ADVANCES IN MICROBIAL ECOLOGY

Edited by M. Alexander

Volume 2

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Preface

The substantial and impressive changes in microbial ecology can scarcely be chronicled in a meaningful fashion, and a review series such as *Advances in Microbial Ecology* can thus not do justice to the numerous studies that have been published in recent years. On the other hand, the mere existence of this series bears testimony to the many and diverse activities.

The growing concern with microbial communities and processes in natural ecosystems is not restricted to scientists in one region and is not limited to particular groups of organisms or to individual theoretical or applied problems. The recent and successful international symposium on microbial ecology held in New Zealand—sponsored in part by the International Commission on Microbial Ecology, as is the *Advances*—and the general microbiology and ecology conferences and congresses have included reports from investigators from all corners of the globe and have explored both new and traditional areas, agricultural and public health problems, individual species and complex communities, and heterotrophs and autotrophs as well as ecosystem models relying on mathematical concepts and environmental processes needing sophisticated chemistry for their definition.

The reviews in the present volume thus can offer only a minute sampling of the multitude of topics being actively explored at the present time. Two of the reviews focus attention on biogeochemical cycles regulated by microorganisms, in particular the way these organisms contribute to or control the levels and identities of chemical substances in the atmosphere. The chapter by Y. Dommergues, L. W. Belser, and E. L. Schmidt deals with specific factors limiting growth and biochemical transformations in terrestrial communities, a subject of broad interest to many ecologists investigating natural ecosystems. Nutrient limitation as viewed from a physiological vantage point, by contrast, is the approach of D. W. Tempest and O. M. Neijssel, and their contribution provides new insights on the application of physiological principles to environmental

phenomena. W. C. Noble and D. G. Pitcher explore our knowledge of a fascinating ecosystem inhabited by a specialized microflora, an ecosystem whose residents have a profound impact on the macroscopic bearer of this microflora. Although microbial populations have been characterized and described in many ways, the review by T. Rosswall and E. Kvillner opens new doors and discloses new means for describing microbial components of a variety of habitats.

We wish to stress that *Advances in Microbial Ecology* is sponsored by international agencies and seeks to satisfy an international audience. We have been gratified by the breadth of the response to this series and hope that suggestions for future directions and reviews will continue to be submitted. By responding to the needs of microbial ecologists and by providing a wide audience for reviews of current importance, this series can serve a critical and useful role.

M. Alexander, Editor
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Principal-Components and Factor Analysis for the Description of Microbial Populations

T. ROSSWALL AND E. KVILLNER

1. Introduction

The ecosystem ecologist and the population ecologist often set out to describe the structure and function of an ecosystem or of a population. The biotic structure is given by a description of the species present and their abundance, while the function of the biotic component of the ecosystem calls for a fairly detailed analysis of the role of individual populations (species or functional groups) in, for example, energy flow or nutrient cycling.

There is often a fundamental difference between botanists and zoologists on the one hand and microbiologists on the other in their approach to studying their respective groups of organisms in an ecosystem. Whereas it is natural for a plant ecologist to start an investigation with a structural description of the plant community, the microbial ecologist often tries to avoid this approach. The nature of the organisms to be studied and the techniques available limit the microbiologist, and autecological studies are rare although certain methods, such as immunofluorescence, have during the past 10 years greatly promoted studies of single microbial species or genera in natural environments. Microbiological studies are instead often process oriented, and measurements are

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made of, for example, respiration, litter decomposition, or nitrogen fixation. Studies of processes are usually more important to the microbiologist than those of organisms, but a knowledge of the organisms responsible is necessary for an understanding of how processes are regulated.

The difference in approach between botanists/zoologists and microbiologists has in some ways hampered the development of multidisciplinary studies since the difficulties seemingly caused by semantic problems have often been left untackled. Perhaps the process-oriented microbiological studies in the ecosystem projects, developed during the International Biological Program (IBP), have brought us to an endpoint with regard to process studies, and we must now turn to microorganism-oriented studies for obtaining a further understanding of the structure and function of ecosystems, communities, and populations.

To the microbiologist, a population description is always a hazardous undertaking. Robert Koch introduced the plate-count technique, which has been used ever since, especially for bacteria, despite its severe limitations (Jensen, 1968; Schmidt, 1973), and a host of papers describing variations in plate-count numbers in different environments and the influence of various external factors on plate counts have appeared.

Pochon and Tardieux (1962) developed the use of the most-probable-number (MPN) technique for determinations of "total numbers" of soil bacteria as well as for various physiological groups, and the technique was later modified by Darbyshire *et al.* (1974) and Rowe *et al.* (1977) by the use of automatic diluters and microtiter plates. Skerman (1969) compiled data on methods available for the selective cultivation of various taxonomic and physiological groups of bacteria.

Comparison between direct microscopic measurements and plate counts have shown that only a minor proportion of the soil bacteria are able to grow on any one isolation medium (see, e.g., Nikitin, 1973). Only a small part of the fungal mycelium seen in an ordinary stained preparation is metabolically active (Söderström, 1977), and the same limitations with regard to selectivity of isolation media apply to fungi. It is thus impossible to isolate a truly representative fraction of the bacteria and fungi living in natural environments for taxonomic purposes. All these factors, together with taxonomic difficulties, make a conventional description of the number and species composition of microorganisms from natural environments difficult, if not impossible.

These inherent difficulties have forced microbiologists to look for nonconventional ways of analyzing populations of microorganisms and their functional relationships. One such approach is the use of principal-components analysis and factor analysis in describing complex popula-

tions of microorganisms from natural environments in functional terms. This chapter describes the use of factor analysis in microbial ecology. It is not intended to be a technical paper, and the reader is referred to references given in each section for details of mathematical and computational procedures.

It is hoped that the presentation will show that these techniques may afford a powerful tool in analyzing the complex interactions between organisms and processes and the possible influence of external factors on them. It is not written for the specialist in the use of numerical methods but for the general microbial ecologist who wishes to obtain a glimpse of the possibilities that factor analysis can offer for the analysis of specific problems.

The chapter focuses mainly on bacteria. Bacteria are more suitable for this type of approach inasmuch as an isolate is more easily identified in relation to its occurrence in nature; microfungi isolated from spores or pieces of mycelia can only with difficulty be related to their functional occurrence in nature.

2. Adansonian Classification and Microbiology

Numerical and multivariate methods have found wide application in such diverse fields as the social sciences (Alker, 1969), psychology (Cattell, 1966), medicine (Baron and Fraser, 1968), archaeology (Clarke, 1968), anthropology (Driver, 1965), atmospheric-pollution studies (Gaarenstroom *et al.*, 1977), and ecology (e.g., Webb *et al.*, 1970). The field of numerical taxonomy has witnessed a rapid evolution, resulting in a second, greatly expanded edition of Sokal and Sneath's (1963) classic textbook on numerical taxonomy after only 10 years (Sneath and Sokal, 1973). A publication solely on the methodology of the use of numerical taxonomy in microbiology has also appeared (Lockhart and Liston, 1970).

The use of numerical methods for classification purposes is based on the assumption that an organism, a population, and a community can be expressed in numerical terms describing the characteristic features. The method was first developed by a French naturalist, M. Adanson, who, during his travels in Senegal, laid the foundation stone of numerical taxonomy (also called Adansonian taxonomy). The principle of Adanson's approach was to take all measurable characters into consideration and to give them all equal weight. The relationship between taxa was thus based on overall similarity, resulting in a phenetic classification. Adanson in this way described the molluscs (Adanson, 1757) and plants (Adanson, 1763) from Senegal, and although his work was unrecognized for nearly

two centuries, he is today regarded as the father of numerical taxonomy (for a further discussion, see, e.g., Sokal and Sneath, 1963).

Sneath and Sokal (1973) point out that the use of numerical methods for taxonomic purposes has generally been more readily acceptable and has provoked far less criticism when applied to bacteria than when used for plants and animals. One reason for this is probably that microbiologists, faced with seemingly insurmountable problems of taxonomy and classification, will grasp at any straw. Microorganisms, especially bacteria, are also suitable as test organisms for the development of suitable methods in numerical studies, since the possibilities for repetition, multiplication, and control of tests are greater than with most, if not all, other organisms (Bonde, 1975).

Numerical taxonomy has been used extensively for the classification of bacteria (see Sneath and Sokal, 1963, 1973, for references), while only a limited number of papers have been published on its use for the classification of microfungi (e.g., Ibrahim and Threlfall, 1966). Numerical methods for classification purposes are based on the assumption that all characters have equal weight. This has been questioned (Adams, 1964) but no practicable alternatives have resulted, and dichotomous (two-state) tests are usually used, although there are exceptions (e.g., Harman and Kocková-Kratochvílová, 1976). The use of multistate tests has been discussed by Beers and Lockhart (1962), Sundman and Gyllenberg (1967), and Lockhart (1970).

In a study on the gram reaction of soil bacteria, Gyllenberg (1968) quantified his observations and ranged the results on a scale from 0 to 1. It should similarly be possible to use quantitative metabolic fingerprints as conceived by Kühn and Hedén (1976) as a basis for a factor-analytical approach, thus avoiding the necessity of only using yes/no, +/−, 0/1 dichotomous tests.

Rapid developments in the use of numerical taxonomy have resulted in techniques suitable for the analysis of large numbers of microbial isolates with regard to their physiological/biochemical capacity. The techniques have mainly been used for bacteria, but the use of similar methods for microfungi should also prove possible.

3. Methods for Collecting Primary Data

3.1. Introduction

Two events have been prerequisite for the development of the use of factor analysis in the study of microbial populations, viz., the introduction of automation and rapid miniaturized techniques for the collection of the

primary results and the development of computers for handling the statistical/mathematical treatment of the primary data.

The new techniques recently developed for the rapid testing of microbial cultures are mainly a result of demands from the clinical microbiology sector (Rosswall, 1976). The growth in the number of publications on the subject of rapid methods and automation in microbiology has resulted in a separate bibliography on this topic (Palmer and LeQuesne, 1976); in addition, two international symposia have been devoted to it (Hedén and Illeni, 1975a,b; Johnston and Newsom, 1976), and a review article has been published (Isenberg and MacLowry, 1976).

The result of these activities has been that a large number of bacterial strains can be investigated for a multitude of physiological and biochemical characteristics in a fraction of the time it would have taken with the conventional test-tube equipment. Although the principles have not changed much, there has been a methodological revolution with regard to the hardware equipment.

3.2. Multipoint Techniques

Multipoint techniques, whereby a number of microbial strains can be transferred from a master plate containing the various cultures to be tested to appropriate test media, is a development of the replica plating technique described by Lederberg and Lederberg (1952) for the selection of bacterial mutants. This technique was based on the use of a velveteen cloth that was pressed gently onto the surface of an agar plate with bacterial colonies. The velveteen was then pressed onto the surface of a sterile agar plate, and the bacteria that could grow on this second plate formed colonies.

Many multipoint inoculation devices have been constructed (see for example Garrett, 1946; Beech *et al.*, 1955; Quadling and Colwell, 1964; Corlett *et al.*, 1965; Ridgway Watt *et al.*, 1966; Seman, 1967; Lovelace and Colwell, 1968; Lighthart, 1968; Hill, 1970; Clarholm and Rosswall, 1973; Joseph *et al.*, 1975). Wilkins *et al.* (1975) have described an inoculator especially adapted for use in an anaerobic glove box.

Although most of the techniques described were geared to work on bacteria, the first multipoint inoculating device described was for use with fungi (Garrett, 1946), and some later techniques were developed for the same purpose (Cooke, 1965; Fusaro, 1972; Littlewood and Munkres, 1972). The further development of techniques suitable for use with microfungi is urgently needed not only for the inoculation step but also for all subsequent steps in the testing procedure.