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Extremophiles and Their Significance in Aquatic Environment

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Abstract

This article is mainly focused on the occurrence, physiology and habitat of various kinds of extremophiles in the environment. These organisms can survive in extreme environments and performs different ecological roles. Since, these organisms can thrive in adverse conditions, the enzymes and other metabolic products produced by them can be effectively used in several biotechnological processes. The biomolecules produced by these organisms can be used for the preparation of cosmetics and medicinal products. So, it is important to study about these microbes to explore their potential benefits and their significance on environment.

Introduction

An extremophile (from Latin *extremus* meaning "extreme" and Greek *philus* meaning "love") is an organism that thrives in physically or geochemically extreme conditions that are detrimental to most life on Earth. Extremophiles include members of all three domains of life, *i.e.*, bacteria, archaea, and eukarya. Most extremophiles are microorganisms (and a high proportion of these are archaea), but this group also includes eukaryotes such as protists (*e.g.*, algae, fungi and protozoa) and multicellular organisms. Archaea is the main group to thrive in extreme environments. Archeans are a group of microscopic organisms that are considered to be intermediate between prokaryotes and eukaryotes. They have the cell size and shape similar to prokaryotes but their metabolic reactions resemble to eukaryotes.

Extreme Environments and Their Microbial Life

Extreme environments can be categorized based on the nature of their physical and chemical extremities. The major extreme conditions taken into account with respect to their microbial life include: highly saline environments with variable salinity, extreme of temperatures (coldand hot), underground environments (ice and rocks) and deep sea vent (extremely high temperature and pressure). Problems relating to the existence and survival of organisms and strategies to overcome extremities are the major issues of studies. The microbes from extreme environments are focusing major attention in recent years. Extremophiles may be divided into two broad categories: extremophilic organisms which require one or more extreme conditions in order to grow, and extremotolerant organisms which can tolerate extreme values of one or more physicochemical parameters though growing optimally at "normal" conditions (Rampelotto, 2013).

Extreme Conditions

This includes,

1. Temperature
2. Salinity
3. Nutritional scarcities
4. Absence of oxygen
5. Radiation
6. Pressure

Classification of Extremophiles

They are classified according to the conditions in which they grow.

1. Thermophiles - Organisms that grow at high or very high temperature conditions.
2. Psychrophiles – Organisms that grow best at low temperature.
3. Halophiles – Organisms that require salt (NaCl) for growth.
4. Barophiles – Organisms that grow best at high temperature.
5. Acidophiles – Organisms that prefer acidic environments to grow.
6. Alkaliphiles – Organisms that grow best at alkaline conditions.

Environmental extremes include high levels of ionizing radiation. This affects cells by damaging DNA directly or by producing reactive oxygen radicals that can cause mutations in DNA or break the strands. However, some organisms can survive these conditions. The bacterium *Deinococcus radiodurans* has been isolated from the environs of nuclear reactors and can survive up to 20,000 Gy of gamma radiation, enough to split its genome into small fragments (Irwin, 2010).

1. Thermophiles

The microorganisms which are able to grow optimally at temperatures above 40 °C are referred as thermopiles. Among Extremophiles, they are the best studied groups. They grow optimally at temperatures between 50-70 °C. They grow slowly below 40 °C. Thermophiles include bacteria, actinomycetes, fungi, protozoa, algae and blue green bacteria. Among the long time contributors to the field of thermophiles, the work of Thomas D. Brock on microbial life in hot springs of yellow Stone National Park in Wyoming (USA) deserves attention. In 1960's, his group identified bacteria, now known as *Thermus aquaticus* (Figure 1) which later became a star candidate for it's extremely heat stable DNA polymerase (Taq polymerase) used in PCR applications.

Hyperthermophiles

The studies on the thermophile were further propelled by the discovery of the eubacteria and archaea bacteria able to grow at temperatures between 80-110 °C. A term, hyperthermophile, was used to group and describe these organisms, which were unable to grow below 60 °C.

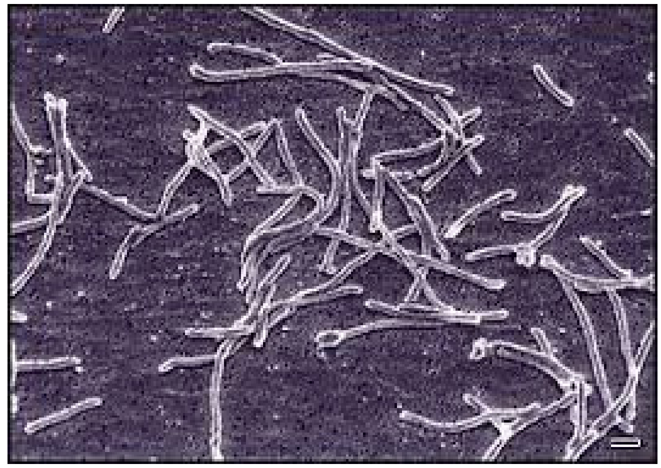

Figure 1: *Thermus aquaticus* - Rod shaped bacterium which can thrive upto 80 °C

Examples: *Pyrolobus fumarii, Thermotoga* sp., *Aquifex* sp., *Pyrococcus* sp., *Pyrodictium* sp., *Thermoproteus* sp. and *Sulfolobus* sp.

Key habitats of thermophiles:

- Hot springs
- Hydrothermal vents
- Geothermal locations
- Acidic solfatara fields
- Anaerobic geothermal mud and soil

Hot Springs

Hot springs are usually located outside the volcanic active zones. Temperature is around 75 °C in this zone. The representative groups belong to both primary producers (autotrophs) and consumer (heterotrophs). *Thermothrixthiopara, Methanobacterium thermoautotrophicum* and *Desulfovibrio thermophilus* are the autotrophs able to use H_2S/O_2, H_2/CO_2 as energy source, respectively and can grow up to 80-85 °C, the optimum temperature and pH being 65-75 °C and 6.8-7.5. Similarly, consumers (heterotrophs) are represented by *Thermus* spp. and *Bacillus* spp. in these habitats. The heterotrophs are able to grow up to 800 °C, with the optimum ranging from 60-80 °C.

Hydrothermal Vents

A Hydrothermal vent is a fissure in a planet's surface from which geothermally heated water issues. They are commonly found near volcanically active places. Under the sea, they may form features called BLACKSMOKERS. Temperatures range from 60 °C upto 464 °C. Temperatures well above 662 °F (350 °C) are not uncommon at vents. The microbes thriving in this environment include *Methanocaldococcus jannaschii*, *Desulfonauticus submarinus,* *Desulfovibrio gigas, Desulfobacterium autotrophicum,* and *Desulfobacter latus, Desulfovibrio vulgaris*. A sub seafloor thermophile isolated from deep-sea hydrothermal vent fluids that eats sulfur and hydrogen and fixes its own carbon from carbon dioxide.

Significance of Thermophiles

Thermophilic organisms, particularly those able to grow at temperatures more than 90 °C can be excellent models for investigating and understanding protein stability at extreme temperatures. Such organisms would have significant biotechnological potential. Certain other specific advantages of such organisms are as follows.

• Higher reaction rates at elevated temperatures.

• Solubility of substrate at elevated temperatures.

• Reduced contamination probability.

• Excellent Biocatalysts such as Glycosyl hydrolases, proteinases, Esterase.

• DNA processing enzyme DNA polymerase (from hyperthermophilic archaea) with high proof reading activity useful in PCR.

2. Psychrophiles

In Greek terminology, Psychrophile means 'cold-loving'. They are found in places that are permanently cold, such as the polar regions and the deep sea. The psychrophiles cover the temperature range between -15 to +15 °C. They are also known as cryophiles. These microbes can be isolated from Arctic and Antarctic habitats. They are also found in icebergs, glaciers, snow fields, *etc*. These microbes maintain their metabolisms quite normal at low temperature. Their cell membranes contain high level of fatty acids which remain fluid even at colder temperatures. They also have developed proteinaceous ANTI-FREEZE MECHANISMS to protect the cell and DNA.

Examples: *Arthrobacter* sp., *Psychrobacter* sp., *Halomonas* sp., *Pseudomonas* sp., *etc*.

Significance of Psychrophiles

• Several cold active enzymes can be extracted from Psychrophiles.

• Cryoprotectants and Anti freeze proteins can be extracted from them.

• These microbes can be used for bio remediation works in colder regions.

• Some microbes are used as Bio sensors for environmental purpose.

3. Halophiles

Halophiles are the organisms able to grow at very high salt concentrations prevailing in diverse hypersaline environments. Depending on their requirement for salt, they are classified as halotolerant (optimum growth, 0-0.3 M salt; growth range, 0-1.0 M salt), moderatelyhalophilic (optimum growth, 0.2-2.0 M salt; growth range, 0.1-5.0 M salt) and extremely halophilic/ hyperhalophilic (optimum growth, 3.0-5.0 M salt; growth range, 2.0-5.5 M salt).

Halophilc organisms are highly diverse groups including heterotrophic and methanogenic archaea, autotrophic and heterotrophic bacteria and heterotrophic eukaryotes. Among the archaeal category, *Halobacterium* and *Halococcus* (from Dead Sea) and *Nitrobacter* and *Nitrococcus* (from Kanyan Soda lakes) can grow between 3-5 M salt and are the most extensively studied organisms.

Significance of Halophiles

• **Bacteriorhodopsin:** The purple membranes from many halophilic archaea could be useful in fabricating biochips for new generation of computers. Artificial membrane capable of converting sun light to electricity can also be a technological possibility.

• **Bio-Plastic and Polysaccharides:** Biopalstics from halophilic bacteria would be biodegradable and resistant to water. Similarly, exo-polysaccharides could be used as stabilizers, thickeners, gelling agents and emulsifiers in pharmaceuticals, paints, oil recovery, paper, textiles and food industries.

• **Compatible Solutes:** Many halophilic organisms accumulate organic compounds e.g. betaine, ectoines and glycerol. These solutes could be quite useful for commercial applications. In this context, the on-going studies on the regulation of gene expression relating to the synthesis of biocompatible solutes are likely to open new area for improved and large scale production of these molecules**.**

• **Enzymes:** Exoenzymes produced by these organisms includes proteases, lipases, amylases and nucleases. These biocatalysts are potentially useful in detergent, food and textile industries. Combination of properties of such enzymes at extreme conditions attracts many novel applications.

• **Biodegradation:** Degradation of toxic industrial residue like organo phenol, heavy metals pollutant containing high salt can be a possibility using halophilic organism. Use of such organisms in bioremediation is a reality. However, processes for large scale bioremediation would have to optimized and developed.

• **Production of Beta-carotene:** Many members of the halophilic group produce beta-carotene which can be used to synthesize value based molecules such as vitamin A.

4. Barophiles

Barophiles are the micro organisms that can survive at immense hydrostatic pressure (Figure 2). They are generally found in ocean floors where pressure exceeds 300 atm (38 MPa). Some have been found at the bottom of the Pacific ocean (Mariana trench - 10,500 m) where pressure often exceeds 117 MPa. These organisms cannot grow at pressure below 400-500 atm. True obligate barophiles also comprises of bacteria which are present in the gut of Holothurins.

Example: *Photobacterium* sp., *Shewanella* sp., *etc*.

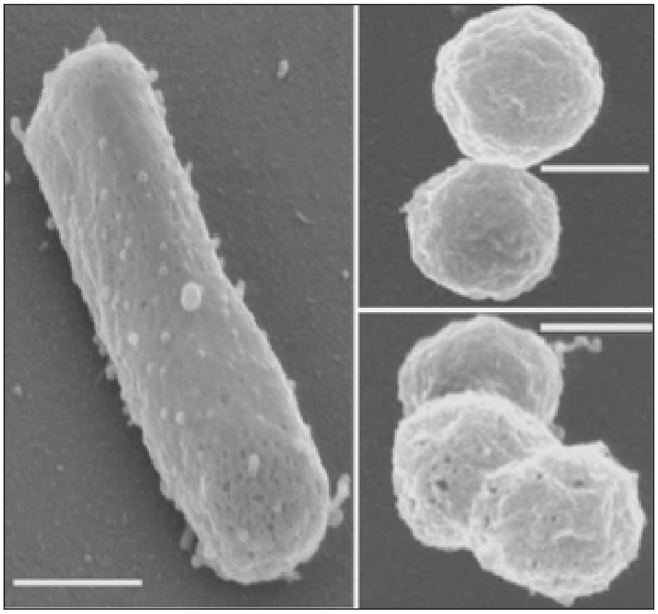

Figure 2: Barophilic microbes isolated from deep sea

Significance of Barophiles

• In oceans, barophiles grow on sediments and support life by providing carbon to benthic animals which ingest bacteria.

• Helps in recycling of nutrients.

• Some decompose organic matter, releasing various minerals like sulphates, phosphates, nitrates which are used by other bacteria.

• Enzymes derived from barophilic microbes can be used in bio-reactors and food processors.

5. Acidophiles

An acidophile is an organism that can or must live in an acidic environment. An acidic environment is one that has a pH below 6. Acidophiles are able to live and thrive to a highly acidic environment, particularly at pH 2.0 or below. Some acidophiles are adapted to an acidic environment because of a membrane system that effectively pumps protons out of the intracellular space and consequently helps keep the cytoplasm at or near a neutral pH. Other acidophiles with acidified cytoplasm have proteins that can attain acid stability.

Some of the organisms considered as acidophiles are as follows.

• *Acetobacter aceti*, *i.e.*, a bacterium capable of producing acetic acid through oxidizing ethanol

• *Acidobacteria* spp., *i.e.*, a phylum of bacteria comprised of bacteria such as *Acidobacterium capsulatum*, *Holophaga foetida*, *Bryobacter aggregates*.

• *Alicyclobacilus* spp., *i.e.,* Gram-positive, spore-forming rods that grow in acidic conditions (pH 2.0-6.0).

Significance of Acidophiles

• Desulphurication of coal using acidophiles involves sulphur oxidation and the process is quite useful in improving the coal quality.

• Similarly, these microbes can also be useful in food industries.

• Other potential applications of the enzymes from such organisms include degradation and synthesis of compounds under acidic conditions. Preparation of animal feed to be used in the animal stomach, where pH is acidic, is one such application.

6. Alkaliphiles

An alkaliphile is an organism that can live and thrive in an alkaline environment, *i.e.*, with pH ranging from 8.5-11. Examples of an alkaline environment are the Soda Lake in Carrizo Plain National Monument, California, the Octopus Spring located in Yellowstone National Park, and the Mono Lake in California's Eastern Sierra. Carbonate-rich soils are also a typical habitat of alkaliphiles. Alkaliphiles are able to survive in an alkaline environment because of a membrane system that actively pumps H^+ across the cell membrane into their cytoplasm and therefore able to maintain pH of about 8.0. Others have evolved pH stable enzymes that help them survive an alkaline environment. Alkaliphiles have developed a negatively charged cell wall, which lowers the pH of the environment just outside the cell. They also produce an acidic secondary cell wall composed of teichurono-peptide and teichuronic acid or polyglutamic acid. These acids attract H^+ and repel OH^-, possibly helping to generate the proton motive force needed to drive ATP synthesis (Coker, 2019).

Alkaliphiles may be grouped according to their alkalinity requirement for growth and survival.

• Obligate alkaliphiles, *i.e.*, those requiring high pH.

• Facultative alkaliphiles, *i.e.*, those that can survive in both alkaline and normal conditions.

• Haloalkaliphiles, *i.e.*, those requiring high salt content for survival.

Examples of alkaliphiles are *Anabaena* sp. and *Microcystis* sp., which are blue-green algal species.

Significance of Alkaliphiles

• Alkaliphiles are particularly significant for their unique enzymes such as cellulases, proteases and amylases. The ability of such enzyme to catalyze the reaction at high pH has attracted several applications and many such enzymes have already been commercialized.

• Alkaliphilic extremozymes are important in fabric industries where stone washed look into the denim fabric can be created by softening and fading fabric.

• Further proteases and amylases from alkaliphilic organisms are quite significant in detergent and leather industries.

• Alkaline proteases may provide potential applications for the management of waste originating from various food processing industries and household activities.

• Proteases solubilize proteinaceous waste lowering down the biological oxygen demand of aquatic systems.

Conclusion

Exploration of newer habitats, particularly the extreme ones, would generate new horizons for their application and generation of novel and unknown value based products. One of the future trends of research relating to extremophiles would be to produce recombinant enzymes after cloning and expression into suitable hosts for investigation of enzymatic properties such as stability and biochemical characterization. Observation of certain unique features among the extremophilic protein/ enzymes, such as resistance against chemical denaturation, indicates towards their application as model system to investigate protein stability, besides attracting unique biotechnological roles.

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