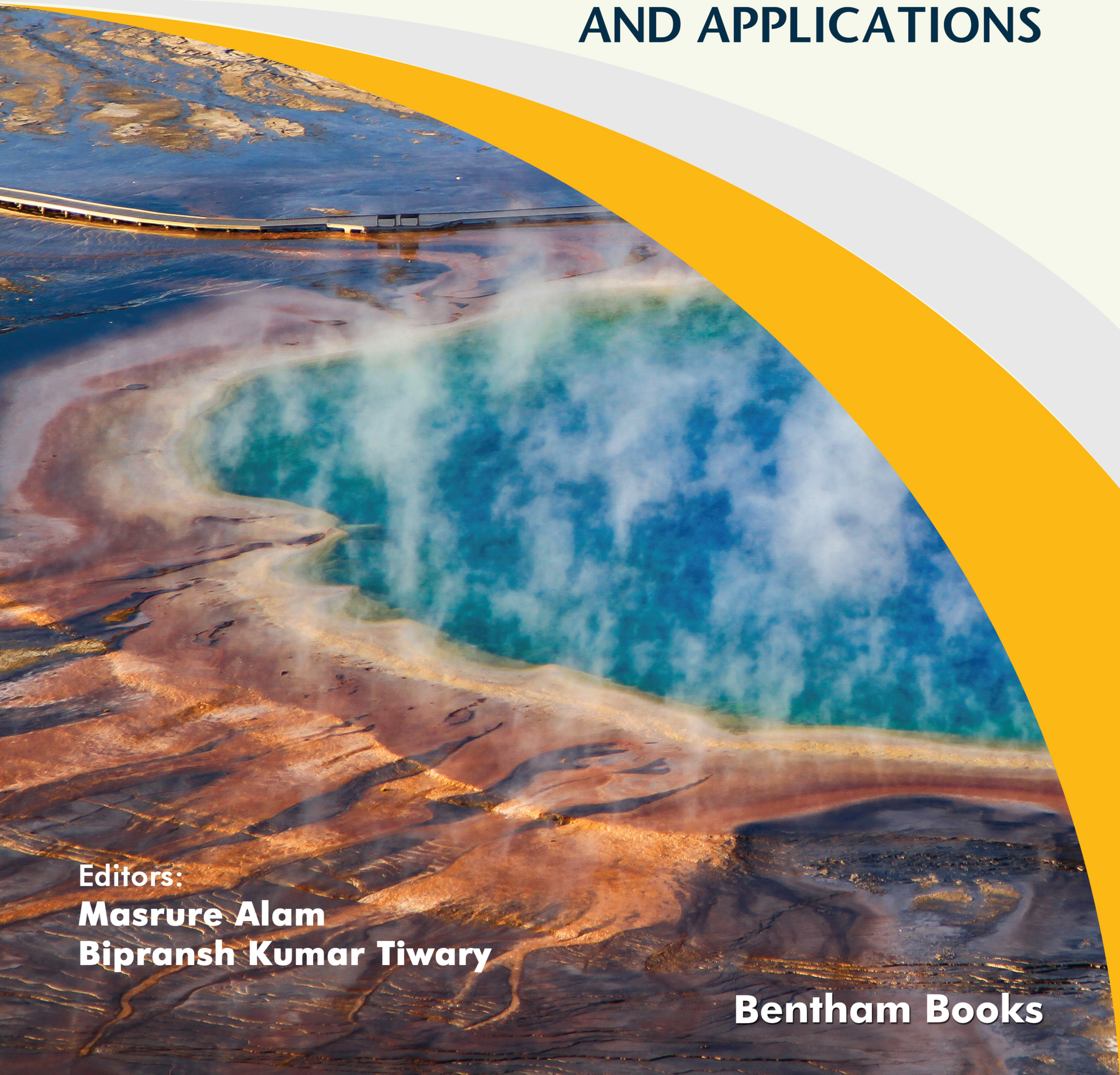
EXTREMOPHILES: DIVERSITY, ADAPTATION AND APPLICATIONS



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Extremophiles: Diversity, Adaptation and Applications

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Extremophiles: Diversity, Adaptation and Applications

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FOREWORD

I am extremely happy to know that a book titled “Extremophiles: Diversity, Adaptations and Applications” edited by Dr. Masrure Alam, Assistant Professor and officiating Head, Department of Biological Sciences, Aliah University, Kolkata and Dr. Bipransh Kumar Tiwary, Assistant Professor and Head, Department of Microbiology, North Bengal St. Xavier’ College, Jalpaiguri is ready for academicians.

The present book covers the most relevant and promising research on “Extremophiles”. This is evident from the content of the book covering overview on Hyperthermophiles, Acidophilic microbes, Alkaliphiles, Xerophytes and various microorganisms including Radiation-Resistant Microorganisms. The book also deals with the diversity and the mechanism of adaptation of predominant bacterial chemolithotrophs in extreme habitats.

In the past also, the research on extremophiles enabled the scientists to understand the challenges in existing biochemistry and molecular biology and helped to know about the new product in the biotechnology industry.

The endeavor in editing the book by both is commendable. I am sure the book will enrich the knowledge of students and faculty of microbiology associated with these fields because this covers all major developments in research on extremophiles.

I would also like to thank all the authors for their dedicated effort in taking up such important fields of microbiology and describing the importance of extremophiles for industrial purposes.

I wish this effort a grand success.

Ramesh Kumar Pandey
Vice-Chancellor
Usha Martin University
&
Former Vice-Chancellor
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PREFACE

Extremophiles are nature's ultimate survivors, thriving in environments ranging from the frozen Antarctic to abyssal hot hydrothermal vents. Interestingly, the discovery of several habitats/environments, resembling that of outer space, on the surface of Earth, and the discovery of organisms that thrive in such extreme environments have given many of the clues to find 'life' outside the Earth's environment. Though life is believed to be originated amidst high temperature, highly reducing and acidic conditions of early Earth, many of these sites on the surface of our planet have been explored only recently, which contain environmental extremes that were unimaginable to our forefathers. The organisms that survive and grow under such harsh environmental conditions, which are uninhabitable to other organisms, are called extremophiles.

High-temperature environments occur in terrestrial hot springs and marine hydrothermal vents where the temperature could reach 100°C to 350°C. Several thermophilic (optima between 55°C and 65°C) and hyperthermophilic (optima between 80°C and 113°C) bacteria and archaea have been isolated that can grow optimally at temperatures above the boiling point of water. Psychrophiles on the other hand can grow optimally below the freezing point of water. Bacteria, like *Deinococcus radiodurans* is one of the most radiation-resistant organisms known and is capable of withstanding acute doses of gamma radiation. Environments with extreme atmospheric pressure, for example, deepest sea floor environments (*e.g*., Mariana trench, 10,898m deep), having a pressure of almost 1200 atm, harbor barophilic/piezophilic microbial community. Xerophiles can tolerate extreme desiccation by entering anhydrobiosis, a state characterized by little intracellular water and no metabolic activity. A number of gram-positive bacteria can form a special resistant, dormant structure called an endospore. These structures are extraordinarily resistant to environmental stresses such as desiccation, heat, ultraviolet radiation, gamma radiation, chemical disinfectants, *etc*. Environments, like the Dead Sea (a salt lake between Israel and Jordan and the lowest lake in the world) and the Great Salt Lake in Utah, despite having saturated salt (NaCl) concentrations, holds extreme halophiles, like *Halobacterium salinarum* which can grow at a salt concentration of 6.2 M. Highly acidic environments, like Rio Tinto river in Spain which has a pH of 1 to 2, and Danakil depression in Ethiopia which has a pH of 0, harbor acidophiles (*e.g*. *Sulfolobus acidocaldarius*, *Ferroplasma acidarmanus* and *Picrophilus oshimae*) which have their growth optimum of pH 0 or closer to it. *Bacillus alcalophilus*, and *Microcystis aeruginosa* on the other hand inhabit natural alkaline soda lakes where pH can reach about 12.0. There are a number of anaerobic bacteria and archaea that can live in complete anoxic environments by using terminal electron acceptors other than oxygen. Some microorganisms, called poly-extremophiles, are adapted to multiple environmental extremities. Thus far hundreds of phylogenetically diverse extremophiles have been isolated or identified from diverse environmental extremes.

In the last few decades, the research on extremophiles has not only provided ground-breaking discoveries that challenge our understanding of biochemistry and molecular biology but also has boosted the biotech industry to search for new products from them. On the applied side, extremophiles and their enzymes have spawned a multibillion-dollar biotechnology industry, with applications spanning biomedical, pharmaceutical, industrial, environmental, and agricultural sectors. The mechanism of adaptation to such environments by the extremophiles has also been well studied in the last few decades. Thus, the book aims to provide the most comprehensive and reliable current state of knowledge on the diversity of extremophiles along with the descriptions of the environments from which they have been isolated, mechanisms of their adaptation to such harsh environments, their applications in human welfare, and future scope. Indeed, the application of extremophiles and their biologically active compounds has opened a new era in biotechnology. However, despite the latest advances, we are just at the beginning of exploring the biotechnological potentials of extremophiles.

This book consists of fourteen chapters that explore the fascinating world of microbes in extreme environments. The first chapter deals with the overview of extremophiles and their strategies of adaptations in extreme environments. The rest of the chapters of the book cover the details including recent scientific information and future prospects of all types of extremophiles, including hyperthermophiles, psychrophiles, halophiles, acidophiles, alkaliphiles, xerophiles, chemolithotrophs, oligotrophs and anaerobic and other extremophiles. Each chapter is organized in such a way to cater to the knowledge of extremophiles (underrated fascinating microbes) including diversity, adaptations, and applications to the scientific community.

This book provides an overview of the current state of knowledge and all major developments in research of all types of the fascinating group of life-forms *i.e.*, extremophiles. This book is an essential read for microbiologists working with extremophiles and their potential biotechnological applications, as well as for all budding microbiologists. The book is also recommended as a reference text for anyone interested in the field of research encouraging readers to reach out to new worlds and discoveries.

Finally, we express our gratitude to Professor Ramesh Kumar Pandey, Vice-Chancellor, Usha Martin University, Ranchi, Jharkhand, India, who has always been an inspirational persona to the young scientific community. With his profound knowledge on the subject, he prudently intuited the importance of the work and kindly wrote the foreword of this edited book. We are also grateful to Prof. Mahammad Ali, Vice Chancellor, Aliah University, Kolkata, India, for providing all necessary facilities and a conducive academic ambience ensuring smooth completion of the book. We are extremely grateful to Fr. Dr. Lalit P Tirkey, Principal, North Bengal St. Xavier's College, West Bengal, India, for his enthusiastic support and encouragement in the completion of the project. We are also thankful to all authors for their hard work and professionalism in making this book a reality. Their expertise in the contributed chapters is acknowledged and appreciated. Our appreciation and credit go to Mrs. Humaira Hashmi, Editorial Manager Publications, Bentham Books for generous assistance, constant support, and patience in materializing the book. Lastly, we thank everybody who helped us in the successful realization of this book and express our apology that their names could not be mentioned personally.

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CHAPTER 1

Extremophiles: An Overview

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Abstract: Earth contains several environmental extremes which are uninhabitable for most of the living beings. But, astonishingly, in the last few decades, several organisms thriving in such extreme environments have been discovered. "Extremophiles", meaning "Lovers of Extremities" are the entities that are especially adapted to live in such harsh environmental conditions in which other entities cannot live. The discovery of extremophiles has not only boosted the biotech industry to search for new products from them, but also made researchers to think for the existence of extra-terrestrial life. The most inhospitable environments include physical or chemical extremities, like high or low temperatures, radiation, high pressure, water scarcity, high salinity, pH extremes, and limitation of oxygen. Microorganisms have been found to live in all such environmental conditions, like hyperthermophiles and psychrophiles, acidophiles and alkaliphiles. Bacteria like *Deinococcus radiodurans,* which is able to withstand extreme gamma radiation, and *Moritella* sp., able to grow at atmospheric pressure of >1000 atm, have been reported. Environments like the *Dead Sea*, having saturated NaCl concentrations, hold extreme halophiles like *Halobacterium salinarum*. Highly acidic environments, like the *Rio-Tinto* River in Spain or *Danakil* depression in Ethiopia harbour acidophiles with growth optima of pH zero, or close to it. *Bacillus alcalophilus*, and *Microcystis aeruginosa* on the other hand inhabit natural alkaline soda lakes where pH can reach about 12.0. A number of anaerobic prokaryotes can live in complete anoxic environments by using terminal electron acceptors other than oxygen. In this chapter, we shall discuss very briefly the diversity of all extremophiles and their mechanism(s) of adaptation.

Keywords: Acidophiles, Alkaliphiles, Anaerobic microorganisms, Halophiles, Hyperthermophiles, Piezophiles, Psychrophiles, Radiation resistant microorganisms.

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INTRODUCTION

Many scientists across the world are focused on subjects like "origin of life" and "life beyond Earth" and are searching for evidences of past or present life forms on Mars, or on Jupiter's moon Europa, or Saturn's moon Enceladus, or anywhere else in our solar system or other parts of the universe. The discovery of several environments on the surface of Earth that may resemble the conditions of outer space, and the discovery of many microorganisms that thrive in such extreme environments, have boosted the confidence of astrobiologists about the existence of life beyond our planet Earth. Several environments, such as high-temperature terrestrial hotsprings, or hydrothermal vents; highly acidic *Rio Tinto* and *Danakil Depression* or highly alkaline soda lakes; highly saline *Dead Sea* and *Salt Lake*; very high atmospheric/hydrostatic pressure of deep sea, *etc.* may resemble the extra-terrestrial environmental conditions. Organisms that live in such harsh environmental conditions, which are uninhabitable to other life forms, are called extremophiles (Roman "*Extremus"* meaning "outermost" plus the Greek "*Philos"* meaning "lovers").

Several thermophilic and hyperthermophilic bacteria and archaea, some of which even grow above the boiling temperature of water, have been isolated from terrestrial hot springs or marine hydrothermal vents where the temperature could reach 100 °C to 350 °C. On the other hand, psychrophiles can grow optimally below the freezing point of water. Deepest sea floors with extreme hydrostatic pressure (*e.g.,* Mariana trench, 10,898m deep, having pressure of almost 1200 atm.) harbour barophilic/piezophilic microbial community. *Deinococcus radiodurans* is one of the most radiation-resistant organisms known so far and is able to withstand a severe dose of gamma radiation. Xerophiles can tolerate extreme desiccation by entering anhydrobiosis, a physiological state that allows the organism to survive with little intracellular water and no metabolic activity. Other environments, like the *Dead Sea* (the lowest lake in the world) and *Great Salt Lake* in Utah, despite being saturated with salt (NaCl), are inhabited by extreme halophiles, like *Halobacterium salinarum* which can grow at a salt concentration of 6.2 M. *Danakil* depression in Ethiopia and *Rio-Tinto* river in Spain with a pH of almost 0 to 2, are inhabited by extreme acidophiles, like *Sulfolobus acidocaldarius*, *Ferroplasma acidarmanus*, *Picrophilus oshimae, etc.* which have their growth optima of pH 0 or closer to it. Several natural alkaline soda lakes around the world where pH can reach about 12.0 are inhabited by alkaliphiles, such as *Bacillus alcalophilus*, *Microcystis aeruginosa, etc.* Some extremophiles, called poly-extremophiles, are adapted to multiple environmental extremities.

HYPERTHERMOPHILES

Diversity

Temperature is one of the most important factors that determine the structure, and thus functionality of cellular components of living beings. Change in temperature causes several changes, from the damage of the structure of biomolecules through the formation of ice crystals at low temperature, to the denaturation or degradation of all the biomolecules towards the higher side. Temperature near or above 100 °C denatures structural proteins, enzymes, nucleic acids and other essential biomolecules, as well as affects the interaction or association among those molecules by hindering most of the noncovalent interactions. Fluidity of cell membrane, thereby cellular function, is also affected by high and low temperatures with a decrease in fluidity towards the lower end and an increase in fluidity, ultimately leading to its denaturation, towards higher. Furthermore, due to the low solubility of essential gases, such as O_2 or CO_2, in water at high temperature, aquatic organisms face problems when the temperature rise. Notwithstanding these damaging effects of high temperature, several organisms have not only been found in the last few decades to endure high temperatures but also to live naturally in environments with temperatures as high as 100 °C or more. Such high-temperature environments are found in terrestrial hot springs and solfataric fields, where the temperature remains high because of the discharge of hot-water heated from underneath magma chambers; and in marine hydrothermal vents where temperature can reach up to about 400 °C because of the discharge of mineral-containing hydrothermal fluids into the surrounding deep sea cold water, building up rock chimneys with temperature gradients [1]. During the 1960s and 1970s Thomas D. Brock isolated numerous thermophilic (organisms which grow optimally at 55 °C to 65 °C with upper limit of up to 80 °C) bacteria and archaea, including *Thermus aquaticus* and *Sulfolobus acidocaldarius* which grow optimally at temperatures up to 75 °C, from thermal hot springs of Yellowstone National Park [2 - 4]. These findings changed the previous knowledge of upper-temperature range at which an organism can live. Later, a team led by K. O. Stetter of the University of Regensburg, Germany isolated several bacteria and archaea from boiling springs, mud pools and hydrothermal vents of several areas one after another with higher and higher temperature limit [5]. The first hyperthermophile (organisms that grow optimally above 80 °C) reported was the methanogen *Methanothermus fervidus* which was found to grow at temperatures as high as 97 °C with optimum being 82 °C [6]. More surprising was the finding of the ability of the archaeon *Pyrodictium occultum*, isolated from a submarine solfataric field on the hot sea floor at Vulcano Island, Italy, to grow above the boiling point of water (100 °C). *P. occultum* was reported to grow optimally at 105 °C with an upper limit of 110 °C [7]. Another archaeon *Pyrolobus fumarii,*

CHAPTER 2

Hyperthermophiles: Diversity, Adaptation and Applications

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Abstract: Hyperthermophiles are microorganisms that love to grow optimally in extremely hot environments, with optimum temperatures for growth of 80 °C and above. Most of the hyperthermophiles are represented by archaea; and only a few bacteria, such as *Geothermobacterium ferrireducens*, and members of the genera *Aquifex* and *Thermotoga* have been reported to grow at temperatures closer to 100 °C. Several archaea, on the other hand, such as *Methanopyrus kandleri*, *Geogemma barossii*, *Pyrolobus fumarii*, *Pyrococcus kukulkanii*, *Pyrodictium occultum, etc.* isolated from terrestrial hot springs, marine hydrothermal vents, or other hyperthermal environments have been reported to grow optimally even above the boiling point of water. The discovery of this astonishing group of microorganisms has not only provided us with the model systems to study the structural and functional dynamics of the biomolecules, and to understand the molecular mechanisms of their adaptation to such high temperature, not even closer to what can be endured by other life forms, but also have boosted the biotechnological industry to search for new products, particularly enzymes with unique characteristics, from them. This chapter has exhaustively reviewed the different hyperthermal environments on Earth's surface and the hyperthermophilic microbial diversity in such environments; mechanisms of adaptation of the hyperthermophiles, especially with regard to the adaptations of the membrane structures, maintenance of the structures of the nucleic acids and proteins; and their diverse applications in human welfare.

Keywords: Adaptation of the membrane structures, Amylases, Cellulases, Extremophiles, Hot springs, Hydrothermal vents, Hyperthermophiles, Lipases, Maintenance of nucleic acid structures, Maintenance of protein structures, Proteases, Polyextremophiles, Pullulanases, Thermophiles, Thermoprotection, Thermostable enzymes.

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INTRODUCTION

Microorganisms that "love" high temperature for optimal growth are known as thermophiles. Different groups of microorganisms have different temperature ranges for growth. Based on the optimal temperature for growth, prokaryotes can be broadly classified as follows: *psychrophiles* (optimum temperature for growth < 15 °C), *mesophiles* (optimum temperature for growth 20 - 45 °C), *thermophiles* (optimum temperature for growth 45 – 80 °C), and *hyperthermophiles* [1, 2]. The fourth group, hyperthermophiles are the microorganisms that love to grow optimally in extremely hot environments, with temperatures of 80 °C or more. During the 1970s and 80s, isolation and characterization of a number of prokaryotic species have extended our understanding of the upper range of temperature on which life can persist. Several archaeal species have been discovered with the ability to grow beyond the boiling temperature of water [3 - 6]. Life beyond the boiling point of water could only be possible under very high atmospheric/hydrostatic pressure, such as in deep-sea hydrothermal-vent environments. Because, liquid water, essential for all living organisms, can be found only in such environments with very high atmospheric pressure [7]. However, the upper range of thermostability of important cellular constituents, such as amino acids, peptides, and nucleobases like ATP, indicates that 150 °C may be the upper limit for life [8 - 10]. Notwithstanding, life beyond boiling temperature of water is surprising as that much high temperature may lead to denaturation of one or the other essential cellular macromolecules such as proteins, enzymes, DNA, RNA, or phospholipids.

The first hyperthermophilic microorganism reported was the methanogen *Methanothermus fervidus*, which was isolated from an Icelandic hot spring and is able to grow optimally at 82 °C (97 °C being the upper range) [11]. Later, the archaea *Pyrodictium occultum*, isolated from a submarine solfataric field at the hot sea floor at Vulcano Island, Italy, and *Pyrolobus fumarii,* isolated from the walls of a black smoker hydrothermal vent at the Mid Atlantic Ridge were reported to grow optimally at 105 °C and 106 °C with the upper limits of 110 °C and 113 °C respectively [3, 4]. The record temperature at which microorganisms have been found to grow is 121 °C, the autoclaving temperature, by *Geogemma barossii* (that is why this bacterium is named as strain 121), which is a Fe (III)-reducing archaeon isolated from a hydrothermal vent along the Juan de Fuca Ridge [5]. This archaeon was also found to remain viable even after exposure to temperatures as high as 130 °C. Another methanogenic archaeon, *Methanopyrus kandleri* strain 116, isolated from a deep-sea hydrothermal habitat in the Kairei hydrothermal field in the Central Indian Ridge, has been found to grow at 122 °C under high hydrostatic pressure [6]. Bacterial counterparts, on the other hand, have very few representatives among the hyperthermophiles and none have been reported to grow above the boiling point of water. Although several Gram-positive bacteria (such as, species of *Bacillus*, *Clostridium etc.*) can survive very high temperatures through the formation of highly heat-resistant endospores, only a few bacteria, like *Geothermobacterium ferrireducens*, *Thermotoga maritima* and some species of the genus *Aquifex*, which have optimum temperatures for growth close to 90 °C, can be considered as hyperthermophiles.

Terrestrial hot springs, geysers and solfataras, or mud pools are the high temperature environments which are inhabited by thermophiles or hyperthermophiles. Thermal hot springs of Yellowstone National Park, North America were the site from where some of the earliest thermophiles, including *Thermus aquaticus* and *Sulfolobus acidocaldarius,* with the ability to optimally grow at temperatures up to 75 °C, were isolated by Thomas D. Brock during 1960s and 1970s [12 - 14]. Depending on the pH of the spring water (alkaline chloride springs or acidic sulfate-springs), terrestrial hot springs may harbor thermophiles or hyperthermophiles that are also alkaliphiles or acidophiles. In marine systems, hot environments exist surrounding the deep-sea hydrothermal vents where the temperature of the venting water may exceed 300 °C. The hot vent-water gets mixed with the surrounding deep-sea cold water creating a thermal gradient in which hyperthermophiles grow at their appropriate temperature zones using reduced inorganic chemicals present in the vent-water as an energy source. Black smoker hydrothermal systems are generally rich in high concentrations of sulfides [7], whereas serpentinite-hosted hydrothermal systems, like the Lost City hydrothermal field, are rich in hydrogen and methane as the source of energy [15]. In addition to these environments, several subsurface and anthropogenically made environments may also be hot. Subsurface hot environments include petroleum reservoirs and geothermally heated lakes and aquifers; and anthropogenically made hot environments may include thermal effluents from power plants, composting piles, water heaters, industrial processing units, *etc.* Microorganisms that live in the deep-sea hydrothermal vent areas can also withstand other environmental extreme conditions. For example, they must also be piezophilic or at least piezotolerant to cope up with high hydrostatic pressure in the deep-sea environment. All organisms living in marine environments also have to endure some degree of (around 3%) salinity. Also, they are adapted to elevated levels of radiation because the natural radioactivity level in the deep-sea hydrothermal vent environments can be 100 times greater than that of the Earth's surface because of the presence of elements like ^{210}Pb, ^{210}Po, and ^{222}Rn [16]. In general, hyperthermophiles are actually polyextremophiles that can cope up with one or the other additional extreme condition.

Till now, no eukaryotic organism has been found to survive above 60 °C. On the other hand, extreme thermophiles and hyperthermophiles cannot live below 60

CHAPTER 3

Psychrophiles

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Abstract: Psychrophiles can be defined as the members of the kingdom *Monera* thriving permanently at the lowest temperature range. Since the majority of our planet is generally cold, psychrophiles are common within a wide range of habitats. Extensive research in the field of genomics, transcriptomics, and proteomics revealed that psychrophiles are endowed with several adaptive features to survive and grow in their cold habitat. Several adaptations in different cellular entities, such as cell envelopes, enzymes, chaperones; protein synthesis machinery, energy generating system, and metabolic pathways have been reported. All these modifications in psychrophiles are found to be indispensable to withstand these harsh environmental challenges. The chapter focuses on the current state of knowledge for understanding the biodiversity and mechanism of low-temperature adaptation of psychrophilic microorganisms. Furthermore, the modified biomolecules in psychrophiles, mainly enzymes and reserved materials, with distinct features, were found to be useful for several applications including molecular biology research, bioremediation, detergent formulations, and the food industry. The biotechnological and industrial significance of the psychrophiles is also discussed in this chapter.

Keywords: Application of psychrophiles, Biodiversity, Cold temperature adaptation, Extremophiles, Psychrophiles.

INTRODUCTION

Living organisms, particularly the microbial community, have evolved with the potential to survive, grow and proliferate in habitats with a wider range of temperatures. Depending upon the temperature of the habitats, two completely different adaptations have been observed in two extreme temperature ends. Thermal adaptation at one extreme end was observed in some members of *Archaea*. These microorganisms were isolated from deep-sea hydrothermal vents and accomplished their growth at temperatures between 85 °C and 121 °C [1]. At the extreme opposite end, cold- adapted microbial species have been described

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around the cold biosphere that represents the largest fraction of the global biosphere. They have successfully colonized all cold habitats including deep sea to mountains and polar areas. These cold-adapted microorganisms are known as "*psychrophiles*" which are able to actively metabolize at low temperature up to -20 °C. In general, psychrophilic microorganisms have been characterized by their ability to grow and multiply at ≤0 °C and growth is restricted above 30 °C [2 - 5]. Depending upon their optimal growth temperature, some cold adapted microorganisms are also known as either *psychrotolerant* or *psychrotroph* [6].

Psychrophiles are microbial species that love cold habitats for their survival and proliferation. They have an optimal temperature for growth at around 15 °C or lower and their normal growth ceases at 20 °C or above. In a strict sense, psychrophiles do not grow above 20 °C [6, 7]. The minimum temperature that allows the proliferation of psychrophilic microorganisms is 0 °C or lower. In contrast, psychrotrophs (also known as psychrotolerant) are cold tolerant microbial species. They are capable of growing at low temperature but their optimal growth temperature is 15 °C. Their growth starts to cease above 20 °C and completely stops at moderate temperature [6, 8, 9]. The laboratory temperature in the United States is around 20-22 °C which is not considered a cold environment. Therefore, the maximum growth temperature for cold-adapted microorganism was set at 20 °C. However, it is well recognised that there is a continuum of cardinal temperatures among different groups of microorganisms categorised based on growth temperature. In this respect, the above-mentioned definitions of psychrophilic microorganism are quite relevant as they help to understand the respective ecological niche of each group. Psychrophilic microorganisms are restricted to permanently cold habitats [8]. The term "psychrotrophic" and "psychrotolerant" are used interchangeably. However, majority of the scientists involved in food and dairy microbiology use the term "psychrotrophic" and the scientists working on environmental microbiology prefer the term "psychrotolerant". In this chapter, we will use a general term "psychrophiles" or "psychrophilic microorganisms" to cover all these microbial species growing in cold habitats.

The first report regarding the psychrophilic microorganism came in 1887. It was isolated from fish preserved by cold temperature [10]. However, the reported organisms were not psychrophiles, instead they were psychrotrophic bacteria. The majority of the studies at that time were not dealing with the extreme cold habitats, and the available reference organisms in culture collection were not true cold loving psychrophiles. Therefore, many debated terms like *cryophile*, *rhigophile*, *psychrorobe* and facultative psychrophile were originated to describe microorganisms isolated from cold environments [6].

In 1962, with respect to the psychrophilic microorganisms, John L. Ingraham (presently an Emeritus Professor at the University of California, Davis) mentioned that "Other authors have felt that the term psychrophile should be reserved for bacteria whose growth temperature optima are below 20 °C if and when such organisms are found" [2]. Considering the foregoing definition, the first true psychrophiles described were *Vibrio marinus* (1962) and *Vibrio psychroerythrus* (1972) [11 - 13]. However, prior to the discovery of these two psychrophiles, a medically important microorganism, *Cytophaga psychrophile*, was isolated which was found to be a true psychrophile [14]. Unfortunately, this work gone unnoticed. The first genome sequence of psychrophiles was obtained from a marine psychrophilic bacterium *Colwellia psychrerythraea* 34H [15]. Subsequently, the genome sequences of several psychrophiles were completed after that. Extensive analysis of the genome and proteome of several psychrophilic microorganisms has revealed some interesting and unique cold-adaptive features in these microorganisms [16, 17].

Psychrophilic microorganisms are endowed with several cold adaptation machineries to survive in the extremely low-temperature habitats. A number of physiological and structural adaptations have been noticed in these organisms. However, the majority of the strategies are still not clearly known. Psychrophiles efficiently respond to the stress associated with low temperature. In some cases, these responses are specific to the low temperature, while in others, the responses are an overall response for several overlapping environmental stressors common in that particular habitat. In this chapter, we will discuss various adaptive strategies of psychrophiles, along with their diverse habitats around the globe. Furthermore, we will provide an overview of the biotechnological applications of these cold-adapted features in psychrophilic microorganisms.

DIVERSITY OF PSYCHROPHILES

Around 20% area of the Earth's surface is covered by ice, which includes polar sea ice, glaciers, permafrost (frozen soils) and snow [18, 19]. The temperature of these regions varied considerably throughout the year. The average temperature of the deep-sea falls within the range of –1 °C to 4 °C, whereas the seawater and sediments of Antarctic and Arctic marine habitats maintained nearly at –1 °C throughout the year. Most remarkably, internal fluids of sea ice remain liquid with a surrounding temperature of –35 °C in the winter season [20]. The temperature at high altitude of the atmosphere (stratosphere and mesosphere) sometimes reaches about –100 °C [21, 22]. Life at such a low temperature is a remarkable characteristic of the living world. For any living object, the lowest temperature limit for their measurable growth is around –18 °C. Furthermore, the metabolic activities like protein and DNA synthesis occur at even lower temperatures.

CHAPTER 4

Acidophilic Microbes: Diversity and Adaptation to Low pH

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Abstract: Acidophiles are the organisms that usually grow at a pH of 3.0 or below. They usually occur in an environment rich in iron and sulfur. These organisms have the ability to oxidize sulfur and iron producing sulfuric acid thus making the environment acidic. The environments where acidophiles are commonly found are termed acid mine drainage (AMD) or acid rock drainage (ARD). The production of acid helps in the dissolution of several minerals present in the environment; hence acidophiles play important roles in bio-metallurgy. Acidophiles are a diverse group of organisms belonging to all three domains of life *viz*. *Bacteria*, *Archaea* to *Eukarya*. Many of them are obligate chemolithotrophs, and few are acidophilic heterotrophs. Usually, the chemolithotrophs are the ones that oxidize ferrous iron and sulfur into ferric iron and sulphate respectively. During their growth, they produce or secrete organic waste products, which are otherwise toxic to obligate chemolithotrophs but are usually scavenged by the acidophilic heterotrophs. Because of the acidic environment, proton concentration $[H^+]$ is always high outside the cell compared to the cytoplasm, thus pH gradient across the membrane is readily generated for these organisms. The pH gradient so generated forms proton motive force (PMF), which is utilized for the coupling of ADP and P_i to generate ATP molecules with the help of ATPase enzymes. However, continuous flow of proton from outside into the cell results in the cytoplasmic protonation or acidification of cytoplasm which may lead to deleterious effects such as denaturation or inactivation of several macromolecules such as DNA or proteins. Thus, the acidophiles must have evolved mechanism(s) to resist or tolerate low pH. Several mechanisms, such as proton impermeability, reverse membrane potential, *etc.* have been proposed to explain their ability to thrive under low pH maintaining the homeostatic balance in their systems. In this chapter, the diversity of acidophilic microorganisms and the mechanisms of their acid resistance are discussed in detail.

Keywords: Acidophiles, Acid mine drainage, Acid rock drainage, Diversity of acidophiles, Extremophiles, Iron oxidation, Lithotrophy, Sulfur oxidation.

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INTRODUCTION

Acidophiles are extremophiles that usually grow in acidic environments, such as acid mine drainage (AMD) or acid rock drainage (ARD), marine volcanic vents, and acidic, sulfur springs. Acidophiles may be divided into three groups based on the range of pH they prefer to grow - (a) extreme acidophiles which grow at or below pH 3.0, (b) moderate acidophiles which grow at pH between 3.0 to 5.0 and (c) acid tolerants which grow optimally at neutral pH or around it, but can also tolerate a pH of around 5.0. The most widely studied acidophiles are the ones that have the ability to oxidize reduced iron and/or sulfur and are capable of catalyzing the oxidative dissolution of metal-sulfide-minerals such as pyrite (FeS_2). The process results in the production of acid (usually sulfuric acid that often decreases the pH below 3.0). The acidophiles are considered an economically important group of microorganisms, because they have successfully been applied in biomining processes to solubilize metals from ores [1]. Moreover, some of their enzymes (extremozymes) have found use in harsh industrial conditions [2, 3].

Several natural and man-made environments with varying degrees of acidity are known to exist on the Earth. Of all these, AMD or ARD is of great interest as in general, the acidic condition of the habitat is the result of microbial metabolism and not a condition imposed by the system as in the case of other extreme environments [4]. The community of microorganisms that are capable of oxidizing sulfur and iron usually produce sulfuric acid resulting in the formation of ARD or AMD systems. ARD is the result of spontaneous oxidation of surface rock outcrops of sulfide masses, whereas AMD is the result of the appearance of effluents produced by mining operations [5]. They are formed when the sulfide ores (often pyrite, iron sulfide – FeS_2) of a mineral comes in contact with oxygen and atmospheric humidity leading to a complex set of reactions resulting in the production of acid. The reaction is accelerated up to 10^6 times in the presence of the chemolithoautotrophic bacterium *Acidithiobacillus ferrooxidans* [6 - 8]. Besides, these environments are very poor in organic matter content and contain high concentrations of heavy metal ions such as lead, copper, *etc.* that render the environment unfavourable for the growth of other organisms. The organisms that can thrive under such acidic environments have been found to be distributed in all three domains of life - *Archaea*, *Bacteria* and *Eukarya* [9, 10].

The acidophiles have developed several mechanisms to adapt themselves to low pH conditions; especially, to maintain homeostatic balance in their protoplasm. Different physiological and molecular mechanisms with the help of metagenomic and metatranscriptomic analyses have been proposed that provide hints for microbial reaction and adaptation mechanisms [2, 11, 12].

DIVERSITY OF ACIDOPHILES

In earlier days, many conventional microbial ecological studies of acid laden metal rich environments (AMDs) have been conducted to study the diversity of acidophiles [13 - 15]. The studies revealed that the most abundantly occurring organisms were chemolithotrophs that could oxidize iron (iron oxidizers) and sulfide minerals (sulfur oxidizers). Recently molecular approaches, such as FISH, 16S rRNA gene libraries, pyrosequencing, T-RFLPs, Q-PCR, and DGGE have been used to examine the microbial diversity of these habitats [16 - 18]. Because of the limited types of substrates available in such environments, the microbial diversity was initially expected to be extremely poor. Cultivation-based studies have, however, revealed a great diversity of the microbial community in AMDs [13, 19].

Traditionally, through culture-dependent methods, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* were recognized as the major chemolithotrophic bacteria responsible for acid production in AMDs [13, 19]. Nowadays, however, cultivation-based analysis is not considered a suitable method rather molecular technique analysis is considered as more reliable method for characterizing microbial diversity [17, 20]. In the study of Tinto River, the most representative bacterial species were found to be *At. ferrooxidans* (23%) and *L. ferrooxidans* (22%) [21]. Since both of them are iron oxidizers, they play an important role in the iron cycle. In most of the cases, *Leptospirillum* sp. is the one that is commonly found to occur in acidic environments. It is actually because of higher affinity of this genus towards ferrous iron and high tolerance to ferric concentration; and being a little thermotolerant (>40°C) they are found to occur in thermal vents also [13, 22]. In the environments rich in sulfidic minerals, members such as *At. thiooxidans, Acidimicrobium ferrooxidans,* have also been found to occur as major microflora [10]. Besides, *Acidithiobacillus ferrivorans* [23], a psychrotolerant and iron-/sulfur-oxidizing bacterium, has been found to be a dominant microflora of some subterranean pyrite mines [24], and ARD of Antarctic [25]. The other species of *Acidithiobacillus* that are found to occur in acidic environments are *At. ferridurans, At. ferriphilus, At. caldus* and *At. ferrianus* [26]. In addition, several iron-oxidizing bacteria such as *At. ferrivorans*, *Acidobacteria* spp., and *Actinobacteria* spp. have been studied in a coal mining area located in the Arctic [27]. In contrast, some acidophiles such as *Acidithiobacillus, Leptospirillum, Ferrimicrobium and Ferroplasma* are found to occur in geothermal waters of the Río Agrío, a volcanic acidic river (pH 1 – 4) also [28].

Other prokaryotes that were found to be the dominant microflora are the members of *Firmicutes*. They fall under three groups *viz*. *Acidimicrobium ferrooxidans,*

CHAPTER 5

Alkaliphiles: Diversity, Adaptation and Applications

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Abstract: Alkaliphiles are some of the major extremophiles which occupy a certain niche of the globe where the pH values are usually two unit higher that the neutrality. Although abundantly found in rare geographical regions, these organisms are of immense importance in terms of their enzymatic activities which enable them to be functional under extreme alkaline conditions and therefore have numerous industrial and biotechnological applications. Their unique mode of adaptation and exclusive ability of resource utilisation make their existence interesting for biotechnological research. The study of alkaliphiles revealed the potential of these microorganisms in the bioremediation of the soda lake, their efficiency to degrade complex organic compounds and a certain class of antibiotics produced by them are of immense importance for the pharmaceutical industries. Recent advancements in genetic studies and recombinant DNA technology allowed the understanding of their genetic modifications which are unique to their taxa and helped researchers to utilise their coding sequence for isolation and purification of commercially important alkaline active enzymes. Despite all the beneficial effects, the isolation, culturing and study of alkaliphiles are among the most challenging tasks and matters of continuous research. This chapter will elaborate on the existence of some important alkaliphilic bacteria in the rare alkaline region of the globe, the diversities among them, their metabolic activities, unique adaptation and modifications in their structural and genomic profile and also summarises the commercially important product isolated from them.

Keywords: Antiporters, Alkaliphiles, Alkaline proteases, Applications of alkaliphiles, Biofuels, Bioplastics, Bioremediation, Cell membrane, Diversity of alkaliphiles, Extremophiles, Fatty acid, Transporters, Transposons.

INTRODUCTION

Extremophiles are the organisms that by virtue of their adaptations choose the most hostile environments of the globe as their natural habitats. These environments can be of high temperature, elevated pressure and zone of high

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acidification, or regions with high pH. Alkaliphiles are the important extremophiles living under environmental conditions where the pH is usually greater than or equal to 9 and where most of the other forms of organisms generally do not survive [1]. These environments can be the result of anthropogenic activities, like soda lakes, or can be of natural origin like ocean sediments, fresh water springs and mining drainages. The uniqueness of these organisms is their adaptive features which enable them to be functional under such harsh environment. This is possible due to certain modifications in their cell membranes, cell surfaces, cytoplasm, extracellular products, electron transport chain, bioenergetics and cellular metabolisms, which are the results of ultimate expressions of a certain class of genes unique to these extremophiles, encoded by either chromosomal DNA or by the extrachromosomal genetic materials like plasmids [2]. The tolerance of the extreme pH is allowed by virtue of their enzymes which are functional under such an elevated pH making the organisms a unique and special component for various industrial and research applications [3]. Due to their adaptations in the extreme geological habitats, it is very difficult to isolate them in culture and study these organisms. Under certain circumstances various alkaliphiles display dual or poly extremophilic characteristics which include halotolerant, thermophilic, psychrophilic and methanogenic alkaliphiles that are much more difficult to culture and study, still are of immense importance in various industries. The diversity of alkaliphiles is not only limited to various types of poly extremophilic bacteria and archaea but it also includes certain eukaryotes, like fungi and micro-algae, which can survive under extreme alkaline environments of certain regions of the globe, including soda lakes, coastal seabed, alkaline columns, *etc.* [4].

The extreme tolerance of these organisms results in certain degree of modifications to their cellular structures, which include the high saturated and branched chain fatty acids in their cell membranes, certain anti-port systems which prevent the loss of H^+ ions from the cytoplasm, and secretion of certain chemicals which enable them to tolerate the extremely alkaline pH of the surrounding environment [5]. Modern PCR technologies and other genomic techniques made it possible to understand the genomic make up of alkaliphiles which show a unique set of genes exclusively present in these extremophiles only. Certain groups of alkaliphiles contain higher percentage of GC sequence, which may be as high as 60%; in addition, these organisms contain a large number of transposable elements in their genome. Along with these modifications, the extrachromosomal genetic elements of the alkaliphiles encode a certain class of transporters which enable metal resistance, provide pH homeostasis under elevated pH of the surroundings, and high expression for protein which can act as osmoprotectants. Although the studies show numerous variations in the genomic sequence of the alkaliphiles, but the database available till date still need to be extended further for more clear understanding of the other aspects of their adaptation. The isolation of alkaliphiles and understanding the exact requirements of the culture conditions for these organisms are very challenging tasks where several environmental microbiologists are involved. Despite of the challenges and difficulties in isolation, culture, design of media for the study of alkaliphiles, the various aspects of these organisms are extremely important for the environment, as well as for a number of different industrial applications where the commercially important products isolated from these organisms are used. Almost all of the enzymes which are obtained from the alkaliphiles are functional at elevated pH and therefore become extremely important for different industries, like paper and pulp industries [6]. The pH tolerant proteases and lipases are immensely used in leather industries for digestion and completion of leather surface from the raw materials. Certain attributes and adaptation of alkaliphiles make them important tools for producing non-fossil fuels, also known as biofuels [7]. Due to their extremophilic nature, the production of biofuels can be achieved even under non sterile condition as other organisms could not be able to withstand the elevated pH. Some of the major industrially important products obtained from, and by the applications of alkaliphiles are summarised in (Table **5. 1**). In addition to all these applications, the alkaliphiles play important roles in the removal of environment pollutants. They can degrade extremely complex and polymeric hydrocarbons found in such extreme environment and therefore are important in natural bioremediation [8]. In this chapter, various aspects of alkaliphiles are discussed which included the adaptation, diversities and applications of alkaliphilic organisms and the several challenges faced while working with these organisms.

Table 5.1. The economic importance of alkaliphilic organisms.

S. No.	Substances/Process	Alkaliphilic Organism	pH Tolerance
1.	Bioplastics	*Enterococcus casseliflavus, Alkalibacterium indicireducens*	At or above pH- 9
2.	Alkaline Protease (for leather Industry)	*Bacillus* sp. MLA64	Upto 12.5
3.	Xylanase (for paper industry)	*Bacillus* sp. 41-M	pH 10.5
4.	Biofuels	*Bacillus marmarensis*	pH 10
5.	Antibiotics	*Bacillus alkalophilshaggy* JY-827	pH 9 and above
6.	Metal reducing (Bioremediation)	*Alkaliphilus metalliredigens*	pH 9.5
7.	Benzoate degradation (Bioremediation)	*Bacillus krulwichiae*	pH 8-10
8.	Anti-fungal compound	*Streptomyces violascens*	pH 8 and above
9.	Lactic acid	*Enterococcus casseliflavus*	pH 9 and above
10.	Biodiesel	*Chlorella vulgaris* BA050	pH 8.3-9

CHAPTER 6

Halophilic Microorganisms: Diversity, Adaptation and Application

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Abstract: Saline environments are one of the most common extreme habitats prevalent in this universe. They are of two primary types, 'thalassohaline' those which arose from seawater, with NaCl as the dominant salt; and 'athalassohaline' of non-seawater origin with different ionic compositions. Organisms from all domains of life have adapted themselves to thrive in environments with salinities ranging from normal to the saturation level. In particular, halophilic microorganisms have developed several adaptive mechanisms to cope up with osmotic stress. While halotolerant or moderate halophiles use efflux pumps, or accumulate neutral compatible solutes in the cytoplasm; extreme halophilic microorganisms accumulate potassium ions, a strategy called 'salting-in' to match the high ionic composition in the external environment. The later predominantly includes archaeal members, except the bacterium, *Salinibacter ruber*. The general adaptive features of halophilic microorganisms also help them to thrive under, and overcome other stressed conditions such as resisting antibiotics, heavy metals and ionic liquids. These microorganisms have wide physiological diversities and include members of oxygenic and anoxygenic phototrophs, aerobic heterotrophs, and those capable of diverse anaerobic respiratory metabolisms. Nano-microorganisms are also reported from saline environments. Their great metabolic versatility, low nutritional requirements, and adaptation machineries, make them promising candidates for several biotechnological applications such as production of pigments, biopolymers, compatible solutes, and salt tolerant hydrolytic enzymes. They are also used in bioremediation, food preservation, and preparation of specialized fermented foods. Understanding the halophiles also paves way for astrobiological research. This book chapter summarizes the present understanding of the diversity, adaptation, and application of halophilic microorganisms.

Keywords: Applications of halophiles, Compatible solutes, Efflux, Halophilic bacteria, Halophilic archaea, Saline environments, Salt stress, Salt-in strategy.

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INTRODUCTION

Saline environments are extensively distributed across the world in the form of oceans, salt lakes, deserts, saline lands, and coastal lagoons. These environments, particularly those with very high salinity are examples of habitats which provide adverse conditions for life. However, several organisms encompassing all realms of life, including *Bacteria*, *Archaea*, *Eukarya* and viruses do thrive in these extreme conditions with unique adaptation strategies. The major challenges faced by these organisms are high ionic composition, osmotic stress, low water activity, alkaline pH, high to low temperature, low oxygen concentration, and the presence of heavy metals [1].

The relationship between halophilic microorganisms and NaCl has been well studied. Classification of halophiles by Kushner and Kamekura based on their optimal growth with respect to salt concentration is the most accepted and used one [2]. They have described halophiles in three groups, *i.e.*, extreme halophile, that grows optimally in 15-30% (2.5-5.2 M) NaCl; moderate halophiles growing in 3-15% (0.5-2.5 M) NaCl; and slight halophiles supported by 1-3% (0.2-0.5 M) NaCl in their environment. Non halophiles prefer to grow in media supplemented with less than 1% (0.2 M) NaCl. Halotolerant organisms optimally grow without any excess NaCl in the medium but can also grow in the presence of high salt concentrations. In contrast, archaeal halophilic members of the family *Halobacteriaceae* require NaCl for their normal growth, cellular functioning and stabilization [3]. In the absence of NaCl, their cell wall eventually lyses because they require Na^+ ions for cell wall integrity.

This book chapter describes in detail the saline environments present across the globe, and the organisms, particularly microorganisms that thrive in these ecosystems and their diversity. In addition, the mechanism of their salt tolerance and bioprospecting avenues are also discussed. Special emphasis has been given to the only inland hypersaline lake present in India, Sambhar Lake.

SALINE ENVIRONMENTS

Among different extreme habitats, saline environments are the most prevalent on earth, as well as in other planets, which are characterized by the presence of salts like sodium chloride, magnesium and calcium sulfates and bicarbonates. Generally speaking, any environment having salinity near or higher than the salinity of sea water (~3.5%) is considered as a saline environment. They are represented by sea water, salt lakes, salterns, and saline soils. In addition, several anthropogenic activities such as leather processing, and food industries also result in varying saline conditions. Some of these environments have very high salt concentrations, which in some instances can reach to salt saturation levels. These hypersaline environments are of two major types, 'thalassohaline' and 'athalassohaline' [4, 5]. Thalassohaline environments are derived from the evaporation of sea water, with ionic composition matching the source *i.e*., NaCl as the dominant salt [4]. In fact, the ionic composition of saline environments is sometimes used to classify them into either of the two types, irrespective of their apparent origin. For example, the ionic composition of Great Salt Lake in Utah, USA, a remnant of an ice age lake with no connection with sea shows resemblance with sea water and is thus considered a thalassohaline environment [6]. On the other hand, athalassohaline environments are originated from non-sea water sources and are dominated by divalent cations like magnesium and calcium. The Dead Sea in Jordan Rift Valley is an athallasohaline salt lake with ionic composition dominated by magnesium and calcium ions compared to sodium ions [7, 8]. Chaotropic ions like calcium and magnesium in high concentration hinder biological activity [9, 10], while kosmotropic ions such as NaCl, stabilize the biomolecules and provide protection against chaotropic agents in saline systems [11, 12]. The proportion of chaotropic and kosmotropic ions determines microbial community composition in these environments [13, 14]. The latter is often the case with soda lakes with salts of sodium, potassium or magnesium being the dominant one, mostly as carbonates.

Hypersaline environments, present mostly as saline lakes and solar salterns are dispersed across continents. Some prominent examples are soda lakes in Egypt (Wadi An Natrun), Dead Sea, Great Salt Lake in Utah, Big Soda Lake and Mono Lake, in California, Lake Chaka in China, Maras salterns in Peru, and lakes in Antarctica [4, 15 - 21].

Among hypersaline environments, hypersaline soda lakes are unique with high salt concentrations and alkaline pH. These lakes are generally characterized by high concentrations of sodium salts, but low levels of calcium and magnesium salts. The lower concentrations of divalent cations, in turn, prevent carbonate precipitation. To add to this, the evaporation of water/brine further increases carbonate concentrations that eventually form sodium carbonate and bicarbonate resulting in an increase in pH (9.5-11.0) [22, 23]. Soda lakes are widely distributed across the globe, such as Lake Picturesque, Lake Tanatar trona, Lake Tanatar, and Lake Bitter-1 in Siberia, Russia [24]; Lake Magadi in east-African Rift Valley [22]; Lake Chagannor in Inner Mongolia, China [25]; Lake Wadi An Natrun in Egypt [20]; Mono Lake in California, USA [16] and Sambhar Lake in India [26].

Sambhar Lake is a large inland hypersaline soda lake in India, located in the middle of a closed depression of the Aravalli schists, in Rajasthan, near Jaipur [27]. The lake is also used for salt production employing several multi-pond solar

CHAPTER 7

Piezophiles: Quiddity of Extreme Pressure Devotees

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Abstract: Piezophiles are a sort of extremophilic organisms that nurture and survive under extreme hydrostatic pressures up to 10 MPa (1450 psi = 99 atm). The diversity of piezophilic organisms can be studied by swotting deep-sea environments that are inhabited by diverse piezophiles from all three domains of life. Information about the physiology and adaptive mechanisms of piezophiles have been obtained by the process of collection and culturing of deep-sea microorganisms. The corporeal adaptations are an absolute requisite for growth under high hydrostatic pressure in these deep-sea environments. Piezophiles possess homeoviscous adaption of lipids and fatty acids which varies with variation in the hydrostatic pressure. However, they contain docosahexaenoic acid (DHA) (22:6n-3), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) as major components, which help to acclimatize such an extreme environment. The ability of piezophiles to tolerate ultra-high pressure, extreme conditions, like low and high temperatures (2 °C– 100 °C) offers numerous applications as discussed in this chapter. This chapter mainly presents piezophilic microorganisms, including their diverse groups, their ability to raise and endure in deep-sea environments with their molecular approaches and their several applications.

Keywords: Application of piezophiles, Barophiles, Diversity of piezophiles, Deep-sea, Extremophiles, High pressure, Piezophiles, Piezophilic organisms, Pressure adaption.

INTRODUCTION

In all of the Earth's severe settings, all three domains of life are represented. However, because prokaryotes are the most abundant organisms on Earth, it should come as no surprise that they have been isolated or discovered from vir-

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tually everywhere on the planet. Even considering the known growth limits - *i.e.*, minus 12 °C (*Psychromoas ingrahamii*) to 129 °C (*Geogemma barossii*) in temperature, pH of less than 0 (*Picrophilus torridus*) to 13 (*Plectonema nostocorum* and *Hydrogenophaga* sp.), pressures of more than 100 MPa (*Shewanella benthica*) [1], beyond saturating conditions of NaCl and KCl (*Halodarex volcanii*), and high levels of ultraviolet (UV) (>100 J/m^2) and gamma (>12KGy) radiations (*Halobacterium* sp. NRC-1 and *Deinococcus radiodurans*) [2] also corroborate this [3, 4]. Additionally, viable bacteria have been discovered from the mesosphere (48 to 77 km above the Earth's surface) [5], the Mariana Trench/Challenger Deep (11 km below the ocean surface) [6] to several kilometres below the Earth's surface (for example, the South African gold mines) [7]. The primary reason of their survival at these extreme conditions is their specialized enzymes already adapted to work under those extreme environmental conditions.

The term "*extremophiles*" has originated from the Latin word "*extremus*" meaning "excessive" and Greek word "*philia*" meaning "love", and is defined as the microorganisms that prefer to grow in a variety of extreme environmental conditions which are uninhabitable for other organisms [8]. Hence, the extremophiles are organisms that not only survive but also thrive in harsh environments. This is in contrast to an organism that can withstand or tolerate extreme environmental conditions while thriving optimally in less extreme ones. From an anthropocentric standpoint, the Earth contains a profusion of conditions that could be characterised as extreme, and the extremophiles thrive in conditions of extreme temperature, pH, salinity, pressure, radiation, or other abiotic growth limitations [9]. Microorganisms found at pH extremes are called acidophiles and alkaliphiles, whereas those developed affinity for the extreme high temperature are called thermophiles; and psychrophiles are those which can thrive at extreme low temperature. Bacteria having the capacity to tolerate the higher salt concentration and UV radiation are called halophiles and radiophiles, respectively, while piezophiles are the microorganisms that thrive in high pressure environments [10, 11].

Thus, piezophiles, also known as barophiles, are a group of microorganisms belonging to extremophiles that thrive in the environments having ultra-high atmospheric/hydrostatic pressure. ZoBell and Johnson first coined the term 'barophiles', which has recently been replaced by the word 'piezophile' as the Greek translation shows the 'baro' means weight; whereas the word 'piezo' means pressure [12], while 'phile' means lover. It is a group of organisms that nurture and survive under extreme hydrostatic pressures up to 10 MPa (1450 psi = 99atm). Almost all of Earth's surface is concealed by oceans and deep-sea environments, however the study of this substantial, united and alive system is still in its early period. Numerous ecological variables, *i.e*., high salinity, low and unpredictable nutrient accessibility and depth-correlated gradients of light, temperature, nutrients and pressure shape the diversity, physiology and ecology of marine species. As waters present an average depth of 3800 m, deep-sea bionetworks epitomise the furthermost mutual ocean-going biological niche [10, 13]. They are found predominantly in the depths of the ocean, which has an average pressure of 38 MPa (megapascals) and up to 110 MPa at its deepest point in the Marianas Trench, in comparison to a pressure of 0.1 MPa at sea level [14, 15].

The pressure adapted first isolate was firstly reported by Yayanos [12]. However, the roots of piezobiology began to be conventional in the late nineteenth century by the exertions of Certes and co-workers, progress in the field lay relatively dormant until the mid-twentieth century [16, 17]. There are many types of piezophiles as described below in Table **7.1**. Most piezophiles are psychrophilic Gram-negative bacterial species that belong to the genera *Shewanella, Psychromonas, Photobacterium, Colwellia, Thioprofundum*, and *Moritella*, but some are archaea derived and can be found among the genera *Thermococcus, Sulfolobus,* and *Pyrococcus* [18, 19].

Origin and Distribution of Piezophiles in Piezosphere

The deep-sea environment inhabited by piezophiles is recognized as "piezosphere" as those environments have more than 1000 m sea depth and with hydrostatic pressures of more than 10MPa [20]. Hydrostatic pressure surges with depth at an estimated rate of 10 MPa (~100 atmospheres/bars) per km in the water column and 30 MPa per km underground [21, 22].

The deep-sea piezosphere is the volume of the deep-sea at depths of 1000 m and higher, with hydrostatic pressures exceeding 100 atm or 10 MPa. The temperature of the piezosphere is generally cold (2–3 °C), but it can get extremely hot (400 °C or greater) around hydrothermal vents. The deep-sea contains 62% of the world's biosphere and accounts for about 75% of the total ocean volume. In this way, the deep-sea piezosphere constitutes a huge biotope on Earth, probably the largest [23, 24]. Deep-sea piezophiles are evidenced to be a valuable source of industrially efficient extremozymes. Due to the unique physiologies and metabolism, as well as the diversity of deep-sea extremophiles, evolutionary adaptations of their extremozymes to the extreme circumstances present in deep-sea habitats, *etc*., have aided in the selection of piezophiles for more robust biocatalysts with unique features not found in other prokaryotes. These extremozymes will be useful in developing new bio-catalytic processes that are more efficient and precise.

CHAPTER 8

Xerophiles

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Abstract: Water is one of the most important substances that are essential for the activity of cellular micromodule and housekeeping functions of a microorganism. However, some microorganisms, known as *xerophiles*, have adapted to their niche and evolved to utilize very less amount of water. Xerophiles are a group of extremophiles, that can grow and proliferate in the presence of very limited water, as low as water activity (a_w) of 0.8. The term xerophiles is derived from the Greek words "xēros" which means "dry", and "philos" meaning "lovers", indicating their affinity to grow in low a_w. The existence of xerophiles is reported from the arid deserts, food spoilage, and highly saline environments, to meteorites and asteroids. Due to the habitation of these organisms in diverse extreme environments, they possess behavioral, physiological, metabolic, and molecular adaptations to survive in those atmospheres. In this chapter, we have discussed diversity and different adaptative mechanisms of xerophiles.

Keywords: Actinobacteria, Adaptation, Biofilm, Carnitine, Compatible solute, Cyanobacteria, Dipicolinic acid, Ectoine, Exobiology, Glycine betaine, Halophiles, Proline, Proteobacteria, Sporulation, Water activity, Xerophiles, Xerotolerant.

INTRODUCTION

Water is the most critical element required for life as it determines the functional activity of all cellular micromodules. During desiccation and hypertonic atmosphere, microbes tend to lose their water, which destabilizes their normal housekeeping functions. Osmotic shock is experienced due to organic solutes or ions. Thus, most of the microbial life cannot proliferate when the water activity (a_w) drops below 0.90 [1]. Few privileged, extremophilic bacterial and archaeal strains uniquely adapted themselves, such that they can divide under severe xeric stress (~0.61 a_w) [2]. The existence of these microorganisms has been recorded in extreme xerotic environments such as dried foods, deserts, plant-associated niches of drylands, dead sea, antique wood, *etc*. collectively known as *xerophiles* [3 - 7].

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The term xerophiles is derived from the Greek words "xēros" which means "dry", and "philos" meaning "lovers", indicating their affinity to grow in lower a_w. Food spoiling xerophilic microorganisms such as *Zygogaccharomyces rouxii*, can proliferate in a medium containing 20% NaCl where the osmotic stress is imposed by ions rather than organic solutes [8]. Arid lands provided a vital biogeochemical cycle to our ecosystem as it covers around 30% of available lands of all continents. Among them, the *Atacama* desert is one of the oldest arid lands which started its formation 150 million years ago [9]. The dynamic metabolism of xerophilic organisms present in arid lands enables them to spend energy more efficiently to harvest water from the surrounding atmosphere. Many of them have adapted themselves to utilize the trace of water trapped inside mineral soils like gypsum, quartz, *etc.* [10]. Moreover, arid regions often serve as a landscape where vegetation ends and the beginning of agriculturally unproductive lands. Therefore, the root exudates from arid land plants, such as *Atriplex halimus*, often attract several xerophiles, which enhance their population and diversity [11]. Additionally, the presence of various secondary-metabolite producing halophilic and xerophilic *Actinobacteria* enhances the metabolic diversity of those specific niches [12]. Various members from the genera *Acidimicrobidae* and *Rubrobacteridae* have exhibited an extreme tolerance towards diverse stresses like heat, desiccation, solute, ionizing radiation, *etc.* which resemble the hostile atmosphere of Mars [13, 14]. Owing to their existence in the most extreme conditions, little knowledge is available to us about xerophiles. In this chapter, we are discussing the diversity, dynamics, metabolism, physiology, and adaptations of xerophilic microorganisms.

DIVERSITY OF XEROPHILIES

Most of the xerophiles adapted themselves to enter the anhydrobiosis state where cells minimize their metabolism to remain only in a viable state. They can survive on the surface and subsurface layers of rocky (Cryptoendoliths and Chasmoendoliths), hyper-arid, and semi-arid atmospheres. Xerotolerant microorganisms, along with heterotrophic bacteria, cyanobacteria, and lichens form a strong biofilm matrix on the top layer of the rock to reduce its weathering. The presence of these microorganisms is not only circumscribed to only arid soil, but they are also capable of thriving in the presence of high salt concentrations. Based on salt-tolerant capabilities they are divided mainly into two groups, *i.e.*, halophilic and halotolerant microorganisms. The salt-tolerant archaea are mainly represented by the members of three families, *i.e.*, *Halobacteriaceae, Methanosarcinaeae,* and *Methanospirillaceae*. The occurrence of cyanobacteria species, like *Microcoleus, Aphanothece,* and *Arthrospira* is predominant in salterns, salt, and soda lakes [14]. The majority of halophilic and halotolerant prokaryotes can be found from *Proteobacteria* (*Halomonas* spp., *Chromohalobacter* spp., *etc*.), sulphate-reducing bacteria (*Desulfovibrio* spp., *Desulfohalobium* spp., *Desulfobacter* spp., *Desulfonatronum* spp.), sulphur oxidizers (*Halothiobacillus* spp., *Halothiobacillus* spp.), anoxygenic phototrophs, firmicutes (low G+C% Gram-positive, such as *Thermohalobacter* spp., *Haloanaerobium* spp., *Halothermothrix* spp., *Natrionella* spp., *etc*.), methanogens (*Methanocalculus* spp., *Methanohalophilus* spp., *Methanosalsum* spp., *etc*.) and actinobacteria (high % G+C Gram-positive bacteria, like *Nocardiopsis* spp., *Actinopolyspora* spp., *Nesterenkonia* spp., *etc*.) [15]. The presence of other members of *Actinobacteria,* such as *Rubrobacteridae, Acidimicrobidae, etc*. was also reported from arid regions which can withstand severe desiccation [13].

MECHANISMS OF ADAPTATION

The process of xerophilic adaptation can be broadly classified into four types:

(a) Behavioral adaptations, (b) Physiological adaptations, (c) Metabolic adaptations and, (d) Molecular adaptations.

Behavioral Adaptations

Dormancy: The common behavioral adaptation of microorganisms under the xeric condition is entering a state of dormancy until the favorable condition returns. This stage is achieved by drastically reducing their metabolic activity; thus, entering an inert non-replicating state. Dormancy involves the emergence of highly resistant spores in bacterial genera like *Bacillus* and *Clostridium* [1, 16]. Sporulation starts with the accumulation of dipicolinic acid (DPA) in the intracellular space. It is one of the main components, occupying about 10% of the dry weight, of the spores. DPA plays a crucial role in making the spores resistant to wet heat by binding and chelating with Ca^{2+} ions to form calcium-dipicolinate, which results in the spore-core getting dehydrated [17]. DPA along with α-type and/or β-type of small acid-soluble proteins (SASPs) which accumulate in the intracellular spore space not only make the cells desiccation-tolerant but also protect them from UV radiation, or extreme temperatures, or also play important roles in protecting the DNA from harmful reactive oxygen species (ROS). α/β-type SASPs are small proteins encoded by monocistronic *ssp* genes that are highly conserved in different *Bacillus* sp. *In vitro* studies have revealed that α/β-type SASPs directly interact with the spore DNA forming α/β-type SASP-DNA complex resulting in the change of conformation of both the DNA and SASP. Electron microscopy-based studies have suggested that SASPs specifically interact with the backbone of the DNA which might be a possible explanation for the DNA backbone to be unsusceptible to cleavage by any chemicals (hydrogen peroxide) and enzymes (DNase, restriction endonucleases). Moreover, the persistence length of the DNA increases several folds due to the enhanced rigidity

CHAPTER 9

Radiation Resistant Microorganisms

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Abstract: Starting from its formation as a cosmic particle, the earth is exposed to various types of radiation. With gradual cooling and environmental modifications, it started supporting life, first in the form of viruses and bacteria. So, radiation-resistant microorganisms are thought to be among the Earth's ancient life forms. But, however, it is relatively an unexplored arena of research today. Though the members are few, radiation-resistant bacteria belong to a phylogenetically diverse community and their degree of withstanding the dose of radiation is also diverse. In most of the cases, the resistance mechanism involved survival from DNA damage and protein oxidation. In this chapter, we will discuss the diversity of radiation-resistant bacteria explored so far with their generalized mechanisms of resistance, along with the basic concept of radiation and radiation-induced damages.

Keywords: : Radiation resistance, *Deinococcus*, Radiation induced DNA damage, DNA repair.

INTRODUCTION

In 1920, Arthur Eddington first hypothesised that the source of solar energy is probably due to the fusion of hydrogen atoms into helium. After the discovery of the nuclear fusion reaction by Hans Bethe in 1930, the hypothesis received better support for its establishment. In the late 20th century, the exothermal fusion reaction of hydrogen atoms into helium has been established as the prime source of solar energy and temperature. This temperature is the source of shortwave electromagnetic radiation that comes into the earth in the form of light and heat. After absorption of solar energy, the ground hits up and emits back longwave radiation. So, the atmosphere may be considered as the dynamic equilibria of shortwave and longwave radiation. Now, following the equation $E= h\nu/\lambda$, the shortwave radiation has more energy that may affect the cellular structure, and integrity at the molecular level.

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Types of Radiation

In physics, radiations may be classified as per their wave, or particulate nature into *ionizing* and *non-ionizing* radiations.

Ionizing Radiation

As per WHO guidelines, ionizing radiations are the radiations that possess enough energy to remove the tightly bound electron from an atom which results in the ionization of the atom. Ionizing radiations may further be subdivided into *particulate ionizing radiation* and *electromagnetic ionizing radiation* with respect to the wave and particulate nature of the radiations.

Particulate ionizing radiations consist of sub-atomic particles, like electrons and protons that gain kinetic energy from their momentum. The α-ray and β-ray are members of this group. Particulate ionizing radiations possess charges and directly ionize the particles with which those interact by Coulombic force. Comparative properties of α, β and γ rays are given in Table **9.1**.

α-Ray: α-ray is a stream of α particles. The α particles have two protons and two neutrons in their nucleus and thus are equivalent to $^4He_2$ ion. These two protons in the nucleus, in the absence of an electron in the orbit, impart net two positive charges to each α particle. Most α particles have energies between 3-7 MeV with low to moderate penetration power.

β-Ray: β particles bear the same mass and charge as that of the electron but the basic difference with the electron is that the β particle emits from the unstable or radioactive atoms, whereas the electrons originate from the outermost orbit of a stable atom. β particles may be positively (positron) or negatively (negatron) charged. However, in most cases in radioactive decay, negatron supersedes positron. Though have less ionizing power than α particles, β particles have more penetrating power due to their smaller size.

Electromagnetic ionizing radiations are electrically neutral radiations that charge the particles indirectly without any direct interaction with the electrons. γ-ray and X-ray belong to this category.

γ-Ray: γ-ray is a highly penetrating electromagnetic radiation with the smallest wavelength and the highest energy among all electromagnetic radiations. On earth, γ-rays are originated from nuclear fusion, nuclear fission and to a lesser extent from all sorts of nuclear decay.

$$^{238}U_{92} \longrightarrow {}^{4}He_{2} + {}^{234}Th_{90} + 2\ {}^{0}\gamma_{0}$$

Example of γ-ray production from α decay.

Because of having high energy, γ-ray possess very high penetrating power and requires several inches of dense material to shield its effect. γ-ray can pass through the human body without striking anything.

X-Ray: X-ray is an electromagnetic radiation with a short wavelength of about $10^{-8} - 10^{-12}$ meter. It is produced from a high-energy accelerating or decelerating atom. Because of its high penetration power, it can pass through the biological tissue and thus is widely applicable in taking the internal image of a biological system on a photographic plate for medical diagnostic purposes. When interacting with atoms, X-ray removes electrons from the outermost orbit of the atom and thereby ionizes the atom in an indirect way.

Table 9.1. Comparative properties of α-, β- and γ-rays.

Properties	α-Ray	β-Ray	γ-Ray
Mass	6.65 X 10^{-27}kg	5.5 X 10^{-4} amu	Negligible
Charge	+2	-1	0
Speed and nature	High-speed helium nucleus	High-speed electron	High-speed electromagnetic radiation
Velocity	5% of the velocity of light	Nearly equal to the velocity of light	Equal to the velocity of light
Penetration power	Low	Moderate, 100 times more than α particle	High, 100 times more than β particle
Effect on magnetic and electric field	Deflected towards the negative plate	Deflected towards positive plate	Not deflected
Ionizing power	Greater than β particles	Low	Nil
Luminescence	Produce fluorescence and phosphorescence	Produce phosphorescence	Produce phosphorescence
Distance travelled	2 – 4 cm	2 – 3 m	500 m

Non-Ionizing Radiation

Non-ionizing radiations refer to those electromagnetic radiations that possess sufficient energy to move the atoms in molecules around, or allow them to vibrate but lack the threshold energy to remove the electron from the particles with which it interacts and thereby is unable to ionize the concerned particle. These radiations

CHAPTER 10

Metallophiles and Heavy Metal Bioremediation

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Abstract: Heavy metals, a group of naturally occurring elements present throughout the earth's crust are known to have wide biological implications. Anthropogenic activities cause constant augmentation of heavy metals having a tremendous negative impact on life forms in the environment with levels beyond safety. Microorganisms invariably are the first group of organisms that are directly impacted by the accumulation of heavy metals in the environment. Heavy metal toxicity is pronounced amongst microbes which impacts change in microbial community composition and function in any ecosystem. The intrinsic and acquired resistance properties have led to the development of resistant bacterial communities in contaminated areas. A large number of heavy metal tolerant bacteria have been isolated from various polluted sites like industrial effluents, aquaculture, agricultural soils, foods, river water and sediments. The determinants of resistance are both plasmid and chromosomal encoded in bacteria. Amongst the various strategies of survival mechanisms employed by bacteria, efflux system and enzyme detoxification are two general mechanisms supplemented occasionally by resistance mechanisms like sequestration or bioaccumulation. These strategies of resistance in bacteria are generally exploited in bioremediation strategies. Due to the persistent nature and non-degradability of heavy metals, it becomes difficult to clean up the pollutant from the environment and moreover, the conventional treatments for heavy metal pollution are complicated and cost-intensive. Therefore, microbial-based technology furnishes effective, economic and eco-friendly applications for the bioremediation of heavy metals from contaminated environments.

Keywords: Bioremediation, Ecological applications, Heavy-metal resistant bacteria, Metallophiles, Metal contamination.

INTRODUCTION

The term 'heavy metal' is arbitrary and very imprecise, although it has been used as a group name for metals and metalloids associated with contamination and potential toxicity to the ecosystem. In 1936, the first scientific definition was pro-

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posed by Bjerrum [1] which was based upon the density of the elemental form of the metals. Since then, several definitions have been proposed in terms of atomic number, atomic weight, chemical properties, *etc*., which rarely have any biological significance. Heavy metals are defined as elements (metals, metalloids, lanthanides and actinides) having a specific weight of more than 5.0 g/cm^3 [2]. However, this term remains obscure when it comes to use in life science as plants cannot detect the density of the metal. The physiological and toxicological effects of heavy metals cannot solely be attributed to the density of the metal [3]. In the last decades, chemists are suggesting the possibility of defining the term based on toxicity assessment. This is based on the fact that each metallic element or its compounds have distinct physico-chemical characteristics which in turn determine its biological and toxicological properties as well as its mobility in the environment [4].

Metals are natural constituents found in the earth's crust and undergo a continuous process of biochemical recycling since the formation of the Earth [5]. The distribution of metals in the environment is greatly influenced by various environmental factors and their compositions show spatial variations [6]. Heavy metals are generally classified as toxic metals which include Hg, Zn, Cu, Ni, Cr, Pb, Co, Cd, As, Sn, *etc*., precious metals like Au, Pt, Ag, Ru, *etc*. and radionuclides namely, Th, Ra, U, Am, *etc.* [2, 7]. Heavy metals like Fe, Mn, Zn, Co, Cu and Ni exert various biochemical and physiological effects on living organisms when present in trace amounts ranging from nanomolar to micromolar concentrations. They are important microelements known to interact with biomolecules and are involved in many oxidation-reduction and catalytic reactions [8]. For example, zinc is an important component of enzymes and DNA binding motif, zinc-finger proteins [9]; iron is important in the respiration system found in the cytochromes, while some anaerobic bacteria use Fe^{3+} as a terminal electron acceptor [10]; nickel plays an essential role in different cellular processes and is incorporated into various microbial enzymes like Ni-Fe hydrogenase, acetyl CoA decarbonylase/synthase, methylenediurease, urease, carbon monoxide dehydrogenase, methyl coenzyme reductase, superoxide dismutases, and some glyoxalases [11]. Cobalt is a key atom in vitamin B_{12} complex and nitrile hydratases are cobalt containing enzymes [12]. Similarly, molybdenum and vanadium are a catalytic centre of various enzymes. These include bacterial nitrogenases having Fe-Mo-Co centre and pterin-based Mo enzymes [8]. Some nitrogen fixers like *Azotobacter chroococcum* are able to form vanadium-dependent nitrogenase when molybdenum is lacking in the environment [13]. Amongst the various oxidation states of manganese, Mn (II) is the most predominant form and is used by many bacteria in anaerobic respiration as a terminal electron acceptor [14]. Likewise, copper serves as an essential cofactor in metalloenzymes like superoxide dismutase, catalase, peroxidase, monoamine oxidase, ferroxidases, and cytochrome c oxidases [15]. These metals are micronutrients, maintaining various physiological and biological functions when present in trace amounts in the environment and have a deleterious effect on various cell functions when the amount exceeds the threshold level, usually above 1 mM concentration [16, 17]. The non-essential metals such as aluminium (Al), antinomy (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In), lead (Pb), lithium (Li), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti) and uranium (U) have no known biological functions [18].

Natural sources of heavy metals in the environment are through the processes like weathering of metal-enriched rocks and phenomena such as volcanic eruptions which increase the heavy metal concentration in the soil [19]. For any metal to enter the living system, it has to be bioavailable. In general terms, bioavailability is the soluble form of a metal that can be absorbed by living organisms [20]. The form and levels of trace metals in soil, sediment or the aquatic environment are determined by the interaction of various factors like pH, redox potential, presence of organic ligands and complexing anions for metals and solid materials [5], as well as soil particle size [21]. Metals in soils can be found in different geological forms and can be characterized as exchangeable, bound to oxides of Fe and Mn, bound to organic matter, bound to carbonate phase and residual metal. The presence of chemicals like potassium ferrate can mitigate the bioavailability and mobility of heavy metals [22]. The impact of varying salinities on the bioavailability of heavy metals has also been studied and results have shown that high salinity can increase the concentration of cations like Na, K, Ca and Mg, by increasing the desorption of heavy metals from the solid phase [23]. Similarly, Li *et al*. [24], showed that desalination of freshwater had a decreasing effect on the availability of heavy metals. Microorganisms can also mediate the distribution and availability of heavy metals in the environment. Sulphate reducing bacteria can methylate the elemental form of mercury forming methyl mercury which is readily bioavailable and enters the food chain and, in the process, they accumulate from lower trophic levels to other higher trophic levels [5].

SOURCES OF HEAVY METAL CONTAMINATION

In recent years, heavy metals have been recognised as emerging environmental contaminants having a tremendous impact on the environment and a major threat to public health. Apart from natural flux, the other major way of heavy metal contribution to the environment is through human activities. The build-up of heavy metals in the environment as a result of anthropogenic activities is a serious concern. Heavy metals find huge industrial, technological, domestic and

CHAPTER 11

Anaerobes

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Abstract: Extremophilicity, or the capability to thrive in environmental conditions considered extreme is generally determined from the human perspective. From that point of view, organisms adapted to scarce, or even the absence of molecular oxygen, can be considered as one of the extremophiles, *i.e*., anaerobes. In this chapter, various aspects of anaerobic microorganisms are addressed, including their different taxa, their phylogenetic distribution, and the environments from where they have been isolated. Since prokaryotic taxonomy is a dynamic process, here we have emphasized the organisms that are validly placed in taxa and have cultured representatives. In this section, *Archaea* and *Bacteria* - the two domains are separately discussed. Similar separation is also maintained while discussing mechanisms of adaptation, as far as possible. Since these two domains share certain properties, the subsequent sections are not separated between these two domains.

Keywords: Anaerobic microorganisms, Anaerobic bacteria, Anaerobic archaea, ATP synthase, Biogeochemical cycle, Chemoorganotrophs, Extremophiles, Facultative anaerobes, Obligate anaerobes, Hydrothermal vent, Microaerophiles, *Proteobacteria*.

INTRODUCTION

"Extremophiles" refer to the organisms that are capable of surviving, or even growing, in extreme environmental conditions, which are otherwise considered as unfavourable to sustain life. There are diverse forms of extremophiles depending on the nature of extreme conditions in which they thrive. For example, acidophiles survive or favour low pH; alkaliphiles survive or favour high pH; halophiles survive or favour high salt concentration; thermophiles survive or favour high temperature; psychrophiles survive or favour low temperature, *etc*. Oxygen requirement is another important property to determine growth condition

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of an organism which is, in turn, related to the metabolic machinery it has. Depending on this property, organisms can be categorized into: (i) *obligate aerobes*, which have an absolute requirement of atmospheric O_2 for growth, (ii) *facultative anaerobes*, for which oxygen is not essential, although growth is better in its presence, (iii) *aerotolerant anaerobes*, whose growth is almost equal in the absence or presence of atmospheric O_2, although they cannot use it. (iv) *microaerophiles*, which require lower O_2 levels (2-10%) and are damaged in atmospheric O_2 level (20%), and (v) *obligate anaerobes*, which cannot tolerate O_2 at all. The major involvement of O_2 dependent metabolism is to satisfy the energy needs by oxidative phosphorylation. The associated electron transport chain components like flavoproteins, other cellular constituents, enzymes, *etc*. promote oxygen reduction and generation of toxic superoxide radicals, hydrogen peroxide, and hydroxyl radicals [1].

Therefore, cells need to set up a mechanism to remove or reduce the toxicity. Usually, obligate aerobes and facultative anaerobes have the enzymes like peroxidase, catalase, superoxide dismutase (SOD), *etc*., that catalyze the destruction of the toxic radicals [1].

Microaerophiles lack most of the enzymes, while obligate anaerobes completely lack them. This metabolic diversity may or may not confer actual evolutionary relation; therefore, a single microbial group often has several types of dependencies on O_2. Photosynthetic protists are mostly obligate aerobes, whereas fungi are generally aerobes, with the exception of yeasts, mostly which are facultative anaerobes. All the above five types of microorganisms are found in prokaryotes and protozoa. In this chapter, anaerobic prokaryotes are on the focus and emphasis is given to the organisms that are validly placed in taxa and have cultured representatives.

DIVERSITY OF ANAEROBES

Archaea, *Bacteria*, *Eukarya* - among these three domains of living forms - this discussion will be focused on prokaryotes. The term "*Archaea*" is derived from the Greek word "archaios", meaning "ancient" or "primitive," because they were originally discovered and described in extreme environments. These unique features made them different from the other two domains of life. Archaeal diversity was addressed in Volume 1 of the 2nd edition of *Bergey's Manual of Systematic Bacteriology.* rRNA sequence based phylogenetic approach classifies them into 2 major phyla- *Crenarchaeota* and *Euryarchaeota*. Both the phyla have extremophiles and are classified into classes, which are further subdivided into orders. The focus of the subsequent section is about anaerobic *Archaea*, and

Figs. (**11.1A** and **B**) draws phylogenetic relationship among them. Table **11.1** summarizes *Archaea* having multiple extremophilicity.

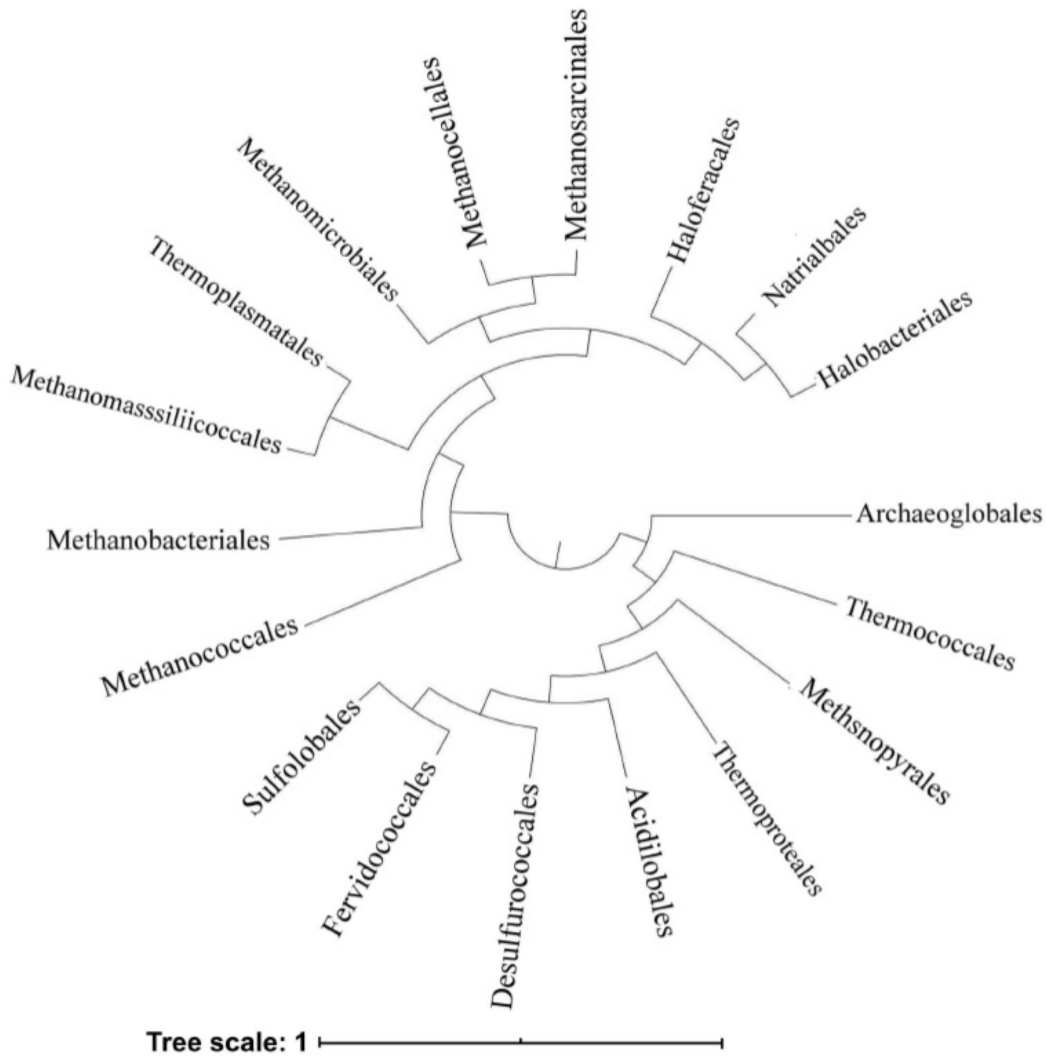

Fig. (11.1A). Dendrogram showing phylogenetic relationship among different orders of *Archaea*. Dendrogram was constructed in maximum Likelihood (ML) method with 16S rRNA sequences retrieved from GenBank, NCBI [https://www.ncbi.nlm.nih.gov/nuccore] using MEGA v5.0 software [https://www.megasoftware.net/]. It was circularized through iTOL web server [https://itol.embl.de/].

CHAPTER 12

Oligotrophs: Microbes at Low Nutrient Levels

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Abstract: Extremophiles are microbes capable of adaptation, survival and growth in extreme habitats that are supposed as adverse or lethal for other life forms. Like various other extreme environments, bacteria are also reported to grow in a minimum medium without additional carbon and energy sources. The microorganisms that can grow in low nutrient concentrations, or in the apparent absence of nutrients, are known as oligotrophs. In contrast, copiotroph bacteria grow fast where the resource or nutrient is abundant. Many of these oligotrophs alter their morphology (surface to volume ratio) with changing nutrient concentrations. The diverse oligotrophs have been isolated from the different low-nutrient habitats, such as marine, soil, desert soil, ultra-pure water, *etc*. The molecular and physiological properties of diverse oligotrophs and their applications in bioremediation are also studied. Oligotrophs would also be suitable for *in situ* bioremediation, because such microorganisms can grow on the contaminated site without additional nutrients. Remarkably, the adaptive capabilities of oligotrophs convert them into an attractive source for industrial purposes. Thus, oligotrophs have a biotechnological potential, orienting researchers to attempt their isolation and studies from various low-nutrient habitats. The objective of this chapter is to discuss the characteristics, adaptations and applications of oligotrophs.

Keywords: Copiotrophs, Extremophiles, Extremozymes, High-throughput culturing (HTC), Low-nutrient conditions, Oligotrophs.

INTRODUCTION

Most forms of life on earth are adapted to survive in moderate environmental conditions with temperatures around 28-30 °C, pH near neutrality, moderate water activity and adequate amounts of basic nutrients. However, some organisms have developed strategies to not only adjust but also to survive and thrive in hostile environmental conditions, such as the depths of oceans, hot springs, hydrothermal vents, xerophilic habitats, *etc*. that are normally challenging for other organisms to survive. These groups of organisms have been termed as 'extremophiles'- meaning 'one who loves extreme environments' [1]. Another related group of

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organisms, called extremo-tolerants, are however able to tolerate such extreme conditions but they show optimal growth only under normal physiological conditions [2]. These extremophiles still remain largely unexplored groups because of the various constraints encountered during their isolation, culture and study. Designing extremophilic microcosm in the laboratory itself is a very challenging task for the researchers. These extremophiles undergo several adaptations that enable them to survive and flourish in forbidding environmental conditions. They possess unique and highly stable enzymes, called ‘extremozymes’, which have a wide range of applications in industry and medicine [3]. The presence of various other unique properties, as compared to their mesophilic counterparts, makes these organisms an excellent model for research, specially to study the origin of life and also to understand the limits of life on earth.

The environment does not always offer a constant and ample amount of nutrients for the growth of microorganisms. There are areas in nature with nutrient scarcity. Depending upon the availability of nutrients and other resources, microorganisms adopt different strategies for survival and growth. They are generally categorized into two types- *oligotrophs* and *copiotrophs*. Oligotrophs are characterized by slow growth and are found in environments with low levels of nutrients. On the other hand, copiotrophs, also called *eutrophs*, are the ones that are normally found in nutrient-rich environments, especially carbon sources, and they exhibit fast growth [4]. These oligotrophs are also a type of extremophiles that have been adapted to grow optimally under low-nutrient conditions where other bacteria cannot survive. Except for a few environments, like the animal gut and open sea, both the types of bacteria are cosmopolitan in habitats.

OLIGOTROPHS: DEFINITION AND CHARACTERISTICS

Oligotrohps (Greek ‘oligos’, means few and ‘trophikos’ means feeding) are defined as the microorganisms that are found to grow and multiply in low or poor-nutrient environments. The term 'oligotroph', introduced by Weber (1907), can be compared with another similar term 'autochthonous' introduced by Winogradsky in 1924 to define those soil bacteria growing in sparse and in the presence of more recalcitrant materials [5]. The term ‘oligotroph’ and ‘oligocarbotroph’ are often used synonymously, the latter refers to the organisms that grow in the presence of trace amounts of organic carbon. However, the ‘oligotrophs’ is rather a general term for any organism that grows in the presence of trace amounts of nutrients and an organism may be classified and named based on the type of specific trace nutrients scavenged by them, such as oligonitrotrophs, oligophosphotrophs, *etc.* [6].

Kuznetsov *et al*., (1979) have defined oligotrophs as microorganisms that can be isolated and cultivated in a minimal media having a carbon source concentration of less than 10 mg/L [7]. However, in addition to this, oligotrophs require various other specific conditions for their isolation. Therefore, the above definition of oligotrophs is still vague. Poindexter (1979) has defined oligotrophs as those bacteria having the ability to multiply in habitats with a low energy flux which is almost fifty times less than that in the copiotrophic environment [8].

The nutrient is the basic requirement for the growth of any kind of organism. In order to survive and thrive in such a challenging environment where the nutrient is perennially scarce, such oligotrophic bacteria employ several unique strategies. They modify their growth accordingly and grow optimally using a low concentration of organic substrates as reported by various workers. They have been found to develop various mechanisms to assimilate nutrients from low-nutrient environments [8 - 13].

The constant nutrient deprivations trigger morphological changes, primarily reduction in size in the starving bacterial species [14]. Oligotrophs have been reported to reduce their size during growth [15]. They are defined by many authors as 'ultramicrocells' as their biovolume was found to be less than 0.1 µm [16 - 18]. Aggregated forms of oligotrophs have been found in certain natural habitats like the free ocean, water, Antarctic rocks, *etc*., where the carbon source usually does not exceed 1-5 mg/L. The oligotrophic microorganisms usually prefer to stay attached to solid surfaces in such habitats with constant famine. Even though such organisms are constantly facing nutrient deprivation, they still manage to synthesize polymers and certain storage products. Alteration in the morphology (surface-to-volume ratio) has also been one of the strategies of adaptation in such poor-nutrient habitats. Some of the literature has reported that oligotrophs secrete exopolysaccharides (EPS) which might assist in sequestering nutrients under nutrient depleted conditions [19, 20], and also has some other useful features [21].

Hirsch *et al*., (1979) have pointed out several characteristics of oligotrophs. The oligotrophs are known for their ability to survive extreme conditions of famine in terms of carbon and energy sources [22]. Under starved conditions, oligotrophic bacteria, like *Arthobacter* sp. or *Nocardia* sp. have been found to survive the longest as compared to the copiotrophic bacteria, like *Streptococcus* sp. and *E. coli*, which died off rapidly as noted by Nelson and Parkinson while comparing the 50% survival time of the two groups [23]. The oligotrophs have also been found to survive on mineral salt media with gaseous substrates. The ability to fix atmospheric carbon dioxide with the aid of ribulose1,5-bisphosphate carboxylase using the energy sources like H_2, CO, methanol, *etc*. has also been observed in

CHAPTER 13

Diversity and Mechanisms of Adaptation of Predominant Bacterial Chemolithotrophs in Extreme Habitats

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Abstract: Bacterial chemolithotrophy is one of the most ancient metabolisms and is generally defined as the ability of some microorganisms to utilize a wide range of inorganic substrates as an energy or electron source. While lithotrophy can itself be considered as extremophily, as only some microorganisms (the rock-eaters) have the ability to utilize diverse inorganic chemicals as the sole source of energy, the phylogenetically diverse groups of lithotrophs can thrive in a wide range of extreme habitats. Apart from their excellent eco-physiological adaptability, they also possess versatile enzymatic machinery for maintaining their lithotrophic attributes under such extreme environments. In this chapter, we have highlighted the diversity of iron, hydrogen and sulfur lithotrophic extremophilic bacteria in various extreme habitats, and their role in maintaining the primary productivity, ecosystem stability and mineral cycling / mineralogical transformations. Moreover, genetic determinants and different enzymatic systems which are reported to be involved in such lithotrophic metabolism also have been discussed. We hope this article will shed some new light on the field of extremophile lithotrophy, which will eventually improve our understanding of the extended new boundaries of life.

Keywords: Diversity of lithotrophs, Extremophilic bacteria, Hydrogen-oxidation, Iron-oxidation, Lithotrophy, Lithotrophy mechanism, Sulfur-oxidation.

INTRODUCTION

In the context of habitats and diversity, prokaryotes, particularly bacteria are ubiquitous in nature and can be found in almost all types of known Earths'

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extreme ecosystems. They have exceptional abilities to utilize different substrates as their energy or electron sources, which can either be light (phototrophic) or chemical compounds (chemotrophic); and carbon sources that include fixation of inorganic CO_2 (autotrophic), or use of organic carbon compounds (heterotrophic). Within both phototrophs and chemotrophs, those that utilize organic and inorganic sources as electron donors are usually considered as organotrophs and lithotrophs respectively (see Fig. **13.1**, for detailed information) [1]. Following the legendary discovery of lithotrophy by Russian microbiologist Sergei Nikolaievich Winogradsky in 1887, a global endeavour had begun for further understanding of this unique metabolism in a diverse group of bacteria [2]. Based on their inorganic energy and electron sources, some important examples of microbial lithotrophs include sulphur-oxidizing bacteria (*e.g.*, *Sulfobacillus acidophilus*), iron-oxidizing bacteria (*e.g.*, *Leptospirillum ferrooxidans*), nitrifying bacteria (*e.g.*, *Nitrococcus mobilis*), hydrogen oxidizing bacteria (*e.g.*, *Sulfurihydrogenibium azorense*), *etc.* [3 - 6]. Additionally, several other groups of extremophilic bacteria are also able to carry out lithotrophy using inorganic compounds, such as carbon monoxide (CO), ammonia (NH_4^+), manganese (Mn) and arsenite (As (III)). Diverse electron acceptors such as O_2 in aerobic respiration, and $(SO_4)^{2-}$, Fe (III), $(NO^3)^-$*etc.* in anaerobic respiration were found to be operational in the diverse bacterial groups which unanimously extend the metabolic capacity/flexibility of this metabolism [7]. Apart from being obligate (utilize only inorganic energy sources as electron donors and inorganic carbon for growth) or facultative (preferentially utilize preformed organics as an energy source, but also able to utilize inorganic sources as electron donors, when preformed organics are limited), lithotrophs can also be mixotrophic (able to utilize both types of energy sources simultaneously) (See Tables **13.1** and **13.2** for detailed information) [2]. Being able to grow in minimum nutritional requirements and able to withstand physicochemical conditions, lithotrophs are usually found in different extreme environments.

Primarily, environments with extreme conditions are considered dead zones and thought to be uninhabitable from anthropogenic viewpoint [8]. However, in the past few decades, a plethora of bacteria have been discovered from deep-sea hydrothermal vents to freezing cold waters in the Antarctic; from acid mine drainages to highly saline waters and several other such extreme habitats (Table **13.1**). They are widely known as extremophiles. Based on their ability to grow in either one or several (polyextremophiles) different physicochemical extremes, extremophiles are broadly categorized as thermophilic, hyperthermophilic, psychrophilic, acidophilic, alkaliphilic, halophilic and barophilic (see Fig. **13.2** for details) [4, 8 - 14]. Moreover, radioresistant (grow under high radiation exposure, *e.g.*, *Deinococcus radiodurans*), heavy metal tolerant (grow at high heavy metal concentrations, *e.g. Alkalilimnicola ehrlichii* use arsenite as an electron donor) and bacteria with high osmotic potential (osmophiles) (*e.g.*, *Acidiferrobacter* *thiooxydans*; grow at 5 Bar) are also considered as extremophiles [15, 16]. Notably, nowadays a collection of deep-sea sediments samples became possible due to the invention of deep submersible vehicles, like *Alvin* and *Nautile* [17, 18].

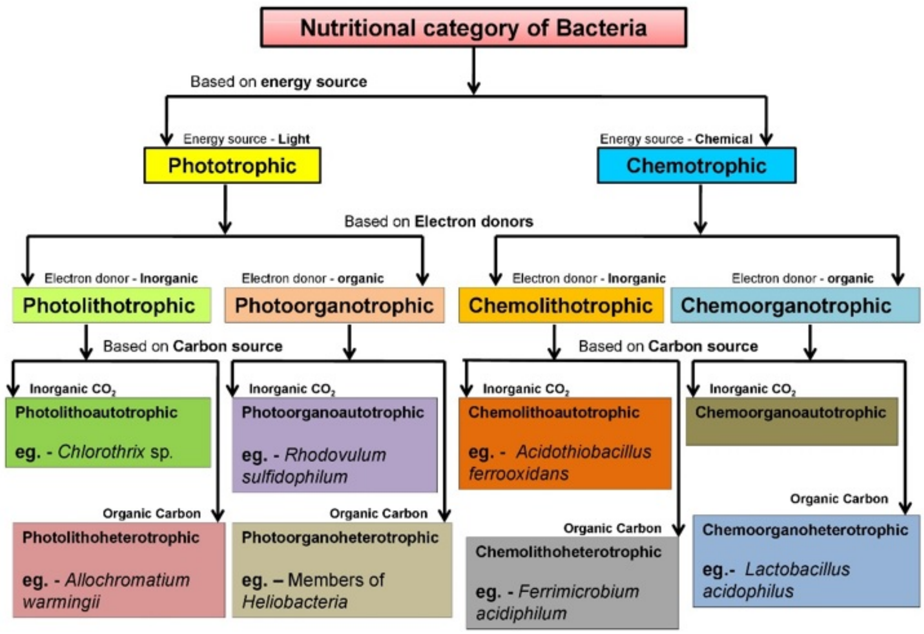

Fig. (13.1). Schematic representation of nutritional category of bacteria.

Eventually, these findings obviously propelled further research for the deeper understanding of diversity, physiology, metabolism and survival strategies of extremophilic microbes and subsequently helped us to elucidate the new boundaries of life.

Microbiological, as well as geological pieces of evidence, indicated that the use of Fe (III) as a terminal electron acceptor by extremophiles to generate energy is considered to be the first form of microbial respiration in Fe (III) rich sediments of hydrothermal vents [19]. Apart from this, they are also involved in changing the Earth's atmospheric composition/condition, biogeochemical cycling of different elements (like carbon, sulfur, iron, hydrogen, nitrogen, *etc*.), speciation of metals and metalloids and availability of minerals in soil sediments and water, and weathering of rocks, *etc*. [18]. However, discussing every aspect starting from diversity, habitats, survival strategies and evolution of all microbial extremophiles is beyond the scope of this chapter. Lithotrophy can itself be considered as extremophily as only some microorganisms (the *rock-eaters*) have the ability to utilize inorganic chemical compounds as the sole source of energy. Thus, in this

CHAPTER 14

Applications of Extremophiles

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Abstract: Extremophiles are organisms that can survive in harsh environmental conditions such as varying ranges of temperature, pH, high levels of salinity, extreme pressure and high doses of radiation. They are distributed throughout the Earth's surface and water bodies. They are classified on the basis of their habitats and extreme conditions they inhabit, like oligotrophs, thermophiles, psychrophiles, halophiles, acidophiles, alkaliphiles, piezophiles and radiophiles. Extremophiles have a huge impact on human life. Enzymes obtained from them are nowadays used in industrial microbiology, agriculture, pharmaceuticals and medical diagnostics, bioremediation, and in many more fields. With enormous commercial benefits and advanced scientific techniques, researchers are investigating extremophiles for a better understanding of their metabolism, and survival strategies for newer applications. This chapter focuses on applications of different types of extremophiles in industry, scientific research, medical science, and other fields.

Keywords: Acidophiles, Alkaliphiles, Bacteriorhodopsin, Cold-active enzymes, Compatible solutes, Extremophiles, Extremozymes, Halophiles, Heavy-metal resistance, Industrial applications, Mycosporine-like amino acids, Oligotrophs, PGPR, Piezophiles, Polyextermophile, Poly-β hydroxyl alkanoates, Psychrophiles, Radiophiles, Thermophiles.

INTRODUCTION

Microorganisms are the most primitive dwellers of Earth and have evidently originated dated back to about at least 3.5 billion years, if not more. They are omnipresent and their growth is not restricted to a certain environment. They are found in diverse and extreme conditions; from freezing Antarctica continent to scorching hydrothermal vents. They can grow in some of the most hostile conditions on earth which are lethal to other living creatures. The microorganisms that can survive, thrive, and reproduce in such extreme conditions are called extremophiles. Extremophiles are present in all three domains of life *i.e.*, *Arc-*

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haea, *Archaea*, *Bacteria*, and *Eukarya* [1, 2]. They are classified based on their habitats and environmental conditions therein, *i.e.* oligotrophs - extremely low nutritional condition; temperature - thermophiles (high temperature ranging from 45 °C to 121 °C), hyperthermophiles (optimal growth temperature above 80°C), psychrophiles (optimal growth temperature ranges from 2 °C to 20 °C); salinity - halophiles (high salt concentration, 2-5 M NaCl); pH - acidophiles (optimal pH for growth < 4), alkaliphiles (optimal pH for growth >9); high pressure - barophiles/peizophiles (high hydrostatic pressure, 80 MPa); radiophiles - survive under ionizing radiations, and many more. Some organisms can tolerate a wide range of extreme conditions and these organisms are called polyextremophiles. For example, many deep-sea organisms are barophiles as well as psychrophiles *i.e.*, they can survive high hydrostatic pressure (> 10 MPa, corresponding to depths over 1000 m) as well as low temperature (< 4°C).

In recent years, scientists have gained keen interest in extremophiles and extensive research is being carried out. Although, only about 1% of microorganisms are isolated, and even less than that can be cultured in the laboratory. Advancement in isolating extremophiles from exotic conditions and culturing in the laboratory is done because of their importance in human welfare as well as for scientific knowledge. Enzymes play a key role in human life since ancient times. Enzymes are used in day-to-day activities, like baking, and brewing as well as for industrial and medicinal purposes. The enzymes produced by mesophilic organisms cannot function optimally under diverse conditions of industrial processes and therefore increase the expense and reduce productivity. These limitations in the use of enzymes are because of their narrow range of diversity in terms of temperature, pH, *etc*. Whereas, extremophiles grow and reproduce in extreme conditions, and their survival is attributed to their metabolic activities and enzymes that are functional in such extreme environmental conditions. Thus, the enzymes produced by extremophiles are known as extremozymes. Extremozymes can withstand extreme conditions and are useful in industrial procedures, increasing reproducibility and productivity on their own. This flexibility is due to the amino acid composition, structure, charge, and hydrophobicity/hydrophilicity of the enzymes. However, not much is known about their metabolism as extremophiles are difficult to culture in the laboratory, Extracellular hydrolytic enzymes such as amylase, chitinase, lipase, cellulase, xylanase, pectinase, protease, and β-galactosidase have diverse potentials of application in biotechnological, agricultural, medicinal, food industry, bioethanol production, pharmaceuticals, molecular biology, and other sectors.

The study of the genetic material of these organisms and advancement in technology with next-generation sequencing allowed scientists to not only understand their genome structure but also have a clear idea of their survival mechanisms which will further help in understanding and altering the molecular elements to exploit in therapeutic and industrial processes. The use of microorganisms has created new possibilities in human welfare along with sustainable approaches for the future. Even though only a small fraction of microorganisms is known today, they still play a very crucial role in modern civilization. This chapter focuses on the diverse applications of extremophiles and their extremozymes in biotechnological and other sectors for a better understanding of their potential role in the present and future of human civilization.

APPLICATIONS OF OLIGOTROPHS

Oligotrophs are microorganisms that have the ability to thrive in extremely low nutrient environments. According to Grime, who designated oligotrophs as 'stress tolerators', habitats in which biomass accumulation rate is restricted by the supply of one or more resources are called oligotrophic habitats and the inhabitants that can manage to grow in those habitats are called oligotrophs. They have a number of important biotechnological, medical and environmental implications.

As their diversity and biomass are dominant in the biosphere, they play an important role in biogeochemical cycles. Their mechanisms to survive in oligotrophic conditions and their roles in ecosystems make them interesting subjects in microbial ecological research. Recently oligotrophs are being used in iatrology to identify pathogenic organisms, and for environmental monitoring and the detection of toxicological contamination [3].

One of the novel applications of oligotrophic microorganisms is there in microbial electrochemical systems (MES). The MES are microbial catalyst driven electrochemical devices that catalyze the electrochemical reactions on electrode surfaces by using a range of organic wastes. In MES, the chemical energy stored inside organic resources is converted into electrical energy or other valuable bio-electro-fuels by microorganisms [4].

Oligotrophic bacteria are isolated by many researchers from a variety of clinical samples but they are not found in routine bacteriological examinations in the hospital laboratory. Though the clinical significance of such oligotrophic bacteria is still uncertain, they can be used as tools to monitor aseptic conditions in hospitals, pharmaceutical production units, *etc*. It is observed that oligotrophic counts in clean rooms exceeded the standard plate counts by up to 2 orders of magnitude [5].

Nowadays microorganisms are successfully used as 'biosensors' for environmental monitoring and detection of various toxic chemicals including

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