PROBLEMS IN STUDYING MICROBIAL ECOLOGY

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Often the macrobial ecologist is surprised and a little annoyed when a microbial ecologist refuses or is reluctant to take part in a general ecological survey. The reason is not that microbial ecologists are uncooperative, but rather they are faced with problems that tend to discourage co-operation. Whether interested in autecology, synecology, or habitat ecology (Alexander 1971) the problems tend to be the same and often seem insurmountable.

The over-riding difficulty is that the microbial ecologist cannot see the organisms he has to study. He is interested in those organisms which belong to the protists and include viruses, bacteria, blue-green algae, the fungi, the smaller green algae and protozoa, all of which must be viewed using a lens system if one is to see individual cells and their structure. Their small size affects attempts to estimate the numbers present, to identify the organisms, and to study their distribution and metabolism.

Perhaps these and other difficulties may be better understood if we consider problems encountered when studying the microbial ecology of soil.

The numbers of bacteria in a soil sample may be counted using direct or indirect techniques, but due to the limitations of these methods only an estimate of the numbers present can be obtained. By direct methods both living and dead cells are counted and it is difficult to distinguish between filaments, which may be bacterial or fungal. Indirect methods, such as the dilution plate-count technique, promote growth of only those bacteria favoured by the media and methods of incubation used.

Whatever the methods, all are subject to the further disadvantage that the estimation of numbers has been made from a very small sample when compared to the soil mass under consideration. Even if random samples are taken over a set area and pooled so that a representative mixture is obtained, the final amount actually studied is small.

The question of sampling poses problems when attempting to study aspects of soil ecology other than numbers of bacteria. For example, how can one study distribution of organisms in soil? At best one can examine only a small sample, as the techniques involve either direct microscopic examination of glass slides, removed from the soil after being buried for a set time, or examination of thin sections cut from soil crumbs set in resin (Jones and Griffiths 1964). Even immunofluorescence methods only allow study of a particular organism in a small area (Bohlool and Schmidt 1968) as do autoradiographic techniques (Brock and Brock 1966). When one considers that the numbers and types of organisms may vary within centimeters, and that variation may be tremendous within slightly greater distances if, for instance, moisture levels differ significantly, it becomes apparent that study of distribution in small samples may be meaningless. The study of distribution of organisms in a sample is further complicated by the fact that one cannot positively identify those organisms which can be seen.

This last point highlights one of the very great problems of microbial ecology. Bacteria, in particular, are difficult to identify. Not only do large numbers of isolates often have to be identified, but rarely can bacteria be identified by morphology alone. It is necessary to subject each isolate to a number of biochemical tests as well as staining techniques. This presents problems in manipulation. The use of multi-point inoculation together with adaptation of test media to allow use of this technique has helped to partially overcome the problem for soil bacteria (Bowie, Loutit and Loutit 1969). Once the results of the identi-

fication tests are known, considerable time is needed to follow through Skerman's Key (Skerman 1967), particularly for large numbers of strains. For this reason computer techniques have been used to help in identification and have proved successful (Loutit, Hillas and Spears 1972). Unfortunately, even after identification tests have been performed, it is not always possible to separate certain isolates from the genera, *Nocardia, Arthrobacter, Corynebacterium, Streptomyces, Brevibacterium* and *Mycobacterium* using Skerman's Key (Skerman 1967) based on Bergey's Manual (Breed, Murray and Smith 1957) which is the classification usually used. Other groups can be equally difficult to differentiate but the example cited serves to illustrate the problem.

The difficulties in estimating numbers and identifying and studying distribution of bacteria coupled with an additional problem makes study of a habitat difficult. The additional complication is that some bacteria reproduce in less than an hour, so that in a relatively short time the changes in numbers or types may be considerable. Such a potential for variation in a short time means any attempt to study the effect of environmental factors becomes extremely involved. To gain a true picture one would need to sample at 24 hour intervals and it should be obvious that to do this would involve handling, in the case of soil, thousands of isolates and subjecting them to a large number of tests. The task is almost an impossible one. At best one could follow the fate of a particular organism over a period of time, but to follow the effect of all the environmental factors listed on Table 1 would be a Herculean task with the techniques at present available.

TABLE 1. *Environmental factors able to affect growth of microorganisms.*

pH
Eh
Oxygen tension
Carbon dioxide and other gases
Water
Temperature
Radiations
Nutrients—inorganic, organic.

In addition to the environmental factors listed there are others peculiar to microbial ecology. For example, the effect of surfaces, either liquid or solid, may affect bacterial distribution and activity. In soils, clay type may affect distribution of a fungus *Fusarium oxysporum* which causes banana wilt (Stotzky and Martin 1963). Further, nitrification rate may be influenced by soil composition where the clay adsorbs to its surface both the ammonium ion and the *Nitrosomonas* organism. This close proximity promotes nitrification (Quastel 1954).

In microbial ecology one needs to know something of the metabolism of the organisms,, what they comsume and what they excrete. Sometimes their excretion products profoundly affect the habitat and may exert an influence on plants and animals present. While metabolic studies on pure cultures are possible and yield a wealth of information, caution has to be exercised in extrapolating from pure culture to natural conditions. Various techniques have been devised which allow metabolic studies on soil samples, but all have limitations. The Warburg technique has been used extensively and, although very small samples are used, it permits many replicates to be studied. The soil perfusion technique (Lees and Quastel 1946) allows studies on a relatively large soil sample, but has the disadvantage that one does not necessarily know which organisms are responsible for the metabolic activities in that sample. Some interesting results have been obtained using pure and mixed cultures of known organisms grown on glass beads which approximate soil particle size (Parr and Norman 1964). Both this technique and studies using irradiated soils offer possibilities for metabolic studies of soil organisms.

In ecology one needs to study interactions either between microorganisms or between micro- and macroorganisms. So far the greatest progress in these fields has been in studies on the latter interaction, particularly those relating to the disease process. This preoccupation with disease of either plants or animals is natural, but the knowledge gained has been at the expense of knowledge of habitats such as soil or water. It is this knowledge which is now so necessary

to an understanding of pollution, and the lack of it may account for the slowness shown in tackling some of the problems of pollution.

Perhaps if some of the enthusiasm shown in the field of molecular biology in recent years is now transferred to microbial ecology, microbial ecology will cease to be the poor relation of microbiology. We may then see more rapid progress in the field, and some of the difficulties I have listed will cease to exist.

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