

## Observations Upon a Strain of *Streptomyces albus* Antagonistic to Certain Phytopathogenic Fungi

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Investigations of the antibiotic activities of the actinomycetes has for the most part been focused upon those organisms which are antagonistic to bacteria and fungi pathogenic to man. The inhibition of phytopathogenic fungi is at present of little more than academic interest since the chemotherapeutic treatment of plant disease is still in rather early stages of practical development, and the inoculation of soil with specific antagonists of soil inhabiting pathogens has not as yet met with conspicuous success. The more significant work in the latter field prior to 1945 has been reviewed by Waksman (8). Records of actinomycete antagonists of phytopathogenic fungi occur scattered through the literature, but very little appears to have been done in the way of extensive investigation. Alexopoulos (1) used a considerable number of actinomycetes in studies of the inhibition of *Colletotrichum gloesporioides*, and later with Herrick (3) tested a more limited group of actinomycetes upon various species of fungi. Meredith (5) reported actinomycetes to be antagonistic to *Fusarium oxysporium* v. *cubense*, causing the Panama disease of bananas, and Tims (7) has noted antagonism of a parasitic *Pythium* by an actinomycete.

The *Streptomyces* which is reported herein was discovered growing in a petri plate culture of the coffee leaf spot fungus, *Omphalia flavidia*, and was presumably an air borne contaminant. It attracted attention because of the clear circular zone of inhibition several centimeters in diameter in the basidiomycete colony, with the colony of the antagonist only a few millimeters wide in the center of the zone. The *Streptomyces* was isolated in pure culture, and stock cultures were made in tubes of sterile soil according to the method of Green and Fred (4).

A selection was made of 28 species of fungi which were chosen to include several species from each class of the Eumycetes with particular attention to soil dwelling phytopathogenic species. One method of testing the antagonistic properties of the *Streptomyces* was that employed by Alexopoulos *et al* (2) in which the actinomycete is inoculated at two points 4 cm. apart on a plate of synthetic agar with maltose as the carbohydrate source. The fungus against which the actinomycete is to be tested is inoculated midway between the two actinomycete colonies after they have been incubated for five days. The inhibitory distance is determined as the average minimum distance between the fungus and actinomycete colonies at the time of first evidence of definite inhibition. Alexopoulos considered "strong inhibitors" to be those which

showed an inhibitory distance of 10 mm. or more. Four plates were inoculated with each fungus tested. This number seemed adequate for a preliminary run since there was little variation within each group, and the purpose of the experiment was primarily exploratory. The observations of principal interest may be summarized as follows:

1. Varying degrees of complete or partial inhibition were demonstrated against 27 of the 28 test fungi. The single exception was *Gliocladium fimbriatum*. Partial inhibition is to be interpreted as marked reduction of normal growth of the fungus in the vicinity of the antagonist without complete prevention of growth. In the case of complete inhibition there is no growth whatever within the area of inhibition.

2. Both resistant and sensitive species were found in each class of fungi, supporting the observation of Alexopoulos and Herrick (8) that there is no general correlation between amount of inhibition and systematic position.

3. None of the fungi tested produced appreciable retardation of growth of the actinomycete.

4. Two phycomycetes (*Pythium debaryanum* and *Phytophthora cactorum*) and two basidiomycetes (*Pleurotus ostreatus* and *Omphalia flavida*) were unable to make any growth under the conditions of this test. These were apparently more marked inhibitions than those observed by Alexopoulos and his associates. When the *Streptomyces* was inoculated 8 cm. apart instead of the usual 4 cm., *Pythium* showed an average inhibitory distance of 3.4 cm.

The inhibitory effect of the *Streptomyces* was next tested against five species of fungi by a slightly modified version of the agar dilution method used by Reilly, Schatz and Waksman (6). The *Streptomyces* was grown on glucose-tryptone medium for eighteen days at room temperature. The culture liquid was then strained through cheesecloth and passed through a Seitz filter. The filtrate was added to melted potato dextrose agar and plates were poured in a series in which the filtrate was diluted from 1:5 to 1:200. The test fungi were then inoculated on the petri plates, and the diameter of the colonies was measured after 64 hours incubation at room temperature. Six plates were inoculated with each fungus at each dilution in addition to a set of controls to which no filtrate was added. It was found that *Helminthosporium sativum* was able to grow at all concentrations employed, although growth was markedly slower than the controls from 1:5 to 1:75. *Rhizopus nigricans*, *Aspergillus niger* and *Trichoderma viride* failed to grow at 1:5 and 1:10 dilutions and showed retarded growth up to 1:50 in the case of the *Aspergillus*, up to 1:100 in the case of *Trichoderma* and at all dilutions in the case of *Rhizopus*. *Pythium debaryanum* failed to grow in all cases. A second series was then set up with dilutions up to 1:1000 using only the *Pythium* as the test organism. Growth failed to occur at dilutions through 1:300 and was markedly retarded even at 1:1000, the *Pythium* colonies averaging 35 mm. in diameter after 48 hours incubation at room temperature in contrast to the 84 mm. diameter of the controls. It should be pointed out that the

fungus inoculum in each case was taken from the periphery of a young colony of the fungus, so that continued mycelial growth rather than spore germination was being tested. These results are presented in greater detail in Table I.

TABLE I. Assay of *Streptomyces* Filtrate by Agar Dilution Method

| Average diameter of test fungus colonies in millimeters<br>after 64 hours incubation |                                    |                                     |                                     |                                                       |                                     |
|--------------------------------------------------------------------------------------|------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------------------------|-------------------------------------|
| Dilution of<br><i>Streptomyces</i><br>filtrate                                       | <i>Aspergillus</i><br><i>niger</i> | <i>Trichoderma</i><br><i>viride</i> | <i>Rhizopus</i><br><i>nigricans</i> | <i>Helmintho-</i><br><i>sporium</i><br><i>sativum</i> | <i>Pythium</i><br><i>debaryanum</i> |
| 1:5                                                                                  | 0                                  | 0                                   | 0                                   | 4                                                     | 0                                   |
| 1:10                                                                                 | 0                                  | 0                                   | 0                                   | 6                                                     | 0                                   |
| 1:20                                                                                 | 6                                  | 9                                   | 5                                   | 15                                                    | 0                                   |
| 1:30                                                                                 | 9                                  | 17                                  | 8                                   | 17                                                    | 0                                   |
| 1:50                                                                                 | 12                                 | 25                                  | 15                                  | 22                                                    | 0                                   |
| 1:75                                                                                 | 11                                 | 32                                  | 20                                  | 22                                                    | 0                                   |
| 1:100                                                                                | 12                                 | 35                                  | 40                                  | 25                                                    | 0                                   |
| 1:150                                                                                | 14                                 | 43                                  | 45                                  | 26                                                    | 0                                   |
| 1:200                                                                                | ..                                 | ..                                  | ..                                  | ..                                                    | 0                                   |
| 1:300                                                                                | ..                                 | ..                                  | ..                                  | ..                                                    | 0                                   |
| 1:500                                                                                | ..                                 | ..                                  | ..                                  | ..                                                    | 36*                                 |
| 1:1000                                                                               | ..                                 | ..                                  | ..                                  | ..                                                    | 52*                                 |
| Control                                                                              | 13                                 | 40                                  | 90                                  | 32                                                    | 90                                  |

\* Incubated for 72 hours.

The antagonistic effect of the *Streptomyces* against *P. debaryanum* was further demonstrated in a somewhat different manner by inoculating the *Pythium* in the center of petri plates containing maltose-mineral salts agar. The plates were incubated for 40 hours, at which time the colonies were about 75 mm. in diameter. The *Streptomyces* was then inoculated at the edge of the rapidly growing fungus colony. Within the following 24 hours the *Pythium* covered the entire surface of the agar, but after a few days an expanding zone could be detected with its center at the site of the *Streptomyces* inoculum. This zone was characterized by a collapsed, water-soaked appearance of the fungus mycelium. Transfers of mycelium from these zones failed to grow when placed on sterile agar slants, indicating that under these experimental conditions the *Streptomyces* was able to cause the death of established *Pythium* mycelium in its vicinity.

The *Streptomyces* has been tested in a preliminary fashion against a number of bacteria commonly used in antibiotic assays, and against certain fungi pathogenic to man. Although a degree of inhibition was manifest in several instances, there was nothing sufficiently noteworthy

to warrant further tests until such a time as it may be possible to concentrate or purify the active principle.

A culture of the *Streptomyces* was sent to Dr. S. A. Waksman who stated that it was similar to *Streptomyces albus* (Rossi Doria *emend.* Krainsky) Waksman and Henrici and that for the present it could be referred to as *S. albus* var. ST6. The varietal designation refers to the writer's culture number.

Morphologically the culture agrees fairly well with the description in the current 7th edition of Bergey's Manual. However it differs in several of its physiological reactions from those described for the species. Milk is not coagulated, a soluble brown pigment is produced in the liquefaction of gelatine, and starch is hydrolyzed. Gelatine liquefaction takes place very slowly. Certain of these properties, however, agree with earlier descriptions of *S. albus*. Abundant drops of exudate varying from nearly colorless to golden yellow were produced upon all media used which supported moderate to strong growth of the organism. The reaction upon litmus milk was quite distinctive. The milk was peptonized without coagulation, and the litmus was reduced. Then after a month or more of incubation the medium becomes blood red in color by transmitted light and increases in alkalinity to about pH 7.8.

#### Literature Cited

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