MICROBIAL ECOLOGY: FUNDAMENTALS AND APPLICATIONS

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PREFACE

A report published in the *News Bulletin* of the American Society for Microbiology in December, 1975 concluded that "the teaching of microbial ecology is sorely in need of a substantive treatment in this field in a single well-documented text on the level of *The Microbial World* (by Stanier, *et al.*)". In writing this book we have responded to this need. We have attempted to write a comprehensive text for the field of microbial ecology. Our aim was to create a thorough and flexible microbial ecology text, useful not only to microbiology majors, but also to students in general ecology, environmental science, sanitary engineering, resource management and numerous other related study programs. A thorough treatment of basic principles and approaches is followed by an extensive discussion of various applied aspects of microbial ecology. In addition to serving as a text, the book should also be useful as an introduction and reference source for scientists in related fields who wish to extend their knowledge and research into microbial ecology, and should help to bridge the communications gap among ecologists and microbiologists.

As a teaching tool, this text is designed for a one-semester course on the senior undergraduate or graduate level, and intends to familiarize the students with the principles, methodology and practical applications and implications of microbial ecology. As a background, we have assumed that the student will have had introductory courses in general chemistry, biology and microbiology.

The coverage of the field of microbial ecology in this book is more complete than any other book in this field. The book includes a short historical introduction tracing the emergence of this new field (chapter 1). Microbial diversity and metabolism are reviewed in chapters 2 and 3. Material normally found in upper level general microbiology textbooks, as far as it is essential to the understanding of microbial ecology, is summarized in these two review chapters. This review considers both prokaryotic and eukaryotic microorganisms. Ecological parameters (environmental determinants, numbers, biomass and activity) and their measurement are discussed in chapters 4 and 5; chapters 6 and 7 are devoted to habitat and community ecology. These topics are usually addressed in general ecology courses, with consideration given to higher plants and animals, but the applicability of ecological principles to microorganisms receives little attention in general microbiology and ecology texts. Interactions among microorganisms, as well as between micro- and macroorganisms, are explored in chapters 8 through 10. These chapters include discussion of the relationship of microorganisms to plant growth and pathology and to animal nutrition and disease. Microbial activities in biogeochemical cycling and applied aspects of microbial ecology are covered in chapters 11 through 15. The role of microorganisms in biodeterioration control, in soil-, water- and waste-management, in various contemporary pollution problems, in resource recovery, in energy production and in biological control receive detailed and up-to-date coverage. Chapters 16 and 17 discuss sampling designs, statistical evaluation of results and modeling approaches that are gaining an increasingly important role in research in microbial ecology. Modeling and the systems approach have received wide use by general ecologists, but hitherto have been given relatively little attention by microbiologists.

Courses in microbial ecology are now being offered at many universities. The students who attend these courses have a wide diversity of backgrounds and career goals. The study of microbial ecology cuts across traditional academic disciplines and is truly an interdisciplinary science. A challenge in writing this book was to accommodate the rather varied backgrounds of students taking a course in microbial ecology. In our experience, some students have strong backgrounds in biology and microbiology, but not in the relevant environmental sciences, such as soil science or limnology. Others have strong backgrounds in ecology, but not in microbiology. Individuals who have taken an introductory course in microbiology may have been exposed almost exclusively to material concerning bacteria with little attention given to algae, fungi, viruses, or protozoa. We, therefore, have included introductory review chapters as "background equalizers". To students with a sound microbiology background, these chapters will merely serve as concise refreshers, but to others they will impart essential background information needed for the subsequent chapters. Depending on the composition of the class, the instructor may choose to emphasize, skim, or skip these two review chapters.

In the chapters of our book that deal with fundamental aspects of microbial ecology, along with the presentation of factual knowledge, we took care to discuss the methodology employed in obtaining this knowledge. The emergence of the field of microbial ecology has gone hand in hand with technical developments, and the field will continue to expand as new experimental tools become available. Experimental approaches need to be emphasized, because the common introductory laboratory courses in microbiology that stress pure culture procedures do little to prepare the student for the investigation of the dynamic interactions of organisms and their environment.

At the option of the instructor, the last two chapters of the book (statistics and modeling) may be treated either as an integral part of the course material, or as an appendix with special relevance for those students and scientists who wish to prepare themselves for making research contributions in this field.

An advanced text should serve as a reference source to current literature, but the style of citations used in most review articles and treatises often inhibits efforts to summarize, simplify and interpret. In order to increase the readability of the text, we chose not to list specific citations for our statements. Instead, we have provided an extensive list of appropriate references, sources, and suggested readings at the end of each chapter. Inclusion of full titles with the references should facilitate ready access to the pertinent literature for anyone interested in pursuing further detail on a particular subject. We suggest that advanced students should be required to examine some of these works so that they become familiar with extracting information directly from the scientific literature. Throughout the book we have liberally illustrated general statements with specific figures and tables taken from recent journal articles; the source articles are cited in the legends and are included among the references. They also provide access points to the relevant literature on specific topics.

The field of microbial ecology has experienced dramatic growth during the past twenty years. While prior to 1960 this area of specialization was virtually unknown, an impressive body of literature has since accumulated in this field. The rapid development of microbial ecology as a scientific discipline was undoubtedly promoted by a coincident resurgence in societal interest in environmental quality. Many of today's environmental problems, as well as their potential solutions, are intimately interwoven with the microbial component of the global ecosystem. Numerous practical implications add relevance and excitement to this new field, and student interest in the subject is increasing. We hope that *Microbial Ecology: Fundamentals and Applications* helps stimulate interest in this exciting field.

We wish to acknowledge our colleagues, particularly Drs. J. Staley, J. C. Meeks, W. Mitsch, G. Cobbs, M. Finstein, D. Eveleigh and D. Pramer for reviewing various sections of this work; their suggestions proved most helpful in improving the final product. We are indebted to the many individuals, societies and companies who generously provided permission to reprint illustrative material, including Drs. R. Blakemore, P. Johnson, J. McN. Sieburth, E. Grula, H. Reichenbach, R. Unz, J. Cairns, the American Society for Microbiology, Springer-Verlag, John Wiley and Sons, Academic Press, McGraw Hill Book Co., Prentice Hall, W.B. Saunders and Williams and Wilkins.

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Louisville, Kentucky R. M. A.
New Brunswick, New Jersey R. B.
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CONTENTS

Part V Biogeochemical Cycling and Applied Aspects of Microbial Ecology 347

PART I

INTRODUCTION

CHAPTER ONE

HISTORICAL DEVELOPMENT AND SIGNIFICANCE OF MICROBIAL ECOLOGY

ECOLOGY AND MICROBIAL ECOLOGY

The term "ecology" is derived from the Greek words of "oikos" (household or dwelling) and "logos" (law). Thus, ecology is the "law of the household" or, by its contemporary definition, the science that explores interrelationships between organisms and their living and abiotic environment. The term was first defined and used in this sense by the German biologist Ernest Häeckel in 1866. The term "microbial ecology" for describing the ecological relationships of microorganisms came into frequent use only in the early 1960s. The current popularity of this term and the rapid development of the field in the last 20 years have obvious connections with a general resurgence of interest in ecology and environmental quality. The unprecedented spell of technological and economic growth which followed the conclusion of the Second World War, and an accompanying mood of boundless expectations, gradually gave way to a feeling of alarm over population explosion, deterioration of the environment, and rapid depletion of nonrenewable natural resources. Mankind, having acquired almost limitless powers to subdue and exploit the earth, appeared neither able to control its own population size, nor to manage the limited resources of "Spaceship Earth" in a wise and sustainable fashion. Grim, but quite credible scenarios of impending disaster were drawn up by groups of scientists and economists who attempted to project current population, consumption and pollution trends into the near future. Proposed remedies stressed population control, limits to technological and economic growth, pollution abatement and increased reliance on renewable resources for energy and raw materials. Such ideas were presented in books understandable to large segments of society and had notable effects on societal attitudes and on legislation; the former was manifest in various types of antipollution, conservation and consumer activism, the latter led to the formation of the Environmental Protection Agency (EPA) and various legislative actions on air pollution, water pollution and strip mining.

The desire of a responsive segment of society to live in harmony with nature rather than to disrupt it changed the once rather esoteric concept of ecology into a common household word and stimulated a broad interest in this branch of biology. Microorganisms, due to their crucial activities on organic and inorganic substrates, occupy a key position in the orderly flow of materials and energy through the global ecosystem. The persistence of various synthetic chemicals and plastics, biomagnification of pollutants, eutrophication, acid mine drainage, nitrate pollution of wellwater, methylation of mercury, ozone-depleting nitrous oxide in the atmosphere and a plethora of other environmental problems reflect unfavorable and unintended interactions of man's activities with the microbial component of the global ecosystem. On the other hand, microorganisms are just as crucial in solving some of our pressing environmental and economic problems, such as the proper disposal of various liquid and solid wastes, relief of nitrogen fertilizer shortage, food, feed and fuel production from byproducts and waste materials, recovery of metals from low-grade ores, biological control of pests, etc. The above listed and numerous other practical implications render microbial ecology a highly relevant and exciting subject for study.

HISTORICAL OVERVIEW

Although the widespread use of the term "microbial ecology" is rather recent, ecologically oriented research on microbes was performed as early as their existence was realized. Much of this research was identified as soil or aquatic microbiology, but general microbiology, too, contributed much to our understanding of microbial activities that are crucial to the balance of nature. We should recognize, however, that some strongly ingrained microbiological approaches, such as the isolation of pure cultures and their cultivation on synthetic media, are not conducive to ecological observations of the type that the general ecologist can readily make on plants and animals in their natural environments. It is, therefore, perhaps not surprising that some important observations relating to microbial ecology either predated the pure culture technique, or were made later by investigators who unwittingly or of necessity often dealt with mixed microbial populations rather than with pure cultures.

The Beginnings of Microbiology

Antonie van Leeuwenhoek (Fig. 1.1), an amateur scientist and microscope maker in Delft (Holland), using his simple but powerful lenses (Fig. 1.2), was the first to glimpse and record the multitude and diversity of the previously hidden world of microbes, including such small forms as yeasts and

Fig. 1.1 Antonie van Leeuwenhoek (1632–1723) developed simple, but powerful, microscopes, published first sketches of microscopic observations of bacteria. (Courtesy of National Library of Medicine.)

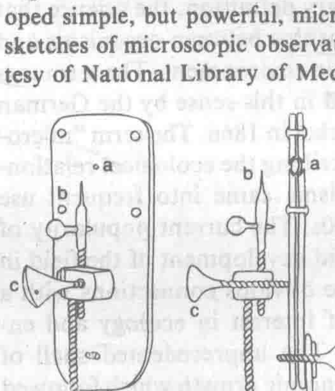

Fig. 1.2 Early microscopes of Antonie van Leeuwenhoek; (a) spheric glass lens held by two metal plates; (b) specimen holder; (c and d) screws for positioning and focusing specimen. (From C. E. Dobell, 1932; reprinted by permission of Russell and Russell.)

some bacteria. His observations, including detailed and recognizable drawings (Fig. 1.3), are recorded in a long series of letters he sent to the English Royal Society, which elected him as Fellow

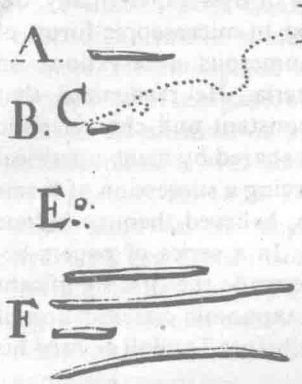

Fig. 1.3 Drawings of microorganisms by Antonie van Leeuwenhoek. Several common bacterial shapes are recognizable: A, F, rods of varying size; B, E, cocci; C, D, path of a short motile rod; G, spiral; H, cluster of cocci. (Courtesy of Royal Society of London.)

Fig. 1.4 Lazzaro Spallanzani (1729–1799) published studies disputing the doctrine of spontaneous generation. (Courtesy of National Library of Medicine.)

in 1680. Through the Proceedings of the Royal Society his discoveries, which otherwise might have been lost, were rapidly disseminated. Little additional progress took place after Leeuwenhoek until the turn of the eighteenth century, when the Italian naturalist Lazzarro Spallanzani (Fig. 1.4), debating the then prevalent view of spontaneous generation, produced experimental evidence showing that putrefaction of organic substances is caused by minute organisms that do not arise spontaneously, but multiply by cell division. Heating destroys these organisms and, in sealed flasks, prevents spoilage indefinitely. Advocates of the spontaneous generation theory, who claimed that microorganisms arise spontaneously and as a result of putrefaction of organic substances, were not convinced and tried to explain Spallanzani's experiments by the partial removal of air during the heat treatment. The heat resistance of bacterial endospores, unknown at that time, contributed to inconsistent and confusing results.

In retrospect the idea may seem rather strange to us, yet in the first half of the nineteenth century even such eminent scientists as the German chemist Justus Liebig (1803–1873) firmly believed that yeasts and other microorganisms were products rather than causative agents of fermentations. Cagniard-Latour (1838), Theodor Schwann (1837) and Friedrich Küntzing (1837) independently came to the conclusion that alcoholic fermentations were caused by living plantlike microorganisms (yeasts). Experiments by Schwann and Tyndall clearly implicated airborne microorganisms as agents of subsequent spoilage in heat-sterilized media, but the controversy dragged on until the classic experiments of Pasteur silenced even the most obstinate advocates of the spontaneous generation theory.

The Pure Culture Period

Louis Pasteur (Fig. 1.5), trained as a chemist, was prompted to study fermentation processes by practical problems of vine growers, brewers, distillers, and vinegar makers. His studies on fermentations

Fig. 1.5 Louis Pasteur (1822–1895), noted for numerous accomplishments in microbiology. He demolished the theory of spontaneous generation, demonstrated the microbial origin of various fermentations and diseases, and contributed to the development of immunization by use of attenuated microorganisms. (Courtesy of National Library of Medicine.)

between 1857 and 1876 clearly established microorganisms as the causative agents of the various fermentation processes. In a beautifully logical series of experiments (1860–1862) he utterly demolished the theory of spontaneous generation. By direct microscopic observation, as well as by cultural methods, he demonstrated the presence of microorganisms in the air and their role in initiating fermentation or spoilage in presterilized media. In his efforts to disprove spontaneous generation he found a powerful ally in the English physicist, John Tyndall (1820–1893), who demonstrated that "optically clear" (particle-free) air does not cause spoilage of sterile media and solved the nagging problem of heat resistant bacterial endospores by discontinuous heat treatments. This technique resulted in germination of endospores and their destruction upon a subsequent heating, thus accomplishing the reliable sterilization of all media at 100 C.

Ferdinand Julius Cohn (Fig. 1.6), professor of botany at the University of Breslau, Germany, developed a strong interest in microscopic forms of life. He contributed numerous observations on algae, fungi, and bacteria. He recognized that microorganisms have constant and characteristic morphology, a view not shared by many uncritical investigators who, observing a succession of forms in their impure cultures, believed them to be one and the same organism. In a series of papers between 1872 and 1875 he made the first significant efforts concerning the taxonomic classification of bacteria. In 1876, a year before Tyndall devised his

Fig. 1.6 Ferdinand Cohn (1828–1893) discovered bacterial spores and their relation to heat resistance, contributed to initial systematic classification of microorganisms. (Courtesy of National Library of Medicine.)

sterilization method by intermittent heating, Cohn described the endospores of *Bacillus subtilis* and their heat-resistant properties. He was also the first scientist to recognize and encourage the work of Robert Koch on the etiology of anthrax.

Pasteur's work clearly established the role of microorganisms in biodegradation of organic substances. He anticipated but failed to prove transformations of inorganic substances by microorganisms in the nitrification process, but proof for such transformations was forthcoming from other investigators. In 1839 DeSaussure reported his observation on the capacity of soil to oxidize hydrogen gas. Since this activity was eliminated by heating, by adding a 25% NaCl solution or by adding a 1% sulfuric acid solution, he correctly concluded that the oxidation was microbial, though hydrogen oxidizing bacteria were isolated only during the first decade of the twentieth century. J. J. Schloesing and A. Muntz reported in 1877–1879 that ammonium in sewage was oxidized to nitrate during passage through a sand column. The nitrification activity of the column was eliminated by treatment with chloroform vapors but was restored by inoculation with a soil suspension. Again, microbial nitrifying activity was the only logical explanation for this experiment, which also pointed to the presence of nitrifying organisms in soil.

All the above early information was obtained without the aid of the pure culture technique, but soon thereafter Sergei Winogradsky (Fig. 1.7), a Russian scientist working at various European Universities and later in his life at the Pasteur Institute in Paris, succeeded in isolation of the nitrifyers (1890–1891). The same scientist during his long and fruitful career described the microbial oxidation of H_2S and sulfur (1887), the oxidation of ferrous iron (1888), and correctly developed the concept of microbial chemoautotrophy even though one of his principal models (*Beggiatoa*) was later recognized not to be a chemoautotroph at all. He also described the anaerobic nitrogen fixing bacteria (1893) and contributed to the studies of reduction of nitrate and symbiotic nitrogen fixation. He originated the nutritional classification of soil microorganisms into the autochthonous (humus-utilizing) and zymogenous (opportunistic) groups, and is regarded by many as the founder of soil microbiology.

Fig. 1.7 Sergei Winogradsky (1856–1953) established the concept of microbial chemoautotrophy, made major contributions to development of enrichment culture technique and to soil microbiology. (Courtesy of Waksman Institute of Microbiology, Rutgers University.)

Equally important was the work of the Dutch microbiologist, M. W. Beijerinck (Fig. 1.8) at the University of Delft, who isolated the agents of symbiotic (1888) and non-symbiotic aerobic (1901) nitrogen fixation, and also isolated the sulfate reducers. He recognized the virtual ubiquity of most microbial forms and the selective influence of the environment that favors the development of certain types. Based on his principle "everything is everywhere, the environment selects" Beijerinck, with contributions from Winogradsky, developed the immensely useful and adaptable technique of enrichment culture. Tailoring culture conditions to