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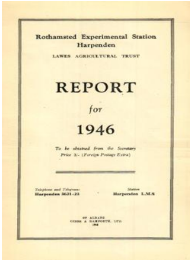

Micro-organisms Capable of the Selective Destruction of Soil Bacteria

H. G. Thornton

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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.

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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 bacterial population. A systematic survey of the types of protozoa in soils was carried out and a method was developed for the approximate enumeration of protozoa in soil samples (1, 13). With the help of this technique it was shown that the numbers of active amoebae and of flagellates in field soil underwent rapid fluctuations and that the rise and fall in numbers of active amoebae found in daily samplings was on the whole inversely related to that of bacterial numbers found in plate counts (3). Experiments also showed that the presence of amoebae kept down the numbers of bacteria in soil stored in the laboratory (2), so that it seemed reasonable to suppose that they control the size of the fluctuating bacterial population in the field. Laboratory experiments with pure bacterial cultures with and without the addition of protozoa showed, however, that the latter do not always depress the biochemical activity of the bacteria. They may stimulate this activity by keeping the bacteria at a lower numerical level than they would attain in pure cultures. This probably results in the culture remaining in a younger and more active condition (4, 5, 8, 9, 11).

The differential feeding of soil protozoa

It is an interesting question as to how far protozoa are selective in consuming certain species of bacteria and not others. There was some previous work suggesting selective feeding, but the question had been little studied.

To investigate this problem B. N. Singh developed an elegant method in which amoebae could be given a choice of bacterial food supplied as streaks, each of a different bacterial species, disposed in a petri dish in star formation at the centre of which the inoculum of amoebae was applied. Bacteria could be classified into (1) species readily eaten (2) those that were slowly eaten but eventually completely consumed (3) those that were only partly consumed and finally (4) an entirely inedible group (14). No correlation could be found between edibility of bacteria and their gram staining reaction (14, 17), but nearly all pigmented organisms other than yellow and orange were inedible. It is thus possible that certain bacterial pigments afford an advantageous protection against amoebal attack (16, 17). In other cases the edibility or otherwise of a bacterium seems to be determined by a fine difference not readily identified, since strains of Aerobacter similar in morphology and physiology differ in edibility (14). This is also the case with strains of *Rhizobium* (16).

Species of soil amoeba differ somewhat in their preference as regards bacterial food both amongst themselves and from the soil flagellate Cercomonas which also feeds selectively. Amoebae grown on plates or in sterilised soil culture can select edible from inedible bacterial food where both are supplied and, in soil they greatly reduce the numbers of edible bacteria (14). This discovery suggests that they are able in field soil to alter the quality of the bacterial flora as well as limiting its total numbers. It seems likely that this is their more important function.

Bacteria that are inedible to amoebae include certain types whose mere presence whether alone or in mixture with edible forms is definitely toxic to the amoebae. These toxic types include

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pigmented forms and there is evidence in some cases that the pigment itself is toxic. This is so in the case of the soluble pigment excreted by *Pseudomonas pyocyanea* and in that of *Chromobacterium violaceum* and *Serratia marcescens* whose violet and red pigments are relatively very insoluble in water (17). It seems possible that the further study of bacterial pigments toxic to protozoa may lead to the discovery of substances of importance in the treatment of protozoal diseases.

The estimation of numbers of protozoa in soil samples

This work on the differential feeding of soil protozoa revealed a serious defect in the previous method used for estimating protozoal numbers in soil and made possible the development of an improved method giving valid estimates. The numbers of protozoa in soil are too small to enable them to be counted directly in stained films of soil nor will they form colonies on platings. The methods used are therefore based on observing their presence or absence in a range of soil dilutions. In the methods previously used, a range of dilutions of the soil sample were made and duplicate plates of nutrient agar were inoculated with 1 c.c. portions of each dilution. From the presence or absence of protozoa at each dilution the numbers were estimated, using the Table worked out by Fisher and Yates (6). In this technique the mixed bacteria added with the diluted soil suspension were relied upon to supply food to any protozoa that might be present at that dilution. The selectivity of protozoal feeding habits shows that this method may give invalid results because an individual protozoan may come to lie on the plate amid inedible or even toxic bacteria and thus fail to grow. Apart from this serious defect the previous method was greatly limited in accuracy by the small replication enforced by the use of an entire petri dish for each culture. In his study of bacterial food supply Singh found that a number of bacterial species were readily eaten by all protozoa tested. Thus by applying the diluted soil suspension to a pure culture of such a bacterium placed on the surface of agar without any added nutrients, growth of inedible bacteria from the suspension was checked and a suitable food supply to any protozoa that might be present was assured. Replication of cultures at each dilution was obtained by the use of 8 glass cells per petri dish in each of which a replicate culture was set up (19, 20). Extensive tests have shown the method to give a reliable estimate of the numbers of amoebae and flagellates in soil samples although recovery tests with amoebae show that the estimates are consistently low owing to about 20 per cent. of the amoebae lacking viability. This method has been applied by Singh and Garcia to samples taken from three plots of Barnfield.

The occurrence and distribution of giant Rhizopods in soils

The use of plain (non-nutrient) agar with a pure bacterial culture as food supply has proved an excellent method for the culture and isolation from soil of several groups of soil organisms that derive their food from bacteria but which have until now been considered rare in soil. Amongst these are the giant amoeboid organisms of the genus *Leptomyxa* described by Goodey (7) but not since studied. By using his method of isolation B. N. Singh has found these organisms to be common and widely distributed in soils. They have been found in all of 26 arable and in 12 out of 33 grassland soil samples collected from 10 counties in Great Britain. They occur in variously manured plots from Barnfield and Broadbalk. Approximate estimates give their numbers as of the order of 1,000 per gram. Since they have an individual volume about 1,000 times that of a typical soil amoeba the numbers of bacteria that they must consume to maintain their numbers are likely to be considerable. They resemble amoebae in being specific in their food requirements. Their life cycle and ecology are under investigation.

Acrasieae

A second group of microorganisms, whose presence as regular soil inhabitants has been revealed by the technique of isolations using pure bacterial cultures as food supply, is the Acrasieae. These organisms were formerly believed to be derived from dung, but a number of strains of the genus *Dictyostelium* developed from dilutions of soil added to non-nutrient agar smeared with bacteria edible to protozoa. B. N. Singh has obtained them from 33 out of 38 arable and from 3 out of 29 samples of grassland soils obtained from localities ranging over 10 counties in Great Britain. They occur in all of the variously manured plots of Barnfield and Broadbalk, most of which receive no dung; they are hence true soil inhabitants. The Acrasieae have a life cycle comprising an amoeba-like stage which forms a "pseudoplasmodium" developing into mucor-like fruiting bodies inside which spores are formed. The myxamoebae resemble true amoebae in being differential in their feeding habits although in some cases different species of bacteria are eaten by myxamoebae and true amoebae respectively. The type of bacterial food greatly affects the morphology and occasionally the colour of the fruiting body formed. On some species of bacteria very abnormal fruiting bodies are formed so that the existing classification of the Acrasieae, based on the morphology and colour of the fruiting body, is only applicable where due regard has been taken to the food supply and cultural conditions. When added to sterilised soil supplied with a suitable bacterial culture, Acrasieae can spread through the soil at an approximate rate of 1 in. in 24 hours and will multiply therein, ultimately producing fruiting bodies on the surface. They greatly reduce the bacterial numbers in the soil culture. There is also evidence that they can multiply in fresh unsterilised soil. It thus seems likely that they are a factor affecting the bacterial population in arable soils (18, 22, 23).

Myxobacteria

Methods similar to those used in isolating giant Rhizopods and Acrasieae have revealed the presence in soils of appreciable numbers of the "higher" types of Myxobacteria of the genera *Myxococcus*, *Chondrococcus* and *Archangium* (21). They have been found in all of the 38 samples of arable and in 21 out of 31 samples of grassland soils collected over the counties of Great Britain. Their occurrence in all the classical plots of Barnfield and Broadbalk again shows them to be true soil inhabitants and not dung organisms as was previously supposed. Counts show that the Barnfield farmyard manured plot contains numbers varying from 2,000 to 76,000 per gram. They are also found in large numbers in compost heaps. The higher Myxobacteria feed on true bacteria by the production of secretions that kill and lyse the latter. Dr Singh and Dr. A. E. Oxford, working in collaboration, found that *Myxococcus virescens*, growing on a cell-free medium, produces two substances, an antibiotic agent that acts most powerfully against gram positive bacteria and a bacteriolytic substance which is a proteolytic enzyme and which lyses dead bacteria. It acts most powerfully against gram negative organisms. A further study of the production and activity of these substances may throw much light on the mechanism of antibiotic activity in general.

Number of bacterial species in soil liable to attack

In assuming the ecological importance in soil of holozoic predators and of organisms producing antibiotic secretions, it is important to form some idea of how many of the numerous and widely different species of soil bacteria are susceptible to attack by one or more of these organisms. Dr. Singh has tested the susceptibility of 84 strains of bacteria nearly all from soil, to attack by five holozoic organisms and to three species of Myxobacteria. The same bacterial strains were used in each test and these comprised a wide range of types differing in morphology, growth habit, gram staining and pigment production and included both common species and others relatively rare in soil. He found that any one species of organism could attack about half of the bacterial species tested, the actual percentages being as follows:—

	Percentages of bacterial strains attacked
Large Amoeba	54·8
Small Amoeba	60·7
Leptomyxa	41·7
Dictyostelium giganteum	57·1
D. mucoroides	61·9
Myxococcus virescens	69·0
M. fulvus	53·6
Chondrococcus exiguus	47·4

But the organisms differ markedly in which particular bacterial strains that they attack. On an average the members of any pair of organisms differ in their reaction to 36 per cent. of the bacterial strains tested. As a result of this specificty in attack all but 6 out of the 84 bacterial strains were attacked by at least one of the 8 organisms although only 6 were attacked by all of them.

This test did not cover the fungi or the numerous actinomycetes in soil that produce antibiotic secretions active against gram positive bacteria. It seems likely that very few species of bacteria exist in the soil that are immune to attack by some other micro-organism.

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