nutrient other than those named above. At the lower glucose concentration very slight inhibition of the fungus occurred. It was not clear whether this slight inhibition should be ascribed to antibiosis or to competition between the organisms for some limiting nutrient, e.g. the small amount of glucose in the culture flasks (c. 0.2 mg./flask).

Table 4. *Inhibition of growth of Fusarium culmorum by Streptomyces albidoflavus in sand moistened with ASP solution containing glucose*

Conc. of glucose in liquid medium (%, w/v)	Type of culture	Period of incubation after inoculation with fungus (days)	
		1	2
		No. fragments fungus mycelium ($\times 10^3$)/g. dry wt. culture	
0.01	Fungus alone	29.7	48.2
	Fungus + actinomycete	24.3	35.7
1.0	Fungus alone	31.1	236.8
	Fungus + actinomycete	1.9	3.6

Under certain conditions, growth of the fungus spores was completely inhibited in a way that cast no doubt on the production of antibiotic by the actinomycete. Two sand flasks each received 1 ml. of glucose asparagine solution (with the same concentrations of glucose and phosphate as the ASP solution with 10 g. glucose/l.) containing spores of the actinomycete. After incubation for 5 days each flask received 1 ml. of medium containing spores of *Fusarium culmorum*. Two control flasks, which had not previously been inoculated with the actinomycete, were also inoculated with the fungus. In the control cultures, fungus spores began to germinate within 3 hr. but, in the presence of the actinomycete, no germination was seen even after 23 hr. and the spores remained unswollen and lost their refractility as they do in the presence of active actinomycete secretions in liquid or agar media. It seemed unlikely that germination had been suppressed by lack of oxygen caused by the growing actinomycete since fungus spores germinated satisfactorily on glucose asparagine agar slopes in sealed tubes in which the oxygen tension had been drastically lowered by alkaline pyrogallol. Also, spores germinated overnight in distilled water so deficiency of nutrients could not have caused failure of germination. Whenever germination of fungus spores is complete, as in this experiment, the production of antibiotic by the actinomycete is certain but when germination takes place and is followed by some suppression of further fungus growth it is difficult to decide how much this suppression is due

to antibiotic actinomycete secretions and how much to competition between the organisms for nutrients. This question will be examined in a later communication.

Inhibition of Fusarium culmorum by Streptomyces albidoflavus with natural organic materials as nutrient sources

To gain some idea of the type of organic matter likely to support growth of the fungus and the actinomycete and to permit antibiotic production by the latter, several natural organic materials, each likely to occur in soils, were tested. Dried grass, wheat straw, leaf mould, root fraction, farmyard manure, peat and lignin were tested by the following method, which was used because it enabled the pH values of the cultures to be kept constant. Thirty-two Petri dishes each containing 15 ml. plain buffered agar medium (pH 7.0) were left overnight at room temperature to allow the agar surfaces to dry. Next day, a straight line with its centre point *c.* 30 mm. from the circumference was drawn in indian ink across the bottom of each dish (the 'base-line'). Sixteen plates (test plates) were then streaked with a suspension of actinomycete spores in such a way that the edge of the streak facing the centre of the plate was immediately above the base-line. When the streaks were dry, the plates were supplied with the organic materials. The material to be tested (finely milled and sterilized) was deposited with a flamed spatula over the whole area between the base-line and the nearer part of the plate circumference. The sixteen uninoculated (control) plates received organic matter in the same way; each test was made in duplicate. Two mixed culture and two control plates received no addition of organic material.

After 5 days of incubation, a suspension of *Fusarium culmorum* spores was streaked on each plate in a line parallel with the base-line and 30 mm. from it. Organic matter (of the same kind as that already present on the plate) was then distributed between the fungus streak and the rim of the dish to provide a source of nutrients for the fungus (Pl. 2). In this way, solid material was provided for both organisms, while leaving the space between them clear of organic particles which would otherwise interfere with microscopic examination and the measurement of fungus growth. Daily measurements of the distance grown by the fungus toward the actinomycete in each mixed culture, and the distance grown toward the base-line in each control culture, are given in Table 5.

Fungal growth towards the actinomycete was virtually arrested after 5 days in the dried grass cultures. Considerable distortion of the marginal fungal hyphae suggested that the inhibition was caused by antibiotic secretions from the actinomycete. Inhibition also took place on the plain buffered agar test plates though this was not sufficient to arrest growth of the fungus or to prevent contact between the organisms after 9 days of incubation. Though this medium was poor in nutrients, extension of the fungus in the corresponding control cultures was rapid though the amount of mycelium formed was obviously very small compared with that in the dried grass controls. It was therefore assumed that the actinomycete produced the antibiotic on the plain

buffered agar. With the remaining materials tested the fungus grew until it approached closely to the actinomycete and inhibition was less than on plain agar. These materials had thus actually decreased the inhibition of fungal growth by the actinomycete, although the latter grew more strongly than in the absence of these materials. This suggested that all the materials except dried grass were able to inactivate to some extent any antibiotic formed from the plain agar base.

Table 5. *Distance grown by Fusarium culmorum toward Streptomyces albidoflavus on buffered agar with various solid organic materials*

Organic material	Inoculum	Period of incubation after inoculation with fungus (days)		
		5	6	7
		Distance grown by <i>F. culmorum</i> towards <i>S. albidoflavus</i> (mm.)		
Dried grass	F + A	13.2	13.5	14.5
	F	24.9	30.0 +	
Wheat straw	F + A	19.8	23.5	25.6 C
	F	23.8	30.0 +	
Root fraction	F + A	22.1	25.7	26.9 C
	F	24.2	30.0 +	
Leaf mould	F + A	20.1	24.1	26.6 C
	F	25.0	30.0 +	
Farmyard manure	F + A	20.7	24.1	26.2 C
	F	22.0	30.0 +	
Peat	F + A	18.7	21.5	24.4 C
	F	21.7	29.4	30.0 +
Lignin	F + A	17.2	19.8	22.6
	F	20.9	28.4	30.0 +
None (plain buffered agar alone)	F + A	16.5	18.9	21.1
	F	20.2	26.7	30.0 +

F = fungus alone; F + A = fungus + actinomycete; 30.0 + = growth of fungus beyond base-line; C = contact made between organisms.

*Inactivation by organic materials of the antibiotic formed
by Streptomyces albidoflavus*

In order to determine the direct effects of some of the natural soil materials used on the antibiotic of *Streptomyces albidoflavus*, an experiment was made with sterile filtrates of liquid cultures of this actinomycete. *S. albidoflavus* was cultivated in glucose asparagine solution (buffered at pH 7.0) for 7–10 days at 25° and a filtrate was prepared by passing this culture through a no. 5 (or no. 50) filter-paper contained in a sterile filtration assembly (Fig. 1). These filtrates usually remained sterile though actinomycete spores sometimes passed through the filter. To prevent renewed actinomycete growth, each filtrate was heated in boiling water for 2 min., a process which decreased its antibiotic activity only slightly.

Ten mg. or 100 mg. portions of dried sterile materials (dried grass, leaf

mould, root fraction and peat) were placed in test tubes. Each tube then received 5 ml. of *Streptomyces albidoflavus* culture filtrate, 1 ml. of fresh medium and an inoculum of *Fusarium culmorum* spores. Control tubes without the organic materials were also set up. Another set of tubes each received 5 ml. of *S. albidoflavus* filtrate, 1 ml. of extract prepared from either 10 mg. or 100 mg. of organic matter/ml. of fresh extracting medium, and the inoculum.

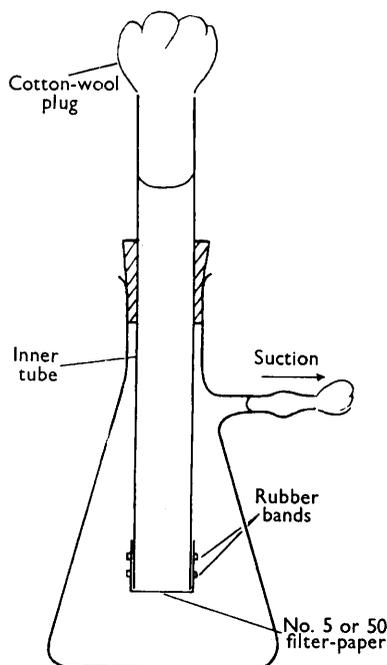


Fig. 1. Apparatus for filtration under sterile conditions. Solutions to be filtered are introduced into inner tube.

The filtrate of *Streptomyces albidoflavus* was able to prevent fungal growth in the controls, but the presence of each of the natural materials in solid form enabled *Fusarium culmorum* to grow. Even extracts of the materials had some effect in neutralizing antibiotic action. This effect varied with the nature of the material, being greatest with root fraction and least with dried grass and peat. This effect seems to be due to the presence of substances which either enable the fungus to overcome the effects of the antibiotic or which destroy it directly. It was interesting to note that dried grass which favoured antibiotic production by *S. albidoflavus* on solid agar medium also contained substances which could, to some extent, inactivate that antibiotic.

DISCUSSION

The experiments with *Streptomyces albidoflavus* and *Fusarium culmorum* growing together in sand suggest that the actinomycete antagonizes the fungus in at least two ways. When actinomycete growth was at first poor in media low

in phosphate the fungus was able to make some mycelial growth before antagonism became noticeable. After a while, however, the actinomycete attacked and grew at the expense of the fungus mycelium. This attack took place under all conditions that allowed fungus mycelium to develop in the mixed cultures. But, where the actinomycete made good growth before the introduction of the fungus it was able to check the latter at an early stage. It remains a question how far this early check was due to antibiotic action or to competition for some limiting nutrient. Early antagonism was intensified by increasing the glucose and phosphate concentration which also increased early growth of the actinomycete. That this effect was at least in part due to antibiotic action is suggested by the observed inhibition of fungus spore germination under these conditions. Moreover, the fact that higher glucose concentration similarly increased the antagonism of the actinomycetes to fungi at a distance on agar plates supports this conclusion.

In natural soil the supply of easily available nutrients is limited and it is an important question whether the nature of these materials is such as to permit actinomycetes to grow and produce antibiotics in sufficient quantities to be effective. Experiments with some natural materials of a type likely to be found in soils showed that dried grass at least was able to support actinomycete growth and antibiotic production, but, on the other hand, that all the materials tested had some effect in inactivating the antibiotics produced. This raises the further question of how far these and other soil constituents can inactivate actinomycete antibiotics and, where they do so, how much antagonism can be exerted by the actinomycete through other means such as competition for limiting nutrients. This question will be considered in a further communication. The present results emphasize one point to which little attention has hitherto been given. This is that when supplies of carbon and energy sources are low, conditions may be unfavourable not only for growth of a potential antibiotic producer but also for that of other micro-organisms susceptible to the antibiotic when produced. The relative importance of such growth-limiting mechanisms as antibiotic production, nutrient competition, and direct attack by one organism on another at low nutrient concentrations has been scarcely studied as yet, and more information on this subject is needed if the complex interactions affecting survival of micro-organisms in soils are to be understood.

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REFERENCES

- CHESTERS, C. G. C. (1947). A contribution to the study of fungi in the soil. *Trans. Brit. mycol. Soc.* **30**, 100.
- ERIKSON, D. (1947). Differentiation of the vegetative and sporogenous phases of the actinomycetes. 2. Factors affecting the development of the aerial mycelium. *J. gen. Microbiol.* **1**, 45.

- FEIGL, F. (1943). *Laboratory Manual of Spot Tests*. New York: Academic Press Inc.
- JONES, P. C. T. & MOLLISON, J. E. (1948). A technique for the quantitative estimation of soil micro-organisms. *J. gen. Microbiol.* **2**, 54.
- ROBISON, R. S. (1945). The antagonistic action of the by-products of several soil micro-organisms on the activities of the legume bacteria. *Proc. Soil Sci. Soc. Amer.* **10**, 206.
- SCHATZ, A. & HAZEN, E. L. (1948). The distribution of soil micro-organisms antagonistic to fungi pathogenic to man. *Mycologia*, **40**, 461.
- SKINNER, F. A. (1951). A method for distinguishing between viable spores and mycelial fragments of actinomycetes in soils. *J. gen. Microbiol.* **5**, 159.
- WAKSMAN, S. A. (1945). *Microbial Antagonisms and Antibiotic Substances*. New York: The Commonwealth Fund.
- WAKSMAN, S. A. (1950). *The Actinomycetes*. Waltham, Mass.: The Chronica Botanica Co.; London: Wm. Dawson and Sons.
- WAKSMAN, S. A., HORNING, E. S., WELSCH, M. & WOODRUFF, H. B. (1942). Distribution of antagonistic actinomycetes in nature. *Soil Sci.* **54**, 281.

EXPLANATION OF PLATES

PLATE 1

Streptomyces albidoflavus and *Fusarium culmorum* growing in sand moistened with ASP solution containing 10 g. glucose/l. Fungus inoculated 7 days after actinomycete.

Fig. 1. Two days after inoculation with fungus. Actinomycete beginning to attack fungus mycelium.

Fig. 2. Ten days after inoculation with fungus. Actinomycete growing on fungus mycelium which has lost its staining property. Water mounts. Stained with acetic acid-aniline blue. Magnification $\times 296$.

PLATE 2

Growth of *Streptomyces albidoflavus* and *Fusarium culmorum* on leaf mould distributed on a buffered agar surface. Fungus growth too transparent to be visible in photograph.

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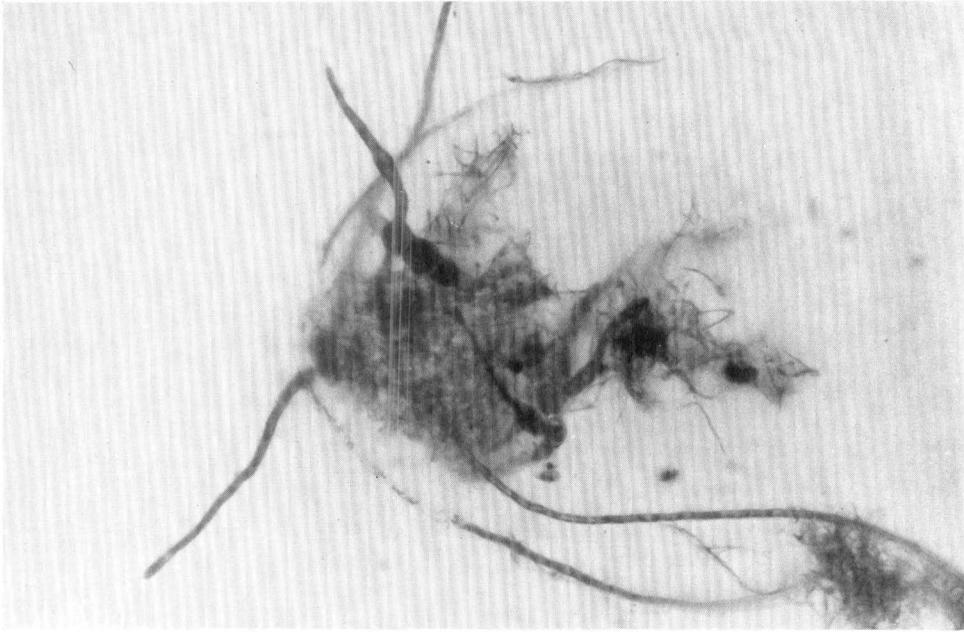


Fig. 1

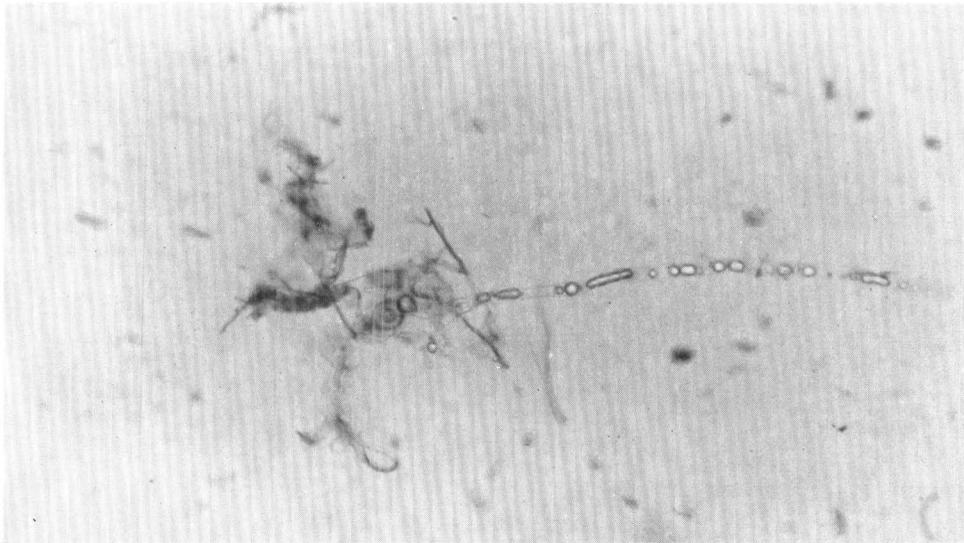


Fig. 2

F. A. SKINNER—ANTIFUNGAL ACTIVITIES OF SOIL ACTINOMYCETES. PLATE 1

(Facing p. 392)

