Microbial Life in Extreme Environments

edited by
D. J. Kushner

Academic Press
London New York San Francisco
A Subsidiary of Harcourt Brace Jovanovich, Publishers

Microbial Life in Extreme Environments

Edited by
D. J. KUSHNER
University of Ottawa

1978

ACADEMIC PRESS

LONDON NEW YORK SAN FRANCISCO
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road
London NW1

US edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003

Library of Congress Catalog Card Number 77–85106
ISBN: 0–12–430250–5

Text set in 10/12 pt Monotype Times New Roman, printed by letterpress,
and bound in Great Britain at The Pitman Press, Bath

In the past, organisms capable of living in toxic environments, intense radiation, or extremes of temperature, pressure, pH or salt concentration, have been studied out of academic interest or because of their importance in food spoilage and preservation. In recent years however, their potential as subjects for the investigation of many fundamental aspects of molecular and cell biology has been fully recognised. They reveal not only the scope of the biochemical and physiological mechanisms that living organisms can use, but also how basic biological processes may be modified for survival in these extreme conditions. Furthermore, understanding of their functioning is essential for consideration of the origin and evolution of life and the possibility of life in outer space.

The contributors to this book, who are specialists in their fields, discuss both the ecological distribution and the physiology and modes of adaptation of the microorganisms that live in extreme environments. The book is designed to be read by specialists, advanced students of microbiology and biochemistry and by others interested in evolutionary biology and the scope and diversity of the microbial world.

Extremely halophilic bacteria growing in salterns in San Francisco Bay have turned the saturated salt water bright red. (See Chapter 8.) (Reproduced with permission from J. Lanyi.)

Contributors

R. E. AMELUNXEN *Department of Microbiology, University of Kansas Medical Centre, Kansas City, KS 66103, USA*
J. A. BAROSS *Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA*
T. D. BROCK *Department of Bacteriology, University of Wisconsin, Madison, WI 53706, USA*
H. L. EHRLICH *Department of Biology, Rensselaer Polytechnic Institute, Troy, NY 12181, USA*
J. L. INGRAHAM *Department of Bacteriology, University of California, Davis, CA 95616, USA*
W. E. INNISS *Department of Biology, Faculty of Science, Waterloo, Ontario, Canada N2L 3GI*
A. P. JAMES *Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6*
D. J. KUSHNER *Department of Biology, Faculty of Science and Engineering, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5*
T. A. LANGWORTHY *Department of Microbiology, School of Medicine, University of South Dakota, Vermillion, SD 57069, USA*
R. E. MARQUIS *Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, USA*
P. MATSUMURA *Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, USA*
R. Y. MORITA *Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA*
A. L. MURDOCK *Department of Microbiology, University of Kansas Medical Centre, Kansas City, KS 66103, USA*
A. NASIM *Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6*
D. W. SMITH *Department of Biological Sciences, University of Delaware, Newark, DE 19711, USA*
M. R. TANSEY *Department of Biology, Indiana University, Bloomington, IN 47401, USA*

To my father and mother,
Sam and Lily Kushner

Preface

It has long been known that certain microorganisms can live under conditions of high and low temperatures, great pressure, high concentrations of solutes or normally toxic substances, acid or alkaline pH values and intense irradiation. These microorganisms were at one time studied as biological curiosities and also because some of them were important in food preservation and spoilage. In the past few years a wider and more fundamental interest in these microorganisms has developed. It has become apparent that they may provide fascinating objects for studying the deepest aspects of cellular and molecular biology, they reveal the scope of biochemical and physiological mechanisms that living things can use and they also help us to understand modes of life in the deep sea. Their study is essential for considerations of the origin of life and of the possibilities of life in outer space.

The chapters in this book, written by a number of specialists, deal with the ecological distribution of microorganisms that live in extreme environments, their physiology and modes of adaptation. The book is designed to be read by specialized workers, advanced students of microbiology and biochemistry and by others interested in the scope and diversity of the microbial world.

D. J. KUSHNER
FEBRUARY, 1978

Contents

Chapter 1

Introduction: A Brief Overview

D. J. KUSHNER

University of Ottawa

Those of us whose biological training and experience is mainly concerned with mammalian biochemistry, or the ways of such "standard" microorganisms as *Escherichia coli* or *Bacillus subtilis*, tend to think of life as a rather cosy affair, taking place at a pressure of one atmosphere, in well-aerated sera or growth media at approximately neutral pH and temperatures near that of our own bodies. Biologists more concerned with life in the outside world realize that "Nature" is much more hostile; that living creatures must contend with temperatures close to freezing (as do most of the forms of life in the oceans), or much colder. It has long been known that life can exist in such harsh environments as hot springs, some of which may also be very acid; salty natural lakes and salterns (ponds from which salt is prepared by evaporating sea water); very acid streams, such as mine effluents, which may also contain high concentrations of toxic heavy metals; in acid laboratory reagents with even higher concentrations of toxic metals; on dry rock surfaces; in deserts and in depths of the sea where the pressure may reach over 1000 atm.

Interest in such forms of life is not confined to microorganisms. Observations on animals, plants and microbes living in cold, hot, salty and deep places of the earth go back for well over one hundred years (some of these are cited in Heilbrunn, 1943; and many other reviews in this volume). When we consider that the brine shrimp, *Artemia salina*, can grow in saturated salt lakes, that invertebrates thrive in the deepest seas, that some fish do very well in sea water at −2°C, that insects and trees survive cooling to −40°C, we might ask, 'What is so special about the ability of microorganisms to come to terms with extreme environments?" In fact, microorganisms are not qualitatively different from other forms of life in this respect. However, they are able to withstand somewhat more stringent conditions. In the hottest parts of hot springs and the saltiest of lakes (the Dead Sea), only microorganisms are found. Only bacteria can survive the very highest temperatures (Brock, 1969). Then, too, with microorganisms, at least the outside of the whole creature is exposed to the environment. We do not have to consider the possibility that special tissues may have been evolved to deal with the external conditions, such as the salt glands of sea birds that permit them to drink sea-water, or the specialized channels in halophytic plants that grow in salty soil. It might be easier to understand the mechanism of adaptation of a microorganism than of a whole animal—though, in fact, a good deal is understood of ways in which several animals adapt to cold, salty or other unusual conditions (Hochachka and Somero, 1973).

A great deal of interest in microbial adaptation to extreme environments has been aroused (and supported) by the search for life on other planets. Even the most likely of these, Mars, has a harsh environment from the terrestrial point of view: low temperatures, which periodically rise above freezing, and very dry conditions. The only comparable earthly habitats are found in the dry valleys of the Antarctic, whose soil is sterile in places (Heinrich, 1976, and Chapter 2 by Baross and Morita). While this is being written, the search for life on Mars by the Viking Lander is going on, and a definite answer for the particular site studied may be obtained by the time this book appears.

Several symposia on microbial life in extreme environments have appeared in recent years (Heinen, 1974; Heinrich, 1976). The last is the report of the 1974 NASA conference on life in extreme environments, which has been held biannually since 1970. The 1976 volume is dedicated to Dr Wolf Vishniac, a pioneer student of such environments, who died in the Antarctic in 1973 while studying the dry valleys. Among the shorter general review articles written on the theme of this book are those by Brock (1969), Alexander (1976) and Kushner (1964, 1966, 1971). The last was part of a symposium dealing with the origins of life, a subject of obvious interest for the search for life on other plants. It is clear that knowing the limits of life on earth helps us to gauge the physical and chemical limits under which life may have arisen on earth and, for that matter, on other planets. The two studies go together and attract similar audiences.

The last few years have seen a gratifying growth of research on life in extreme environments. This is evidenced by the proliferation of reviews on specialized aspects of this subject, especially reviews on life in hot, cold and salty conditions. This book is an attempt to present a more integrated treatise on the subject, bringing together detailed essays on its different facets. For the most-studied subjects; life in hot, cold and salty conditions, separate chapters were obtained for the physiological and environmental aspects. There is necessarily some overlap between such chapters, as well as between chapters on different major topics. Microorganisms living in acid hot springs are considered in chapters on high temperature and pH extremes. Those that live in acid mine waters are also exposed to high concentrations of normally toxic metal ions. Effects of temperature and pressure are interrelated. Both Chapter 2 and Chapter 4 by Marquis and Matsumura discusses the ecology of microorganisms in the deep sea, take note of the fact that high pressures can have a much greater effect on microbial growth at low temperatures. Some of the coldest places on earth, the dry valleys of the Antarctic, are, as it happens, also some of the dryest.

Neither here nor in the succeeding chapters is an attempt made to define "extreme" precisely, except to point out that many of the conditions discussed would seem quite extreme to man. Rather, we are dealing with the ranges of environmental conditions that microorganisms can withstand and with the mechanisms by which they do so. These ranges are limited by the nature of matter and by the physical nature of the Earth. Liquid water is needed for all forms of life. Of all stresses to which living things can be exposed, they are probably most sensitive to drying. It is very doubtful that any organism can grow if its internal water activity (a_w) is less than 0·6, that is, less than 60% of the activity of pure water, and this limit is reached by only a few fungi. Most microorganisms need considerably higher values. The actual temperature range in which living organisms can grow is that in which liquid water can exist, approximately 273–373 K. (I speculated on conditions that might permit liquid water to exist at much lower temperatures, such as those prevailing on some of the moons of Jupiter (Kushner, 1976) but these were admittedly extremely fanciful.) In absolute terms, the most heat-resistant microorganisms can live at a temperature only about 37% greater than the lowest temperature of the most cold-resistant ones—which still does not hinder us from regarding boiling water as an extreme condition.

Much wider environmental variations are possible. Life is possible over concentrations of H^+ ions, varying by several orders of magnitude, and some individual microorganisms can grow over a range of 10 pH units or more. Many microorganisms can easily stand hundred-fold or greater variations in pressure. Indeed, the highest pressure possible in the depths of the sea is only moderately inhibitory to growth of many microorganisms. Heavy metal concentrations as low as 10^{-8}M can inhibit the growth of some microorganisms, while others may be resistant to concentrations a million-fold greater. Different microbial species show thousand-fold differences in susceptibility to irradiation.

With such a diverse subject, any order of presentation is somewhat arbitrary. To emphasize that extreme environments are not small, specialized niches, I have chosen to begin with the chapter by Baross and Morita, which deals with the microbial ecology of cold environments. These include soils, the atmosphere and the oceans, indeed the great bulk of our biosphere. Most of the seas' waters are near zero degrees. Many of the microorganisms in the atmosphere are also exposed to very low temperatures, though it is uncertain if any of them grow there. This subject is of special interest in considering the forms of life that might exist on the giant plants which have only gaseous portions in which conditions at all compatible with life exist (Ponnamperuma, 1976). Other cold environments are considered in detail, especially the Antarctic, which has been a region of increasing study in the last few years.

In Chapter 3, Inniss and Ingraham examine reasons why microorganisms are able to grow in cold conditions or require such conditions. The latter may be explained in terms of lesions in protein synthesis, in cell envelopes or in other vital parts and processes that occur at moderate temperatures. As for the former, there is no obvious reason that the growth of many mesophiles stops, instead of just slowing down, at low temperatures above zero. This has been investigated most precisely and elegantly with cold-sensitive mutants of *E. coli* and other mesophiles. This work points out that, below about 10°C, misfunction in regulatory enzymes or in ribosome formation may prevent growth. Modification of membrane lipids, and hence of membrane function, is also a very important aspect of temperature adaptation, though it is not always clear that a failure to make such modifications can set the lowest temperature at which growth is possible.

Many microorganisms that live in the sea are subjected not only to low temperatures but to pressures that, in the extreme deeps, can go to over 1100 atm. The average pressure is almost 400 atm. It has been known for some years, nevertheless, that many living organisms are found in sediment from all these depths. However, combinations of low temperature and high pressure are so highly inhibitory to microbial growth that the role of microorganisms found in the sea bottom in biodegradation is still mysterious. Recently, the intriguing possibility has arisen that microorganisms in the guts of deep sea amphipods, and other marine animals, are especially active in biodegradation. These matters, as well as the history of barobiology, and the effects of pressures—including those of several thousand atmospheres—are reviewed in Chapter 4. Pressure effects at the subcellular level have been studied in detail. The well-known ability of high pressures to dissociate hydrophobic bonds and thus disassemble cell structures poses the problem, still little explored at the cellular level, of the ways in which organisms that live in great pressures, or over a wide range of pressures, can adapt.

The fact that certain microorganisms can live at high temperatures has long caught the imagination of biologists and, for some time, this was the most studied extreme environment. Ecological relations were examined, especially in hot springs and other volcanic effluents. Work on these is reviewed briefly in Chapter 5 by Tansey and Brock, but the most detailed survey is made of the ecological distribution of the thermophilic fungi. The chapter shows that there is no lack of additional hot places in the world, including such diverse ones as the upper layers of the soil, compost heaps, hay, wood chip piles, coal dumps, slag heaps, alligator dung and cooling towers from nuclear reactors. Except for the first and the last examples, the high temperature in such sites, which may reach 60°C or more, is caused by microbial action. This is the first instance cited of an extreme condition caused by microorganisms themselves; others are cited in later chapters.

Mechanisms of resistance to high temperatures have been studied in more detail than of resistance to any other extreme condition. In Chapter 6 by Amelunxen and Murdoch, changes in metabolic rates and in the structure of membranes, ribosomes and individual proteins are considered as causes of temperature adaptation. Though all are important, it seems generally agreed that the key to high temperature adaptation lies in changes in protein structures. Many enzymes of thermophiles maintain both activity and regulation at the high temperatures at which these organisms grow. Several such enzymes have been highly purified, and they would seem to offer an excellent opportunity to correlate chemical properties with temperature adaptation. Despite this, and despite a great deal of work, the structural attributes that determine the ability of proteins to function at high temperatures are still uncertain. Thermophily in enzymes does not seem due to a major change in proportions of nonpolar amino acids or any other class of amino acids. The key seems to lie in more subtle structural differences, which may become evident only through three-dimensional analysis of these proteins. Studies of such proteins emphasize dramatically the great dependence of protein function on subtle changes in structure.

Many hot springs are also acidic, and a number of other acidic regions exist. In some of these, such as bogs and acid mine waters, the low pH is caused by microbial action. Though less studied, zones of high pH exist, and some very curious microorganisms may be found in them. One cause of high pH, urea decomposition to ammonia, is also due to microbial action. Chapter 7 by Langworthy points out that it has long been known that many microorganisms can grow over a range of pH in which their internal enzymes cannot function. The inference has been that they maintain their internal pH constant in face of a varying external pH. In recent years, this has been confirmed for a number of organisms, especially those living in acid conditions. Extracellular structures of such organisms must adapt directly to pH extremes, and a few have been shown to do so. The external layers of microorganisms that live in hot, acid conditions seem to have a distinctive chemical composition which may be needed for them to withstand such conditions. Some have rigid membranes with very low lipid content; in this, and in the properties of their lipids, they bear some relation to the membranes of extremely halophilic bacteria.