



**CHARACTERIZATION OF STRESS RESPONSE IN SELECTED
EXTREMOPHILES**

Project Report

Submitted on the partial fulfillment of requirements for the degree of
M.Sc. in Biotechnology(Hon's)

Submitted by:

Sri Lakshmi Ajit (11609259)

Under the Guidance of

Dr. Gyanesh B Singh

Associate Professor

SCHOOL OF BIOENGINEERING AND BIOSCIENCE

LOVELY PROFESSIONAL UNIVERSITY

PHAGWARA, PUNJAB-144411

DECLARATION

I hereby declare that the project entitled “**Characterization of Stress Response in Selected Extremophiles**” is an authentic record of our own work carried out at School of Bioengineering and Biosciences, Lovely Professional University, Phagwara for the partial fulfillment of the award of Master of Science in Biotechnology (Hon’ s) under the guidance of **Dr. Gyanesh B Singh**.

This work is our original work and has not been submitted for any degree/diploma in this or any other University. The information furnished in this report is genuine to the best of my knowledge and belief.

Place:

Sri Lakshmi Ajit (11609259)

Date:

CERTIFICATE

This is to certify that Sri Lakshmi Ajit(11609259) have completed the project, entitled **“Characterization of Stress Response in Selected Extremophiles.”** under my guidance and supervision. To the best of my knowledge, the present work is the result of their original investigation and study. No part of the report has ever been submitted for any other degree at any University. The report is fit for submission and the partial fulfillment of the condition for the award of M.Sc. in Biotechnology.

Date:

Supervisor Signature:

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Introduction

Much similar to mammal the microscopic organisms also need to cope up with stress. In any case, stress for microorganisms is not quite the same as that of mammals. Stress in case of microbes is in form of dangerous and harmful radiations, or physically unfavorable conditions, such as heat, pH, salinity, can cause adverse effects on the microbes. It can also harms cellular macromolecules including proteins and nucleic acids. A restricted supply of nutrients can likewise be viewed as stress. Microscopic organisms have created alternative mechanisms and machineries, which intend to increase its resilience limits. These stress reactions are specific for a specific form of stress. Some stress reactions encourage bacterial change from a free living being to a host-attacking pathogen. Bacterial versatile reactions incorporate advancement of spores and ability of initiation of motility, combination of anti-microbial and proteases, and changes in vitality creation framework. Tweaking of respiratory electron exchange courses (ETC) and coupling of organelles functioning in order to increase the capacity of microscopic organisms to adapt to varying oxygen and supplement supply. How single celled living beings adjust to various sorts of stress, utilizing the bacteria such as *Deinococcus radiophiles*, *Halobacterium halobium*, *Bacillus alcalophilus*, *Planococcus antarcticus*, *Psychrobacter frigidicola*, etc as the central model creatures is been studied. These reactions are learned at various levels that incorporate growth rate variations, protein-condition, protein- protein interactions, protein-DNA associations and RNA expression changes.

In last few decades the discovery as well as evolution of Archaea to become new domain of life apart from bacteria and eukarya have paved way to many exciting developments in the portrayal of a large range of strange and previously unknown microorganisms and their ability to survive in a really wide range of environmental conditions that were previously recognized as unfavorable for survival and proliferation of life. These associated components make them different and allow them to combat with these conditions like, properties and composition of major components such as plasma membranes, enzymes structure, enzyme activity, and proteins composition, protein folding of these Archaea were found to be different and participate an important roles in maintenance of the archaea bacterial stability in seemingly inhospitable environments. The extremophiles for better cellular protein stability and activity under extreme conditions in which they survive and flourish, have made a number of adaptations. Each extremophiles have developed unique protein characters that are developed in accordance to each environmental conditions in place of having a single set of basic adaptations that can adapt for a wide range of environmental stress **Reed C Jet *al.*(2013).**

Microorganisms have an assortment of developmental adjustments and environmental adaptation components that enable these organisms to survive and stay dynamic even with natural stress. Physiological reactions to stress have resulted in changes at organism level, which has resulted in modified biological system, vitality, nutrient uptake etc. These huge scale impacts result from coordinated consequence for development of dynamic physiological changes and by controlling the composition of the arechaea. They have discussed about the general attributes of how organisms encounter natural stress and how they react to them. They have also discussed about the effects of various essential biological system level stress, like variations in temperature, pH, salinity, nutrient restriction, etc on physiology and structure of these organisms. When these

microbes react to stress, the physiological stress forced on organisms is strong enough to cause vast variation in the gene expression and diverse protein synthesis. For instance, for organisms to orchestrate the osmolytes they have to survive low moisture condition, where they can encounter osmolytic damage. While adjusting to freezing conditions these organisms convert the Arctic tundra soils from immobilizing Nitrogen around the developing season to mineralizing it around the winter. It is believed that all the more successfully incorporating microbial environment into biological system, nature will require a more complex coordination of microbial physiological biology, populace science, and process ecology. **Schimel J *et al.* (2007).**

Scope of the Study

The study of stress response on various extremophiles enables us to understand the resistance of these bacteria to various conditions. These enable us to further study and understand the mechanisms utilized by these bacteria to overcome stress. These can help us to understand and apply these mechanisms at various industrial levels such as.

The enzymes produced by thermophilic bacteria help in the catalysis of reactions where the high thermodynamic and kinetic conditions are favorable apart from that maintenance of such conditions help in the prevention of contamination during the process. Unsworth L D, Oost J V D, and Koutsopoulos S (2007).

In food industries, the high pressure help in prevention of contamination as well as preservation of colour and taste of the product so under such conditions the psychrophilic enzymes can be helpful. Abe F and Horikoshi K (2001).

Acidophilic enzymes are useful in biofuel production and in ethanol production as they help in the proper utilization of carbon source in acidic conditions Sharma A, Kawarabayasi Y, and Satyanarayana T (2012).

Psychrophilic enzymes are used in the field of biotechnology apart from commercial detergents Tutino M L, Prisco G D, Marino G, and Pascale D D (2009).

Objective of Study

- To study the stress response of selected extremophiles towards pH, salinity,
- To study the stress response of selected extremophiles towards H₂SO₄, H₂O₂, CuSO₄
- To study the stress response of selected extremophiles towards formaldehyde, ethanol.

Review of Literature

Few recent works on Extremophiles include:

P. Babu *et al.*, (2015) “Survival Mechanisms of Extremophiles”

In their work they have discussed about various strategies adapted by extremophiles to survive under extreme environmental conditions in order to stay alive and proliferate. These organisms survive under harsh environmental conditions like temperature extremes, high acidity, salt, high pressure etc by changing homeostasis, by the production of extremolytes, by an alteration in genes and proteins, by evolutionary diversity, by amino acid accumulation, by increased catalytic activity, by resistance to cell death, by aggregation resistance strategies, by the use of heat shock proteins, or by activation of the nuclear factor and cellular compartmentalization etc.

These mechanisms can be studied and utilized in therapeutic and medical applications, and it could also help to figuring out the mechanism by which their molecular elements (i.e., proteins and genes) could be genetically engineered and utilized towards the therapeutic applications. One such survival pathway was studied in the radiation-resistant microorganism *Deinococcus radiodurans* in the above paper.

Kumar *et al.* (2010) has discussed about the stabilization of enzymes under cold stress as well as the freeze drying, and protection from the oxidative protein damages in the immune toxin therapy.

Buommino *et al.* (2005), Singh and Gabani (2011), Ortenberg *et al.* (2000) has given an insight on protection of immune cells in skin from UV radiation and how the enzymes are stabilized against heat, freeze, and dry conditions. The paper has also discussed the protection of the skin barrier against dehydration and dryness, it also focuses on the block of UV A induced ceramide release in human keratinocytes by Acidophiles/ alkaliphiles.

Baker Austin and Dopson (2007), Horikoshi (1999), Bordenstein (2008) discussed that how to maintain the intracellular pH circumneutral by the continuous pumping of protons inside and

outside of the cytoplasm across the membrane. Apart from that, the cell membrane containing acidic polymers, passive regulation employed for the cytoplasmic pools of the polyamine groups and low membrane permeability are important acidophilic adaptations.

Berger *et al.* (1996), Feller and Gerdey (2003), D'Amico *et al.* (2006), Chakravorty and Patra (2013) studied the Psychrophiles and their translational changes in psychrophilic enzymes, the paper also discussed about partial protein structures exhibiting increased flexibility, and presence of cold shock proteins and nucleic acid binding proteins, and the exhibition of the reduced package of the acyl chains within in the cell membranes in psychrophiles

Arena *et al.* (2009), Kambourova *et al.* (2009), Barbara *et al.* (2013) gave an insight on mucoidal layer enveloping cell colonies and biofilm formation as stress response to extreme environmental conditions

Lauro and Bartlett (2007), Yano *et al.* (1998), Rothschild and Mancinelli (2001), Kato *et al.* (1995 1996a, b), Kato and Bartlett (1997), Marteinson *et al.* (1999) works discussed. The barophile's Homeoviscous adaptation, and altered lipid membrane Packaging, and increase in the levels of unsaturated fatty acids. The membrane fluidity is maintained by polyunsaturated fatty acids. The paper also discussed about the advanced DNA repair systems and the presence of highly conserved pressure regulated operons along with heat shock proteins

Jorda J and Yeates T O (2011) explained about the Ability of Archeal Extremophiles to thrive in habitat exceeding 100C. The problem with high temperature is that it disrupts the structure of proteins. Protein structure is maintained by forces and effects, which differ with different proteins and organisms, like increased atomic packing, ionic interactions, shorter loops, and hydrophobic interactions provide non covalent bonding whereas disulfide bonding a very strong covalent

bonding for stabilization in these proteins. Apart from these, the enzymes such as adenylosuccinate lyase, etc, in the thermophilic and hyperthermophilic organisms such as *Pyrobaculum aerophilum* form three disulfide bonds in six cysteines protein thus proving the importance of Disulphide bonding in stabilization of proteins in thermophiles and hyperthermophiles. These discoveries also throw light on the altered cytosolic proteins that synthesis disulfide bonds as part of cellular redox signaling mechanisms. In the above article the comparative genomics studies signifies the presences of disulfide oxidoreductase (PDO) in thermophiles with high intracellular disulfide bonding content in it. The importance of disulfide bonding in thermophiles was noticed in genomic sequences of twenty five dynamic prokaryotes, of which seven were archaea. Though archaeal species constitute only a small set the eukaryotic discoveries but upcoming discoveries ensure an update of assessment of thermophilic protein disulfide bonding as an important and diversified adaptation towards the survival in extreme conditions by these dynamic organisms.

Soppa J (2011) reveled the genome sequences and several comparative genomic in silico studies performed on *Haloferax volcanii* reveal the mechanism of protein export, RNA modifications, Small Archaeal Modifier Proteins, ubiquitin-like proteins, etc. Above article discusses about the functional genomics methods and results regarding the transcriptional, protein and metabolic studies. The studies on *Hfx. volcanii* is along with *Halobacterium salinarum* revealed translationally regulated genes that is highly efficient genetic system which enables the application of libraries and parallel generation of genomic deletion mutants in haloarchaea. Genetic approaches are recently used to study biological aspects from replication to post translational modification and selected results are discussed

Koga Y (2012), In the above article the physiological characteristics like chemical stability of lipids in the thermophilic organisms, there altered composition by increased proportion of few

dynamic lipids and change in the lipid bilayer membrane properties help the membranes to function at high temperatures that differ the archaeal and bacterial lipids. Apart from protein adaptations the chemical stability of lipid by itself is responsible for thermophiles survival at high temperatures. For lipid membranes to maintain their function effectively, the property of a high temperature permeability barrier and a liquid crystalline state is a must, which is achieved by the isoprenoid chains.

Cavicchioli R (2006), the archaea are extremophiles, and survive at dynamic temperatures ranges, at dynamic pressure ranges and at concentrated acidic environments. Highest diversity of archaea exists in cold environments and archaea represent a significant fraction of biomass in such areas. In spite of the fact that psychrophilic archaea have been somewhat ignored by researchers, the investigation of these microorganisms is starting to become more important. This paper throws a focus on the nature, adjustment science and special science that is being acknowledged on psychrophilic archaea.

Nath A I V *et al.* (2011) gives us an insight about the dynamicity in extremophiles habitat that ranges from the ice cold waters of arctic and Antarctic to the superheated hot springs in hydrothermal vents. Detailed study of their biochemical, evolutionary and ecological aspects along with their cellular machinery that is responsible for higher flexibility to survive in such harsh environments can help us learn about their tolerance level physiologically and variation in the natural protein conformation in the cell. Stress proteins (heat shock proteins/cold shock proteins/salt stress proteins/ pressure proteins) have crucial part in the adaptation of extremophiles. The present review focuses on the *in vitro* aspects, developed on the transcriptional and translational changes in case of stress proteins in extremophiles. The survey features the quality of

extremophile to combat all the stress conditions, such as salt/osmotic stress, by employing unique changes in transcripts and proteins under psychrophilic condition.

Archaea can be categorized into several groups based on the kind of environmental conditions they thrive in like:

Thermophiles are bacteria that are adapted to extreme heat conditions and have developed the thermophilic proteins with increased and well established hydrophobic core and high electrostatic interactions to maintain their activity at high temperatures **Reed C Jet al. (2013)**. The hyperthermophilic enzymes and phosphotriesterase is tightly packed since there are chances of favorable hydrophobic interactions at the dimer interface **Vecchio P Vet al. (2009)**. In Pf amylase

the lack of quaternary structure and N-terminal loop Increases stability by altering substrate specificity in extreme conditions **Vihinen M (1987)**. Though salt-bridges destabilize proteins in mesophiles it is prominent feature of thermophilic enzymes and enhance their stability **Karshikoff A and Ladenstein R (2001)** **Hendsch Z S and Tidor B (1994)**. High temperature decreases the solubility and entropy with ion pairing in salt bridges and thus become the structural stabilizer favoring charge interactions. This increases the thermal capacity of such enzymes **Chan C H, Yu T H, and Wong K B (2011)**. The thermostable proteins tend to increase the charged residues on their surfaces. **Fukuchi S and Nishikawa K (2001)**. The replacement of polar charged amino acid residues in place of polar uncharged surface amino acid residues help in the increasing of stability. As the temperature increases, polar uncharged amino acid like asparagine and glutamine deaminizes thus reducing the stability of the protein. Therefore, the archaeal protein replaces them with polar charged amino acids such as aspartate and glutamate for better stability. **Fukuchi S and Nishikawa K (2001)**. Near and far ranges of charge interactions increases by replacement of such thermo labile amino acids from the protein structure enabling the extremopliles to attain stability by prevention of thermal denaturation. **Lee C F, Makhatadze G I, and Wong K B (2005)**.

Halophiles survive in extreme salt conditions, and have halophilic proteins with high acidic amino acid content and peptide insertions therefore having high negative surface, charges with which it compensates for the extreme ionic conditions **Reed C *Jet al.*(2013)**.

Radiophiles having high intracellular Manganese/Ferrous concentration proportions that shield proteins from oxidative carbonylation i.e., The addition of carbon monoxide group on to native amino acids such as histidine cystein and lysine to convert itself to their carbonyl derivatives (aldehyde or ketones) under oxidative stress **Daly M J, Gaidamakova E K, Matrosova V Y *et al.* (2007)**. Along with it, these organisms utilize vacuolar type H⁺ ATP syntheses which are also

found in eukaryotes that help in the intracellular acidification, facilitating the Mn redox cycling by providing H⁺. **Makarova KS, Aravind L, Wolf YI, et al. (2001) Kane PM (2006).**

Psychrophiles in contradiction to the thermophiles have reduced their hydrophobic core in protein and developed low charge on protein surfaces to maintain flexibility of protein structures and their activity under cold temperatures **Reed C Jet al.(2013)**. A significant part of the Earth's surface, both marine and earthbound, is either intermittently or for all time cool. In spite of the fact that natural surroundings that are to a great extent or persistently solidified are for the most part thought to be unwelcoming to life, psychrophilic creatures have figured out how to survive in these situations. This is because of their intrinsic versatile ability to adapt to frosty and other related cold climates. They compared different natural, physiological and sub-atomic adjustments that psychrophilic microorganisms use to flourish under unfavorable conditions. They examined the effect of present day "omic" advancements in building up an enhanced comprehension of these adjustments, featuring late work in this developing field. **Schimel Jet al. (2007)**

In case of Alkalophiles, the cell wall is the most important component in maintenance of intracellular pH range 7- 8.5, against the alkaline environment around the cell wall. Although the adaptation mechanism is not clear it is noticed that protoplasts of alkaliphiles is unstable in alkaline environment. **Horikoshi K (1999)**. It has been suggested that the changes in chemical composition of the cell wall of alkalophiles helps that. The cell wall of alkalophilic strains of Bacillus differ from that of the mesophilic strains. Their cell wall, in addition to peptidoglycans, contains acidic polymers, like galacturonic acid, glutamic acid, gluconic acid, phosphoric acid, and aspartic acid. **Horikoshi K (1971).**

Bacillus alcalophilus (mutant strains) showed loss of Na⁺/H⁺ (sodium ion/ proton) antiport action and Na⁺ (sodium ion) coupling for solute transport, and lowered concentration of cytochromes and chromophore. Proof that these progressions could come about because of a solitary transformation is displayed. Obligate alkalophilic bacilli become just in the exceptional pH scope of 9.0 to 12 **Guffanti et al., (1978, 1980)**. Over this scope of pH esteems for development, the cytoplasmic pH is kept up at pH 9.5 or beneath **Guffanti et al., (1978, 1980)**. Upon stimulation, disengaged film vesicles can likewise deliver a pH slope, corrosive in, just if Sodium ions (Na⁺) is available (Mandel et al., 1980). Investigations of sodium ions (Na⁺)and protons (H⁺) developments in cells **Guffanti et al., (1980) Krulwich et al., (1979)**, the averted vesicles as well as the right-side-out vesicles **Mandel et al., (1980)** show the nearness of an electrogenic Sodium ions/protons(Na⁺/H⁺) antiporter which ferments the cytoplasmic as well as the intravesicular spaces with respect to the milieu present outside. The above perceptions prompted the possibility that the reduced uptake of alkalophiles may be expected to the antiporter action, i.e. the ability of cytoplasm to take up the proton may be reduced or seized beneath the pH 9.0. For sure, non-alkalophilic strains that could develop in the impartial pH extend had lost the capacity to develop above pH 9.0, and displayed none of the sodium ions or protons(Na⁺ or H⁺) developments is credited to the sodium ion/proton (Na⁺/H⁺)antiporter **Guffanti et al., (1980) Krulwich et al., (1979) Mandel et al., (1980)**. In *Bacillus alcalophilus*, loss of Sodium ion/proton (Na⁺/H⁺) antiporter action was joined by lost the Sodium ion (Na⁺) -reliance of solute transport frameworks. (Na⁺/solute) Sodium ion/solute symport is a typical transport instrument in *Bacillus alcalophilus* as well as in a wide range of alkalophiles **Guffanti et al., (1980) Kitada & Horikoshi, (1977, 1980)**. A few lines of proof show that, in *Bacillus alcalophilus*, both the loss of sodium ion (Na⁺) -coupling for to solute transportation as well as the loss of antiporter action have remarkable effect

of the survival mechanism of *Bacillus alcalophilus* as well as a wide range of extremophiles **Guffanti et al., (1981)**. Comparable discoveries have been accounted for in *Escherichia coli* **Zilberstein et al., (1980)**. They recommended that the change to non-alkalophilily may influence a (Na⁺) sodium ion-translocating the component that is regular to (Na⁺) sodium ion-coupled symport and antiport frameworks in *Bacillus alcalophilus* **Guffanti et al., (1981)**. With regards to this proposal and option speculations, it was considered the proof of further pleiotropic qualities of non-alkalophilic mutants. They demonstrate that each of the properties show up as an indissoluble total in numerous autonomous non-alkalophilic strains and are reestablished totally in revertants. **Lewis et al. (1982)**

Acidophiles on other hand have adaptations similar to thermophiles as most explored acidophiles are thermophiles but they have enzymes that can change their charge to avoid protonation of the amino acid residues in them thus protecting their enzymes from denaturation **Reed C Jet al.(2013)**, **Huang Y, Krauss G, Cottaz S et al.(2005)**

Diverse parts of stress reaction of *Lactobacillus acidophilus* were explored. To start with, heat, bile, and sodium chloride stresses at lethal and sub lethal levels were resolved. They were 0.05% and 0.5% (bile), 53 degrees C and 60 degrees C (heat), and 2% and 18% (NaCl), individually. To assess the impact of each stress at log stage, log-stage of the *Lactobacillus acidophilus* cultures were tested straightforwardly with the lethal level on each stress (as well as on control) and were contrasted with log-stage of the *Lactobacillus acidophilus* cultures that were pre-presented to the sub lethal level preceding the presentation at the lethal level (test). A few, if not most, of the cells were executed in the control *Lactobacillus acidophilus* cultures against each of the three stresses. In any case, in the test *Lactobacillus acidophilus* cultures, the quantity of cells that had survived expanded fundamentally. It creates the impression that *Lactobacillus acidophilus* is fit for showing

versatile reaction to stress. The versatile reaction to one stress was likewise appeared to give cross-insurance against various stresses tried. The impact of each stress on stationary-stage lactobacillus acidophilus culture was additionally examined. As opposed to log-stage culture, stationary-stage culture was naturally impervious to stress. **(Kim et al. 2001)**

Piezophiles that live in extreme hydrostatic pressure conditions with varying temperature conditions usually in deep Ocean Fang J, Zhang L, and Bazylinski D A (2010) have both thermophilic and psychrophilic adaptations are observed along with extremely compact as well as dense hydrophobic core, small multimeric amino acids connected by hydrogen bonds Reed C Jet al.(2013)Hay S et al. (2009) Boonyaratanakornkit B B et al. (2002) Giulio M D (2005) Mombelli E et al. (2002). There are abundance of small amino acids in these proteins and large hydrophobic amino acids such as tyrosine and tryptophans etc are replaced by small amino acids to provide tight packing to core protein unlike other thermophilic proteins therefore creating more pressure stable protein due to their highly tight packing Giulio M D (2005). Than barrel-shaped multimeric structure the dodecameric structure of the piezophilic protein, TET3 peptidase increased stability when subjected to high pressure conditions Rosenbaum E et al. (2012). This structure also provides more compact structure to the protein thus enabling the less penetrative capacity of water molecules into the core of protein when exposed to very high pressures as these trapped water molecules create entropy within the protein molecule which lead to disruption of structure of protein and thereby denaturing it. Boonyaratanakornkit B B et al. (2002) Rosenbaum E et al. (2012)

Radiophiles are the organisms that can survive the very extreme conditions of radiations by having high intracellular manganese/ ferrous (Mn/Fe) concentration ratios that protects from the oxidative carbonylation of i.e., addition of a carbon monoxide group Daly M J et al. (2007). Along with it

these organisms utilize vacuolar type H⁺ ATP synthase which is also found in eukaryotes that help in the intracellular acidification, facilitating the Mn redox cycling by providing H⁺ **Makarova KS et al. (2001) Kane PM (2006).**

The articulation level of protein DR1199 is seen to increment significantly in the radiation -safe bacterium species like *Deinococcus radiodurans* upon illumination. This protein has a place with the DJ-1 superfamily, which includes the proteins with assorted capacities, for example, the bacterial chaperone (Hsp31), the archaeal proteases (PhpI and PfpI), the infection related proteins such as the human Parkinson's related protein (DJ-1), and hyperosmotic push proteins (YhbO). The proteins of this superfamily are oligomeric in nature, but from protein to protein the oligomerization interface differs. Despite the fact that for a considerable lot of these proteins, their capacity stays dark, a large portion of them are associated with cell assurance against natural anxieties. The structure of DR1199 to a determination of 2.15 is decided. And, its capacity and concentration in these parts in the reaction to illumination is at peak and all the more increased by large to oxidative stress in *D. radiodurans*. The protein is a dimer showing an oligomerization interface like that watched for the YhbO and PhpI proteins. In this structure the cysteine in the synergist set of three (Cys 115) is oxidized, like alterations found in the relating cysteine of the DJ-1 protein. The oxidation happens unexpectedly in DR1199 precious stones. In arrangement, no proteolytic or chaperone movement was recognized. Outcomes, proposed that DR1199 may function, instead of acting as a peptidase it may act as an stress protein: engaged against the oxygen receptive species for detoxification of the cell in *D. radiodurans*. **Fioravanti, Eet al. (2008)**

The generation of superoxide dismutase (SOD) fluctuated in the *Deinococcus radiophilus*, the radiation (UV) safe bacterium, and contingent on various periods of development, UV illumination, and superoxide treatment. A progressive increment in all out SOD movement

happened till the stationary stages of growth. The electrophoretic determination of the SOD in cell concentrates of *D. radiophilus* at every development stage uncovered the event of Mn SOD all through the development stages. The exponential stage SOD profiles of *Dinococcus radiophilus* exhibited oxidative stress because to the potassium superoxide treatment or UV light likewise uncovered the event of a solitary SOD. In any case, these medicines caused an expansion in SOD action. The information emphatically recommend that *D. radiophilus* has just a single types of SOD as a constitutive compound, which is by all accounts a film related protein. **Yun and Lee (2003)**

The creation of two sorts of catalase- peroxidase (catalase-2 and catalase-3) by the *Deinococcus radiophilus* shifted relying on development stages and oxidative stress. A continuous increment in all out catalase movement happened amid in the exponential stage as well as the stationary stages of growth. Determination of these catalases by means of electrophoretic determination in *Deinococcus* cells extricate the uncovered uniform event of catalase-2 and the presence of catalase-3 just amid the late exponential stage and the stationary stage. A significant increment in absolute catalase was seen in either hydrogen peroxide-or UV-treated cells. Checking of *D. radiophilus* catalase movement in the oxidative stressed and non-treated cells by gel electrophoresis took after by densitometry uncovered the few crease increment in catalase-3, which is over the consistent level of catalase-2. The event of catalase-3 and catalase-2 uncovered by fractionation of sucrose-stunned cells proposes that catalase-3 is a cytosolic inducible compound while catalase-2 is the film related constitutive protein. **Yun and Lee (2000)**

Apart from the fact that these organisms can live and survive in extreme conditions through their protein adaptations, these unique archaeal adaptations in response to the drastically changing biomes have generated a special interest in their potential biotechnological applications. In the

current scenario the increased demand of developing more economically as well as environmentally suitable alternative methods of processing, have also given an important contribution to the flourishing research and designing of the various applications of Archaea and their metabolites. For example, applications proposed based on the preexisting applications of bacterial as well as eukaryotic homologues in areas of mariculture, agriculture, medicine, and electronics towards the utilization and engineering of these proteins for various biotechnological, industrial as well as environmental applications that require their activity in extreme conditions like

The enzymes produced by thermophilic bacteria help in the catalyzation of reactions where the high thermodynamic and kinetic conditions are favorable apart from that maintenance of such conditions help in the prevention of contamination during the process **Unsworth L D, Oost J V D, and Koutsopoulos S (2007)**.

In food industries the high pressure help in prevention of contamination as well as preservation of colour and taste of the product so under such conditions the psychrophilic enzymes can be helpful **Abe F and Horikoshi K (2001)**.

Acidophilic enzymes are useful in biofuel production and in ethanol production as they help in the proper utilization of carbon source in acidic conditions **Sharma A, Kawarabayasi Y, and Satyanarayana T (2012)**.

Psychrophilic enzymes are used in the field of biotechnology apart from commercial detergents **Tutino M L, Prisco G D, Marino G, and Pascale D D (2009)**.

Materials and Methods

Isolation and culture of Extremophiles

Samples from various sources with extreme conditions were taken and suspended into 1 ml of Tris-buffered saline (TBS, pH 7). After centrifugation at 3000 rpm for 5 min, supernatant was collected and diluted 1000 times in TBS. 100 ul of diluted supernatant was plated on Nutrient agar plates. Next day, single colonies were be selected from these plates, and cultured in liquid media and agar plates in presence/absence of various kinds of stress such as extreme temperature and pH range, salt concentrations etc.

Optical density analysis:

For observation of any change in growth of bacteria, OD_{595-600 nm} to be taken at different time points(with and without stress).

Media preparation

To prepare 1000 ml of nutrient Agar media, suspended 28 grams of nutrient agar in 1000 ml purified/distilled water. This was autoclaved at 15 lbs pressure and 121°C temperature for 30 minutes. Poured the sterilized media into sterile petriplates inside a working laminar hood under aseptic conditions and let them sit till they were cooled down and set.

To prepare 1000 ml of nutrient broth, suspended 13 grams of nutrient broth in 1000 ml purified/distilled water. Media can be heated, if necessary, to ensure media is completely dissolved. Dispensed the media in test tubes/ conical flask and plugged them tightly with a cotton plug. This was autoclaved at 15 lbs pressure and 121°C temperature for 30 minutes.

To prepare 1000 ml of luria bertani agar, suspended 40 grams of nutrient agar in 1000 ml purified/distilled water. This was autoclaved at 15 lbs pressure and 121°C temperature for 30

minutes. Poured the sterilized media into sterile petriplates inside a working laminar hood under aseptic conditions and let them sit till they were cooled down and set.

To prepare 1000 ml of luria bertani broth, suspended 25 grams of nutrient broth in 1000 ml purified/distilled water. Media can be heated, if necessary, to ensure media is completely dissolved. Dispensed the media in test tubes/ conical flask and plugged them tightly with a cotton plug. This was autoclaved at 15 lbs pressure and 121°C temperature for 30 minutes.

Table 1: List of extremophiles used

MTCC Number	Name of bacterial strains
4465	<i>Deinococcus radiophilus</i>
2852	<i>Halobacterium halobium</i>
7913	<i>Bacillus alcalophilus</i>
3854	<i>Planococcus antarcticus</i>
3707	<i>Psychrobacter frigidicola</i>

Table 2: List of the stress used on extremophiles.

Stress	Organisms exposed to stress			
	Control	Extremophiles		
pH	<i>E.coli</i>	<i>D.radiophilus</i>	<i>H.halobium</i>	<i>B.alcalophilus</i>
Alkalinity	<i>E.coli</i>	<i>D.radiophilus</i>	<i>H.halobium</i>	<i>B.alcalophilus</i>
CuSO₄	<i>E.coli</i>	<i>D.radiophilus</i>	<i>H.halobium</i>	<i>B.alcalophilus</i>
H₂O₂	<i>E.coli</i>	<i>D.radiophilus</i>	<i>H.halobium</i>	<i>B.alcalophilus</i>
H₂SO₄	<i>E.coli</i>	<i>D.radiophilus</i>	<i>H.halobium</i>	<i>B.alcalophilus</i>

Table 3: Optical Density readings of extremophiles (with and without stress) at various time points: TABLE 3.1:

Blank													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
pH 7	0	0.335	0.357	0.308	0.432	0.422	0.452	0.368	0.309	0.291	0.422	0.450	0.435
Added	1	0.595	0.568	0.556	0.876	0.778	0.805	0.695	0.638	0.675	0.675	0.698	0.743
Salt	2	0.845	1.098	0.962	1.397	1.387	1.606	1.483	1.012	1.339	1.352	1.168	1.163
0%	3	1.037	1.512	1.118	1.381	1.500	1.675	1.409	1.112	1.384	1.348	1.067	1.172
	4	0.665	1.398	0.826	1.145	1.381	1.483	1.684	1.250	1.230	1.571	1.109	1.233
	5	0.111	0.806	0.522	0.715	1.086	1.021	1.333	1.166	1.183	0.877	1.453	1.011
	6	0.106	0.752	0.431	0.609	1.032	0.964	1.183	1.635	1.853	0.852	1.635	0.853
	7	0.092	0.185	0.136	0.201	0.849	0.832	0.601	1.093	1.372	0.684	0.841	0.646

Table 3.2:

Salinity													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
pH 7 Added Salt 3%	0	0.112	0.074	0.089	0.109	0.121	0.393	0.194	0.179	0.163	0.060	0.021	0.047
	1	0.202	0.179	0.184	0.209	0.221	0.593	0.396	0.377	0.369	0.259	0.214	0.237
	2	0.326	0.261	0.266	0.375	0.382	0.828	0.726	0.716	0.713	0.686	0.556	0.557
	3	0.348	0.387	0.297	0.470	0.415	1.037	1.019	0.785	0.827	1.026	0.701	0.902
	4	0.540	0.474	0.519	0.449	0.446	0.130	1.100	0.887	0.956	1.657	1.160	1.545
	5	0.865	0.653	0.538	0.524	0.559	1.559	1.288	1.081	1.095	1.638	1.491	1.617
	6	0.863	0.628	0.476	0.432	0.485	1.397	1.237	1.028	1.007	1.780	1.603	1.603
	7	0.574	0.584	0.601	0.525	0.672	1.445	1.235	0.939	1.013	1.760	1.790	1.474

Table 3.3

Salinity													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
pH 7 Added Salt 5%	0	0.089	0.091	0.061	0.077	0.081	0.069	0.157	0.132	0.145	0.178	0.179	0.173
	1	0.279	0.344	0.293	0.242	0.260	0.211	0.397	0.387	0.373	0.515	0.531	0.510
	2	0.381	0.404	0.318	0.306	0.418	0.357	0.805	0.801	0.875	1.318	1.588	1.065
	3	0.379	0.527	0.445	0.516	0.434	0.454	1.688	1.191	0.850	1.713	1.459	1.777
	4	0.530	0.540	0.524	0.456	0.369	0.749	1.713	1.627	1.244	0.896	1.859	1.746
	5	0.513	0.685	0.606	0.427	0.457	0.721	0.924	1.104	1.118	1.131	1.337	1.419
	6	0.789	0.770	0.782	0.499	0.467	0.698	0.670	0.827	1.111	1.132	1.280	1.336
	7	0.335	0.609	0.592	0.344	0.538	0.677	0.362	0.453	0.531	1.121	1.199	1.151

Table 3.4

Salinity													

OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
pH 7 Added Salt 11%	0	0.131	0.148	0.158	0.091	0.076	0.115	0.121	0.151	0.139	0.103	0.149	0.160
	1	0.215	0.239	0.244	0.101	0.149	0.215	0.301	0.396	0.363	0.251	0.292	0.331
	2	0.261	0.275	0.426	0.314	0.367	0.415	0.734	0.916	0.852	0.508	0.542	0.594
	3	0.221	0.259	0.369	0.435	0.443	0.479	0.922	1.073	0.940	0.694	0.610	0.749
	4	0.232	0.345	0.364	0.300	0.377	0.386	1.018	1.176	1.113	0.735	0.878	0.741
	5	0.217	0.352	0.799	0.470	0.577	0.726	1.240	1.234	1.269	0.922	0.923	1.619
	6	0.192	0.348	0.747	0.566	0.507	0.749	1.104	1.169	1.119	1.181	1.272	1.447
	7	0.122	0.301	0.475	0.669	0.504	0.699	1.624	1.729	1.862	1.793	1.805	1.428

Table 3.5

pH													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
pH 2	0	0.109	0.131	0.216	0.267	0.271	0.258	0.240	0.291	0.281	0.019	0.289	0.111
	1	0.297	0.296	0.307	0.318	0.311	0.310	0.367	0.353	0.350	0.221	0.442	0.339
	2	0.538	0.577	0.999	0.745	0.625	0.836	0.753	0.613	0.992	0.419	0.536	0.546
	3	0.742	0.752	1.386	0.933	0.848	1.562	1.116	0.971	1.021	0.690	0.884	0.812
	4	0.918	0.895	0.796	1.343	1.086	1.460	1.553	1.718	1.508	0.854	1.475	1.623
	5	0.882	1.101	1.211	1.621	1.538	1.512	1.231	1.473	1.584	1.383	1.479	1.643
	6	0.689	0.876	0.500	1.828	1.402	1.282	1.819	1.591	1.446	0.918	1.282	0.770
	7	0.238	0.677	0.199	0.445	0.725	0.836	0.753	0.713	0.992	0.519	0.636	0.446

Table 3.6

pH													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
pH 4	0	0.087	0.049	0.258	0.451	0.321	0.350	0.367	0.441	0.448	0.032	0.275	0.366
	1	0.363	0.176	0.601	0.987	0.963	0.768	0.702	0.989	0.976	0.218	0.523	0.618
	2	0.694	0.569	0.945	1.437	1.067	1.199	1.178	1.374	1.408	0.579	0.945	1.152
	3	1.396	1.214	1.222	1.680	1.153	0.940	2.034	1.650	1.788	0.981	0.865	0.792
	4	1.168	0.968	1.193	0.904	0.889	0.966	1.718	1.179	1.248	0.718	0.522	0.214
	5	1.281	1.048	1.295	0.966	0.922	0.984	2.132	1.376	1.364	0.792	0.722	0.532
	6	0.963	0.797	0.994	0.776	0.779	0.929	1.868	1.032	1.011	0.491	0.377	0.131
	7	0.187	0.149	0.258	0.251	0.221	0.550	1.667	0.541	0.548	0.203	0.275	0.066

Table3.7

pH													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
pH 10	0	0.258	0.149	0.187	0.315	0.275	0.208	0.101	0.203	0.3	0.191	0.066	0.110
	1	0.422	0.308	0.330	0.667	0.541	0.548	0.382	0.511	0.629	0.392	0.222	0.371
	2	0.796	0.560	0.598	0.989	0.748	0.716	0.694	0.981	0.881	0.640	0.531	0.622
	3	1.169	0.822	0.885	1.488	1.184	1.015	0.949	1.313	1.183	0.855	0.613	0.825
	4	1.193	1.058	0.622	1.330	1.049	0.806	1.775	1.839	1.305	1.634	0.920	1.748
	5	0.053	0.339	0.203	1.098	0.809	0.524	1.893	1.809	1.080	1.492	0.904	1.087
	6	0.680	0.538	0.208	1.115	0.808	0.527	1.905	1.860	1.015	1.698	0.990	1.322
	7	0.287	0.126	0.098	0.921	0.610	0.259	0.521	1.550	1.809	0.508	1.816	0.948

Table 3.8

pH													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
pH 12	0	0.163	0.176	0.101	0.287	0.163	0.168	0.102	0.189	0.276	0.18	0.023	0.018
	1	0.431	0.494	0.419	0.534	0.405	0.430	0.434	0.481	0.614	0.300	0.277	0.146
	2	0.792	0.557	0.586	0.851	0.895	0.965	0.833	0.681	0.728	0.717	0.600	0.834
	3	1.062	0.974	0.709	0.982	0.765	0.869	1.205	1.087	0.779	1.082	1.118	1.113
	4	1.088	0.856	0.979	1.034	0.850	0.968	1.130	1.024	0.885	1.130	1.207	1.120
	5	1.008	0.808	0.412	1.055	0.813	0.597	1.146	0.960	0.567	1.055	1.102	1.112
	6	1.121	0.915	0.487	0.710	0.929	0.301	0.896	0.509	0.333	0.889	0.717	0.714
	7	0.538	0.677	0.099	0.445	0.525	0.136	0.453	0.213	0.092	0.219	0.336	0.326

Table 3.9

Blank													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
CuSO₄	0	0.335	0.357	0.308	0.432	0.422	0.325	0.368	0.309	0.291	0.422	0.450	0.287
	0%	0.743	0.733	0.341	0.718	0.776	0.417	0.690	0.730	0.364	0.888	0.932	0.356
H₂O₂	2	1.624	1.604	0.277	1.446	1.199	0.363	1.594	1.190	0.468	0.937	1.055	1.084
	0%	0.929	1.493	0.643	0.710	0.777	0.346	0.896	0.982	1.605	1.123	1.295	1.303
	4	0.315	0.526	0.807	0.288	1.013	0.746	0.317	0.678	0.685	0.042	0.644	0.734

Table 3.10

CuSO₄													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		

CuSO₄ 3%	0	0.348	0.378	0.298	0.408	0.290	0.322	0.326	0.290	0.367	0.380	0.283	0.387
	1	0.991	1.063	0.653	0.113	1.260	1.684	0.197	1.991	0.359	0.145	1.998	0.322
	2	1.278	1.262	1.114	1.909	1.889	1.158	1.433	1.589	0.491	1.584	1.474	0.384
	3	1.104	1.135	1.110	1.677	1.753	1.170	1.358	1.486	0.773	1.436	379	0.576
	4	0.373	0.790	0.781	0.561	0.743	1.241	0.373	1.155	1.067	0.211	1.155	1.819

Table 3.11

CuSO₄													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
CuSO₄ 5%	0	0.450	0.231	0.406	0.444	0.301	0.416	0.360	0.304	0.349	0.384	0.281	0.381
	1	0.460	0.383	0.501	0.457	0.372	0.513	0.447	0.366	0.455	0.477	0.428	0.692
	2	0.387	0.275	0.386	0.390	0.276	0.372	0.352	0.835	1.061	0.865	1.462	1.482
	3	0.464	0.374	0.451	0.784	1.080	0.983	1.670	1.640	1.526	1.588	1.326	1.757
	4	1.038	0.599	0.182	1.233	0.906	0.676	1.196	1.607	0.802	1.647	1.723	0.769

Table 3.12

CuSO₄													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
CuSO₄ 11%	0	0.292	0.267	0.413	0.290	0.285	0.405	0.288	0.334	0.391	0.284	0.319	0.371
	1	0.620	0.775	0.999	0.628	0.724	0.972	0.324	0.435	0.514	0.495	0.429	0.423
	2	1.255	1.328	1.485	1.242	1.218	1.399	0.370	0.566	0.613	0.641	0.576	0.567
	3	1.241	1.247	1.383	1.248	1.158	1.285	0.602	0.849	0.756	0.682	0.820	0.712
	4	1.292	1.563	1.486	1.218	1.879	1.431	1.245	1.703	0.966	1.336	1.223	0.892

Table 3.13

H₂O₂

OD _{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
H ₂ O ₂ 1%	0	0.265	0.380	0.417	0.279	0.386	0.427	0.239	0.305	0.357	0.253	0.327	0.377
	1	0.361	1.536	1.582	0.384	1.386	1.543	0.366	0.896	0.969	0.397	0.932	0.966
	2	0.222	1.982	1.861	0.234	1.943	1.750	0.241	1.224	1.209	0.746	1.239	1.238
	3	0.398	1.991	1.920	0.887	1.968	1.904	1.564	1.371	1.361	1.720	1.370	1.427
	4	0.557	1.582	0.482	0.853	0.921	0.794	1.114	1.151	1.133	1.248	1.360	1.270

Table 3.14

H ₂ O ₂													
OD _{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
H ₂ O ₂ 3%	0	0.252	0.332	0.380	0.215	0.345	0.374	0.243	0.294	0.366	0.234	0.331	0.345
	1	0.392	0.776	0.856	0.352	0.690	0.826	0.360	0.434	0.603	0.351	0.456	0.551
	2	1.010	1.097	1.096	0.929	0.916	1.056	0.254	0.270	0.755	0.340	0.558	0.711
	3	1.432	1.293	1.180	1.211	1.036	1.268	0.586	0.568	1.084	0.645	0.736	1.036
	4	1.256	1.801	1.310	1.280	1.622	1.407	1.134	1.134	1.872	1.016	1.036	1.338

Table 3.15

H ₂ O ₂													
OD _{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
H ₂ O ₂ 5%	0	0.376	0.496	0.347	0.375	0.497	0.340	0.296	0.375	0.265	0.319	0.400	0.259
	1	1.172	1.521	0.617	1.408	1.554	0.637	0.889	0.910	0.448	0.933	0.981	0.475
	2	1.921	1.725	0.833	1.780	1.736	1.107	1.120	1.101	0.567	1.181	1.174	0.819
	3	1.570	1.576	1.576	1.673	1.72	1.159	1.296	1.345	1.241	1.397	1.540	1.469
	4	0.560	0.290	0.370	0.954	1.465	0.377	1.145	1.777	1.138	1.215	1.674	1.410

Table 3.16

H₂O₂													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
H₂O₂ 11%	0	0.322	0.437	0.108	0.324	0.439	0.259	0.317	0.423	0.256	0.285	0.410	0.227
	1	0.771	0.835	0.329	0.769	0.864	0.466	0.527	0.646	0.453	0.451	0.525	0.359
	2	1.025	1.939	0.052	1.031	1.998	0.319	0.582	0.653	0.324	0.515	0.566	0.237
	3	1.341	1.467	1.422	1.337	1.468	1.122	0.939	0.992	0.857	0.818	0.848	0.831
	4	1.345	1.163	1.434	1.531	1.301	1.430	1.373	1.213	0.350	1.515	0.254	0.135

Table 3.17

H₂SO₄													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
H₂SO₄ 1%	0	0.292	0.275	0.243	0.331	0.374	0.275	0.338	0.274	0.235	0.363	0.296	0.268
	1	0.379	0.449	0.431	0.452	1.405	0.381	0.470	0.424	0.417	0.446	0.449	0.421
	2	0.859	0.542	0.375	1.086	0.496	0.451	0.934	0.514	0.434	0.929	0.564	0.423
	3	0.923	0.635	0.502	1.163	0.647	0.635	1.022	0.664	0.617	1.00`	0.698	0.589
	4	0.530	0.518	0.575	0.602	0.520	0.544	0.506	0.523	0.510	0.514	0.548	0.501
	5	0.282	0.279	0.256	0.333	0.574	0.296	0.327	0.288	0.238	0.368	0.301	0.281

Table 3.18

H₂SO₄													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
H₂SO₄ 3%	0	0.346	0.280	0.265	0.302	0.320	0.278	0.302	0.283	0.277	0.324	0.287	0.271
	1	0.447	0.395	0.392	0.409	0.440	0.413	0.412	0.406	0.409	0.307	0.349	0.334
	2	1.031	0.485	0.460	0.887	0.357	0.486	0.909	0.481	0.368	0.742	0.455	0.377
	3	1.112	0.629	0.629	0.956	0.495	0.639	0.964	0.609	0.723	0.800	0.544	0.486
	4	0.507	0.484	0.517	0.472	0.395	0.537	0.477	0.489	0.459	0.405	0.437	0.436
	5	0.349	0.293	0.272	0.301	0.337	0.294	0.303	0.295	0.287	0.300	0.281	0.274

Table 3.19

H₂SO₄													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
H₂SO₄ 5%	0	0.231	0.221	0.243	0.264	0.384	0.381	0.269	0.252	0.269	0.290	0.282	0.260
	1	0.340	0.336	0.356	0.423	1.519	1.501	0.432	0.436	0.449	0.447	0.460	0.435
	2	0.462	0.378	0.458	0.405	0.422	0.362	0.436	0.385	0.409	0.473	0.429	0.391
	3	0.593	0.539	0.563	0.626	0.654	0.620	0.638	0.616	0.657	0.672	0.642	0.620
	4	0.495	0.479	0.473	0.515	0.501	0.537	0.512	0.533	0.531	0.542	0.525	0.527
	5	0.239	0.230	0.247	0.277	0.334	0.392	0.279	0.265	0.270	0.305	0.296	0.258

Table 3.20

H₂SO₄													
OD_{600nm}		<i>E.coli</i>			<i>D.radiophilus</i>			<i>H.halobium</i>			<i>B.alcalophilus</i>		
H₂SO₄ 11%	0	0.264	0.252	0.261	0.273	0.312	0.259	0.260	0.260	0.260	0.282	0.243	0.249
	1	0.404	0.405	0.418	0.419	0.472	0.432	0.404	0.420	0.427	0.334	0.290	0.300
	2	0.468	0.448	0.400	0.498	0.474	0.478	0.436	0.395	0.390	0.499	0.429	0.460
	3	0.665	0.667	0.637	0.682	0.677	0.698	0.661	0.588	0.601	0.587	0.578	0.503
	4	0.552	0.551	0.538	0.576	0.546	0.611	0.516	0.502	0.532	0.492	0.475	0.587
	5	0.280	0.263	0.264	0.285	0.322	0.262	0.283	0.266	0.257	0.297	0.247	0.259

Table 4: Effect of the stress used on extremophiles.

Stress	Organism	Effects
pH	<i>Escherichia coli</i>	Could not survive at both acidic and alkaline pH
	<i>Deinococcus radiophilus</i>	Exhibited moderate growth at both acidic and alkaline pH
	<i>Halobacterium halobium</i>	Exhibited moderate growth at acidic pH and high growth alkaline pH
	<i>Bacillus alcalophilus</i>	Exhibited high growth at both acidic and alkaline pH

Stress	Organism	Effects
Salinity	<i>Escherichia coli</i>	Exhibited low growth at various levels of salinity
	<i>Deinococcus radiophilus</i>	Exhibited low growth at various levels of salinity
	<i>Halobacterium halobium</i>	Exhibited high growth at various levels of salinity
	<i>Bacillus alcalophilus</i>	Exhibited high growth at various low levels of salinity and moderate growth at high salinity

Stress	Organism	Effects
CuSO₄	<i>Escherichia coli</i>	Exhibited low growth at various concentrations of CuSO ₄
	<i>Deinococcus radiophilus</i>	Exhibited moderate growth at low concentrations and low growth at high concentrations of CuSO ₄
	<i>Halobacterium halobium</i>	Exhibited moderate growth at low concentrations and low growth at high concentrations of CuSO ₄
	<i>Bacillus alcalophilus</i>	Exhibited highest growth at various concentrations of CuSO ₄

Stress	Organism	Effects
H₂O₂	<i>Escherichia coli</i>	Exhibited moderate growth at various concentrations of H ₂ O ₂ .
	<i>Deinococcus radiophilus</i>	Exhibited highest growth at various concentrations of H ₂ O ₂ .
	<i>Halobacterium halobium</i>	Exhibited low growth at various concentrations of H ₂ O ₂ .
	<i>Bacillus alcalophilus</i>	Exhibited low growth at various concentrations of H ₂ O ₂ .

Stress	Organism	Effects
H₂SO₄	<i>Escherichia coli</i>	Exhibited low growth at various concentrations of H ₂ SO ₄
	<i>Deinococcus radiophilus</i>	Exhibited low growth at various concentrations of H ₂ SO ₄
	<i>Halobacterium halobium</i>	Exhibited moderate growth at low concentrations and low growth at high concentrations of H ₂ SO ₄
	<i>Bacillus alcalophilus</i>	Exhibited moderate growth at low concentrations and low growth at high concentrations of H ₂ SO ₄

Results and Discussion

The Culturing and the stress response towards pH, Salinity, CuSO_4 , H_2O_2 , H_2SO_4 of *Deinococcus radiophilus*, *Halobacterium halobium*, *Bacillus alcalophilus* was performed and effect of these stresses on extremophiles growth was observed by spectrophotometer at 600 nm.

From the above experiments we have observed that *Bacillus alcalophilus* is more resistant over *Deinococcus radiophilus* & *Halobacterium halobium* in case of pH 10-12 (Table 3.7- 3.8) and *Deinococcus radiophilus* & *Halobacterium halobium* exhibited moderate level of resistance at low pH (Table 3.5-3.6).

In case of salinity stress (3%, 5%, 11% NaCl) experiment, the *Halobacterium halobium* & *Bacillus alcalophilus* exhibited high level of resistance over *Deinococcus radiophilus* (TABLE 3.2-3.4).

Deinococcus radiophilus & *Halobacterium halobium* exhibited moderate level of resistance and *Bacillus alcalophilus* showed high resistance to CuSO₂ (TABLE.3.10-3.12).

The level of growth was low in case of *Halobacterium halobium* and *Bacillus alcalophilus* where as *Deinococcus radiophilus* showed highest level of resistance in H₂O₂ (TABLE 3.13-3.16)

Moderate level of resistance was exhibited by *Halobacterium halobium*, *Bacillus alcalophilus* at low concentrations of H₂SO₄ (TABLE 3.17- 3.18) At High concentration of H₂SO₄ the growth was low for all the bacteria used (TABLE 3.19- 3.20).

Summary and Conclusion

We conclude that *Bacillus alcalophilus* is more resistant at high pH, and *Deinococcus radiophilus* and *Halobacterium halobium* are moderately resistant at high pH. *Halobacterium halobium* and *Bacillus alcalophilus* are resistant towards salt stress. In case of chemical resistance, the *Deinococcus radiophilus* and *Halobacterium halobium* exhibited moderate level of resistance and *Bacillus alcalophilus* showed high resistance to CuSO_4 , *Deinococcus radiophilus* is resistant to H_2O_2 , and, *Halobacterium halobium*, *Bacillus alcalophilus* are resistant at low concentrations of H_2SO_4

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