

The background of the cover is a composite of two microscopic images. The top half features a dense field of pink, rod-shaped bacteria, likely Bacillus pasteurii, against a dark background. The bottom half shows blue, rod-shaped bacteria, possibly Bacillus subtilis, also in a dense field. The text is overlaid on these images.

Physiological and Biotechnological Aspects of Extremophiles

Edited by

**Richa Salwan
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Preface

The present book on “*Physiological and Biotechnological Aspects of Extremophiles*” describes the role and importance of extremophiles under extreme conditions. Extremophiles can be found in all domains including Bacteria, Archaea and Eukarya and adapt themselves to survive in extremities which are important for evolutionary differences across different ecological areas. Extremophilic organisms are blessed with ability to tolerate and survive extremes of pH, salt, pressure, heavy metals, organic solvents, and growth in presence of toxic wastes and other habitats which are harsh for normal survival. Extremophiles are differentiated into a variety of groups including psychrophiles, psychrotrophs and thermophiles, alkaliphiles and acidophiles, peizophiles, metal and radiation tolerant. Different extremophiles are source of inspiration and often explored for getting a deep insight into the physiological adaptations. Further, the genomic cockpit of these microbes is known to encode information which helps these microbes to survive under harsh conditions.

Extremophiles have vital importance in industrial applications as they are known for the production of enzymes and metabolites produced under extremes of environmental conditions. These enzymes and metabolites are being applied in detergent, food, leather, paper and pulp, pharmaceutical, textile and agricultural industries. These metabolic products offer properties such as high stability and catalytic efficiency, high salt and alkalinity, oxidant and bleach stability, low water activity and shelf life. The higher catalytic efficiency of their encoding gene products offers industrial advantages over their contemporaries under normal environmental conditions. The thrust for these microbes offers vast potential in present climatic scenario for developing efficient processes. The recent advancements in technology like whole genome sequencing and gene/ genome editing coupled to bioinformatic tools have enhanced the pace of mining microbial diversity of extremophiles and their genome plasticity for human welfare.

The book comprises of 25 chapters that covers both physiological and biotechnological aspects of extremophiles. Chapters on physiological aspects like mechanisms and adaptations of metal tolerance, halotolerant, peizophiles, marine and Antarctic microbes are included. On the other hand, biotechnological aspects cover role of extremophiles in the production of enzymes such as lipases, carbonic anhydrases and thermophilic hydrolases as well as advances in molecular tools such as CRISPR-Cas, metagenomics, SNPs in *Pseudomonas* and adaptations in plants including Nickel Hyperaccumulation. Written with the cooperation of leading international experts with already published research and a strong background in relevant field from academia, government institutions or industries, it will contribute towards interdisciplinary knowledge and a common resource platform on extremophiles at global level. Overall, this book volume seeks to spur the role of extremophiles in bioremediation, industries and ecosystems. The book will be beneficial to the scientific community including students pursuing their doctoral studies, researchers and scientists working in the area of Microbiology in various research institutions and academic Universities.

Acknowledgments

This book is the first of its kind to offer a comprehensive and up-to-date discussion on several aspects of extremophiles. It describes the adaptations underlying survival mechanisms under extreme conditions, diversity of extremophiles and their applications in various industrial processes. This is the first book edited by the authors and we are as dependent as ever on the wisdom of others, begins one, and another, plucked at random from a Barnes & Noble new-arrival shelf: The creation of this book has removed any notion I may have had of it being a solo endeavor. I express my thanks to God for providing me his Holy Spirit and covering all my sins.

Dr. Richa extends her heart-felt gratitude to her soul mate for continuous motivation to come forward and bringing this book as an execution. This book was not possible without his valuable inputs, suggestions and kind support. He always encouraged independent and critical thinking, and shared his valuable research and professional insight to inculcate in me the attributes of an independent researcher. Working with him has been a great experience in learning.

We acknowledge all the authors residing in India and Abroad who have contributed wonderful chapters for the successful publication of this book. The chapters contributed by the authors residing in countries including Brazil, Canada, France, Iceland, Ireland, Italy, Japan, Tunisia, Turkey and United States are highly acknowledged for timely submission. We are highly thankful to Elsevier Editorial Team members for their generous and constant support in finalizing this book and other content. Special thanks are due to Samuel Young and Swapna Praveen for timely intimation, suggestions and planning of tasks. My sincere thanks are due to Mr. Samuel Young for spending his time for repeated plagiarism check for every chapter. The Editors of this book Dr. Richa Salwan and Dr. Vivek Sharma do acknowledge Science for Equity, Empowerment and Development (SEED) Division, Department of Science and Technology, India for the financial support provided under the project SP/YO/125/2017.

Overview of extremophiles

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1.1 Introduction

Extremophiles are living organisms that have the ability to grow under conditions where normal organisms are not able to survive. These extremophilic organisms are always attracted towards conditions like extremely high and low temperature, extreme acidic or basic pH, high exposure to radiations, high salinity, low and high pressure, growth in presence of toxic wastes, organic solvents, heavy metals and other habitats which are harsh for normal survival. According to the growth conditions, extremophiles are categorized into extremophilic and extremotolerant organisms. The extremophilic category includes organisms which have the ability to grow under one or more extreme environmental conditions whereas extremotolerant includes organisms that normally grow under optimal conditions but can also survive on exposure to extreme environmental conditions [1]. The extremotolerant organisms are also known as extremotrophs [2]. Besides this, there are organisms which can tolerate more than one extreme condition like extreme temperature and pH, radiations, metals etc are known as polyextremophiles.

Extremophiles include prokaryotic bacteria and archaea as well as eukaryotic organisms. Among prokaryotes, most archaeobacteria are extremophilic because of their high versatility and adaptive behavior towards extreme conditions. These archaea are salt loving, high temperature and acid tolerant, and strictly anaerobic. Archaea such as *Pyrolobus fumarii* are also known as hyperthermophiles as they can tolerate temperature up to 121 °C whereas bacteria *Geothermobacterium ferrireducens* can tolerate up to 95 °C [3,4]. Archaeobacteria identified as *Methanopyrus kandleri* and *Picrophilus torridus* grow at high temperature 122 °C and 0.06 pH, respectively. Similarly, cyanobacteria are highly adaptive in combating extreme environmental conditions by forming mats with other organisms. These cyanobacteria can tolerate extremes of salt and metal concentrations, alkalinity and less water in dry areas but can't tolerate low pH conditions [1]. *Gloeocapsa* spp. is an extremotolerant which can withstand extreme conditions in space such as temperature shifts, radiation and vacuum exposure. Similarly, spiral shaped *Helicobacter pylori* can survive extreme acidic environment of stomach. Previously, extremophile term was used to include organisms which are unicellular and prokaryotic but studies have reported that all extremophiles are not unicellular organisms [5].

1.2 Eukaryotic extremophiles

Eukaryotic multicellular organisms including fungi have well adapted physiology to survive in extreme conditions. Various microorganisms such as *Chlamydomonas*, *Dunaliella*, *Euglena* and *Ochromonas*, zooplankton, fungi and protists can grow and tolerate acidic and metal-rich conditions [6–11]. The fungal species can also thrive in acidic and alkaline environments, salt and metal tolerant but they cannot tolerate extremely high temperatures as they do not grow above 60 °C [12]. Species of *Exophiala* and *Cladophialophora* have the capacity to metabolize hydrocarbons to obtain energy and survive in polluted environments [13]. Micro-algae can also withstand extremophilic conditions as they are resistant towards light, high temperature, acidic or alkaline pH, CO₂ and metal concentration [14]. In similar studies, a red algae *Cyanidioschyzon merolae* can adapt to extreme environmental conditions by regulating the expression of 35% genes in blue and red light [15]. Moreover, lichens such as *Usnea antarctica* and *Umbilicaria cylindrica* representing algal and fungal association can also tolerate extremes of low temperatures [16]. These lichens have the ability to photosynthesis at subzero temperature and protects photosystems from damage [17].

Eukaryotic diversity has also been reported in acid mine drainage and certain aquatic environments [8,18–21]. A microscopic invertebrate *Tardigrade* also known as polyextremophilic organism can tolerate $-272\text{ }^{\circ}\text{C}$ temperature, dry and dehydrated conditions, high pressure and radiation exposure. Tardigrades undergo a process known as cryptobiosis to survive in extreme environmental conditions by suspending their metabolism. Tardigrades can survive in such extreme conditions for several years and become active during the onset of favorable environmental conditions [22]. Similarly, *Artemia salina* also known as Sea Monkey can survive in extreme of salt concentrations. *Poecilia mexicana*, a viviparous teleost can grow in environment when there are low oxygen availability and high hydrogen sulfide concentrations [23].

1.3 Prokaryotic extremophiles in diverse habitats

Microorganisms constitute the major component of the earth's biodiversity. The species biodiversity under extreme conditions such as hot and springs, saline and alkaline lakes, hot and cold deserts, and ocean beds is mainly limited to microbes as these extremes are harsh for the existence of life. Even in space where harsh environment like extreme radiation, extreme temperatures, altered gravity, extreme salinity and nutrients restrict the growth of other organisms but do allow the growth of these microbes. Nearly 70% of the earth's biosphere like Arctic, Antarctic, and moderately cold regions are having temperature below $5\text{ }^{\circ}\text{C}$ [24–27]. Such cold environments are occupied by microorganisms categorized into psychrophiles and psychrotrophs. Psychrophiles are known to show optimum growth at or below $15\text{ }^{\circ}\text{C}$ but are able to show growth maximum and minimum growth within $0\text{--}20\text{ }^{\circ}\text{C}$. Psychrotrophs show optimum growth at or above $20\text{ }^{\circ}\text{C}$ but even tolerate a temperature below $5\text{ }^{\circ}\text{C}$ [28–30]. Psychrophilic microorganisms inhabit environments such as deep seas mountains and Polar Regions which are permanently cold whereas psychrotrophs inhabit environments where temperature fluctuates [29,31–34].

Similarly, thermophilic microorganisms are found in hydrothermal vents, hot springs and heated mud flats. Extremely thermophilic *Thermus thermophilus*, *Thermoanaerobacter tengcongensis* and *Thermotoga maritima* have wide biotechnological potential and importance in studying structural biology [35–37]. Similarly, bacteria such as *Pyrodictium abyssi* can survive in hot boiling water [38] and *Desulfuridis audaxviator* which lives in groundwater below the Earth's surface. These microbes can survive without oxygen, light and can resist heat. Besides hot and cold areas, there are certain environments which have high salinity, high pressure, low water content, high radiations exposure and metals ions which are also considered as extreme environments (Fig. 1.1). Extremophilic microbes that can tolerate high metal concentrations such as arsenic, cadmium, copper and zinc are generally adapted to acidic environments, hot springs or from bio-oxidation/bioleaching processes where metal concentration is high.

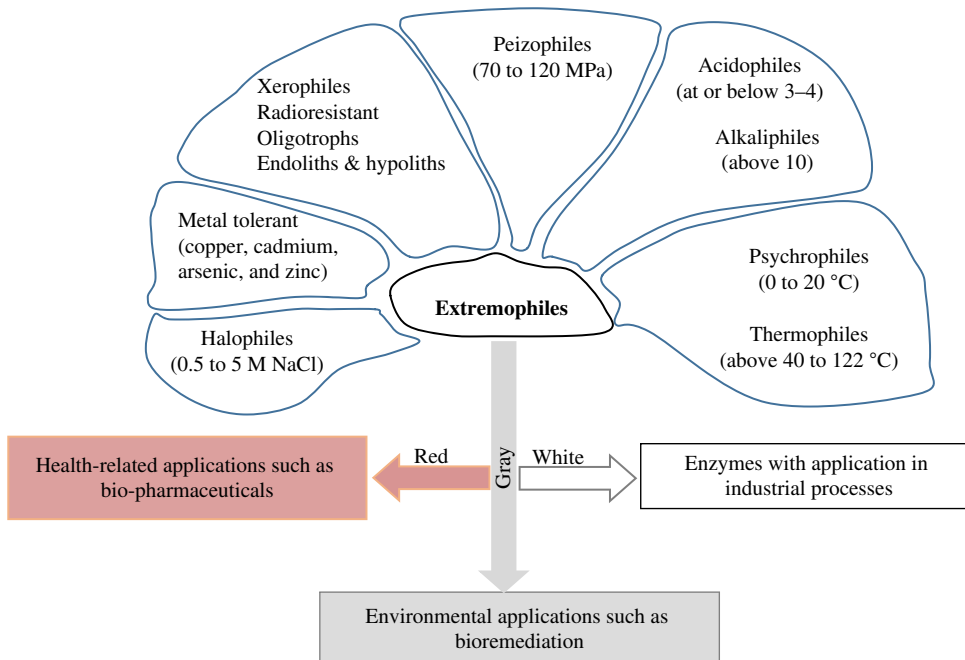


FIGURE 1.1 Categories of microorganisms including psychrophiles, thermophiles, halophiles, alkaliphiles, acidophiles, peizophiles, metal tolerance and others based on temperature, pH, salt, pressure, metal and other type of stress conditions. The metabolic products of these extremophiles find applications in red, white and gray biotechnology.

Extremophiles possess many systems that are common to all microbes likewise *Acidithiobacillus ferrooxidans* showed resistance towards heavy metals cadmium and copper, *Thermus thermophilus* towards mercury, *Ferrimicrobium acidiphilum* towards iron and zinc, and *Geobacillus stearothermophilus* towards cadmium [39–42]. Various organisms such as *Thiobacillus* and *Thiobacillus thioparus* have capacity to oxidize reduced sulfur to harmless state and hence play important role in the biogeochemical cycling of sulfur. Sulfur oxidizing bacteria also finds applications in bioleaching, bioremediation, biofertilizer, biofilters, biosensors and biodeodorizers and rubber recycling [43–49].

Halophilic microorganisms another category of extremophiles thrive in saline environments. These organisms have the capacity to tolerate high sodium chloride concentrations ranging from 0.5 to 5 M [50]. Diverse prokaryotic organisms have been reported from Dead Sea, Salterns, Great Salt Lake and solar lakes in USA, Europe and Africa [51]. Halophilic organisms *Haloarcula marismortui*, *Halofrex volcanii*, *Halorubrum lacusprofundi*, *Natronomonas pharaonis* and square archaeon *Haloquadratum walsbyi* have been reported from Dead Sea, Antarctic lake and Soda Lake [52–55]. Halotolerance has been reported in several yeasts such as *Hortaea werneckii* [56–58] and plants *Chenopodium quinoa*. Both of these are adapted to osmotic stress and excessive salinity [59].

There are some environmental areas where pressure remains very high. These areas are occupied by the microorganisms that can tolerate ambient to high pressure ranging from 70 to 120 MPa and are designated as strict or obligate piezophiles. Piezophiles excel in sustaining pressure conditions beyond the usual limits for humans. Piezophilic bacteria *Shewanella benthica*, *Colwellia marinimaniae*, *Pyrococcus yayanossi*, and *Photobacterium profundum* have well adapted proteins, lipids and genes for tolerating stress due to high pressure [60–63].

Moreover, microorganisms also survive in environments with limited nutrients like carbon, iron, nitrate, and phosphate source which plays important role in biogeochemical cycles for biomass production and nutrient cycling. These microorganisms are known as oligotrophs. Several organisms including *Deinococcus peraridilitoris* survive under extreme desert conditions. Besides this, various organisms can live inside rocks known as endoliths and some lives inside rocks in cold deserts called as hypoliths. Some organisms such as *Dienococcus radiodurans* can resist high levels of ionizing radiation called as radioresistant [64] and organisms able to withstand damaging agents including organic solvents called as toxitolerant. Some organisms are capable of tolerating desiccation known as xerophiles.

1.4 Biotechnological potential of extremophiles

The presence of extremophiles in extremes of environments has evolved biotechnologically suitable additives, enzymes, proteins and other metabolites. The capacity of extremophiles to perform better in harsh environment such as salinity, alkalinity and/or low water activity has opened exciting opportunities in industrial processes as compared to mesophilic counterparts. In today's world, emphasis is given on biological products such as biofuels, bioplastics and biosurfactants to overcome the high production costs and hazardous aspects of chemically produced products. All types of extremophiles including thermophiles, alkaliphiles, halophiles and psychrophiles offer applications in white, gray and red biotechnology. Extremophiles mostly offer applications which are enzymes based but biomolecules such as antifreeze proteins, lipids, and other molecules also find applications in industrial processes. For examples, various proteases have been reported from *Acinetobacter* sp., *Bacillus cereus*, *Colwellia* sp., *Curtobacterium luteum*, *Exiguobacterium undae* Su-1 and *Stenotrophomonas* sp. for detergent industry [65].

Metabolic products known as extremozymes including enzymes such as amylases, cellulases, esterases, keratinases, lipases, pectinases, peroxidases, proteases and xylanases finds applications in agriculture, beverages, detergent, food and feed, pharmaceuticals, textiles, leather, pulp and paper industries. These extremozymes have characteristic properties like high stability and catalytic efficiency under varied temperature and pH conditions, salinity, low water activity, low oxygen and more shelf life [66,67]. Enzymes such as *Taq* DNA polymerase obtained from thermophilic *Thermus aquaticus* is widely used and finds applications in molecular biology [68]. Similarly, ligases, alkaline phosphatases, restriction enzymes and other thermostable polymerases have been reported from various extremophilic organisms [69]. The enzymes responsible for cellulose degradation including cellobiohydrolase, endoglucanase and β -glucosidase have been reported from thermophilic *Thermotoga maritima*, *T. neapolitana* and *Pyrococcus furiosus* [68]. Genencor International first commercialized cellulase from alkaliphile with applications in textiles and detergents [69].

Besides the production of extremozymes, extremophiles also produce organic compounds known as extremolytes under stressed conditions. These extremolytes include polyols, carbohydrates like trehalose, mannose and their derivatives like mannosylglycerate and mannosylglyceramide, glucosylglycerate (GG), glucosylglucosylglycerate, and amino acids [70]. Other derivatives including phosphodiester di-myoinositol-1,1'-phosphate, cyclic 2,3-diphosphoglycerate and α -diglycerol phosphate and trianionic pyrophosphate are produced by archaeobacteria [71,72]. Other compounds including bacterioruberin, ectoines, melanin, scytonemin and mycosporin-like amino-acids (MAAs) have been reported

from UV-resistant extremophilic bacteria [73–75]. These extremolytes find applications in pharmaceutical sector like cosmetics, therapeutics for developing pharmacophore with antiproliferative and anti-inflammatory activity and chemopreventive agents [76,77]. For example, *Pseudomonas* has been reported for the production of pyochelin, an iron binding compound with antifungal activity against *Candida* and *Aspergillus* spp [78]. Extremophiles are also known for the production of metabolites like exopolysaccharides biosurfactants, biopolymers and peptides with diverse industrial potential [79]. Biosurfactants are mostly employed as adjuvants for herbicides, pesticides formulations, bioremediation processes and biocontrol agents [70]. In different studies, halophiles are explored for the production of polysaccharides made of fructans with applications in food, medical, pharmaceutical, chemical and cosmeceutical industries [80–82]. Several halophiles are reported for poly-hydroxyalkanoate as biodegradable plastic, exopolysaccharides as emulsifiers, osmotic solutes as stabilizers and bacteriorhodopsin in energy conversion [83]. Additionally, extremophilic organisms such as *Bacillus licheniformis* are efficient in degrading complex materials produced by industrial wastes and effluents, sewage and petroleum hydrocarbons [84]. *Planococcus* is a halophile having the capacity to tolerate up to 25% NaCl can degrade BTEX in oil-contaminated soil. Similarly, studies have reported that halophilic archaea degrade phenol, pyrene and naphthalene and produce biosurfactants [85]. Similarly, β -carotene from halophilic microalgae *Dunaliella* is used as supplements in food products, pharmaceutical industries for colorant and antioxidant properties [86].

1.5 Molecular approaches like metagenomics and whole genome sequencing (WGS) of extremophiles

In the present scenario, extremophiles are being considered as attractive candidates for studying their adaptations with respect to physiological, biochemical and other fundamental cellular processes. As extremophiles are being adapted to extreme environmental conditions, it becomes difficult to cultivate such organisms in laboratory conditions. Therefore, molecular techniques like recombinant DNA technology for cloning and heterologous expression in a suitable host either bacteria or cell lines using vectors provide easy way for genetic manipulation and successful commercialization of gene products. Molecular approaches like ligation-independent cloning [87] and hybridization cloning [88] have also been used to obtain recombinant proteins for studying structure-function relationship of proteins and other enzymes.

Omic based approaches such as comparative genomics, proteomics, transcriptomics, metallomics and secretomics are preferred for understanding mechanisms underlying physiological, biochemical and structural adaptations of extremophiles [89–94]. To obtain the adaptations of extremophilic microorganisms, it is imperative to obtain the complete genome sequence of a particular microbe. Studies have reported biomass degrading genes in *Bacillus cellulosilyticus* [95], *Cellulomonas* spp. [96], *Dictyoglomus turgidum* [97] and *Fibrobacter succinogenes* [98,99] by WGS. Similarly, the genomes of halophilic strains belonging to *Halomicrobium*, *Chromohalobacter*, *Haloferax*, *Haloarcula*, *Halorubrum*, *Natronomonas* and *Haloquadratum* have been reported for various metabolites profiling such as membrane lipids, cell wall components and bacteriorhodopsin related to high salt concentrations from various habitats. The whole genomes of these halophiles have been explored to identify the possible role of genes DNA polymerase, thioredoxin reductase, cytochrome oxidase, multiple TATA binding proteins (TBP), transcription factors involved in adaptation to hyper saline environments [100]. Various studies on piezophilic bacteria *Shewanella*, *Photobacterium profundum*, *Moritella profunda* and *Saccharomyces cerevisiae* have properties that can withstand high pressure. DNA binding protein such as RecD and other proteins such as Hsp60, Hsp70, OmpH, RecA, F1F0 ATPases, Cct, Tat2 and Ypr153w have important role in adaptations for tolerating high pressure [101]. Moreover, dihydrofolate reductase from piezophilic *Moritella profunda* is involved in tolerance towards high pressure upto 50 MPa [63,102–104]. Psychrophilic organisms *Exiguobacterium antarcticum*, *Pseudoalteromonas arctica*, *Pseudoalteromonas haloplanktis* and *Aquasplillum arcticum* contain enzymes such as β -glucosidase, protease, malate dehydrogenase, DNA repairing enzymes which are adapted to stress conditions such as low temperature [105–109]. By obtaining the knowledge of gene sequences in whole genomes, engineering of proteins and genes with precision for desired property can be done.

Similarly, microbial community's analysis has been studied to evaluate their potential to produce different metabolites and their interaction among extreme environments using metagenomic approaches. Molecular phylogenetic analysis such as metagenomics provides the total genetic pool and arrangement of genes of microbes in a culture-independent manner among particular environments. Studies have revealed most predominating phyla across the extreme environments and prediction of the role of genes responsible for various functional aspects. Metagenomic library from alkaline hot spring revealed predominance of bacterial phyla Acidobacteria, Aquificae, Chloroflexi, *Deinococcus-Thermus*, Firmicutes and Proteobacteria and presence of genes encoding for enzymes such as galactosidases, lipases proteases and xylanases with biotechnological potential [110]. In similar studies, metagenomics has been reported for new and novel biocatalysts from hypersalted biotopes, cold environments, hot springs and deep thermal vents with potential attributes for industries.

Various enzymes like halophilic cellulases, proteases, xylanases and lipases have been reported from soil microbial consortia with suitability in food, detergent and textile industrial processes [111].

Besides omic technologies, genome wide editing tools such as Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated proteins (CRISPR-CAS) are becoming more significant as it includes an array of short palindromic repeats (CRISPR) and CRISPR associated genes (Cas) initially discovered for its role in bacterial immunity acts together to protect against foreign attacking agents [112]. The CRISPR-CAS uses a combination Cas systems and CRISPR to edit genome/gene (s). This technique includes improve or modify the properties of target gene products which can be more efficiently applied to biotechnological, industrial and agricultural applications.

1.6 Conclusion

Microorganisms are ubiquitously distributed in extremes of environment with respect to high or low temperature, acidic or alkaline pH, high or low pressure and radiation and metals. Various studies have revealed abundance of microbes residing in these extremes. But these extremophilic microbes are hidden repository and their metabolic potential is still under explored. In the post-genomics era, the advances in genomics, transcriptomics and metagenomics tools have enabled us to explore and characterize the microbial diversity and metabolic potential of microbial diversity residing in extreme environmental environments. Presently, a large number of whole genomes are available in the database for prediction and annotation but to completely understand the adaptations underlying survival of extremophiles both protein structure and biochemical properties need to be studied.

In this book, different chapters have covered the physiological, biochemical and molecular aspects of different classes including, halophiles, thermophiles, psychrophiles and peizophiles.

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