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### Soil Microbiology Department

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## SOIL MICROBIOLOGY DEPARTMENT

By H. G. THORNTON

GENERAL SOIL MICROPOPULATION

### *Counts of micro-organisms in Broadbalk plots*

The survey undertaken by Dr. Janet Brind, Mr. P. C. T. Jones and Mr. F. A. Skinner, in which direct microscope and plate counts were compared, is completed. Results have been analysed and a paper on the results is in the press and will appear in the Journal of General Microbiology.

### *Partial sterilization—Amphill plots*

Miss L. M. Crump has continued the sampling and bacteriological study of the Amphill Forest Nursery plots, in collaboration with the Chemistry Department. Work this season was limited to a comparison of plots treated with formalin with untreated plots. The results confirmed those found in the two previous years in both of which the weather had been abnormal. In the treated plots maximum numbers of bacteria were found about eight weeks after the application of formalin, but numbers were still significantly higher than control more than six months after application. Qualitative differences were again found after formalin treatment particularly in increased numbers of denitrifying bacteria and in those resistant to formalin.

The study by Dr. Janet Brind of the effect on the fungal population of steam and formalin sterilization and of acid treatment of the Amphill plots has been concluded and the results prepared for publication. The main findings were (1) the persistent effect of steaming in reducing the numbers of fungi, which, after initial elimination, had after two years, increased only to half the numbers of the control plots and (2) the early recolonization of the formalin treated plots by *Trichoderma viride* and its sustained dominance there. Many of the most frequently occurring fungi have been identified from the control plots, as well as the early recolonizers of the treated plots. Plating dilutions of soil samples from a second experiment on an adjacent area showed that the general reactions of the fungal population to steam and formalin followed the same general pattern both qualitatively and quantitatively. Laboratory experiment has shown that *Trichoderma viride* is more tolerant to concentrations of formalin than some of the other frequently occurring fungi.

Dr. B. N. Singh has followed the effect of the formalin treatment of the numbers of amoebae in these plots.

The persistent effects of partial sterilization on the soil microflora are clearly complex and, in analysing them, two possible actions of the sterilizing agent need investigation. One is the direct and specific elimination of certain groups of organisms followed by recolonization by organisms such as *Trichoderma* whose competitive ability, possibly aided by the production of antibiotics, prevents the re-establishment of the original micropopulation. The other is the possible change produced in the soil nutrients available to the microflora which may provide substances utilizable by specific

groups of organisms. Studies in different connection of antibiotic organisms are being made in the case of Actinomycetes and Myxobacteria and of the effect of specific nutrients in the case of bacteria attacking aromatic halogen compounds.

#### ACTINOMYCETES ANTAGONISTIC AGAINST FUNGI

The question how far the antibiotic secretions of micro-organisms exert an effect on the equilibrium of the soil population and whether antibiotic organisms can be used to control root diseases, cannot be answered till we know more about the rate of production and persistence and activity of antibiotics in soil. An important cause of lost activity is absorption by the soil itself. This factor has been particularly studied in the case of antibiotic secretions of actinomycetes.

Mr. F. A. Skinner has continued work on the inhibition of *Fusarium culmorum* by a soil actinomycete. In particular, further investigations have been made on the absorption of the actinomycete antibiotic by clays and by solid organic materials. Tests made with liquid actinomycete cultures and culture filtrates have shown that absorption can be affected not only by bentonite but also by kaolin, samples of Rothamsted clays, lignin and precipitated humic material. Some humified materials can serve both as nutrient sources for the actinomycete and as absorbents for the antibiotic. There is some evidence to indicate that the addition of extracts of some naturally occurring organic soil materials to solutions known to contain the antibiotic enable *F. culmorum* to grow in these solutions.

Some preliminary experiments have been made to find out whether wheat roots have any stimulating action on the growth either of *F. culmorum* or on actinomycete antagonistic to it. No such stimulation has been recorded.

#### DECOMPOSITION OF AROMATIC HALOGEN COMPOUNDS BY SOIL MICRO-ORGANISMS

The increasing use of organic halogen compounds for various agricultural purposes, makes a study of the persistence of such substances in soil of some importance. Experiments have been carried out by Dr. Norman Walker to investigate whether monochlorophenols can be attacked by soil micro-organisms and some work has also been done with monochlorobenzoic acids and 2:4-dichlorophenol. Three main experimental methods have been used; the percolation of solutions of chlorophenols through a column of soil crumbs, using either the Audus type soil-percolator or a simpler modification of it which is easily sterilized, and employing a bromine-titration method for estimating the chlorophenol concentration; attempts to secure enrichment cultures of soil bacteria capable of utilizing chlorophenols by using culture media in which the chlorophenol is the sole carbon source; and finally, studies of the oxygen uptake of suspensions of likely bacteria in Warburg manometers using chlorobenzoic acids as substrates. Up to date, the chief results of this work has been to show that *o*-chlorophenol or *o*-bromophenol can be decomposed by biological means in soil. There is some evidence that *m*- and *p*-chlorobenzoic acids may be oxidized by a soil *corynebacterium*. No reliable evidence of the biological decomposition of *p*-chlorophenol or of 2:4-dichlorophenol

in soil has yet been obtained, and even if these compounds are attacked, they are, in any case, much more persistent in soil than *o*-chlorophenol. *p*-Chlorophenoxyacetic acid suffers some decomposition in soil under conditions of storage and there is evidence that enrichment of soil with appropriate organisms can be obtained by repeated treatments with increasing doses of this compound. A yeast species has been isolated from soil, which is capable of limited growth in a liquid medium in which phenol is the sole carbon source.

#### REDUCTION PROCESSES IN SOIL

It was observed that some soils when incubated aerobically after treatment with carbon tetrachloride and the addition of a solution of sucrose and ammonium sulphate, evolved hydrogen sulphide. As the literature suggested that the formation of hydrogen sulphide from sulphate is due to obligate anaerobes this primary observation was extended to find if anaerobic sites were developing in the aerobically incubated soil. The results of this work indicated that hydrogen sulphide was evolved from soils of low buffering capacity against acid after the soil microflora had been modified by carbon tetrachloride, ether, chloroform or toluene. The hydrogen sulphide produced from the added ammonium sulphate in the experiments was not formed by anaerobic organisms but by an aerobic *Bacillus sp.* High concentrations of oxygen did not prevent the formation of hydrogen sulphide in soil. The conclusion is that hydrogen sulphide formation from ammonium sulphate in soil is not necessarily indicative of anaerobic conditions.

The carbon tetrachloride etc. inhibited the development of fungi. Antagonism on synthetic media between fungi isolated from the untreated soil and the above mentioned *Bacillus sp.* has not been observed although the fungus inhibits the evolution of H<sub>2</sub>S by this *Bacillus* in soil.

The solution and reduction of ferric hydroxide by soil microorganisms has been studied in an attempt to find out how an insoluble inorganic compound can be transformed into a form which may be suitable for plant uptake. A number of pure cultures of facultative anaerobic bacteria have been isolated which can reduce ferric hydroxide during the breakdown of sucrose. Reduction has been observed in cultures which have a final pH of 5.5. This work has been carried out by Mr. S. M. Bromfield.

#### NITRIFYING BACTERIA

Dr. Jane Meiklejohn has investigated the iron requirements of *Nitrosomonas* and the phosphate requirements of this organism and of *Nitrobacter*. She found that Jensen's strain of *Nitrosomonas* is stimulated by as little as 0.1 mg per litre of iron, the stimulation increasing with doses up to 6 mg per litre. The effect of iron shows itself even in the presence of other trace elements; it is most marked in the early stages of cultural growth. In spite of the stimulating action of iron over the above dosage range, the minimal concentration essential for growth is less than 0.05 mg per litre. The phosphate requirements of both organisms are extremely small. Jensen's strain of *Nitrosomonas* has so far been passed through three serial transfers of medium lacking any phosphate.

#### MYCORRHIZA

Dr. Janet Brind's study of the Mycorrhizal associations in clover and wheat roots was undertaken in order to obtain evidence bearing on the claim that such associations are beneficial to crop plants.

A comparison of the growth of clover plants with and without infection of their roots by the mycorrhizal fungus *Rhizophagus* but in a soil of similar microbiological flora has been possible by adding soil dilutions to a sterilized artificial soil medium. At a dilution of 1/5 pots with infected and uninfected plants were obtained, whose dry weights could be compared. A similar experiment was done on wheat. Only at the lowest dilution used (1/5) were some pots found with infected plants. A comparison of the dry weights of these plants with uninfected plants from replicate pots of the same dilution series showed no significant differences between the two series, with either clover or wheat. The results of this work thus offer no support to the view that clover and wheat receive benefit from association with the fungus.

#### SOIL AMOEBAE

Work with soil amoebae has hitherto been handicapped by lack of systematic knowledge of or ability to identify the species of these organisms found in soil. Dr. B. N. Singh has completed his detailed study of the life history and nuclear division stages of nine species of soil amoebae and has proposed a new system of classification for the amoebae based on nuclear division. He has a paper on this subject in the press which will appear in the *Philosophical Transactions of the Royal Society*. In the course of the work he showed that serious errors exist in some previous descriptions of mitotic division in amoebae and that contrary to these descriptions, the course of mitosis closely resembles that normal in metazoa. He has also made a comparative study of the process of mitosis in the amoeboid stages of several species of *Acrasieae*.

Miss Lettice M. Crump has continued her investigation of the behaviour, particularly as regards excystment, of a number of soil amoebae, several of which require the presence of bacteria to enable their cysts to hatch.

#### MYXOBACTERIA

Dr. B. N. Singh has continued his study of the physiology of species of *Myxobacteria* that feed by the lysis of certain species of *Eubacteria*.

#### WORK ON NODULE BACTERIA RHIZOBIUM AND LEGUMINOUS PLANTS

This season's work has been mainly concerned with the action of legume root secretions on infection by *Rhizobium*. This comprises an investigation by Dr. P. S. Nutman of inhibitory action of a legume plant on the nodule formation of other legume plants growing near it and a study by Miss Hilary Purchase of the influence of the near presence of roots on the multiplication of *Rhizobium* in sand.

#### *Plant interaction*

Work on the mutual reduction of nodule formation on clover plants growing together has been extended to include a study of the interactions between species of legumes belonging to different cross-

inoculation groups and between legumes and non-legumes. Lettuce and flax were shown to be only slightly inhibitory (when growing in a medium containing no combined nitrogen) to nodule formation on clover, and to have no influence on the amount of growth made by the clover.

The cross-inoculation group relationships in plant interaction were studied using the following four species. *Trifolium pratense*, *Medicago sativa*, *Vicia hirsuta* and *Anthyllis vulneraria*. These were grown singly and in all combinations of pairs with either one or both host species receiving the appropriate strain of bacteria in the inoculum.

Nodule formation on clover was found to be uniformly reduced (to about half the number of nodules found in single plant cultures) by the presence of a second plant of any kind whether or not the companion plant was inoculated with its own strain of bacteria. Neither the number of roots formed on the clover nor its size, as determined by dry weight, was uniformly depressed by a companion plant of a different species. In most cases no effect was discernable and in one comparison, i.e. with uninoculated lucerne as companion plant, a marked and highly significant increase was observed in the number of lateral roots formed on the clover.

Clover was also distinguished from the remaining host species in causing a depression of infection rate, rooting and growth of all the other plant species when it was itself inoculated but it was without effect on the other species when unnodulated.

A further general result was the inhibition of nodule formation, rooting and growth of each species of host plant when grown in pairs with its own kind.

Among the other interactions no clear relationships could be deduced; on the one hand lucerne was shown to be unaffected by the presence of uninoculated or inoculated *Vicia* whereas the rooting and growth of *Vicia* was stimulated by uninoculated lucerne and inhibited by inoculated lucerne.

The complex pattern of these interactions suggest that more than one inhibitory root secretion may be involved, the host species varying in their susceptibilities to these secretions. Preliminary tests by Miss Purchase have also shown that where two strains of bacteria are present in a culture, one may become dominant and the altered balance of the bacteria may affect the number of nodules formed.

Previous work has shown that the numbers of nodules formed on a clover root is affected by (1) the genetic constitution of the host (2) the character of the strain of bacteria used as inoculum (whether effective or ineffective in fixing nitrogen) and (3) the time at which the inoculum is added to the seedling culture (moderate delay with effective inoculation leading to an increased rate of nodule formation). Further experiments on the effect of these factors on single and paired plant cultures have shown that in all cases they act independently of inhibition.

Experiments with plants which have been selected for early or late primary infection have given clear evidence of differences in inhibition. Plants which form their first nodules early in seedling growth are more inhibitory to nodule formation of another plant,

than those which develop nodules late. This result might indicate identity between the inhibitor and the root secretion postulated by Thornton to be stimulatory to infection.

As yet experiments made with the object of concentrating the inhibitory principle or to elute it from chemical adsorbents added to the culture medium, have not been successful.

#### *Growth of Rhizobium in the root surroundings*

The depressing influence of root secretions on nodule numbers found by Dr. Nutman raised the question whether this could be in part explained by any depressing action that they might have in the numbers of bacteria in the proximity of the root. There is also the possibility that where nodule bacteria of types unable to infect the plant are also present these may be stimulated to increase in competition with the infective strain. To study these possibilities Miss Hilary Purchase carried out an experiment in sand culture with clover and lucerne, using three strains of bacteria from clover, two from lucerne and two from pea each added to sand with and without clover and lucerne plants. The presence of any host plant caused a rise in bacterial numbers regardless of whether the bacterial strain was capable of nodulating the particular plant species. The possibility of bacterial competition at the expense of the infective strain is thus not ruled out.

There was a larger rise in bacterial numbers in the immediate proximity than at a distance from the roots. This result thus does not suggest any harmful influence of root secretions on the bacterial population that might have accounted for the depression in nodule numbers found by Dr. Nutman. After an initial rise bacterial numbers remained constant till the end of the experiment (60 days).

Miss Purchase has also made a large number of serological tests of strains of clover nodule bacteria from various parts of Great Britain in connection with our study of their geographical distribution.

#### *Formation of $\beta$ -indolylacetic acid by Rhizobium*

Previous work has suggested that the substance secreted by nodule bacteria that is responsible for the deformation of the host root hairs before infection, was  $\beta$ -indolylacetic acid. Dr. Norman Walker has found evidence that Rhizobium can in fact produce this compound, some strains producing it from *l*-Tryptophane, others from *d*-Tryptophane and others from both isomers.

### WORK ON RHIZOBIUM BACTERIOPHAGE

#### *Rhizobium bacteriophage*

Work on these phages has by now produced a considerable literature. This interest is due in part to the influence that they may have on nodule bacteria in the soil but also because the unusually slow process of infection and multiplication characteristics of these phages makes them particularly suitable material for the study of phage behaviour in general.

Previous work has shown that while with a young culture of Rhizobium a single phage particle may suffice to produce infection, the chance that it will do so decreases with the age of the bacterial

culture. It was thought possible that this might be due to some inhibitory action of the polysaccharide produced by the bacteria. A purified sample of this polysaccharide was prepared, but when diluted phage was plated with the bacteria in agar in the presence of 0.25 per cent of the polysaccharide the latter produced no decrease in plaque count as compared with a control to which no polysaccharide was added. Nor did this concentration of polysaccharide in liquid medium influence the plaque count when phage and bacteria were incubated in it for a few hours before dilution. Thus the reduced chance of infection with an old bacterial culture can scarcely be attributed to an accumulation of the polysaccharide. On the other hand with longer incubation of the phage and bacteria in liquid culture, the rate of phage multiplication was reduced by the presence of the polysaccharide regardless of whether this was obtained from the host bacterial strain or from a strain resistant to the phage.

Studies have also been made on the inactivation of Rhizobium phage by ultra violet light. No evidence was found that several irradiated phage particles could multiply in a bacterial cell where a single particle failed to do so, as has been reported with Coli phage. The effects of ultra violet light in inactivating the Rhizobium phage in a culture continue after the irradiation has been stopped, since phage thus exposed falls off in activity at a rate greater than that of phage not irradiated. The addition of phage inactivated with ultra violet light to an active phage culture of the same strain checks the multiplication of the latter in the host bacteria. There is complete inhibition for the first three to five hours of incubation at room temperature. The effect seems to be specific since very little inhibition is produced by the addition of irradiated phage of a different strain to which the bacteria are resistant. Rhizobium phage that has been inactivated with ultra violet light can be partially reactivated if a mixture of such phage with the host bacteria is exposed to visible light. This phenomenon of "photo reactivation" has been recorded with Coli phage and also with other biological material. This work with Rhizobium bacteriophage was carried out by Dr. J. Kleczkowska in collaboration with Dr. A. Kleczkowski of the Plant Pathology Department.