Chapter I

Introduction

Extreme environmental habitat exhibits diverse group of microorganisms capable of growing in harsh conditions, their molecular diversity make the organisms to survive in extremities such as salt, pressure, pH, heavy metal contaminated environment and temperature; such microorganisms are called polyextremophiles. Microorganisms encountering only to high salt concentration are known as halophilic bacteria and these bacteria have attracted the attention because of their peculiar molecular mechanism to tolerate hypersaline conditions making them to grow even at 4 M (NaCl) concentration in such extreme conditions. Such microbes can tolerate the external stress by intracellular accumulation of biomolecules.

Hypersaline conditions lead to the study of mechanisms of molecular adaptations of halophilic organisms. They appear either to exclude salts by synthesis of equally high concentrations of uncharged compatible solutes or osmolytes or contain stable macromolecules that can withstand denaturing effects of salts. The genes involved in synthesis and accumulation of compatible solutes and their regulations have become the focus of recent investigations. Early studies showed the intracellular concentration of salts to be extremely high, but with few exceptions, their highly acidic salt-resistant proteins have not been studied in detail in halobacteria.

In case of hypersaline environment, the high osmotic stress on microorganisms is tolerated by accumulation of various biomolecules like inorganic solutes (potassium and chloride ions; Galinski and Truper, 1994; Roberts, 2005). The compatible solutes or osmolytes are low molecular mass organic compounds that accumulate in halophiles which are usually amino acids and polyols, glycine betaine, ectoine,
sucrose, trehalose and glycerol, which do not disrupt metabolic processes and have no net charge at physiological pH. A major exception is for the halobacteria and other extreme halophiles, which accumulates KCl equal to the external concentration of NaCl.

Halotolerant yeasts and green algae accumulate polyols, while many halophilic and halotolerant bacteria accumulate glycine betaine and ectoine. Ectoine is the most abundant compatible solute produced intracellularly in high concentrations by halophilic or halotolerant bacteria (Roberts, 2005; Oren, 2008). Compatible solutes accumulation may occur by biosynthesis, de novo or from storage material or by uptake from the medium. Ectoine are an important class of biomolecules that has gained increasing interest in biotechnological applications like protection of enzymes (Lippert and Galinski, 1992), DNA and whole cells against stresses such as freezing, drying and heating (Louis et al., 1994; Welsh, 2000). Moreover, ectoine can be used as moisturizer in cosmetics or skin care products, as chiral building block and also as protecting agent for healthy cells during chemotherapy (Lentzen and Schwarz, 2006).

Microorganisms respond to external osmotic stress by accumulating either through uptake or de novo synthesis, osmotically active solutes. These solutes are inorganic ions which act as a type of osmoadaptation results in high cytoplasmic concentrations of salts. To tolerate this, specially adapted enzymes and cell structures are required. A second osmoadaptation strategy involves accumulation of organic solutes called ‘compatible solutes’; this is a more flexible mode of adaptation since compatible solutes can be accumulated at high intracellular levels without disturbing vital cellular processes. This strategy is widespread among halophilic and halotolerant
aerobic eubacteria (Galinski, 1995; Da Costa et al., 1998). When organic osmolytes are present in the culture medium, the uptake is usually preferred over de novo synthesis.

Several classes of compatible solutes have been characterized, one of the most abundant osmolytes of aerobic chemoheterotrophic halophilic and halotolorant eubacteria is ectoine (1, 4, 5, 6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid) (Severin et al., 1992). Apart from its purely osmotic function, ectoine also exhibits a protective effect on proteins, nucleic acids and membranes against a variety of stress factors and a wide range of applications in biochemical, medical and cosmetic sector which can be envisaged (Sauer et al., 1995). Here, we report the on-factors affecting the accumulation of ectoine in *Brevibacterium epidermis* DSM 20659. Ectoine is the main compatible solute in the genus *Brevibacterium* (Bernard et al., 1993; Nagata & Wang, 2001).

**Halophiles and their habitat**

An important review by Larsen (1962) outlined a scheme that has relevance today. Non-halophiles are those microorganisms that grow best below 2 % salt. Slight, moderate, and extreme halophiles are those that grow best in media containing 2 to 5%, 5 to 20 %, and 20 to 30 % respectively. Kushner (1968) then distinguished between obligate moderate halophiles and obligate extreme halophiles as which requires 0.5 to 3.5 M and 3.0 M to saturated salinities respectively. Kushner later in 1978, added a definition for borderline extreme halophiles that grow best at 0.5-2.5 M salinity. Halotolerant organisms then are nonhalophiles that can grow at high salinities (15 % w/v or 2.5 M). Facultative halophiles require high salt only under certain environmental conditions.
Halophiles are a group of microorganisms that live in saline environments and in many cases require salinity to survive. Halophiles include a great diversity of organisms like moderately halophilic aerobic bacteria, cyanobacteria, sulphur-oxidizing bacteria, heterotrophic bacteria, anaerobic bacteria, archaea, protozoa, fungi, algae and multicellular eukaryotes. Microorganisms that are able to grow in the absence and presence of salt are designated as halotolerant.

**Moderately Halophilic Bacteria**

The occurrence of non-pigmented halotolerant bacteria is said to be first mentioned in 1919 by LeFevre and Round, in their study on microbiology of cucumber fermentation brines. Halophilic bacteria are found in a variety of salt environments like marine ecosystems, salted meat, salt evaporation pools and salt mines. Most of the important groups of bacteria are able to live in concentrations up to 15 % salt and others can adapt to conditions even at higher salt concentrations. They form a versatile group and are adapted to life at the lower range of salinities and have the ability to adjust rapidly to changes in the external salt concentration.

**Mechanism of tolerance to salt**

Halotolerance is referred to the adaptation of a microorganism to saline conditions for living. Proteins of microorganism adapted to halotolerance are mostly acidic in nature, most of the halophiles does contain acidic amino acids at high salt concentration, slight and moderate halophilic protein require the salt for their activity and extreme halophiles functionally and structurally stable only in the presence of salt, membrane proteins of the halophile are acidic in nature and do not allow the denaturation and precipitation of protein in harsh conditions. The adaptation to the
salinity has begun century ago, which has its roots in evolutionary significance. In addition, information of halotolerance including osmotic changes can also be significant to understanding tolerance to extremes in moisture or temperature.

**Mechanism of Osmoregulation**

Many microorganisms respond to increase in osmolarity by accumulating osmotica in their cytosol, which protects them from cytoplasmic dehydration. Osmophily refers to the osmotic aspects of life at high salt concentrations, especially turgor pressure, cellular dehydration and desiccation. Halophily refers to the ionic requirements for life at high salt concentrations. Organisms live in a range of salinities essentially from distilled water to saturated salt solutions. Halophilic microorganisms usually adopt to either of the two strategies of survival in saline environments: ‘compatible solute’ strategy and ‘salt-in’ strategy. Compatible solute strategy is employed by the majority of moderately halophilic and halotolerant bacteria, some yeasts, algae and fungi. In this strategy cells maintain low concentrations of salt in their cytoplasm by balancing osmotic potential through the synthesis or uptake of organic compatible solutes. Hence these microorganisms are able to adapt to a wide range of salt concentrations. The compatible solutes include polyols such as glycerol, sugars and their derivatives amino acids and their derivatives and quaternary amines such as glycine betaine and ectoines.

The salt-in strategy is employed by true halophiles, including halophilic archaea and extremely halophilic bacteria. These microorganisms are adapted to high salt concentrations and cannot survive when the salinity of the medium is lowered. They generally do not synthesize organic solutes to maintain the osmotic equilibrium. This adaptation involves the selective influx of K+ ions into the cytoplasm. All enzymes
and structural cell components must be adapted to high salt concentrations for proper cell function.

**Potentiality of Halophilic Bacteria for Biotechnology**

The industrial and environmental applications of halophilic microorganisms have been reviewed by Oren, 1998. The salient features of halophiles, including their highly successful applications in production of β-carotene by *Dunaliella* and ectoine synthesis using *Halomonas* and other moderately halophilic bacteria. The potential use of bacteriorhodopsin, the retinal protein proton pump of *Halobacterium* is being explored in photochemical processes. Other possible uses of halophilic microorganisms include treatment of saline and hypersaline wastewaters and production of exopolysaccharides, poly-β-hydroxyalkanoate, bioplastics and biofuel.

Halophilic bacteria offer potential applications in various fields of biotechnology. Although moderately halophilic bacteria have many industrial applications, only a few studies have been carried out concerning this aspect. These microorganisms can be used as a source of metabolites, compatible solutes and other compounds of industrial value. Novel halophilic biomolecules may also be used for specialized applications, bacteriorhodopsin for bio-computing, pigments for food colouring and compatible solutes as stress protectants. Biodegradation of organic pollutants by halophilic bacteria and archaea has been recently reviewed. These microorganisms are good candidates for the bioremediation of hypersaline environments and treatment of saline effluents. Understanding the degradation process would also shed light on the enzymes involved and also on the regulation of metabolism.
Chapter I  Introduction

Halophilic bacteria are a potential source of extracellular hydrolases like proteases with a wide array of industrial applications. These enzymes exhibit stability over a range of saline conditions. Halophilic bacteria constitute excellent models for the molecular study of osmoregulatory mechanisms. Naturally halotolerant plants or microorganisms could be developed into useful agricultural crops or fermentation organisms. This has possible application in agriculture, where conventional agricultural species could be made more halotolerant by gene transfer from naturally halotolerant species.

**Polyhydroxy Butyrate (PHB)**

Biomaterials include chemically unrelated products that are synthesized by microorganisms or part of them under different environmental conditions and biomaterials include chemically unrelated products that are synthesized by microorganisms or part of them under different environmental conditions and Poly (D-3 hydroxybutyrate) is the most ubiquitous and most intensively studied PHA. The content of PHA could be as high as 80 % or higher for the sake of efficient recovery and these polymer-filled bacteria are called “Plastic Bacteria” (Lee, 1996b) in terms of molecular weight, brittleness, stiffness, melting point and other physical properties.

PHA is compared to some of the more common petrochemical-derived thermoplastics. Therefore in certain applications, PHB can directly replace some traditional, non-biodegradable polymers. Because PHB is resistant to water and UV radiation and is impermeable to oxygen, it is specifically suited to use as food packaging (Grothe et al., 1999). Because PHAs are thermoplastics with biocompatible properties, they are being developed as new absorbable materials for implantable
medical applications (Du and Yu, 2002). Other applications of PHAs include packaging material, osteosynthetic material in stimulation of bone growth, raw material for production of stereo regular compounds (PHAs being stereospecific), as much films in agricultural fields and as hot melt adhesives.

PHAs can also be depolymerized to optically active bi-functional hydroxyl acids. A good example in that context is Merck’s Anti-glaucoma drug “Truspot” which is synthesized from hydrolysis of PHB to R-hydroxybutyric acid and processing (Reddy et al., 2003). The best advantage of PHAs is that, on disposal they are completely degraded by microorganisms in various environments such as soil, sea, lake water and sewage.

There are two types of PHB polymers: Native and Denatured PHB granules. Native PHB granules containing lipids and proteins and are rapidly hydrolysed by intracellular PHB depolymerase. On the other hand, denatured granules which are partially crystalline are hardly hydrolysed by intracellular depolymerases but can be degraded by extracellular PHB depolymerases in water soluble products.

**Extracellular biological Synthesis of Nanoparticles from *Halomonas organivorans***

Biological entities and inorganic materials have been in constant touch with each other ever since inception of life on the earth. Due to this regular interaction, life could sustain on this planet with a well-organized deposit of minerals. Recently scientists become more and more interested in the interaction between inorganic molecules and biological species. Studies have found that many microorganisms can produce inorganic nanoparticles through either intracellular or extracellular routes. This section describes the production of various nanoparticles via biological methods.
following the categories of metallic nanoparticles including gold, silver, alloy and other metal nanoparticles, oxide nanoparticles consisting of magnetic and nonmagnetic oxide nanoparticles, sulfide nanoparticles and other miscellaneous nanoparticles.

**Metal Nanoparticles**

**Silver Nanoparticles.**

Silver nanoparticles, like their bulk counterpart, show effective antimicrobial activity against Gram-positive and Gram-negative bacteria including highly multi-resistant strains such as methicillin resistant *Staphylococcus aureus* (Pananck *et al.*, 2006). The secrets discovered from nature have led to the development of biomimetic approaches to the growth of advanced nanomaterials.

Recently, scientists have made efforts to make use of microorganisms as possible eco-friendly nano-factories for the synthesis of silver nanoparticles. Various microbes are known to reduce the Ag$^+$ ions to form silver nanoparticles, most of which are found to be spherical particles (Mukharjee, 2001; Ahmed, 2003). Klaus and co-workers have shown that the bacterium *Pseudomonas stutzeri* AG259, isolated from a silver mine, when placed in a concentrated aqueous solution of silver nitrate, played a major role in the reduction of the Ag$^+$ ions and the formation of silver nanoparticles (AgNPs) of well-defined size and distinct topography within the periplasmic space of the bacteria (Klaus, 1999). AgNPs were synthesized in the form of a film or produced in solution or accumulated on the surface of its cell when fungi *Verticillium, Fusarium oxysporum,* or *Aspergillus flavus* were employed (Jain, 2011; Vigneshwaran, 2007; Bhainsa, 2007; Senapati, 2004).
Gold Nanoparticles

Gold nanoparticles (AuNPs) have a rich history in chemistry, dating back to ancient Roman times where they were used to stain glasses for decorative purposes. AuNPs were already used for curing various diseases centuries ago. The modern era of AuNPs synthesis began over 150 years ago with the work of Michael Faraday, who was possibly the first to observe that colloidal gold solutions have properties that differ from bulk gold (Hayat et al., 1989). Biosynthesis of nanoparticles as an emerging biotechnology (the intersection of nanotechnology and biotechnology) has received considerable attention due to a growing need to develop environment-friendly technologies in materials synthesis. Sastry and coworkers (2001) have reported the extracellular synthesis of gold nanoparticles by fungus *Fusarium oxysporum* and actinomycete *Thermomonospora* sp. respectively (Mukharjee, 2002; Ahmed, 2003). They reported the intracellular synthesis of gold nanoparticles by fungus *Verticillium* sp. as well (Mukharjee et al., 2001) have demonstrated that gold particles of nanoscale dimensions may readily be precipitated within bacterial cells by incubation of the cells with Au$^{3+}$ ions (Southan and Beveridge, 1996).

Applications of Nanoparticles

Nanomedicine is a burgeoning field of research with tremendous prospects for the improvement of the diagnosis and treatment of human diseases. Dispersed nanoparticles are usually employed in nanobiomedicine as fluorescent biological labels drug and gene delivery agents and in applications such as bio-detection of pathogens, tissue engineering, tumor destruction via heating (hyperthermia), MRI contrast enhancement and phagokinetic studies. A plethora of reviews and research papers studying the applications of nanoparticles in biomedicine have been published,
while the field of biosynthesized nanoparticles is relatively new, researchers have already started exploring their use in applications such as targeted drug delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, enhancing reaction rates, separation science and MRI.

Delivering the drugs precisely and safely to their target sites at the right time to have a controlled release and achieve the maximum therapeutic effect is a key issue in the design and development of novel drug delivery systems. Targeted nano-carriers must navigate through blood-tissue barriers to reach target cells. They must enter target cells to contact cytoplasmic targets via specific endocytotic and transcytotic transport mechanisms across cellular barriers, because of their small size, nanoparticle drug carriers can bypass the blood-brain barrier and the tight epithelial junctions of the skin that normally impede delivery of drugs to the desired target site. Secondly, as a result of their high surface area to volume ratio, nano-carriers show improved pharmacokinetics and bio-distribution of therapeutic agents and thus minimize toxicity by their preferential accumulation at the target site. They improve the solubility of hydrophobic compounds and render them suitable for parenteral administration. Furthermore, they increase the stability of a variety of therapeutic agents like peptides and oligonucleotides.

Applications in Biotechnology

Although current commercial uses of halophiles are quite significant (fermentation of soy and fish sauces, β-carotene production, aquaculture). The novel and unique properties of many of these organisms suggest that they have even greater potential for biotechnological applications (Rodriguez-Valera, 1992). Halophiles can
survive and flourish in environments that limit the growth of most other organisms. Hypersaline environments are ubiquitous and they are spreading as a result of irrigation and other uses of fresh water. Many natural geological formations such as petroleum reserves are associated with hypersaline brines. Many industrial processes also use salts and frequently release brine effluent into the environment. Halophiles are likely to be useful for bioremediation of contaminated hypersaline brine. Halophiles produce a large variety of stable and unique biomolecules that may be useful for practical applications. Halophilic microorganisms produce stable enzymes (including many hydrolytic enzymes such as DNAases, lipases, amylases, gelatinases and proteases) capable of functioning under conditions that lead to precipitation or denaturation of most proteins. Halophilic proteins compete effectively with salts for hydration, a property that may result in resistance to other low-water-activity environments, such as in the presence of organic solvents. Novel halophilic biomolecules may also be used for specialized applications, bacteriorhodopsin for bio-computing, gas vesicles for bioengineering floating particles, pigments for food colouring, and compatible solutes as stress protectants.

**Industrial Enzymes**

The most widespread commercial use of products and processes derived from halophilic and alkaliphilic microbes are in industrial enzymes and this is probably the largest economic sector. These industrial products used on large scale quantities as process aids in various industrial processes in industries such as food processing, textile manufacture and the grain and starch processing industry. Additionally, industrial enzymes are used directly as active ingredients in consumer products like
laundry detergents or in animal feed to improve the nutritional value. Although accurate figures are often difficult to obtain and it is often unclear whether industrial analysts are referring to value in dollars or in volume, the industrial enzymes market is currently estimated to be worth between 1.5 and 1.8 billion US dollars.

The four main commercial sectors are grain and starch processing, textiles, food and cleaning. The concentration on the use of these enzymes have had the most significant impact on the cleaning and textile business areas, since these are the products of alkaliphiles and halophiles.

The availability of halophilic hydrolases in significant amounts for bioprocessing evaluations could be improved in the future using recombinant techniques; on the other hand, the construction of new recombinant enzymes by site-directed mutagenesis of specific residues in order to increase the activity and/or stability will allow the use of these enzymes in different commercial applications. In the last decade, diverse genetic tools for moderate halophiles have been developed (Ventosa et al., 1998), facilitating their genetic manipulation that will make possible the development of economical bioprocesses of industrial interest.

Compatible solutes-Ectoine and Betaine

Although halophilic microorganisms have attracted much attention in recent years, most studies have been performed in halobacteria. However, moderately halophilic bacteria represent an excellent model of adaptation to frequent changes in extracellular osmolality and constitute an interesting group of microorganisms from a biotechnological point of view (Ventosa et al., 1998). Thus, many of them accumulate intracellular organic osmolytes named “compatible solutes” which can be used as
stabilizers of enzymes and whole cells (Da Costa et al., 1997; Ventosa et al., 1998; Nieto et al., 2000) and they produce halophilic exo-enzymes that could be of commercial interest and could be used in biodegradation processes (Ventosa et al., 1998).

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Apart from osmotic function, ectoines have gained much attention in biotechnology as protective agents for enzymes, DNA, membranes and even entire cells against stress conditions such as heating, drying and freezing (Lippert and Galinski, 1992; Louis et al. 1994; Welsh, 2000). Many publications have shown the superior ability of ectoines as protective agents for enzymes against stress conditions, thereby increasing the shelf life and activity of enzyme preparation (Andersson et al., 2000; Borges et al., 2002; Kolp et al., 2006; Lippert and Galinski, 1992). For this reason they are sometimes termed as “molecular chaperones”.

The study on the effect of protective effects of various compatible solutes (glycine betaine, trehalose, maltose, sucrose, ectoine, and hydroxyectoine) on the model enzymes lactic dehydrogenase (LDH) and phosphofructokinase (PFK), the results indicated that all compatible solutes tested were able to protect the enzymes against
the stresses of freezing, heating and drying (Lippert and Galinski, 1992). Hydroxyectoine showed the highest efficacy for the protection of LDH against a freeze-thaw treatment and heat stress, whereas ectoine was the most effective freeze-stabilizing agent for PFK (Lippert and Galinski, 1992). A comparative investigation of the thermostabilizing properties of some compatible solutes (mannosylglycerate, trehalose, ectoine, hydroxyectoine, di-\textit{myo}-inositol phosphate, diglycerol phosphate and mannosylglyceramide) on the model enzyme LDH showed that hydroxyectoine was the best stabilizer, whereas ectoine protected the enzyme to a lower extent (Borges \textit{et al.}, 2002).

\textbf{Fig 1. Mode of Action of Osmolytes}

\textbf{(Mode of action of osmolytes} the hydrated globular protein in aqueous solution is \textbf{a) Stabilized in the presence of Extremolyes} \textbf{b) Which Build solute hydrate cluster (Galinski \textit{et al.}, 1997) that are preferentially excluded from hydrate shell of protein (Arkawa and Timasheff 1985). This leads to the macro compact tertiary structure with reduced surface area. c) Thus the solute does not interact directly with the protein, the stabilizing effect are rather due to modification of the solvent (Water) properties.)
Ectoine and hydroxyectoine were also shown to protect the zymogens against activation, and ectoine was the most potent solute in reducing the formation of trypsin and chymotrypsin (Kolp et al., 2006). Hydroxyectoine has been seen to be able to protect LDH from metal-catalysed oxidation as well as from oxidation by hydrogen peroxide (Andersson et al., 2000). Ectoine and hydroxyectoine have also been shown to provide protection against pH stress. The alkaline active xylanase from Bacillus halodurans retained a significantly higher activity in the presence of ectoine and hydroxyectoine during incubation at pH 4.5 and at pH 12. Furthermore, hydroxyectoine provided a higher stabilizing effect as opposed to ectoine. Besides acting as protective agents for enzymes against stress conditions, some osmolytes can be used for PCR amplification of DNA with a high G+C content and therefore with a high melting temperature.

Ectoine and betaine, for example, have been shown to decrease the melting temperature of double-stranded DNA (Lapidot et al., 1999; Schnoor et al., 2004). Ectoine and hydroxyectoine also increase the thermal stability of DNA polymerases at elevated temperatures, thereby demonstrating that they could be used to improve primer extension and polymerase chain reactions (Lapidot et al., 1999). Ectoines do not only stabilize proteins and other macromolecules, they are also potential cell protectants. Louis et al., (1994) showed that the addition of ectoine and hydroxyectoine prior to freeze-drying increased the survival rate of the two E. coli strains tested. Ectoine can be used in fermentation technology to increase the osmotolerance and yield in the production of amino acids by coryneform bacteria (Yasuhiko et al., 1997). Manzanera et al., (2004) showed that E. coli dried in hydroxyectoine exhibited a high
degree of desiccation tolerance, similar to that achieved when using trehalose as an extracellular protectant.

In addition, the results obtained for the gram-negative soil bacterium *Pseudomonas putida* suggested that hydroxyectoine was superior to trehalose as a desiccation protectant, thus offering a significant potential as a drying excipient for live microorganisms (Manzanera *et al.*, 2002). Cytoprotection by ectoines is however not limited to bacteria: it can also be applied to eukaryotic cells. The effect of ectoine on membranes was tested with a red blood cell (RBC) assay and the results suggested that damage of the cell membrane by surface active substances was significantly reduced or prevented by a pre-treatment with ectoine (Bunger *et al.*, 2001). Ectoine has also been found to protect human skin against harmful ultraviolet irradiation (Bunger and Driller, 2004).

The introduction of ectoine or its derivatives into cosmetic preparations increases their humidifying activity and provides a stabilization of the skin (Bunger *et al.*, 2003). For these reasons, a major application area for extremolytes established today is in cosmetics where ectoine (Ectoine™) is now used in a growing range of skin care products (Lentzen and Schwarz, 2006).
Table 1. Shows the Applications of osmolytes

<table>
<thead>
<tr>
<th>Application</th>
<th>Extremolyte(s)</th>
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<tr>
<td>Protection of biological macromolecules</td>
<td>✷ Hydroxyectoine</td>
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<td>Protection of oxidative protein damage (LDH)</td>
<td>✷ Hydroxyectoine</td>
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<tr>
<td>Stabilization of enzymes against thermal stress and freeze drying</td>
<td>✷ Mannosylglycerate</td>
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<tr>
<td>Stabilization of recombinant nuclease</td>
<td>✷ Mannosylglycerate</td>
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<tr>
<td>Enzyme stabilization against heating, freezing, and drying</td>
<td>✷ Ectoine</td>
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<td>Protection of LDH against heat and freeze-thawing</td>
<td>✷ Ectoine</td>
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<td>Protection against proteolytic cleavage of antibodies</td>
<td>✷ Ectoine</td>
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<td>Thermostabilization of proteins</td>
<td>✷ DGP</td>
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<td>Stabilization of rubredoxin</td>
<td>✷ DGP</td>
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<td>Cutinase unfolding and stabilization</td>
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<td>Inhibition of insulin amyloid formation</td>
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<td>Protection against freeze-thaw stress of immunotoxins</td>
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<td>Reduction of VLS in immunotoxin therapy</td>
<td>✷ Hydroxyectoine</td>
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<td>Expression of functional recombinant proteins</td>
<td>✷ Hydroxyectoine</td>
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<td>Stabilization of retroviral vaccines</td>
<td>✷ Mannosylglycerate; Hydroxyectoine</td>
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<td>Reduction of apoptotic cell death induced by MJD gene product</td>
<td>✷ Ectoine</td>
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<tr>
<td>Inhibition of aggregation and neurotoxicity of Alzheimer’s beta-amyloid</td>
<td>✷ Ectoine, Hydroxyectoine</td>
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<tr>
<td>Protection of cells</td>
<td>✷ Ectoine; Hydroxyectoine</td>
</tr>
<tr>
<td>Stabilization of E. coli during drying and storage</td>
<td>✷ Hydroxyectoine</td>
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<td>Induction of thermotolerance in E. coli</td>
<td>✷ Ectoine</td>
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<tr>
<td>Protection of P. putida against anhydrobiotic stress</td>
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<td>Osmoprotection of lactic acid bacteria</td>
<td>✷ Ectoine</td>
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<tr>
<td>Stabilization of tobacco cells against hyperosmotic stress</td>
<td>✷ Ectoine</td>
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<tr>
<td>Block of UVA-induced ceramide release in human keratinocytes</td>
<td>✷ Ectoine</td>
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<td>Protection of human RBC membranes</td>
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<td>Protection of mitochondrial DNA in human dermal fibroblasts</td>
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<td>Protection of skin</td>
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<tr>
<td>Protection of the skin barrier against water loss and drying out</td>
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<td>Protection of skin immune cells against UV radiation</td>
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<td>Reduction of UV-induced SBCs</td>
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<td>Prevention of UVA-induced photoaging</td>
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<td>Cytoprotection of keratinocytes</td>
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18
Food Industry

Potentially halophilic biomolecules can be used to enhance flavor of salted foods. *Carnimonas nigrificans* is the sole representative of the genus *Carnimonas* and it is responsible for the presence of black spot on the surface of food products in connection with organisms of different origin (Garriga *et al.*, 1998). *Cobetia sp.* and *Halomonas sp.* have been identified as predominant spoilage bacteria in water-boiled salted duck during storage (Liu *et al.*, 2010). Furthermore, a recent study carried out by Essghaier *et al.*, (2009) used moderately halophilic bacteria, including *Halomona elongata*, in the biological control against the grey mold caused by *Botrytis cinerea*, an economically important disease of strawberries in Tunisia and worldwide. The use of such bacteria may constitute an important alternative to synthetic fungicides and can be exploited in commercial production and application under storage and greenhouse conditions.

Exopolysaccharides

Microbial exopolysaccharides (EPSs) have aroused great interest among biotechnologists because of their wide potential range of applications in the fields such as medicine, pharmacy, foodstuff, cosmetics and the petroleum industry, where emulsifying, viscosifying, suspending, and chelating agents are required (Sutherland, 1998). To date many species of the family *Halomonadaceae* have been described as producers of EPSs. Due to their great biotechnological interest, several studies related to the isolation of exopolysaccharide-producing bacteria from different areas such as Spain, India, Morocco and Chile have been carried out (Bejar *et al.*, 1998; Bouchotroch *et al.*, 1999).
The first studies on EPS’s from halophilic microorganisms were performed in *Halomonas maura* (Bouchotroch et al., 2001) and *Halomonas eurihalina* (Calvo et al., 2002). *Halomonas* species able to produce EPSs are: *H. nitroreducens* (Gonzalez-Domenech et al., 2008a), *H. cerina* (Gonzalez-Domenech et al., 2008b), *H. sabkhae* (Kharroub et al., 2008), *H. alkaliphila* (Romano et al., 2006) and *H. Ventosae*. These EPSs give moderately viscous solutions if they are suspended at neutral pH but the viscosity increases enormously in the acidic pH range (Calvo et al., 1995). Another important property of the biopolymers synthesized by *H. eurihalina* is their ability to emulsify crude oil and other hydrocarbons much more effectively than do Tween 20, Tween 80 or Triton X-100 (Martinez-Checa et al., 2007). Perez-Fernandez et al., 2000) have described the effect of EPS V2-7 on the proliferation in vitro of human peripheral blood lymphocytes. The carAB genes, which encode carbamoyl- phosphate synthetase in *H. eurihalina* have been cloned and characterized; this enzyme is involved in the pathway for the synthesis of EPSs in strain F2-7 (Lamas et al., 2003). With respect to EPSs produced by *Halomonas maura* they have both viscosifying and emulsifying properties, particularly from strain S-30. EPS S-30, which has been designated as mauran, gives highly viscous solutions and in addition it is resistant to osmotic stress, changes in pH and to freezing–thawing processes (Arias et al., 2003). The epsABCJ genes that are involved in the biosynthesis of mauran have been cloned and characterized (Arco et al., 2005). Recently, a highly exopolysaccharide-producing *Halomonas* strain has been reported as causing epizootics in larval cultures of the Chilean scallop *Argopecten purpuratus* (Rojas et al., 2009).
The abundant potentiality of halophiles as model industrial organisms with vast applications in the biotechnological industries, cosmetics, therapeutic and agriculture, led us to take up this topic for the Ph.D programme with the following objectives as shown below: The Materials & Methods and Results & Discussion regarding these have been represented and discussed in Chapter wise for each biomolecules in this thesis.

**Objectives**

- Screening and characterization of halophiles
- Molecular characterization of Biomolecules
- Optimization and purification of Biomolecules
- Proteomic analysis of Biomolecules
- Characterization and Industrial applications of Biomolecules