Chapter 1
Introduction

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Abstract This introductory chapter provides a brief historical perspective on the field microbial ecology and its aims. After dispelling the misconception that "extreme" environments represent just a small portion of the biosphere, the discussion highlights the importance of understanding the function and ecological roles of microbial communities in every environment and concludes by putting the current knowledge in the context of potential new discoveries of enzymes and activities with applications in industry, medicine and biotechnology.

1.1 Microbial Ecology

Ecology is the study of organisms in their natural environment and starts with the observation of natural systems, which becomes the foundation of hypotheses and theories.

Antonie Van Leeuwenhoek was a curious observer and, one could say, the first microbial ecologist. More than three centuries ago, using a rudimentary microscope he saw what he called "animalcules" and the field of microbiology was born. For the following two centuries, the field advanced mostly with laboratory experiments and using cultured isolates. The focus was on understanding the role of microorganisms in a mechanistic way, particularly in disease. Yet the pioneering work of the likes of Louis Pasteur and Sergei Winogradsky struggled to define the relevance of microbial communities and their interactions with the natural environment. In fact, the real extent of microbial diversity was not really appreciated until the advent of molecular ecology pioneered by Carl Woese in 1977 (Woese and Fox 1977) This

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opened the door to characterize not just those microbes that could be cultured, but many others, which had never been seen before on a Petri dish.

As with all new discoveries, microbiologists were left with more questions than answers: were 16S phylotypes really the most appropriate biological unit for Bacteria and Archaea and the best proxy for a microbial "species"? Moreover, how did individual phylotypes relate to functional traits and ecological niches? The widespread use of molecular phylogenetics suddenly allowed for studies of taxonomic diversity, but the advent of meta-omics technologies in the mid-2000 provided a completely new perspective. Microbial assemblages could be understood in terms of communities of genes, functions and biological activities rather than communities of taxa. Every new environmental sample yielded millions of new putative genes, mostly with unknown functions. And the increased sequencing throughput revealed the universal presence of a rare biosphere (Sogin et al. 2006). The role of these low-abundance taxa was thought to represent a permanent seed-bank, providing support to the Baas Becking hypothesis (Becking 1934) that "everything everywhere and the environment selects". Yet even this notion was soon considered too simplistic (Baltar et al. 2015) and that a combination of many biotic and abiotic factors had to be responsible for the establishment and persistence of the rare biosphere (Lynch and Neufeld 2015).

It is clear that with molecular data accumulating at an ever-increasing pace, microbial ecology requires a solid theoretical framework: do the same principles of ecological theory described by macroecologists apply and, if so, at what scale? What defines a microbial species or an ecotype? What are the rules governing the assembly of multiple taxa in complex communities? With careful observations, extreme environments provide an opportunity to address some of these questions with carefully designed natural experiments. They also represent an endless source of innovation for enzymes and compounds for the benefit of mankind.

1.2 Extreme Environments Are "The Norm"

Extreme environments are those environments where at least one physical (temperature, radiation, pressure) or chemical (pH, salinity) property is outside the normal range for human survival. Some examples of extreme environments include extremely cold environment such as those found in the Arctic and Antarctic or high pressure environment such as the deep ocean. Other severe environments include hydrothermal vents, hypersaline lakes and pools, alkaline soda lakes, dry deserts and clouds. Many environments can also be considered "extreme" under more than one parameter. For example, deep-sea hydrothermal vents are both under high hydrostatic pressure and low pH.

Contrary to popular belief, extreme environments are the norm rather than the exception. Indeed, 80% of the biosphere is permanently at low temperature (Gounot 1999) while 70% is under high pressure (Picard and Daniel 2013). These extreme environments are also home to the majority of the Earth's biomass. They support a

large diversity of microorganisms, called extremophiles, which thrive in extreme physical and or chemical conditions. For example, the bacteria *Planococcus halocryophilus* Or1, isolated from high Arctic permafrost, grows at −15 °C (Mykytczuk et al. 2013) while the chemolithoautotrophic archaea *Pyrolobus fumarii* isolated from a deep sea vent can live at 113 °C (Cowen 2004).

Some of the challenges that extremophiles must face include: changes in membrane fluidity, effects on transcription and translation and DNA damage. Indeed, nucleic acids are especially vulnerable to high temperature, radiation, oxidative damage and desiccation. However, to adapt to their environment, extremophiles have developed unique mechanisms that enables them to remain viable and active. For example, *Deinococcus radiodurans* is able to withstand an acute dose of 5000 Gy of ionizing radiation, which is 1000 time the amount that would kill a human. The radiation-induced double-strand breaks in DNA are repaired with a mechanism that consist of re-assembling fragmented DNA with remarkable accuracy (Battista 1997). Another example is the development of adaptations to deal with the denaturation and chemical modification that DNA encounters at high temperature (>70 °C). Indeed, hyperthermophiles are believed to contain more stable DNA due to the presence of monovalent and divalent salts. These salts screen the negative charges of the phosphate groups and protect the DNA from depurination and hydrolysis (Marguet and Forterre 1998).

1.3 "Extremozymes" and Their Biotechnological Interests

Microbial communities that live in extreme environments represent an innovative source of novel enzymes, which can be used for a wide range of products and industrial processes. These extremophile–derived enzymes, also known as "extremozymes", perform the same enzymatic function as their non-extremophilic homologs but with greater versatility and adaptability to extreme conditions.

The field of molecular biology has benefited from many extremophile-derived enzymes (see Table 1.1 for example). Arguably the most famous "extremozyme" is Taq polymerase, the heat resistant enzyme commonly used for Polymerase Chain Reaction (PCR). Taq polymerase was first described in the thermophilic bacterium *Thermus aquaticus*, isolated from a hot spring in Yellowstone National Park (USA) (Chien et al. 1976).

Cold-adapted enzymes are another class of "extremozymes" with applications in molecular biology. These enzymes carry the advantage that they can be selectively inactivated in a complex mixture simply by increasing the temperature of the reaction. One example is an alkaline phosphatase (AP) derived from an Antarctic bacterium (Kobori et al. 1984; Sarmiento et al. 2015). In molecular biology, alkaline phosphatases remove 5′-terminal phosphates from linearized DNA molecules, preventing self-ligation for a higher cloning efficiency. Once the activity is no longer needed, the psychrophilic AP can be easily heat-inactivated. Cold-adapted DNA ligases also confer advantages in comparison to mesophilic homologs.

Table 1.1 Few examples of potential biotechnological application derived from microbial organisms isolated from extreme environments

Organism	Group	Environments	Product/application	References
Galdieria sulphuraria 074G	Thermophilic cyanobacteria	Hot, acidic springs (T > 40 °C and pH 1–3)	Blue pigment phycocyanin (PC) used as a fluorescent marker in histochemistry	Sloth et al. (2006)
Raphidonema sp.	Psychrophilic algae	Arctic snow and permafrost	α-Tocopherol (vitamin E) and xanthophyll cycle pigments	Leya et al. (2009)
Thermus aquaticus	Thermophilic bacteria	Hotspring, Yellowstone National Park	Heat-resistant enzyme Taq polymerase	Chien et al. (1976)
Chlorella sorokiniana UTEX 2805	Thermophilic green algae	Wastewater stabilization ponds	Wastewater treatment (Ammonium removal)	De-Bashan et al. (2008)
Ralstonia sp.	Bacteria	Various environments	Biosensors for heavy metals	Nies (2000)
Antarctic psychrophile HK47	Psychrophilic bacteria	Antarctic seawater	Alkaline phosphatase (AP)	Kobori et al. (1984)
Pseudoalteromonas haloplanktis	Psychrophilic bacteria	Antarctic sea water	DNA ligase	Georlette et al. (2000)
Glaciozyma antactica strain PI12	Yeast	Antarctic	Serine protease (detergents)	Alias et al. (2014)

The function of DNA ligase is to join DNA fragments with the formation of a phosphodiester bond but at high temperature residual nuclease activity can interfere with the ligation. The use of a cold-adapted enzyme has the advantage of maintaining high specific activity at low temperature while concurrently minimizing nuclease interference (Sarmiento et al. 2015). The DNA ligase from the psychrophilic bacterium *Pseudoalteromonas haloplanktis* offers great potential given that it is active at temperatures as low as 4 °C (Georlette et al. 2000). Cold-adapted enzymes are also of interest for improving laundry and dishwasher detergents. Some of these potential enzymes include a high-performance lipase isolated from *Pseudomonas stuzeri* PS59 which has optimal activity at 20 °C (Li et al. 2014) or a serine protease isolate from Antarctic yeast *Glaciozyma antactica* strain PI12 with optimal activity also at 20 °C (Alias et al. 2014).

The food and beverage industry is also taking advantage of the discovery of new extremozymes. To date, there are many examples of commercially available thermostable enzymes especially for the starch-processing industry. Indeed, enzymes isolated from thermophilic microorganisms are optimal between 80 and 100 °C and at pH levels from 4.0 to 7.5, which are the optimal conditions for starch liquefaction (Niehaus et al. 1999).

1.4 Conclusions

In the various chapters of this book we describe the aspects of the microbial ecology from different extreme environments including polar desert soil, hyperthermophilic environments and clouds. Some of these chapters also highlight some of the potential industrial or biotechnological applications.

References

Alias N, Ahmad Mazian M, Salleh AB, Basri M, Rahman RNZRA (2014) Molecular cloning and optimization for high level expression of cold-adapted serine protease from antarctic yeast glaciozyma antarctica PI12, Enzyme Res (Hindawi Publishing Corporation, Cairo)

Becking LGMB (1934) Geobiologie of Inleiding tot de Milieukunde. In: Van Stockum WP, Zoon. (ed) The Hague

Baltar F, Palovaara J, Vila-Costa M, Salazar G, Calvo E, Pelejero C, Marrasé C, Gasol JM, Pinhassil J (2015) Response of rare, common and abundant bacterioplankton to anthropogenic perturbations in a Mediterranean coastal site. FEMS Microbiol Ecol 91:1–37

Battista JR (1997) Against all odds: the survival strategies of *Deinococcus radiodurans*. Annu Rev Microbiol 51:203–224

Chien A, Edgar DB, Trela JM (1976) Deoxyribonucleic acid polymerase from the extreme thermophile *Thermus aquaticus*. J Bacteriol 127:1550–1557

Cowen D (2004) The upper temperature of life-where do we draw the line? Trends Microbiol 12:58–60

De-Bashan LE, Trejo A, Huss VAR, Hernandez J-P, Bashan Y (2008) Chlorella sorokiniana UTEX 2805, a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater. Bioresour Technol 99:4980–4989

Georlette D, Jonsson ZO, van Petegem F, Chessa J-P, Van Beeumen J, Hubscher U, Gerday C (2000) A DNA ligase from the psychrophile *Pseudoalteromonas* haloplanktis gives insights into the adaptation of proteins to low temperatures. Eur J Biochem 267:3502–3512

Gounot AM (1999) Microbial life in permanently cold soils. In: Margesin R, Schinner F (eds) Cold-adapted organisms. Springer, Berlin Heidelberg, pp 3–15

Kobori H, Sullivan CW, Shizuya H (1984) Heat-labile alkaline phosphatase from Antarctic bacteria: rapid 5' end-labeling of nucleic acids. Proc Natl Acad Sci 81:6691–6695

Leya T, Rahn A, Lutz C, Remias D (2009) Response of arctic snow and permafrost algae to high light and nitrogen stress by changes in pigment composition and applied aspects for biotechnology. FEMS Microbiol Ecol 67:432–443

Li X-L, Zhang W-H, Wang Y-D, Dai Y-J, Zhang H-T, Wang Y, Wang H-K, Lu F-P (2014) A high-detergent-performance, cold-adapted lipase from *Pseudomonas stutzeri* PS59 suitable for detergent formulation. J Mol Catal B Enzym 102:16–24

Lynch MDJ, Neufeld JD (2015) Ecology and exploration of the rare biosphere. Nat Rev Microbiol 13:217–229

Marguet E, Forterre P (1998) Protection of DNA by salts against thermodegradation at temperatures typical for hyperthermophiles. Extremophiles 2:115–122

Mykytczuk NCS, Foote SJ, Omelon CR, Southam G, Greer CW, Whyte LG (2013) Bacterial growth at −15 °C; molecular insights from the permafrost bacterium *Planococcus halocryophilus* Or1. ISME J Nature Publishing Group 7:1211–1226

Niehaus F, Niehaus F, Bertoldo C, Bertoldo C, Kähler M, Kähler M, Antranikian G, Antranikian G (1999) Extremophiles as a source of novel enzymes for industrial application. Appl Microbiol Biotechnol 51:711–729

Nies DH (2000) Heavy metal-resistant bacteria as extremophiles: molecular physiology and biotechnological use of *Ralstonia* sp. CH34. Extremophiles 4:77–82

Picard A, Daniel I (2013) Pressure as an environmental parameter for microbial life—a review. Biophys Chem 183:30–41

Sloth JK, Weibe M, Eriksen NT (2006) Accumulation of phycocyanin in heterotrophic and mixotrophic cultures of the acidophilic red alga *Galdieria sulphuraria*. Enzyme Microb Technol 38:168–175

Sarmiento F, Peralta R, Balmey JM (2015) Cold and hot extremozymes: industrial relevance and current trends. Front Bioeng Biotechnol 3:148

Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored 'rare biosphere'. Proc Natl Acad Sci USA 103:12115–20

Woese CR, Fox GE (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc Natl Acad Sci USA 74:5088–5090