

Prospecting Microbial Extremophiles as Valuable Resources of Biomolecules for Biotechnological Applications

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Abstract: *Life has been said to exist in virtually all habitats. Of great astonishment is existence of life in habitats that were considered to be lethal and from which viable organisms were found to survive and even proliferate. Organisms that are able to tolerate these environments are referred to as extremophiles. Examples among many others include thermophiles and hyperthermophiles (tolerant to high temperature), psychrophiles (tolerant to low temperature), barophiles (tolerant to high atmospheric pressure), xerophiles (tolerant to dryness), metallophilic (tolerant to heavy metals). The developments of distinctive biomolecules and machineries that can function under these acute situations have allowed extremophiles to tolerate, adapt and survive. The studies of these organisms, their habitats, biomolecules and products have been of great potential in numerous biotechnological applications, though many biotechnological processes are yet to adopt the use of extremophiles in their production. This article thus seeks to harness the various factors involved in microbial extremicity, current and possible future biotechnological applications of inherent potentials of these extremophiles and their biomolecules, towards sustainability of human life and his environments.*

Keywords: Biomolecules, Extreme habitat, Environmental factors, Extremophiles, Lethal, Microbial diversity.

1. Introduction

Life is ubiquitous on earth, with various microorganisms surviving and thriving in the environment they find themselves [1]. In order to maximize their privilege of existence, each of these microorganisms develops means through which they can tolerate and adapt in their surroundings [2]. As a result of their adaptation, these microorganisms can be grouped based on various parameters. One of such parameters groups microorganisms as either mesophiles or extremophiles. **Mesophiles or neutrophilic** microorganisms survive and thrive in moderate environments, with conditions like 25°C - 40 °C as optimal temperature and 6.5 - 7.5 as neutral pH [3]. Most microorganisms are associated with such environments. However, some species of microbe survive or thrive in environments with physiological conditions lethal to the physio-chemical characteristics of mesophilic cells, these microbes are classified as **extremophiles** and their environment categorized as extreme environments, such as deep-sea hydrothermal vents, heights of the Himalayas, boiling waters of hot springs, saline/soda lakes and the cold expanses of Antarctica [1, 2]. These habitats classifies the microbes that have inhabited and adapted to them, such as temperature adaptation (psychrophiles to hyperthermophiles), high salinity adaptation (halophiles), pH adaptation (acidophiles and alkaliphiles), and pressure adaptation (barophiles), among others [3, 4].

The ability for life to be ubiquitous on earth is due to the fact that some organisms possess the ability to stabilize globular proteins at various environmental conditions [2]. The conformational stability of globular proteins can be defined by the free-energy difference between the folded and unfolded states ($\Delta G_N \rightarrow D$), under physiological conditions which is usually in order of the value 45 ± 15 kJ/mol; which

reflects only marginal stability of native proteins [5]. Thus, in order for microorganisms to thrive in an extreme environment, adaptation can be accomplished by shifting the optimum curve such that similar $\Delta G_N \rightarrow D$ values are obtained at the respective optimum conditions [6].

Life in extreme environments, over the years, has been studied intensively, with attention on the diversity of organisms and the molecular and regulatory mechanisms involved [7]. Products obtainable from extremophiles such as proteins, enzymes (extremozymes) and compatible solutes have been found to be of great use in many biotechnological applications and consequently attracted great attention [8]. Although prokaryotes are the dominant organisms in extreme habitats, higher organisms such as plants, vertebrate and invertebrates animals are also present, such as cacti, scorpions and polar bears [9]. These organisms do not just inhabit these niches but the extreme factors are essential for their survival and proliferation [10]. Thus there is a difference between organisms that are only tolerating an extreme factor(s) (i.e extremotolerant organisms) from those that are obligatory dependent on extreme factor(s) to survive; these are the real extremophiles.

Many bioprocesses are yet to tap from the great resources that extremophiles have to offer. This article thus provides an overview of extreme environments and extremophiles associated with them. The relevance of these organisms in current biotechnological applications and the future prospects towards improvement of biotechnological processes and sustainability of human life and his environments are also discussed

2. Microbial Diversity

Microorganisms are known to be life antecedents from which all other forms of life are said to have evolved from, and possess the essential resources and machineries required driving biological functions for their existence [11, 12]. The huge potential imbibed in the microbial world are being exploited and translated into various biotechnological applications by Man by the use of resources that lie within, such as their biomolecules (nucleic acids, proteins, enzymes), products or the whole cell organism [13-15].

The microbial community is basically underexplored and poorly understood compared to the diversities of the plant and animal kingdoms which have been well studied [15, 16]. This is as a result that only a minute fraction (less than 1%) of the diversity of microbial life has been identified and studied [13, 17-19]. Thus there is need for more intense search and study of this community, more importantly on extreme habitats that has the potential to harbor organisms that will be of biotechnological interest.

In the studies by Kirk et al. [20], various hindrances towards the studying of microbial diversities were highlighted, which include:

- the inability to assess the innate heterogeneity of environmental samples as a result of the sampling methods and how the component organisms are spatial and temporal distributed
- the inability to assess the huge phenotypic and genetic diversity of metabolically active viable but unculturable soil microorganisms.
- the taxonomical ambiguity in which the definition of prokaryotic species is yet to be defined because the standards available are designed for higher organisms and are essentially not applicable to microbial species.

However, recent technologies in analytical chemistry, computational biology, and metagenomics which have considerably helped in alleviating some of these issues, have been used in microbial ecology studies, thus offering new insights into the role of microorganisms within their ecosystems [12, 21]. The identities of a significant number of formerly unidentified microorganisms have thus emerged using their molecular architecture. The three domains of cellular life; Bacteria, Archaea and Eukarya (Figure 1; Table 1) have also been verified by these technologies and the richest collection of molecular and chemical diversity in nature is now acknowledged to exist in the microbial world [11, 12, 21].

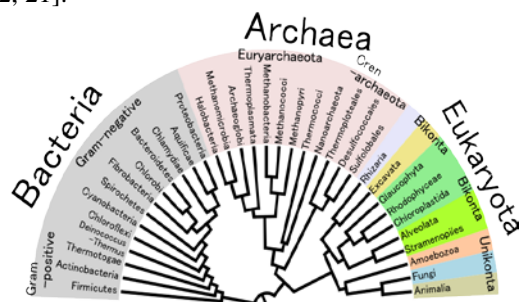


Figure 1: The three domains of cellular life as indicated by metagenomic analyses; adapted from [22].

Table 1: Comparison of some characteristic features of the three domains of life

Feature	Bacteria	Archaea	Eukaryota
Cell wall peptidoglycan	Present	Absent	Absent
Nuclear envelope	Absent	Absent	Present
Introns	Absent or very rare and are in a specific sequence	Present in some genes and are in a specific sequence	Present and are in diverse sequences
RNA polymerase number	One, (4 subunits)	Several, (8-14 subunits)	Several, (12 subunits)
Amino acid that introduces protein synthesis	Formyl-methionine	Methionine	Methionine
Membrane bound organelles	Absent	Absent	Present
Membrane lipid structure	Unbranched	Branched	Unbranched
Histones associated with DNA	Absent	Present in some species	Present
Circular chromosome	Present	Present	Absent
DNA	Circular	Circular	Linear
DNA sequence at transcription initiation site	Diverse type	Sequence similar to TATA box	TATA box
Binding of ribosome	SD sequence	mRNA cap	mRNA cap
Poly A addition	Absent	Present	Present
Ribosome structure	30S,50S	30S,50S (looks like eukaryote)	40S,60S

3. Environmental Factors Involved in Microbial Extremicity

There are several factor(s) that affect the physiological condition of an environment, which makes it to be extreme and such factor(s) plays a big role in maintaining the environmental status. Some of these factors are discussed below.

3.1 Temperature

Several environments exist with fixed temperature range, and these are used in classifying the microorganisms present in them. At temperatures tending towards 100°C, mesophilic cells often experience denaturation of their cellular proteins and nucleic acids, increased fluidity of membranes, degradation of chlorophyll in the case of plant cells and depriving aquatic organisms dissolved gases such as oxygen [23, 24]. From the illustration of Becktel and Schellman [25], protein stability curve of free energy is shown as a function of temperature (Figure 2). From the curve, important thermodynamic parameters were determined similar to the Gibbs-Helmholtz equation:

$$\Delta G(T) = \Delta H_m (1 - T/T_m) - \Delta C_p \times [(T_m - T) + T \ln(T/T_m)]$$

Where;

$\Delta G(T)$ = free energy at a temperature; T

ΔH_m = enthalpy change at T_m

ΔC_p = change in heat capacity associated with the unfolding of the protein

T_m = melting temperature or temperature at midpoint of transition from native to denatured state

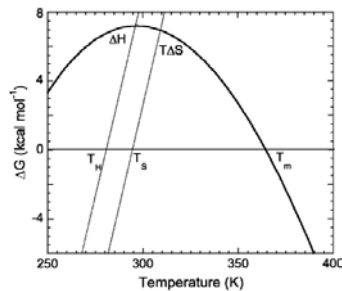


Figure 2: Protein stability curve of free energy as a function of temperature; adapted from [25].

On the other hand, temperatures tending towards 0°C causes mesophilic cells to experience reduced enzyme activity, decreased membrane fluidity; altered transport of nutrients and waste products, decreased rates of transcription, translation and cell division, protein cold-denaturation, inappropriate protein folding and intracellular ice formation [2, 6, 26].

Nevertheless, microorganisms exist in environments of both high temperatures (>50°C) and low temperatures (<4°C) where they survive and thrive [2]. Microorganisms that survive at temperatures >50°C are classified as **thermophiles**, while microbes that survive and prefer temperature range of 85°C - 106°C are known as **hyperthermophile** [27]. With *Pyrolobus fumarii* (Crenarchaeota) from archaea, as the microbe that survives the highest temperature (113°C), several searches have been made on how these extremophiles cope with such condition [2]. Studies on 10 thermophilic (and hyperthermophilic) serine hydroxymethyltransferases and 53 mesophilic homologs focusing on their structural features and homology modeling revealed the various mechanisms employed by these extremophiles in order to adapt to high temperatures [23].

It was discovered that thermophiles possess high ion-pair content which forms higher-order oligomers, allowing them to attain normal flexibility at high temperatures [28]. Also at these temperatures there exists high internal hydrophobicity as a result of inclination of the α -helices residues resulting in short length of surface loops of secondary elemental structures, optimal electrostatic and hydrophobic amino acids interactions [6]. Monovalent and divalent salts were also been discovered to be present in thermophiles, thereby making them attain a more stable nucleic acid [23]. This was attributed to the scavenging activity of these salts (such as KCl and MgCl₂), by mopping up negative charges of the nucleic acid phosphate groups, consequently protecting them from depurination and hydrolysis [29]. In spite of their extremicity, it has not been reported that thermophiles have high level of G-C interaction in their nucleic acid; even if such bonds adds an advantage against high temperatures due to the triple hydrogen bonds [27]. Examples of thermophiles among many others include *Anoxybacillus amylolyticus*, *Symbiobacterium thermophilum*, *Geobacillus thermoleovorans*, *Geobacillus thermoleovorans* subspecies

stromboliensis, *Geobacillus toebii* subspecies *decanicus*, *Bacillus thermantarcticus* and *Thermus oshimai* [30, 31].

Apart from extremophiles that tolerate high temperatures, there are also some that tolerate low temperatures, and are classified as **psychrophiles**. Psychrophiles adapt to cold environments by containing higher levels of unsaturated, polyunsaturated, methyl-branched fatty acids and shorter acyl-chain length that introduce steric constraints, which reduces membrane interactions and thus increases membrane fluidity [32]. Some psychrophiles also possess large lipid head groups with decreased non-polar carotenoid pigment [4]. Psychrophilic enzymes are discovered to adapt to cold environment by increasing their structural flexibility [33]. Psychrophiles also possess antifreeze proteins that bind complementary to surface of the ice crystals, reducing the effect of the cold [23]. Studies on the genome of *P. haloplanktis* showed gene clusters that enhances membrane fluidity via the degradation of steroids [33]. β -keto-acyl-CoA synthetases and *cis-trans* isomerase were observed in *C. psychrerythraea*, where they aid in increasing membrane fluidity [34]. *C. psychrerythraea* and *D. psychrophila* both possess high efficient antioxidant capacity as a result of large quantities of catalase and superoxide dismutase. These enzymes are important owe to the fact that at low temperature gas solubility is high and production of reactive oxygen specie (ROS) increases [33]. *P. haloplanktis* adapts to cold temperature by avoiding metabolic pathways that lead to large production of ROS such as the molybdopterin metabolic process [34]. Other examples of psychrophiles include *Alteromonas haloplanctis* A23, *Moritella profunda*, *Ps. fluorescens*, *Pseudomonas-Moraxella-Acinetobacter*, *Ps.alcaligenes*, *Ps. Syncyanea* [35, 36].

3.2 Power of Hydrogen Ion Concentration (pH)

Biological processes considered to be normal occur at physiological pH conditions of about 5 to 8.5 [1]. Many organisms are known to function best at pH values close to neutrality. The metabolic activities of cells are very sensitive to inorganic ions and metabolites and these are subject of the pH values [4]. **Acidophiles** lives in extremely acidic environment (i.e pH <2) while **alkaliphiles**; also called **alkaliphiles** lives in environment where pH > 10 [37]. Studies have revealed that the internal environments of acidophiles and alkaliphiles are kept constant at neutral pH, with no need for their enzymes to adapt to extreme pH; however their extracellular proteins are exposed to the unusual pH level [1, 38].

When **neutrophilic** microorganism are exposed to extreme pH, their polar charged residues acquire charges and become protonated leading to free intracellular protons which could impair processes such as DNA transcription, protein synthesis and enzyme activities [2]. This is because the influx of protons through the F₀F₁ ATPase produces ATP, resulting in cellular protonation and dissipation of the pH gradient (Podar and Reysenbach, 2006). However its been shown that **acidophiles** adapt to such low pH by possessing negatively charged amino acids (i.e acidic amino acids) on the surface of enzymes and proteins at neutral pH [6]. This was confirmed through a study on endo- β -glucanase from

Sulfolobus solfataricus which is most active at pH 1.8 [39]. Glutamate and aspartate were discovered to be the most common amino acid surface residue, and at neutral pH, the enzyme was found to be inactive due to high negative charges resulting in repulsion in the cellular environment, however at lower pH, the interaction was stable due to lower isoelectric point and the enzyme functioned at optimal performance [6, 40]. However, not all proteins in acidophiles such as ATP dependent DNA ligase from *Ferroplasma acidarmanus* are tolerant to acidic environment, and this is because the DNA (which is the enzyme substrate) exists at neutral pH, and its architecture will be compromised if in an acidic environment [41]. Other studies show that pH gradient across acidophilic cytoplasmic membrane is intrinsically linked to cellular bioenergetics as it is the major contributor to the proton motive force [38].

Alkaline-adapted micro-organisms are classified as **alkaliphiles** (also called alkalophiles) or alkalitolerants [2]. The term alkaliphiles is generally restricted to those micro-organisms that actually require alkaline media for growth [38]. The optimum growth rate of these micro-organisms is observed in at least two pH units above neutral [2]. Whereas Organisms capable of growing at pH values more than 9 or 10, but with optimum growth rates at around neutral are referred to as alkalitolerant [42]. Alkaliphiles also keep a neutral pH in their interior, and therefore show no need for adaptation of internal physiology [6]. Studies on α -galactosidase in the internal environment of the alkaliphile *Micrococcus sp.* showed that the enzyme had optimal performance when the pH was around neutral whereas the external environment had pH level tending towards 10 [43]. This shows that the cell wall has the ability to prevent the intracellular pH level from being compromised [1]. Studies on various alkaliphilic *Bacillus sp.* has revealed that alkaliphile survive high pH by possessing negatively charged acidic nonpeptidoglycan polymers such as aspartic, galacturonic, gluconic, glutamic and phosphoric acids; these residues reduce the pH at the cell surface, making the cell to absorb Na^+ and H^+ and repel OH^- [44]. In addition, the peptidoglycan of alkaliphiles also tends to have more of glucosamine, muramic acid, D- and L-alanine, D-glutamic acid, meso-diaminopimelic acid, and acetic acid when compared with neutrophiles [45]. The plasma membrane also play a role in controlling the pH level of the intracellular environment of the cell by using Na^+/H^+ antiporter system, K^+/H^+ antiporter and ATPase-driven H^+ expulsion [45]. Examples of acidophiles among many others include *ferroplasma sp.*, *Cyanidium caldarium*, *Acidithiobacillus ferrooxidans*, *helicobacter sp.*, *thiobacillus sp.*, while *Natronomonas pharaonis*, *Clostridium paradoxum* *Thiohalospira alkaliphila* and *Halorhodospira halochloris*, *Bacillus sp.*, *Haloburum sp.* are examples of alkaliphiles.

3.3 Pressure

Due to the conducive atmospheric pressure of 1atm on land, organisms with gas-filled compartments such as lungs and swim bladders find it easy to survive and thrive at this pressure, but at environments such as 1,000 meters above the sea level, where the pressure is as high as 110atm, organisms with gas-filled spaces find it impossible to survive because

their gas-filled chamber becomes compressed as a result of the high pressure [46]. Nonetheless organisms still thrive in environments of high pressure; such organisms are referred to as **barophiles** or **barotolerant** [47, 48]. Microorganisms that only survive and thrive at elevated hydrostatic pressure are barophilic (also called piezophilic), barotolerant are microorganisms that grow optimal at 1atm but can also survive at elevated hydrostatic pressure [6].

Barophilic microorganisms possess the ability to withstand pressure of about $1,400 \times 10^5$ Pa with most of them associated with the deep sea habitat [46]. Studies pertaining to barophiles began when Yayanos et al. [49] harvested some isolates at the depth of 10.5km with the isolates growing at pressures greater than 100Mpa and 40Mpa at 2°C and 100°C respectively. With most barophilic microorganisms being psychrophilic, some have shown to be also thermophilic, such as *Methanococcus jannaschii* which thrives under 500atm at 130°C, other include *Thermococcus profundus* and *Pyrococcus horikoshii* [50, 51].

The adaptive mechanisms employed by these barophilic microorganisms in response to high pressure include the possession of polyunsaturated fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) that help in the maintenance and proper fluidity of lipid membranes, possession of electrostatic and hydrogen bond in place hydrophobic interactions, and expression of pressure-regulated genes, such as *ompH* and *ompL* such as found in *Photobacterium sp. SS9* [5, 50, 52].

3.4 Salinity

Saline environments such as saltern evaporation ponds worldwide, Great Salt Lake, the Dead Sea, saline lakes in Inner Mongolia, African soda lakes, deep-sea brines, and many others are usually characterized with low diversity and high community densities. Microorganisms that survive and thrive in these saline environments are referred to as **halophiles** and includes Archaea (*Haloquadratum*), bacteria (*Salinibacter*) and eukarya (*Dunaliella salina*). They categorized into slight, moderate, and extreme depending on the level of their halotolerance. Slight halophiles are found in 0.3-0.8M, moderate halophiles are found in 0.8-3.4M and extreme halophiles are found in 3.4-5.1M NaCl [53, 54]. Moderate halophilic bacteria constitute a heterogeneous physiological group of microorganisms which belong to different genera. These moderate halophiles include *Vibrio (Salinivibrio)* *Costicola*, *Micrococcus (Nesterenkonia)* *Halobius*, *Paracoccus (Halomonas)* *Halodenitrificans*, *Flavobacterium (Halomonas)* *Halmephilum*, *Planococcus (Marinococcus)* *Halophilus*, and *Spirochaeta halophila* [53, 54].

Halophiles survive in saline and hypersaline environment by their ability to accumulate inorganic ions (such as K^+ and Cl^-) until their cellular ionic concentration is similar to that of the external environment [54]. Also, majority of these prokaryotes cope with increasing osmolarity by the uptake or synthesis of compatible solutes, which are small, highly soluble, organic molecules (such as sugars and amino acids),

which do not interfere with the central metabolism, even if they accumulate at high concentrations [55]. Other mechanism involves the intake of potassium ion (K^+) selectively into the cytoplasm and pumping out of sodium ion (Na^+) using the sodium potassium pump [54].

3.5 Water

Water is one of the fundamental requirements for life as it aids all metabolic activities in living organisms. However, some organisms possess the ability to survive in desiccated environments with water activity < 0.80 [56]. Water activity (a_w) is a measure of the amount of water in a medium available to an organism for growth support and development [57]. It is the ratio of water vapor pressure of the substrate to that of pure water under the same conditions and expressed as fraction [56].

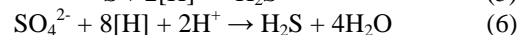
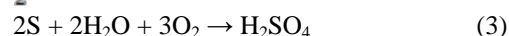
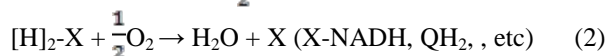
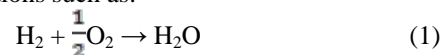
These organisms are classified as **xerophiles**. Desiccated environments range from large desert and dry valleys in the Antarctica to dried water bodies, such as ponds and little streams. Some xerophiles such as insects, tardigrades, crustacean and nematodes worms have been reported to survive 17, 20, 16 and 39 years respectively in desiccated environments [2]. Xerophiles survive desiccation by entering a state of metabolism called anhydrobiosis, characterized by little intracellular water and no metabolic activity, thereby inhibiting water loss until they are rehydrated [57]. They do this by retaining intracellular moisture against a concentration gradient by accumulating or synthesizing compatible solutes internally to a high concentration, in so doing maintaining cell turgor [58]. These compatible solutes are small organic molecules such as polyols, sugars and amino acids that protect enzymes from unfolding during dehydrated conditions, allowing normal cellular activities to continue [59]. However, survival depends on the water activity of the environment during this period and the rate of moisture loss by organism to the environment [58]. Xerophiles on rehydration undergo tissue repair in order to achieve optimal metabolism; enabling them grow and reproduce effectively on rehydration [57].

The lowest a_w value recorded for growth was reported for *Xeromyces bisporus* (a_w value of 0.62) having internal osmotic pressure of -70 MPa [60]. Reports have also shown that viable bacteria have been isolated from sediments dating from 10,000 to 13,000 years [60]. Examples of xerophiles include *Nostoc sp.*, *Bread mold*, *Aspergillus favus*, *Aspergillus ochraceus*, *Eurotium chevalieri*, *Chrysosporium fastidium*, *Wallemia sebi* and *Xeromyces bisporus* [61].

3.6 Oxygen

Despite numerous debates on the atmosphere of early earth, it is widely accepted that carbon dioxide and nitrogen gas are the dominant constituents. However, with the rise of oxygenic photosynthesis; approximately 2.7 Ga, oxygen production has drastically increased [62]. This rise in atmospheric oxygen has led to the evolution of aerobic respiration, which utilizes oxygen for the production of large amounts of energy in the form of ATP [2].

With variations in the availability of atmospheric oxygen around the earth, microorganisms have thus far evolved with different adaptations for oxygen utilization, and such as **aerobic**, **facultative**, **obligate** and **microaerophilic** organisms have evolved [63]. These will therefore involve different redox reactions such as:



Microorganisms that require reactions (1) – (2) utilize molecular oxygen as a terminal electron acceptor, where (2) symbolizes aerobic growth under heterotrophic condition; whereas microorganisms that require reactions (3) – (7) are said to be involved in chemolithotrophic growth. Reactions (5) – (7) shows how microorganisms such as *Halobacteria* and *Sulfolobales* derive ATP and extrude protons [62].

With facultative microorganism possessing the ability to thrive in the presence or absence of oxygen, obligate organisms only survive in the absence of oxygen, utilizing anaerobic respiration that involves the use of inorganic molecules, such as sulfate, nitrate or elemental sulphur as terminal electron acceptors in place of oxygen such as depicted in reaction (5) – (7) [58, 64].

Microaerophiles are microorganisms that survive and thrive in environment with extreme narrow availability of oxygen ($< 10\%$) and can't survive in environment with normal atmospheric oxygen concentration (21%), example include *Wolinella recta*, *Wolinella curva*, *Bacteoides ureolyticus*, *Bacteoides gvacilis*, *Treponema pallidum* and *Desulfoarculus baarsii* [63]. They utilize superoxide reductase as antioxidant enzyme [64]. The fact that microaerophiles do not ferment and therefore metabolize energy aerobically distinguishes them from obligate anaerobes [65].

3.7 Radiation

Radiation involves the emission of radioactive particles (such as neutrons, protons, electrons, alpha particles or heavy ions) or electromagnetic waves (gamma rays, X-rays, UV radiation, infrared, microwaves or radiowaves) [2, 7]. Extreme exposure to radiation could result to a variety of mutagenic and cytotoxic DNA lesions that leads to different forms of cancer. An example is Ultraviolet-B (290-320nm) and Ultraviolet-C (200-290nm) which causes pyrimidine dimerization in DNA, and terrestrial ionizing radiation, which is mostly from nuclear decay that leads to the generation of reactive radicals [58]. These reactive radicals among other things attack proteins containing iron-sulfur or heme groups, cysteine residues and cation-binding sites leading to various complications [66]. However, terrestrial lives are shielded from such exposure as a result of the ozone

layer and the planetary magnetic field, making them experience only 2mGy of ionization radiation each year [58].

Microorganisms residing in environments such as deep sea hydrothermal vents where radiation exposure can be up to 198 mGy terrestrial ionizing radiation per year, has led to the emergence of radioresistant microorganisms called **radiophiles** [7]. These microorganisms thrive in such extreme environments due to their defensive mechanisms provided by primary and secondary metabolic products, usually characterized by extremolytes and extremoyes [67]. These products play a role in absorbing wide spectrum of radiation thereby preserving the organism's DNA. Examples of these metabolites include biopterin, phlorotannin, porphyra-334, palythine, shinorine, mycosporine-like amino acids and scytonemin [68]. Other mechanism employed by these microorganisms includes the use of polyploidy strategy (presence of identical duplicates of chromosomes), this prevents total chromosome damaged in such that there is an extra chromosome available should one be destroyed as a result of radiation, resulting in the survival of the microorganisms [7, 68]. In addition to the polyploidy strategy, microorganisms also have a conventional DNA repair mechanism; an example is *Deinococcus radiodurans* that utilizes RecA-independent double strand break repair mechanism identified as synthesis-dependent single strand annealing [58, 69].

Furthermore, studies have also reveal that manganese (Mn^{2+}) and iron (Fe^{2+}) intracellular level play a role in making radioresistant microorganisms have resistant to radiation [66]. It was found that in *Deinococcus radiodurans* ionizing radiation leads to oxidative modification of proteins, consequently destroying them [7]. However it was shown that Mn^{2+} protects these proteins by forming a complex with phosphate, reducing the reactive superoxides to peroxides, while iron Fe^{2+} helps in the repair and reannealing of the damaged DNA fragments [67].

Further study on *Deinococcus radiodurans* shows that after exposure to extreme radiation, the shattered DNA each function as a template, the damaged ends are removed and fragments are join with corresponding nucleotide sequence by the help of the enzyme PolA. The newly formed single stand then utilize the Waston and Crick theory of base pairing, and thereby nucleotide thymine is annealed with 5-bromodeoxyuridine specifically in order to have a functional set of chromosomes [69].

3.8 Presence of heavy metal

The ratio of the specific gravity of the metal versus the specific gravity of water at 1 to 4°C is the major parameter used to distinguish a metal as heavy [70]. Heavy metals, based on biological functions are either classified as essential or non-essential [71]. Essential heavy metals play important role in the physiological function of microorganism whereas non-essential heavy metals play no role in biological systems, thereby making them toxic even at low concentration [23, 40]. Essential heavy metals at high concentration may also be toxic to biological systems [24]. Non-essential heavy metals are majorly classified as soft metals as they possess high

affinity to react with sulfhydryl (thiol) groups, thereby interrupting the activity of functional proteins [70].

Microorganisms able to tolerate heavy metals more than other microorganisms in extreme conditions have led to the emergence of heavy metals tolerant species and are referred to as **metallophiles** [40]. These tolerant microorganisms handle heavy metals by either carrying out homeostasis on essential heavy metals like every other microorganism or detoxify non-essential heavy metals, as well as excess essential heavy metals [71]. They detoxify by using an efflux pump mechanism which pumps out these heavy metals via essential metal transporters and/or by biotransformation or precipitation of heavy metals using their cellular proteins [6].

Studies on the ascomycetous group (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Candida albicans*) have given an idea on how extremophiles adapt to lethal exposure to heavy metals [70]. The most important means of adaptation of these extremophiles involves the chelation of these lethal heavy metals with thiolated peptides such as metallothioneins, glutathione and phytochelatin, thereby preventing the heavy metals from inducing damaging effects such as binding with thiol group of Cys residue, impairing normal function [71]. The formed metal-thiolated peptide complexes is then extruded to the external environment or accumulated in the vacuole [6]. Other means of detoxification involves the action of Glutathione (GSH), a tripeptide (L- γ -Glu-Cys-Gly) [70]. It is the main antioxidant agent in yeast as well as some other microorganisms and prevents oxidative stress, induced by heavy metals. They do this by reacting with the heavy metal through their Cys residue in a low affinity manner forming a GS-metal complex [72]. However, a mechanism that does not involve utilization of thiol group was found in *Saccharomyces cerevisiae*, and involves the use of *Pca1*; a plasma membrane transporter that expels heavy metals [73].

3.9 Polyextremophiles

Some organisms have adapted to survive simultaneously under multiple environmental extreme conditions. These organisms are called **polyextremophiles**. Examples are organisms that have live and adapted to inside of deep hot rocks (which are under intense pressure and heat), desert regions (adapted to the strong UV irradiation, high desiccation, low water and nutrients availability), hypersaline and alkaline salt/soda lakes, acidic hot springs, high altitude with low oxygen availability. *Picrophilus oshimae* is an example of an *acidophilic, halophilic thermophile*; lives in acidic hot springs at temperature above 65°C and pH 0.06 – 4, *Natronobacterium gregoryi* is an alkaliphilic halophile that lives in soda lakes at pH range 8 – 12, *Acidithiobacillus ferroxidans* is a sulphur oxidizing acidophilic metallophile used in recovery of metals and de-sulphurification of coal [74, 75].

The extreme nature of an environment is also largely influenced by fluctuations that may be transient or protracted as such caused either by man made or natural. Thus the concept of extreme environment and its inhabitants thereof reveals that indeed, **“one mans meat is another mans**

poison”, as oxygen require by most organisms to flourish is actually a poison to obligate anaerobes. A short overview of some of some few types of extremophiles and the environment conditions which they thrive in is as presented in Table 2.

4. Exploring Extremophiles Biomolecules for Biotechnological Applications

Over the last two decades, there has been a spontaneous emergence of biotechnology that has out phased other forms of production processes due to the fact that biotechnology tends to be more eco-friendly, cost effective and reliable [1].

With the ever increasing biotechnological applications, concerns have been on providing suitable biocatalyst that suits these processes [23]. Thus scientists have been exploring extreme environments in order to find suitable extremophiles which may be used in biocatalysis or act as source of biocatalysts [76]. Unlike most enzymes produced from mesophilic organisms that are unstable at extreme conditions of temperature, ionic strength and pH, etc., extremophiles are good sources of biomolecules with extreme stability, which can be applied in numerous industrial environmental, medical and/or pharmaceutical bioprocesses.

Table 2: Overview of some extremophiles

Environment	Unique environmental condition	Diversity of extremicity	Examples of thriving microorganisms	References
Soil, growth media contaminant	121°C, 15 psi	High-temperature survival	<i>Moorella thermoacetica</i> (spore)	Poli et al., 2009
hydrothermal vent, terrestrial hot spring	T _{max} 113°C	High-temperature growth (thermophile/hyperthermophile)	<i>P. fumarii</i>	Stetter, 2006
Snow, frozen water bodies	-17°C	Cold temperature (psychrophile)	<i>Vibrio</i> , <i>Moritella profunda</i> , <i>Ps. fluorescens</i> , <i>pseudomonas</i> , <i>methanogenium</i>	Chintalapati et al., 2004
Dry solfataric soil	pH _{opt} 0.7 (1.2M H ₂ SO ₄)	High acid (acidophile)	<i>Cyanidium caldarium</i>	Le Romancer et al., 2006
Saline lakes, evaporation ponds, dead sea	Saturated salt (≥ 5.2M)	High salt (halophile)	<i>Halobacterium sp</i> and <i>Halorubrum sp</i>	Ma et al., 2010
Soda lakes	pH _{opt} >10	High alkaline (alkaliphiles)	<i>Bacillus sp.</i> , <i>clostridium paradoxum</i> , <i>haloburum sp</i>	Ulukanli and Diurak, 2002
Deep sea	Deep open ocean or submarine vent (e.g pressure in mariana trench is 1.1 tons cm ⁻²)	High pressure (barophile or piezophile)	<i>photobacterium sp.</i> , <i>pyrococcus sp.</i>	Raghukumar, 2006
Toxic waste sites, industrial sites; organic solutions and heavy metals	Substance-specific (e.g benzene-saturated water)	Toxicity (toxitolerant)	<i>Rhodococcus sp.</i>	Cavicchioli, 2002
Rock surfaces (poikilohydrous), hypersaline, organic fluids (e.g oils)	Water activity (a _w)<0.96 (e.g X. bisporus 0.6 and Halobacterium 0.75)	Low water activity (xerophile)	Particularly fungi (e.g xeromyces bisporus) and Archaea (e.g Halobacterium sp.)	Leong and Schnürer, 2013
Pelagic and deep ocean, alpine and Antarctic lakes, various soils	Growth with low concentrations of nutrients (e.g < 1 mg L ⁻¹ dissolved organic carbon) and inhibited by high concentrations	Low nutrients (oligotroph)	<i>S. alaskensis</i> , <i>Caulobacter sp.</i>	Cavicchioli, 2002
nuclear reactor water core, submarine vent	High γ, UV, x-ray radiation (e.g > 5000Gy γ radiation and > 400 J m ² UV)	Radiation (radiation-tolerant)	<i>D. radiodurans</i> , <i>Rubrobacter sp.</i> , <i>Kineococcus sp.</i> , <i>Pyrococcus furiosus</i>	Singh and Gabani, 2011
Upper subsurface to deep subterranean	Resident in rock	Rock-dwelling (endolith)	Methanobacterium subterranean, <i>Pseudomonas sp.</i>	Cavicchioli, 2002

The discovery of thermostable enzymes which allow thermophiles to survive under high temperatures and which were thought may be exploited for industrial processes ignited the interest in studying biotechnological potentials of other extreme environments [14, 74]. This interest was further promoted by the increasing demand for new antibiotics to fight life threatening infections and the need for biocatalysts to drive industrial processes in a more cost-effective and eco-friendly manner [14].

In recent times, several microbial extremophiles have been isolated and screened for extremozymes (enzymes obtained from extremophiles), compatible solutes and metabolites [14]. However, some of the challenges faced by scientist in

introducing extremophiles into biotechnology include the availability of these organisms, difficulty in setting up culture medium for these microbial extremophiles and the low expression level of extremozymes from these extremophiles [23, 77-79]. These challenges have also ignited a spontaneous search in tackling them, such as the emergence of advanced bioreactors that maintains suitable environments for these extremophiles in so doing increases their biomass production [80]; the cloning and expression of extremozymes in mesophilic cell factories leading to the development of modified biocatalysts [23]. As a result, scientists work by identifying utilizable extremophile and concurrently finding out how they can be implemented [81].

However, only few scientific reports gave an idea on how extremophiles have been implemented in biotechnology, this is because most reports only emphasize on the properties of these extremophiles with little to nothing on how it can be successfully employed in biotechnology in a cost effective manner [79, 82]. Hence the inherent potential and possible ways by which extremophiles have been implemented in some biotechnological applications are further discussed in this section.

4.1 Industrial Biotechnological Applications

Biotechnology emphasizes on the utilization of natural products and many extremophiles have been used in numerous bioprocesses. One of these extremophiles is the alkaliphile *Bacillus sp.* in which enzymes such as protease, cellulase (CMCase), α -amylase, lipases and debranching enzymes have been isolated and manufactured in large scale and incorporated into house-hold and industrial detergents [83]. These enzymes are active and effective regardless of the presence of chemical ingredients such as surfactants, phosphates and chelating agents, present in the detergent. With the relevance of detergent in day to day activities of individuals, there has been keen interest in improving their action; such as making it more human and eco-friendly. In addition to increasing convenience, psychrophilic alkalotolerant extremoenzymes such as amylases from *Polaromonas vacuolata* has also been used, thereby making laundry possible and efficient at low temperatures, therefore saving energy and the wear and tear of textile [14, 84].

There have been great concerns over the loss incurred as a result of food spoilage and loss of fragrance during processing. The introduction of extremozymes from psychrophiles has made it possible to preserve heat-sensitive substrates and effectively control the food processes, consequently reducing loss in quality and extending the food longevity. Some of these extremozymes include metalloprotease from *Sphingomonas paucimobilis*, serine peptidase from PA-43 subarctic bacterium, lipase from *Aspergillus nidulans* WG 312, β -galactosidase from *Carnobacterium piscicola* BA and chitinase A from *Arthrobacter sp.* TAD20 [4, 85-89].

Many livestock farmers have resulted in purchasing highly processed grain feeds for their flocks so they could produce at higher yields. However these feeds do not come cheap, farmers do have to pay far more than the normal price. The introduction of acidophilic extremozymes such as cellulases, amylases from *Fructobacillus sp.* which adapt to low pH have been utilized in aiding digestion in these animals and consequently increasing their yield [1, 90].

Leather processing industries have resulted to the use of alkaliphile in its processes in order to curb issues pertaining to cost and waste management [76]. Proteases from alkaliphiles like *Bacillus sp.* and *Vibrio sp.* are used at pH 8 to 10 for hide-dehairing, preventing the use of chemicals like lime and sulfide, thereby preventing massive odor and the quantity of sulfide and soluble keratin present in the wastewater [91, 92]. This is classified as economical, since the protease such as protease R-11 functions by degrading

the proteins at the base of the hair and hairs harvested may be used in making other products like wigs, jacket furs etc. [92].

The photographic industry has also adapted the use of proteases from alkaliphiles in recovering silver and polyester from used x-ray films [45, 93]. The conventional means of recovery usually involved the burning of films which lead to the loss of the polyester base film as well as the release of unwanted environmental pollutants [45]. Alkaliphilic *Bacillus sp.* B21-2 a thermostable alkaline protease has been used in hydrolyzing the gelatin base at 50°C and pH 10, in so doing recovering 1.5% - 2.0% silver as well as the polyester film, since the degradable gelatin is bound to both the silver and the polyester [93].

Hydrogen gas is classified as a clean energy material as it does not produce carbon dioxide on combustion; as a result it has been widely adopted as source of fuel [94]. With its sustainable mode of production from biomass, extremophiles have been implemented in making the process efficient and cost effective [95]. Bio-hydrogen gas have been produced *in vivo* by use of glucose as substrate, and glucose dehydrogenase (GDH) and hydrogenase enzymes from extremophiles *Thermoplasma acidophilum* and *Pyrococcus furiosus* respectively at 82°C [96]. GDH oxidizes glucose to gluconic acid via lactone with NADP⁺ as the electron acceptor, producing NADPH [97]. NADPH concurrently reduces hydrogenase which then produces hydrogen gas molecule [96]. The stoichiometric yield shows that 1mol of sugar produces 1mol of hydrogen gas [98], however when hydrogenase was included with the enzymes of the oxidative cyclic pentose phosphate pathway, 2mols of hydrogen gas were produced [96].

With the demand for biodegradable biobased plastics, extremophiles have been adopted for the production of Polyhydroxyalkanoates (PHA) which are good alternatives for oil-derived thermoplastics [99,100]. PHA is intercellular bacterial reserve for carbon and energy, as a result of accumulated hydroxyl fatty acids complexes stored as lipid inclusions [1, 101]. PHA are majorly found in halophiles such as those belonging to the genera *Haloferax*, *Haloarcula*, *Natrialba*, *Haloterrigena*, *Halococcus*, *Haloquadratum*, *Halorubrum*, *Natronobacterium*, *Natronococcus*, and *Halobacterium* [102]. PHA are degraded by several microorganisms such as *Alcaligenes faecalis*, *Aspergillus sp.*, *Acidovorax delafield* and *Pseudomonas sp.* by incorporation of Poly (3-hydroxybutyrate) as substrate into the culture [100, 102]. Other halophiles such as *Halobacterium salinarum* and *Halobacterium distributum* were found to produce exopolysaccharides [102]. Exopolysaccharides are emulsifiers with high melting temperatures, pseudoplasticity and resistant to salt, colour and thermal break down, and they have also been exploited for various biotechnological applications [100].

The use of large quantities of chlorine and other chlorine derivatives in bleaching treatment of pulp as a result of presence of xylan, the state of environmental pollution has been a great concern [104]. In a bid to alleviate this, xylanase from extremophile have been used to degrade the heterogeneous molecule xylan, which constitutes the main

polymeric compound of hemicellulose [104]. Xylanases from *Dictyoglomus thermophilum* and *Thermotoga thermarum* have been employed in this fashion. These are alkali thermostable, low molecular weight xylanases that do not have cellulolytic activity [105, 106]. These properties allowed them to penetrate in the pulp fibres and not to hydrolyze the cellulose fibres [107].

The applications of extremophiles and their enzymes in bioethanol production is rapidly developing. Thermophiles that can withstand the harsh fermentation and pretreatment processes (in case of second generation ethanol) such as *Zymomonas mobilis*, *Bacillus subtilis*, *Geobacillus thermoglucosidasius*, *Clostridium thermocellum*, *Thermoanaerobacter ethanolicus*, *Thermoanaerobacter mathranii* are currently been employed [103, 108-114]. The use of these organisms in the fermentation processes eliminates the cool step before distillation, as fermentation and distillation processes are run concurrently. Other advantages includes their ability to utilize both pentose and hexose sugars, *Clostridium thermocellum*, which has the ability to ferment cellulose directly to ethanol [109, 110, 115], and *Zymomonas mobilis* that has the ability to tolerate about 120g/l of the ethanol product [108].

4.2 Environmental Biotechnological Applications

The use of biotechnological processes in the sustenance of key resources such as water bodies, soil and energy have been a great benefit to human and his environment. The traditional application of environmental biotechnology among others include bioremediation, biodegradation, biosensor.

• Bioremediation

This involves the use of extremophiles or extremoenzymes to correct or remove environmental issues such as ground water or soil contamination, restoring them to their natural status before the advent of pollution. The microorganisms employed usually target the contaminant, degrading them into a less or non toxic form. For example, polluted water from textile industries that contains azo dye, phenol, and toxic anions have been reported to be remediated by the halophiles *Salinicoccus iranensis* and *Halomonas* [116, 117]. Also, *Halobacterium* sp. NRC-1 and *Nesterenkonia* sp. MF2 were reported to remediate arsenic and chromate from wastewater respectively [118, 119]. *Alcanivorax borkmensis*, *Alcanivorax borkmensis* and *Haloferax mediterranei* remediate n-alkanes, producing biosurfactants glucose and lipid as the by products [116-119]. *Deinococcus radiodurans* has been engineered in the remediation of radioactive waste [120]. Psychrophilic microorganisms have been harnessed for their bioremediation of water pollutants in cold regions [6]. Alkaline protease from alkalophilic *B. subtilis* has been used in the bioremediation of effluents from households and food industries [91].

The use of biosurfactants in the remediation of oil-contaminated soil and water has been of great help to the environment. Biosurfactant producing halophiles such as marine *rhodococci* produces trehalose lipids in the presence of n-alkanes [121].

Biosurfactants are amphiphilic complexes mostly produced on microbial cell surfaces, or extracellularly excreted from the cell. Their hydrophobic and hydrophilic moieties help in reducing surface and interfacial tensions between two immiscible liquids. They therefore aid in increasing the solubility and mobility of hydrophobic hydrocarbons, hence they help in promoting the remediation of oil-contaminated soil and water [122, 123].

• Biodegradation

This involves the breaking down of organic matter or recycling wastes into nutrients that other organisms can make use of. It is carried out by mostly by bacteria, fungi and any other organisms that have ability to consume dead materials and re-process them into utilizable products. Halophiles have been shown to degrade hydrocarbon from oil wastes into smaller absorbable products, example include *Acinetobacter*, *Pseudomonas*, *Arthrobacter* that degrade hydrocarbon into alkane and aromatic compounds; *Marinobacter*, *Haloferax*, *Halorubrum*, *Leucosporidium watsonii* and *Rhodotonia* species that degrade benzene, benzoic acid, toluene, phenol, ethylbenzene and xylenes [124, 125]. Halophilic *Pseudomonas aeruginosa*, *Bacillus flexus*, *Exiguobacterium homiense*, and *Staphylococcus aureus* have been employed in biodegradation in tanning industries [126].

Due to its eco-friendly nature, biohydrometallurgy using autotrophic extremophiles has been greatly adopted as a replacement to pyrometallurgical ore extraction process [127, 128]. Example of such organism is *Thiobacillus ferrooxidans* in direct mineral dissolution from ores [127, 128]. *Thiobacillus ferrooxidans* has the capacity to extract copper and gold directly from their ores due to its ability to utilize sulphur/iron as energy source [127, 128].

• Biosensor

Bacteriorhodopsin - an integral membrane protein has been employed in biotechnology. It is a retinal based pigment found in the halophilic archaeon *Halobacterium salinarum*. It is part of a unique photosynthetic apparatus and functions as a light-driven proton pump [129]. The protein is fuelled by solar energy (500-650 nm) and helps in the transfer of information and materials across cell membranes. It is an ideal model for energy conversion and its biotechnological applications include optical information recording, spatial light modulation and holography [129, 130].

4.3 Genetics and Medical Applications

Numerous approaches in genetics and medicine are currently been improve by exploring what extremophiles have to offer. Example of this is the use of Halophile; *Halomonadaceae* as a more reliable cell factory alternative to *E. coli* and *Bacillus* [131]. This is as a result of the inherent abilities of *Halomonadaceae* that allow it to withstand non-sterile environments and grow fast with little nutritional requirement, utilizing any carbon source for energy [132].

At the inception of polymerase chain reaction (PCR), DNA polymerase I from *E. coli* was the main enzyme employed in the amplification [133]. Due to its liability to heat (as PCR processes involved heating to high temperature so as to

denature and unwind the DNA strands) the search for a solution led to the discovery of Taq polymerase, which was first isolated long ago from the extremophile *Thermus aquaticus* [134]. Taq polymerase only possesses 5'-3'-exonuclease activity and lacks 3'-5'-exonuclease activity; making it impossible to excise mismatches leading to poor base-insertion fidelity and resulting into high amplification errors in PCR products [133]. In view to this, several thermostable proof reading polymerase such as DNA polymerase from *T. maritima*, Pwo polymerase from *P. woesei*, Pfu polymerase from *P. furiosus*, Deep Vent polymerase from *Pyrococcus* strain GB-D and Vent polymerase from *T. litoralis* have been described [135-139]. However, the low extension rates of these polymerases among other factors make Taq polymerase still to be the prominently used polymerase in many PCR systems [105].

Low molecular weight organic solutes (such as glucosylglycerol, proline, diaminoacids, betaines and ectoines) have found to be accumulated by halophiles, in a bid to attain osmotic balance, stabilizing and protecting their enzymes, nucleic acids, and organelles against diverse stress factors [1, 132, 140]. As a result of this, some of these organic solutes like betaines and ectoines are been presented as potent stabilizers of enzymes, membranes, nucleic acids, antibodies, even whole cells against various lethal stress factors [141, 142]. Thus they are been seen to assist in producing high yield during expression of functional recombinant proteins, in optimization of PCR system, act as salt antagonists, stress protective agents, incorporated into moisturizers and for therapeutic purposes [132]. As example, ectoine has been used in cosmetics industries in manufacturing of creams that prevent the skin from dryness [143]. In addition, the discoveries of the genes responsible for the syntheses of these organic solutes are also been utilized in the production of transgenic plants such as in rice, tobacco and *Arabidopsis thaliana*, where drought and salinity tolerant varieties have been produced [144].

Several improvements in fluorescence protein-sensing technology such as new fluorophores and new sensing-specific proteins analytes have led to the use of biosensors engineered from thermophilic binding-proteins [145]. An example is Ph-SBP which is glucose binding protein obtained from *Pyrococcus horikoshii* [146]. The ease of use and sensitivity to fluorescence make it useful in medical testing and drug delivery [147]. Other biosensor such as I-leucine dehydrogenase has also been obtained from psychrophiles [148].

Alkaliphiles have been shown as potent manufacturers of antibiotics. Some of these alkaliphiles also showed tolerance for saline environments [149]. Examples include an alkaliphilic actinomycete *Nocardiosis* strain that produces phenazine [42]. Alkaliphilic *Streptomyces* strain produces Pyrocoll which is an antibiotic and also has antiparasitic and antitumour activities [150]. *Streptomyces microflavus* produces fattiviracin; an antibiotic and antitherapeutic compound [149]. *Streptomyces spp.* from marine produces chinikomycin and lajollamycin, which are antibiotics with antitumour activity [45,151].

Psychrophiles have been shown to produce polyunsaturated fatty acids (PFA) which are useful as dietary supplements, help in sustaining cellular and tissue metabolism, aids cholesterol and triglycerides transports, reduce plaques aggregation, assist in inflammatory processes and nervous system [152, 153]. The most important PFA are omega-3 fatty acids, eicosapentaenoic acid and docosasaenoic acid have been reported to be produced by psychrophilic organisms such as *S. frigidimarina*, *S. gelidimarina*, *S. olleyana*, *S. hanedai* and *S. benthica* strains [154, 155].

Halophilic *Dunaliella*, when dry serve as food supplement with antioxidant properties, and its antifreeze proteins act as cryoprotectants for frozen organs [6]. Thrombolytic agent from the alkaline protease of *Bacillus* sp. strain CK 11-4 has been reported to have fibrinolytic activity [156]. Elastoterase produced by alkaliphilic *B. subtilis* 316M have also been employed in treating purulent wounds, burns, carbuncles, furuncles and deep abscesses [157]. Oral administration of protease obtained from alkaliphilic *Aspergillus oryzae* has been used to correct some lytic enzyme deficiency [158].

5. Pathway to Extremophiles in Biotechnology

Ever since search for the potentials of extremophiles and their products, their incorporation into active industrial processes is yet to be fully actualized. Reports have been made on which biotechnological process extremophiles could prove useful; such as the use of hyperthermophilic *Pyrococcus furiosus* in bioethanol production, which could greatly prevent media contamination, promote self-distillation, reduce viscosity and costly management of mesophilic enzymes [159]. Also the potential use of *Salinicoccus iranensis* and *Halomonas* in the bioremediation of textile pollutants is yet to be fully exploited [116]. Outline below are other important prospective wherein extremophiles potentials could be harnessed, though further experimental work may be needed in validating these views.

5.1 Extremophiles in trehalose production for biotechnological applications

Trehalose, usually classified as a non-reducing disaccharide composed of two α -1, 1 linked glucose moieties is grouped as a carbohydrate energy source capable of protecting living organisms against physical stress such as desiccation, anoxia, cold and heat due to its high water-holding ability [160, 161]. Trehalose exist as either α , β -1,1-, β , β -1,1-, and α , α -1,1-, however only α , α -1,1- have found to be produced by bacteria, fungi, and plants; but yet to be identified in mammals [162]. The non-reducing properties came from its lack of acetals at two glucose moieties by 1,1-glycosidic ether, making them unavailable for reduction [163]. It possesses a bond energy less than -4.2kJ, and has ability to withstand extreme temperatures, pH, and organic solvents [121, 162]. These properties make trehalose thermodynamically and kinetically most stable non-reducing natural disaccharide. Thus it has been used to stabilize enzymes, vaccines and antibodies [164]. Organisms that produces trehalose tend to store them in quantities ranging from 10% - 20% of their dry weight. Macrocyts of

Dictyostelium mucoroides and ascospores of *Neurospora tetrasperma* shows 7% and 10% of trehalose at dry weight respectively [162].

Initially natural α , α -1,1- trehalose was produced from vegetable and fungal sources, involving extraction with ethanol [164]. However this process is difficult and costly, hence another means that involving the use of *Arthrobacter sp.* Q36 or *Arthrobacter ramosus* S34 has led to the production of malto-oligosyltrehalose synthase (MTSase) and malto-oligosyltrehalose trehalohydrolase (MTHase) that produces trehalose via enzymatic saccharification of starch. The thermal instability of these enzymes were enhanced by the presence of other enzymes such as soamylase, cyclomaltodextrin glucanotransferase (CGTase), glucoamylase and α -amylase, increasing the yield of trehalose [162].

The implementation of thermoacidophilic crenarchaeon such as *Sulfolobus shibatae* (optimum growth at pH 2 and 80°C) in the production of trehalose may be able to produce MTSase and MTHase that are tolerant to high temperature, since the enzymatic saccharification of starch is optimal at high temperature [165, 166]. Moreover, the complementary thermophilic enzymes that will be produced by this organism will further assist the enzymes leading to greater yield of trehalose compare to what is been obtained using *Arthrobacter sp.* Since the presence of trehalose is usually associated with occasions when organisms are under stress, it suggests that more biotechnological processes should employ the use of trehalose in production process as it may assist the biomolecules in the system to tolerate adverse conditions that may be occurring in the production system, in so doing improving yield and allowing the biomolecules to be active for longer period.

Studies have shown that mammals possess trehalase which degrades trehalose to glucose residues in the small intestine [167]. However, mammals lack the necessary proteins required to synthesize trehalose. Owe to its health benefits trehalose has been used as food supplement in human [162]. Thus it will be of great scientific finding if evolutionary studies are conducted to ascertain whether trehalose was once synthesized by mammals during the evolution cycle since the disaccharide helps to tolerate stress, and if another molecule similar to trehalose exists. Since the disaccharide trehalose stabilizes cell membranes and also prevents transition of materials across cellular membranes [167]. In-depth research could be carried out to see if trehalose may act as therapeutic agents that could prevent or protect lethal infections caused by viral, fungal, bacterial pathogens.

5.2 Extremophiles in polymerase chain reaction (PCR)

The study of DNA polymerases (EC 2.7.7.7) as key enzymes in the replication of cellular information existing in all life forms has led to the creation of PCR in the vein of carrying out studies and creating proteins of need [105]. The primary aim of polymerase chain reaction is to produce gene sequence in large quantities as needed [168]. PCR led to a great breakthrough in molecular biology with several readily

available reviews explaining its history, technique and process [169].

Some of the challenges associated with PCR are usually concerned with mismatching and synthesizing of non-specific templates prior to thermal cycling [105]. The synthesis of non-specific templates led to the development of a barrier between the DNA and the enzyme, such as neutralizing antibodies to inhibit Taq polymerase at mesophilic temperature coupled with the use of heat-mediated activation of the immobilized enzyme [170]. The accuracy of Taq polymerase has also been improved through the addition of thermostable, archaeal proof-reading DNA polymerases with 3'- 5' exonuclease activity. The extension ability has also been enhanced by adding gelatine, Triton X-100 or bovine serum albumin to stabilize the enzymes and mineral oil to avoid evaporation of water in the reaction mixture [105].

Despite the fact that Taq polymerase possesses the highest extension ability, other thermostable polymerases are also employed in specific replication activities [168]. As a result of the various characteristics shared among these thermostable polymerases, exploring extreme habitats may yet provide a solution by the discovery of extremophiles that will have DNA polymerase and which may possess all the necessary abilities that may be require of a polymerase for a successful PCR, without the need of additives.

5.3 Extremophiles in bioethanol production

Bioethanol is been current been generated from three main sources, which are from crop grains and tubers (term 1st generation); from agricultural, industrial and municipal wastes (term 2nd generation); and from sea weeds and blue green algae (term 3rd generation). Several physico-chemical and enzymatic processes are usually involved in generation of bioethanol, most especially in the 2nd and 3rd generation where very harsh physico-chemical treatments are employed in the liberation of sugars before enzymatic fermentation to ethanol [171]. Most of the microorganism employed in the breaking down and fermentation processes are often sensitive to the negative effects of these processes such as high temperature and low pH, presence of high concentrations of chemicals, organic solvents and by-products, which inhibit their growth and metabolic capacity and usually result in need for additional nutritional requirements, in so doing increases the cost of production and often low ethanol yield [171-173].

The use of long-term course adaptation and genetic engineering of existing mesophilic fermentative organisms have been proposed [111, 174], however using metagenomic approach in biomining diverse extreme environments for extremophiles with good fermentation efficiencies will go a long way in providing solutions to the fermentation challenges. These organisms through the use of their innate metabolic strategies often produce enzymes and other biomolecules that are potentially stable and active at harsh conditions often similar to the fermentation system. Thus the development of a sustainable and cost-effective bioethanol generation will involve screening, selection and utilization of highly vigorous fermentative extremophiles. Although some

extremophilic fermentative organisms such as *Zymomonas mobilis*, *Bacillus subtilis*, *Escherichia coli* strain LY168, *Pyrococcus furiosus*, *Geobacillus thermoglucosidarius*, *Clostridium thermocellum*, *Flavobacterium indologenes*, *Thermoanaerobacter ethanolicus*, *Thermoanaerobacter mathranii*, *Methylobacterium extorquens* [103, 108-110, 111-114, 159, 175], are said to have tolerance to some of the fermentation challenges, however their fermentation efficiencies still needed to be improved on.

6. Conclusion

Exploration of nature is the best and most resourceful way of sourcing new compounds that can be utilized for biotechnological purposes. Man for a while has been exploiting the metabolic wealth of microbial world for his needs. Initial screenings for bioactive molecules focused mostly on mesophilic terrestrial habitats [3]. However biomolecules isolated from organisms from these habitats are generally active at moderate conditions. Thus exploration was extended to the extreme environments, and this has opened new frontiers for the discovery of extremophiles with alternative options to the fast diminishing resources of normal environment with lots of biotechnological potentials such as robust biocatalytic properties, unique metabolic capabilities and novel biomolecules that can withstand extreme conditions [2].

Despite increasing study of microbial extremophiles, only limited information of their physiology, biochemistry and/or genetics exist. Even the existing knowledge of their enormous potentials is still not been fully employed into biotechnological applications. Thus there is a need for more rigorous engaging of these extreme microorganisms' virtues and also finding means of implementing them into newly or already established biotechnological processes.

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