ROTHAMSTED RESEARCH

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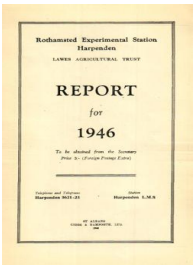

Soil Microbiology Department

H. G. Thornton

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DEPARTMENT OF SOIL MICROBIOLOGY

By H. G. THORNTON

The following main lines of research were carried out during the year:—

A. WORK DEALING WITH THE SOIL MICROPOPULATION

1. *Direct examination of organisms in soil*

Estimates of the total numbers of bacteria in soil cannot be obtained from plate counts owing first, to the uncertainty as to whether colonies are developed from single cells or from clumps of bacteria in the soil suspension, and secondly, to the fact that no single nutrient medium will support the growth of all soil bacteria. Total estimates can only be made by the direct counting of bacteria in stained films of soil. A difficulty in preparing such films has been the disturbance of the soil suspension due to surface tension forces during drying, which resulted in an uneven distribution of microorganisms over the dried film. If, however, melted agar be used as a diluent, gelation of the suspension preserves the original distribution of the soil particles and organisms. Moreover such films can be made of known thickness by setting the suspension in a haemocytometer so that the volume of suspension in a measured area of film is readily calculated. The details of the method finally developed by P. T. C. Jones and Janet Mollison are as follows:—

A known quantity of soil is added to 5 c.c. of sterile distilled water and ground in a small sterile crucible. The resulting suspension is poured into a sterile flask. The soil remaining in the crucible is washed in a further 5 c.c. distilled water and the suspended matter added to the flask. The soil suspension is made up to 50 c.c. with melted 1·5 per cent. agar previously filtered and sterilised. The suspension in the flask is well shaken and a drop is placed on a haemocytometer slide of 0·1 mm. depth, immediately covered by a coverslip and allowed to set. The coverslip can be removed under distilled water and the film gently floated on to a microscope slide and allowed to dry. The dried films are then stained for one hour in the following solution:—

Phenol (5 per cent. aqueous)	15 c.c.
Aniline Blue (water-soluble) (1 per cent. aqueous)	1 c.c.
Glacial acetic acid	4 c.c.

(This mixture must be filtered about an hour after preparation.)

The films are washed in 95 per cent. alcohol, dehydrated in absolute alcohol and mounted in Euparal. Counts of microorganisms are made in 20 random microscope fields on each of 4 replicate slides. Since the volume of 20 fields is known the number of organisms per gram of soil is readily obtained. For observation with an oil immersion it has been found that a suitable density of soil suspension is obtained by taking an initial quantity of soil such that 0·00000005 gram are contained in the volume represented by 20 microscope fields.

Statistical analysis of bacterial counts from replicate fields shows that the method gives a valid estimate of the soil microflora.

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Recovery counts of bacterial suspensions added to sterilised soil have given 93 to 98 per cent. recovery. Total numbers of bacterial cells found in natural soils have ranged from 3,000 million to 8,000 million per gram. The method can be adapted to give counts of fragments of fungus mycelium in soil.

2. *Classification of soil bacteria into nutritional groups*

Following the line of work developed by Lochhead in Canada, Mr. A. V. Garcia has studied the bacteria and proactinomycetes that formed colonies on platings from the three plots of Barnfield receiving (Plot 8, O) no manure, (Plot 1, O) farmyard manure, and (Plot 4, A) complete minerals and ammonium sulphate.

Plate counts made from samples taken on eight occasions covering a period from April till November, using several different agar media, invariably gave the highest counts from the farmyard manure plot and the lowest from the unmanured plot. The organisms that grew on platings were classified according to their growth requirements. The soil from the farmyard manure plot was characterised by an especially large percentage of bacteria that require yeast for their growth. The plot with mineral manurng contained a majority of bacteria that could grow on simple media without growth substances.

These studies on the bacteria from the three plots were accompanied by counts of amoebae carried out by Mr. A. V. Garcia and Dr. B. N. Singh.

3. *Microbial decomposition of resinous substances in soil*

Mr. P. C. T. Jones has continued this investigation, carried out at the request of the Road Research Board (D.S.I.R.) and having the practical object of stabilising resins that are added to soil in order to produce a temporary road base resistant to water absorbtion. A number of bacteria and fungi capable of attacking such resins were isolated from soil and classified according to their morphology and biochemical reactions. The breakdown of compacted blocks of soil was found to be correlated with the increase in numbers of such organisms in the blocks. The effects of various antiseptics on the organisms were studied.

4. *Nitrifying bacteria*

Dr. Jane Meiklejohn has commenced work on nitrigying bacteria. The object of the work is to discover what organisms are mainly responsible for nitrification in Rothamsted soils and what effect organic substances have on this process.

5. *Soil actinomycetes*

Mrs. Dagny Oxford's work on this group has been mainly concerned with the investigation of two problems, (1) the factors which affect the development of aerial mycelium and determine the morphology of actinomycetes in soil, in which the type of growth is generally quite uncharacteristic of their species, (2) the lipoid nature of the outer wall of the aerial mycelium and of the spores. The difficulty in wetting the spores, due to this, makes it very difficult to obtain a spore suspension and hence affects the value of actinomycete colony counts obtained from a suspension of soil.

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6. *Mycorrhiza*

Dr. Janet Mollison has surveyed the occurrence of mycorrhizal fungi in clover and in wheat. They were found almost universally in clover and seasonally in wheat. Their importance to the plant has not so far been ascertained since attempts to isolate the fungus in pure culture have not yet succeeded.

7. *Myxobacteria*

Considerable advance in the study of soil myxobacteria has been made during the year by Dr. B. N. Singh whose work is described more fully below and by Dr. R. Y. Stanier who investigated a group of these organisms found to attack chitin.

8. *Conditions influencing the excystation of amoebae*

Miss Lettice Crump has found that the type of bacterial food supplied to soil amoebae influences not only their reproductive rate but also their excystation. This discovery may explain the rapid changes in the proportion of cysts to active forms previously found in soil.

B. Work dealing with the nodule bacteria (*Rhizobium*) of leguminous plants

9. *Dissociation produced in Rhizobium by bacteriophage*

Dr. Janina Kleczkowska studied the influence of bacteriophage on the strain called F12, a "variant", ineffective in fixing nitrogen in the clover plant, which was derived from the effective strain A. Treatment with bacteriophage usually gives rise to the appearance of 'phage resistant variants which also differ from the susceptible parent strain in other characters such as colony appearance and more rarely in behaviour towards the host plant. Fifty variants of F12 obtained by 'phage treatment were tested on the clover plant. Of these one was consistently effective, three were consistently intermediate in nitrogen fication, three others gave inconsistent results on replicate plants and 43 strains remained ineffective. These characters were maintained after several passages through the plant. Similar treatment of the ineffective strain C with bacteriophage produced a variant giving effective and ineffective responses on different replicate plants.

10. *Geographical strains of clover nodule bacteria*

The testing of samples and classification of results continued during this season.

Further tests were also made of the effect of season on nitrogen fixation in continuation of last year's work.

11. *Competition between strains of nodule bacteria*

Mr. S. Bhaduri continued the study of competition between two strains of *Rhizobium*. There is evidence that this is acute between certain strains but not others. No evidence of competition between *Rhizobia* from different "inoculation groups" could be found.

12. *The interaction of the clover plant with Rhizobium*

Dr. P. S. Nutman continued investigations along the following lines:—

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(a) *Genetics of the clover plant*

Confirmation was obtained of the work suggesting that resistance to infection in clover was due to the interaction of a recessive gene with a maternally inherited factor. The inheritance of the effectivity response as regards nitrogen fixation appears to be of two kinds (1) a polygenetic inheritance that is apparently not specific to a given bacterial strain and (2) a single gene effect that is highly specific to the bacterial strain concerned. The behaviour of one of these single genes has been exhaustively studied and those of four others have been incompletely investigated. The results suggest that the genes concerned may be allelomorphic.

(b) *Physiological studies*

These have been concerned, first, with the physiologically homologous nature of lateral roots and nodules and, secondly, with the partial inhibition of nodule formation on plants growing in association. A paper on the first of these investigations is being prepared.

Publications (including Summaries), page 96.

Micro-organisms Capable of the Selective Destruction of Soil Bacteria

By H. G. Thornton

Introduction

The maintenance of satisfactory biochemical activity in a field soil depends on the establishment in it of a population of those types of micro-organisms that produce desirable chemical changes therein. This will be brought about only if the soil environment is suitable. An important factor in this environment is the existence and activity of other micro-organisms that limit the numbers of useful organisms either by competing with them for nutrients, by harmfully changing the chemical environment (as by producing toxic secretions), or by directly eating them. The competition between related strains of nodule bacteria (*Rhizobium*) studied in this department appears to be an example of the first type. The production of antibiotic secretions by fungi and actinomycetes, many of them derived from soil, has been much studied elsewhere and has given rise to a vast literature. Their production by certain of the Myxobacteria, however, has received little previous attention. Some recent investigations in this field are summarised below. There is in soil a considerable and active population of protozoa and related organisms that feed directly on bacteria. This group has been the subject of investigation at Rothamsted for many years.

In the years between 1919 to 1939 the General Microbiology Department carried out many investigations concerning the protozoan fauna of soil whose object was to elucidate the ecological importance of protozoa in soil and particularly their effect on the

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