Advances in MICROBIAL ECOLOGY

Volume 8

Edited by

K. C. Marshall

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Volume 8

ADVANCES IN MICROBIAL ECOLOGY

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Preface

Advances in Microbial Ecology was established by the International Committee on Microbial Ecology (ICOME) as a vehicle for the publication of critical reviews selected to reflect current trends in the ever-expanding field of microbial ecology. Most of the chapters found in *Advances in Microbial Ecology* have been solicited by the Editorial Board. Individuals are encouraged, however, to submit outlines of unsolicited contributions to any member of the Editorial Board for consideration for inclusion in a subsequent volume of *Advances*. Contributions are expected to be in-depth, even provocative, reviews of topical interest relating to the ecology of microorganisms.

With the publication of Volume 8 of *Advances* we welcome to the panel of contributors Martin Alexander, the founding editor of this series, who discusses the range of natural constraints on nitrogen fixation in agricultural ecosystems. Ecological aspects of cellulose degradation are discussed by L. G. Ljungdahl and K.-E. Eriksson, and of heavy metal responses in microorganisms by T. Duxbury. In his chapter, A. Lee considers the gastrointestinal tract as an ecological system, and comments on the possibility of manipulating this system. The complex interactions among aerobic and anaerobic sulfur-oxidizing bacteria are discussed in terms of natural habitats and chemostat culture by J. G. Kuenen, L. Robertson, and H. van Gemerden. Finally, J. A. Robinson presents the advantages and limitations in the use of nonlinear regression analysis in determining microbial kinetic parameters in ecological situations.

K. C. Marshall, Editor
R. M. Atlas
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J. H. Slater

Contents

Chapter 2

Determining Microbial Kinetic Parameters Using Nonlinear Regression Analysis: Advantages and Limitations in Microbial Ecology

Joseph A. Robinson

Chapter 3

Neglected Niches: The Microbial Ecology of the Gastrointestinal Tract

Adrian Lee

Chapter 4

Ecological Constraints on Nitrogen Fixation in Agricultural Ecosystems

Martin Alexander

Chapter 5

Ecological Aspects of Heavy Metal Responses in Microorganisms

Trevor Duxbury

1

Microbial Interactions among Aerobic and Anaerobic Sulfur-Oxidizing Bacteria

J. GIJS KUENEN, LESLEY A. ROBERTSON, and HANS VAN GEMERDEN

1. Introduction

Life on the earth is dependent on the balanced recycling of various elements. Well-known examples are the carbon, nitrogen, and sulfur cycles. Although these cycles are often discussed separately, they are, in fact, closely linked. This is due not only to the fact that organic matter contains these elements, but also to the role that nitrate or sulfate can play in replacing oxygen as an electron acceptor for the mineralization of organic compounds. Thus, during the breakdown of organic matter, nitrate is reduced to ammonia or nitrogen, and sulfate to sulfide. Both the ammonia and the sulfide can be reoxidized. Since this chapter is mainly concerned with the ecology of bacteria involved in the sulfur cycle, a brief discussion of this cycle is appropriate (Pfennig and Widdel, 1982; Kuenen, 1975; Trudinger, 1982). Sulfate serves as the sulfur source for the biosynthesis of organic sulfur compounds by plants and microorganisms using the process known as assimilatory sulfate reduction (Fig. 1). In biological materials, sulfur is usually present in its most reduced form (e.g., as sulfide in amino acids such as cysteine). During the decomposition of this material under aerobic conditions, the organic sulfide is initially oxidized and subsequently released as sulfate. Under anaerobic

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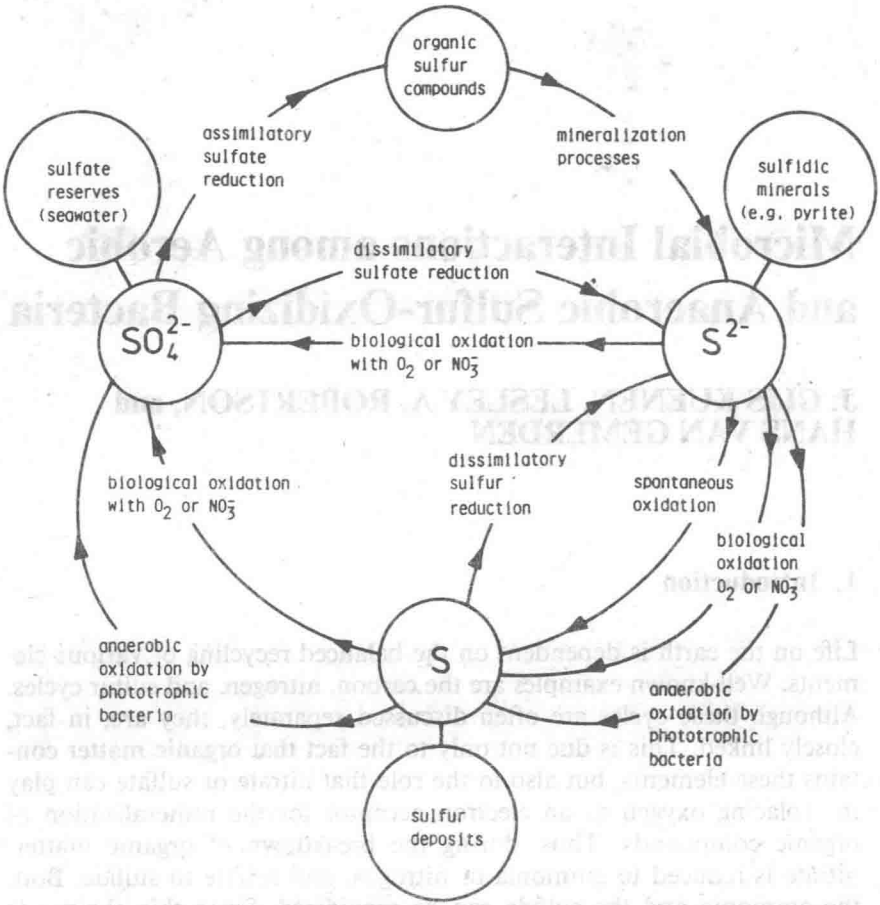

Figure 1. The sulfur cycle. [Adapted from Bos and Kuenen (1983).]

conditions, the sulfide is liberated as hydrogen sulfide. Another important source of hydrogen sulfide is from dissimilatory sulfate reduction, whereby sulfate-reducing bacteria can use sulfate as their electron acceptor for the oxidation of organic compounds or molecular hydrogen. Some sulfide precipitates as ferrous sulfide or forms pyrite, but much of it is reoxidized via elemental sulfur to sulfate (Bos and Kuenen, 1983). This can be done under anaerobic conditions in the light by phototrophic sulfur-oxidizing bacteria, which use the sulfide as an electron donor in the generation of reducing power for the reduction and assimilation of carbon dioxide (Trüper and Fischer, 1982). By this process, the electrons produced in mineralization are rechanneled into organic compounds.

Elemental sulfur, which may be formed as an intermediate, can also be reduced to sulfide by sulfur-reducing heterotrophs. Under aerobic or denitrifying conditions hydrogen sulfide can also be spontaneously oxidized by oxygen or nitrate if these substances interact at high enough concentrations. At lower concentrations of oxygen (or nitrate), sulfide is usually oxidized by the colorless sulfur bacteria to give sulfate (Kelly, 1982). During this process, the majority of the electrons from the sulfide are used to reduce oxygen or nitrate to give water or molecular nitrogen, respectively. Some electrons can be used for CO_2 reduction and be recycled into organic compounds. Thus, it is the complementary action of the two types of oxidative bacteria with the sulfur and sulfate-reducing bacteria that maintains most of the global sulfur cycle. However, it should be remembered that industrial pollution and geothermal processes also contribute substantially.

Both the phototrophic and colorless sulfur bacteria comprise large, heterogeneous groups of organisms. Whereas the difference between the two is obviously based on the possession or lack of photosynthetic pigments, the groups of different species can be further subdivided by their degree of physiological specialization or versatility, type of photosynthetic pigmentation, and other characteristics, for example, the ability to denitrify. These subdivisions are dealt with in Section 2, and are summarized in Tables I and II.

The sulfide-oxidizing bacteria are dependent on reduced sulfur compounds for growth, and therefore are found in environments where sulfate reduction occurs or where a geological source of sulfur compounds is available. Since most of the colorless sulfur bacteria are dependent on oxygen, they often live at the interface between aerobic and anaerobic zones where low concentrations of oxygen and sulfide can coexist. Examples of such environments are the aerobic surfaces of otherwise anaerobic freshwater marine sediments and the interface between the aerobic and anaerobic zones of stratified bodies of water. An example of such an interface is found in Solar Lake (Sinai), where sulfide and oxygen coexist over a depth of a few centimeters (Fig. 2). In sediments, this layer can be as narrow as 0.1 mm or less (Jørgensen, 1982). Under special circumstances, blooms of colorless sulfur bacteria (e.g., *Beggiatoa* mats) can be found on the surface of anaerobic sediments. When light can reach these interfaces, the phototrophic sulfur bacteria may thrive, and in many stratified lakes annual blooms of a variety of these phototrophs occur. These blooms can produce intensely colored green, brown, or red layers sometimes as thick as 1 m. Blooms of these organisms can also be found in the top layers of light-exposed sediments, where they can, for example, form a discrete layer in the complex microbial communities of algal mats.

In our laboratories we have been investigating the occurrence and

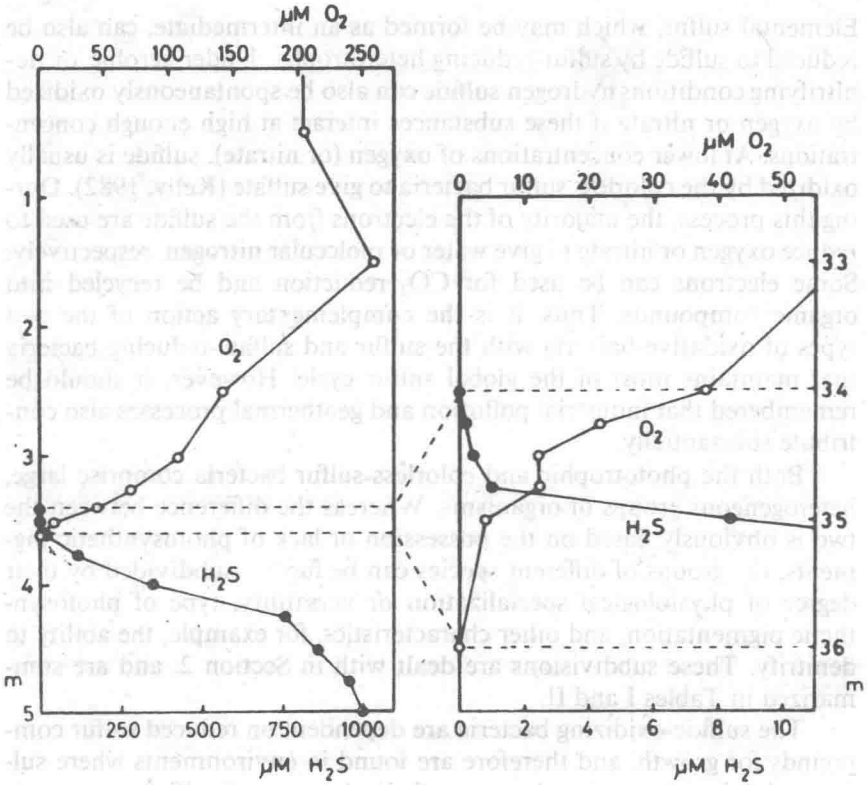

Figure 2. Concentrations of sulfide and oxygen in Solar Lake (Sinai) as a function of depth. Oxygen and sulfide can be seen to coexist at the interface. [Jørgensen *et al.* (1979).]

ecological niches of the seemingly endless variety of species among the colorless and phototrophic bacteria. In addressing these questions, we have tried to consider the abiotic environmental parameters together with some of the biotic variables, especially the interactions that occur among the phototrophic and colorless sulfur bacteria as well as between the two groups. Our approach has been through an ecophysiological study of pure cultures of these organisms under conditions that may be relevant to their existence in nature. As a second step, we have studied mixtures of the pure cultures of these organisms, and in a few cases we have also done some field studies to verify predictions made in the laboratory.

One of the most important environmental pressures imposed on microorganisms is that of nutrient or light limitation. An understanding of bacterial response to these limitations and their survival under such conditions is crucial to a better knowledge of microbial ecology. For the

study of the ecophysiology and interactions of microorganisms under nutrient limitation in the laboratory, continuous cultivation in a chemostat has been an indispensable technique. In our investigation of the interactions between the different types of sulfur bacteria, this technique has allowed us to study the physiology of test organisms under a variety of nutrient limitations, and also to use the chemostat as a device by which the selective pressures exerted on species competing for limiting nutrients or light can be simulated and amplified.

In Section 4 we describe a selected number of examples of microbial competition involving the sulfur-oxidizing bacteria. It will become clear that many of the phototrophs and the colorless sulfur bacteria are very well suited for use as model organisms for the exploration of the basic principles that determine the survival value of different metabolic strategies in the struggle for existence.

2. The Types of Bacteria

2.1. The Colorless Sulfur Bacteria

The group of organisms known as the colorless sulfur bacteria make up a heterogeneous collection of Gram-negative bacteria, which includes intensively studied species, such as some members of the genus *Thiobacillus,* and others that have not been obtained in pure culture and have only been studied superficially, such as the genus *Thiobacterium* (Vishniac, 1974; la Rivière, 1974). The various physiological types represented within the group are shown in Table I. Colorless sulfur bacteria are found at a wide range of temperatures, pH values, and degrees of aerobiosis or anaerobiosis. Some are obligate chemolithoautotrophs (e.g., *T. neapolitanus*), some can only oxidize sulfur compounds if they are supplied with an organic carbon source (e.g., *T. perometabolis*), while others are capable of autotrophic, heterotrophic, or mixotrophic growth (e.g., *T. novellus*). The group is commonly subdivided on the basis of the degree of physiological specialization shown by the various species (Tables I and III).

2.1.1. The Obligate Chemolithoautotrophs

These highly specialized species can only grow autotrophically. They must use an inorganic source of energy and obtain cell carbon from carbon dioxide fixation via the Calvin cycle. Most, however, are able to utilize small amounts of exogenous organic carbon (Matin, 1978), which can serve as a source of carbon, but not energy. The citric acid cycle in these organisms is inoperative, its enzymes only serving for biosynthesis. With