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Extremophiles

Microbiology and Biotechnology

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This book is dedicated to my late father, Vittorio Anitori,
who was not a scientist by profession,
but certainly possessed the mind of one.

Foreword

How do we define an extreme environment and its inhabitants, the extremophiles? I first made an attempt to do this over 40 years ago, in the pre-genome days (Brock, 1969). However, I think this definition still is valid today:

> It is not appropriate to define [an extreme environment] anthropocentrically, as we should be the first to admit that human life is not everywhere possible. More appropriate is its definition as a condition under which some kinds of organisms can grow, whereas others cannot. If we accept this definition it means that an environmental extreme must be defined *taxonomically*. Instead of looking at single species, or groups of related species, we must examine the whole assemblage of species, microbial and multicellular, living in various environments. When we do this we find that there are environments with high species diversity and others with low species diversity. In some environments with low species diversity we find that whole taxonomic groups are missing. For instance, in saline and thermal lakes there are no vertebrates and no vascular plants, although they may be rich in microorganisms, and very high in the numbers of the species that do live there. In many extreme environments we find conditions approaching pure cultures, with only a single species present.

When I first began to study the biology of Yellowstone hot springs, I was struck by the very visible evidence that there was an upper temperature for photosynthetic life that was *lower* than the upper temperature for microbial life in general. Detailed observations of a large number of hot springs, and an extensive review of the literature, showed that this was a general phenomenon. My work eventually led to a summary of the relationships between taxonomy and the upper temperature for different groups that is shown in Table 1. As far as I know, the relationships developed in this table are still valid.

This table raises some interesting questions. Why, for instance, is there an upper temperature for eukaryotic life at about 60°C, whereas prokaryotes (even phototrophic ones) can function well at considerably higher temperatures? Why are microorganisms able to live at considerably higher temperatures than multicellular ones?

Interestingly, for another environmental factor, low pH, a completely different set of relationships exist (Table 2). Some animals and plants can live well at fairly low pH values, whereas the prokaryotic phototrophs (cyanobacteria) have a distinct lower pH limit of around 4. Indeed, for low pH, many eukaryotic phototrophs thrive at pH values well below those of the cyanobacteria. (Brock, 1973). Even certain multicellular animals and plants will grow at lower pH values than cyanobacteria.

Why are eukaryotes able to thrive at very low pH values, but not at high temperature? Why can heterotrophic and lithotrophic bacteria and archaea grow well at temperatures of 100°C and higher, whereas phototrophic life does not exceed 70–73°C? These are evolutionary questions that derive from a careful study of the ecology of extreme environments and the extremophiles that inhabit them.

As this book shows, there are other environmental factors that can be considered extreme,

Table 1 Upper temperature limits for growth of various taxonomic groups

Major group	Group	Approximate upper temperature limit (°C)
Animals	Fish and other aquatic vertebrates	38
	Insects	45–50
	Ostracods (crustaceans)	49–50
Plants	Vascular plants	45
	Mosses	50
Eukaryotic microorganisms	Protozoa	56
	Algae	55–60
	Fungi	60–62
Prokaryotic microorganisms		
Bacteria	*Cyanobacteria* (Oxygenic)	70–73
	Phototrophic bacteria (anoxygenic)	70–73
	Chemolithotrophic bacteria	>90
	Heterotrophic bacteria	>90
Archaea	Chemolithotrophs	121
	Heterotrophs	110

Modified from Brock (1978, p. 40).

Table 2 Lower pH limits for various taxonomic groups

Major group	Group	Lower pH limit[1]	Examples
Animals	Fish	4	Carp
	Insects	2	Ephydrid flies
Plants	Cyanobacteria	4	*Mastigocladus*, *Synechococcus*
	Vascular plants	2.5–3	*Eleocharis*, *Sellowiana*, *Carex*, Ericacean plants
	Mosses	3	*Sphagnum*
Eukaryotic microorganisms	Protozoa	2	Amoebae, Heliozoans
	Eukaryotic algae	1–2	*Euglena mutabilis*, *Chlamydomonas acidophila*, *Chlorella*
		0	*Cyanidium caldarium*
	Fungi	0	*Acontium velatum*
Prokaryotic microorganisms			
Bacteria		0.8	*Thiobacillus thiooxidans*
			Sulfolobus acidocaldarius
		2–3	*Bacillus*, *Streptomyces*

[1]Lower pH limits are only approximate.

Table based on Brock (1978, p. 392).

and the evolution of organisms capable of thriving (or at least surviving) raises further questions. As Bakermans indicates in Chapter 3, adaptation to low temperatures (psychrophily) is not an uncommon trait, and cold-adapted organisms are found throughout all three domains of life. In fact, several distinct mechanisms for adaptation to low temperatures have evolved.

On the other hand, high ionizing radiation is not a common natural environmental factor. Only since the rise of nuclear physics has this factor existed. As Gwin and Battista discuss in Chapter 2, there is no obvious selective advantage to being resistant to ionizing radiation. Yet a significant number of microorganisms (only microbes; no higher organisms) exhibit this interesting characteristic. For this environmental factor, resistance is probably a chance consequence of another evolutionary pathway.

Finally, high hydrostatic pressure is a very common environmental factor, but difficult to study because of the remote regions where piezophiles (barophiles) live, and the complicated equipment needed to maintain this extreme factor (Kato, Chapter 10). Because the deep oceans are also cold, the piezophiles are also psychrophiles. Piezophiles have been identified in many bottom regions of the world's oceans, and significant advances have been made in understanding the mechanisms of piezophily.

I think that the interesting evolutionary questions raised by extremophily are valid, and I would hope that in this genome age that research on these topics will be carried out. Extreme environments and extremophiles are of enormous biological interest, initially for ecological and evolutionary relationships, and now for biotechnological reasons as well.

Many extremophiles have important practical uses, and the biotechnological aspects discussed in this book have not been neglected by the scientific community and private industry. Although the best example is *Taq* polymerase from *Thermus aquaticus*, this enzyme is only one of a large variety of important economic uses for which extremophiles have been harnessed. It has become a watchword that unique microbes are found in unique environments. It is probable that biotechnology has only scratched the surface in its search for new micro-organisms of practical use.

Thomas D. Brock

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Preface

Living cells are truly astounding. That is what I like to tell my undergraduate microbiology students. Not just the processes and reactions that occur, but the microscopic scale at which they occur. I therefore think that the term mind boggling would not be inappropriate to describe extremophiles, those microorganisms that conduct these processes and reactions under chemical and physical extremes that are usually lethal to cellular molecules. How can extremophiles possibly cope with, and even thrive, under these conditions? A major part of the 'microbiology' element of this book is devoted to reviewing the latest insights into the mechanisms used for survival by these fascinating organisms, from the ability of acidophiles to maintain a neutral intracellular pH (see Chapter 11 by Dopson), to the way that psychrophiles 'loosen up' their proteins at low temperatures (Bakermans, Chapter 3), to other equally ingenious adaptations in other classes of extremophiles (see Chapter 2 by Gwin and Battista, and Chapter 10 by Kato). Living an extreme lifestyle also imposes metabolic constraints on microbes, and has led to an astounding array of metabolic strategies, as exemplified by those lovers of high temperatures, the (hyper)thermophiles (see Chapter 6 by Bonch-Osmolovskaya, and Chapter 9 by Kashefi).

Tough microbes produce tough molecules. Since their discovery, the practical, biotechnological promise of extremophiles and their molecules has therefore been front and centre for both science and industry. The 'biotechnology' component of this book covers both established and recent, novel applications. Can extremozymes improve on their thus far relatively minor penetration of the enzyme market (Taylor *et al.*, Chapter 1)? Will extremophiles play a significant role in the production of sustainable energy in our current 'green' era (Vishnivetskaya *et al.*, Chapter 7)? How can the oil industry contribute (Chapter 8, Kotlar)? It will be fascinating to follow these and other biotechnology-related issues in the coming years.

The chapters in this book are self-contained, and hence need not be read in the order in which they appear. Most chapters are general review articles, whilst a few (e.g. those by Moissl-Eichinger *et al.*, Chapter 4, and Nevalainen *et al.,* Chapter 5) provide a more focused discourse on specific examples of extremophilic microbes. It is my hope that, taken as a whole, or as individual chapters, they will serve as helpful, up-to-date reference guides to their subject matter. The 'Future trends' and 'Web resources' sections located at the end of each chapter will help the reader keep up-to-date with new developments.

This editing adventure, my first, has been at times challenging and daunting, yet ultimately rewarding. The challenge has not been mine alone, so I would like to thank Julie and Lilyanne for dealing with my numerous excursions into the world of 'the book'.

I am confident that the audience to which this book is targeted, graduate students and researchers, are not immune to the sense of wonder elicited by extremophiles. My hope is that the information in the chapters herein will not only inform and educate, but also astound.

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