THE ANTAGONISTIC ACTION OF THE BY-PRODUCTS OF SEVERAL SOIL MICROORGANISMS ON THE ACTIVITIES OF THE LEGUME BACTERIA

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In recent years the main emphasis has been placed by the various investigators (2, 9) on the importance of bacteriophage as the factor which is responsible, in certain cases, for the decline of the legume bacteria in the soil. In addition it has been stated that as a result of this decline, symbiotic nitrogen fixation may be reduced. Data submitted by other workers (4, 5, 6) indicated in only the briefest way that the decline of the legume bacteria is due to the presence of a competitive microflora in the soil. Some of the organisms that exert inhibitory effects on the legume bacteria growing on agar medium, according to these workers, are _Actinomyces flavus, Bacillus mesentericus vulgaris, Escherichia coli_ var. communior, _Bacillus subtilis_, and certain species of fungi. No investigators have been able to show, however, that such organism were active against the legume bacteria when both were growing together in the soil.

The more recent work of Trussell and Sarles (8) has directed the attention of soil microbiologists to a field of research opened by Fleming when he discovered the organism that produces penicillin. In a study of the effect of some of the more common antibiotic substances in liquid culture, Trussell and Sarles found that actinomycin, gramicidin, tyrothricin, and tyrocidine hydrochloride inhibited the growth of certain strains of the legume bacteria.

The work presented here was started to ascertain the importance of the phenomenon of antagonism as a factor affecting the growth and longevity of the root nodule bacteria in the soil.

METHODS

Six antagonists of the legume bacteria were used in this study. They were obtained from various sources, such as from petri dishes in which the legume bacteria were being isolated from nodular tissue, and from samples of soil. These antagonists were:

- Isolate No. 1—_Aspergillus wentii_
- Isolate No. 2—_Aspergillus fumigatis_
- Isolate No. 3—_Pseudomonas_ sp. ?
- Isolate No. 4—_Pseudomonas_ sp. ?
- Isolate No. 5—_Actinomyces_ sp. ?
- Isolate No. 6—_Actinomyces_ sp. ?

Strains of the legume bacteria isolated from _Medicago sativa, Lotus uliginosus_, and _Vicia faba_ (Windsor bean) were used as the test organisms.

The agar medium used to culture these organisms and isolate the antagonists of the legume bacteria is designated as medium 100. Its composition is:

- Dextrose .................... 10.0 grams
- Sucrose ..................... 10.0 grams
- Bacto-Asparagine ............ 2.0 grams
- KH₂PO₄ .................... 0.6 gram
- K₂HPO₄ ..................... 0.3 gram
- MgSO₄ ..................... 0.2 gram
- Agar ........................ 20.0 gram
- Distilled water' .............. 1,000 cc

The pH of this medium after sterilization was approximately 6.5.

The method used to isolate these antagonists of the legume bacteria was the giant colony inoculation technique illustrated in Fig. 1. A giant colony was produced on the surface of the above agar medium seeded with a strain of the legume bacteria by adding fresh growth of the organism to be tested for its ability to inhibit the growth of the legume bacteria. The petri dishes were incubated at 27°C for 48 hours. At the end of this time, observations were made for the presence of an antagonist. The criterion used to label an organism as an antagonist was the production of a zone of inhibition, or retarded growth, between the organisms growing together (Fig. 1).

![Fig. 1.—The giant colony inoculation technique as used to isolate antagonists of the legume bacteria. Lower center, the inhibition of the legume bacteria by isolate No. 4.](image-url)