Chapter 1

Introduction
Driven by increasing industrial demands for biocatalysts that can cope with industrial process conditions, considerable efforts have been devoted to the search for such enzymes. Compared with organic synthesis, biocatalysts often have far better chemical precision, which can lead to more efficient production of single stereoisomers, fewer side reactions and a lower environmental burden [Van der Burg, 2003]. Despite the fact that to date more than 3000 different enzymes have been identified and many of these have found their way into biotechnological and industrial applications, the present enzyme toolbox is still not sufficient to meet all demands. A major cause for this is the fact that many available enzymes do not withstand industrial reaction conditions. As a result, the characterization of microorganisms that are able to thrive in extreme environments has received a great deal of attention: such extremophiles are a valuable source of novel enzymes [Gomes and Steiner, 2004].

Extremophiles are bizarre microorganisms that can grow and thrive in extreme environments, which were formerly considered too hostile to support life. The extreme conditions may be high or low temperature, high or low pH, high salinity, high metal concentrations, very low nutrient content, very low water activity, high radiation, high pressure and low oxygen tension. Some extremophiles are subject to multiple stress conditions. Extremophiles are structurally adapted at the molecular level to withstand these harsh conditions. The biocatalysts, called extremozymes, produced by these microorganisms, are proteins that function under extreme conditions. Due to their extreme stability, extremozymes offer new opportunities for biocatalysis and biotransformation. Examples of extremozymes include cellulases, amylases, xylanases, proteases, pectinases, keratinases, lipases, esterases, catalases, peroxidases and phytases, which have great potential for application in various biotechnological processes. Currently, only 1-2% of the microorganisms on the Earth have been commercially exploited and amongst these, there are only a few examples of extremophiles. However, the renewed interest that is currently emerging as a result of new developments, in the cultivation and production of extremophiles and success in the cloning and expression of their genes in mesophilic hosts will increase the biocatalytic applications of extremozymes [Gomes and Steiner, 2004].

Halophiles are extremophiles which are distributed in hypersaline environments all over the world, mainly in natural hypersaline brines in arid, coastal,
estuarial and deep sea locations like hydrothermal vents as well as in artificial salterns [Hunter - Cevera et al., 2005].

Halophilic proteins employ different adaptation mechanisms. Proteins from halophilic organisms have a biased amino acid composition in order to remain stable and active at high ionic strength. Halophilic proteins typically have an excess of acidic amino acids (i.e. glutamate and aspartate) on their surface. These negative charges on the halophilic proteins bind significant amounts of hydrated ions, thus reducing their surface hydrophobicity and decreasing the tendency to aggregate at high salt concentration [Hough and Danson, 1999].

Halophilic microorganisms must offset an osmotic gradient across their membrane to survive in saline conditions. This is accomplished by the intracellular accumulation of organic compounds known as compatible solutes like ectoine, hydroxyectoine, glycine and betaine [Gomes and Steiner, 2004].

Under most conditions, these solutes do not inhibit the function of intracellular enzymes, and are capable of providing osmotic balance in a saline environment. Cells accumulate compatible solutes by either synthesis or environmental uptake. Uptake is a more energetically favorable option [Gomes and Steiner, 2004]; however, the proper solutes or precursor compounds are not always available in the environment. Transport systems, proteins, and enzymes catalyzing the uptake and de novo synthesis of compatible solutes are affected by changing environmental conditions, defining the organism’s ability to survive under a range of salinity. In addition to their function in the maintenance of osmotic strength, compatible solutes play roles in protein stabilization and solubility thus protecting cells against stresses like high temperature, desiccation and freezing [Gomes and Steiner, 2004]. The ability of halophiles to thrive in high salinity makes them candidates for industrial and bioremediation applications [Aston and Peyton, 2007].

Accelerating technological development has made it possible for humans to reach to one of the most remote parts of the Earth, the deepest areas of the sea, also called the Earth’s last frontier. About 15,000 natural products have so far been discovered from marine microbes, algae and invertebrates [Synnes, 2007].

Over billions of years, the ocean has been regarded as the origin of life on Earth. The ocean includes the largest range of habitats, hosting the most life-forms
and rich source of biological and chemical diversity. Marine microbes represent the greatest percentage of un-described marine species constituting as much as 10% of total living biomass carbon of the biosphere. However, only 300000 marine species have been discovered and described. Marine organisms are the key to earth’s habitability and have been a source of unique chemical compounds with the potential for industrial development, such as pharmaceuticals, molecular probes, enzymes, cosmetics, nutritional supplements, and agrochemicals. Each of these classes of marine bioproducts has a potential multi-billion dollar market value [Pomponei, 1999; Synnes, 2007].

Marine microbes also carry out many of the steps in these biogeochemical cycles, making them the workhorses of the biosphere [Hunter-Cevera et al., 2005]. Competition amongst microorganisms for space and nutrients in the marine environment is a powerful selective force, which has led to evolution. The evolution prompted the marine microorganisms to generate multifarious enzyme systems to adapt to the complicated marine environments. Therefore, marine microbial enzymes can offer novel biocatalysts with extraordinary properties. Enzymes have been isolated and purified from microorganisms, animals and plants; among them microorganisms represent the most common source of enzymes because of their broad biochemical diversity, feasibility of mass culture and ease of genetic manipulation. Marine microorganisms have been attracting more and more attention as a resource for new enzymes, because the microbial enzymes are relatively more stable and active than the corresponding enzymes derived from plants or animals [Zhang and Kim, 2010].

Marine enzyme biotechnology can offer novel biocatalysts with properties like high salt tolerance, hyperthermostability, barophilicity, cold adaptivity, and ease in large scale cultivation. The oceans represent a virtually untapped resource for the discovery of even more novel compounds with useful activity. A marine enzyme may be a unique protein molecule not found in any terrestrial organism or it may be a known enzyme from a terrestrial source but with novel properties [Ghosh et al., 2005]. These enzymes are used in pharmaceuticals, as cleansing agents, food additives, beverages, in waste water treatment, in control and removal of pollutants, for biotransformation and production of fine chemicals [Ghosh et al., 2005; Zhang and Kim, 2010]. In recent years, researchers have isolated a variety of enzymes with
special activities from marine bacteria, actinomycetes, fungi and other marine microorganisms, and some products have already been used in industrial applications. In particular, some marine microbial enzymes have yielded a considerable number of drug candidates. Marine microorganisms, whose immense genetic and biochemical diversity is still in its infant stage, are of considerable current interest as a new promising source of enzymes with unsuspected application potentials [Zhang and Kim, 2010].

Industrially important enzymes from marine bacteria have been extensively studied all over the world because of their tremendous potential as described below [Ventosa and Nieto, 1995; Ventosa et al., 1998; Coronado et al., 2000; Porro et al., 2003].

- Protein fragility is the main drawback in enzyme technology and hence enzymes from halophilic bacteria are sturdy and have capacity to function in stressful conditions.
- Enzymes from halophiles are stable not only to high salinity but also to high temperature.
- Halotolerant enzymes can favor enzymatic reactions carried out in organic solvents.

Importance of enzymes like phosphatase, arylsulphatase, asparaginase, glutaminase isolated from microbes of Indian marine environment has been reported by Chandrasekaran, [1997]. Mohapatra et al., [2003], have reported production of enzymes like amylase, carboxymethyl cellulose and protease by bacteria isolated from marine sedentary organisms of Bay of Bengal. Purification and characterization of solvent and detergent tolerant protease from marine bacteria isolated from Sundarbans was reported by Sana et al., [2006]. Studies on marine enzymes; carrageenase, agarase, protease and xylanase from Gujarat coastline have highlighted their potential biotechnological applications [Kambhaty et al., 2007; Kambhaty et al., 2008; Shah et al., 2010; Menon et al., 2010].

‘White’ biotechnology or industrial and environmental biotechnology is a broad and expanding field that includes making enzymes with a variety of industrial uses that include the manufacture of bioplastics and biofuels using micro-organisms and plants for the treatment of wastes and abatement of pollution, a process known as
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Chapter – 1

Introduction

The advent of PCR and other bio molecular techniques have led to a plethora of work related to biocatalytic abilities of enzymes and the source organisms. Amongst the novel methods for industrial enzyme discovery, directed evolution (DE) may be the most powerful. DE is a fast and inexpensive way of finding variants of existing enzymes that work better than naturally occurring enzymes under specific conditions. DE mimics natural evolution in that it depends upon the selection of fitter ‘individuals’, in this case enzymes, from a diverse population. DE is ‘directed’ in the sense that the researcher selects the variant enzymes that better meet some criterion via a series of step-wise improvements [Marrs et al., 2002].

Global enzyme demand will rise 6.3 percent annually through 2013, driven by strong demand in the specialty enzymes segment and good growth in animal feed and ethanol markets. North America and Western Europe will see healthy market gains while the fastest growth remains in developing countries. The world market for enzymes will recover from a difficult 2009 to reach $7 billion in 2013 [http://www.reportlinker.com/p0148002/World-Enzymes-Market.html].

Gujarat has a coastline of 1600 km with a rich unexplored microbial diversity. This provides a strong ground for conducting a study for isolating marine microbes producing extra-cellular industrially important enzymes.

Objective

The present investigation is directed to screening and identification of marine microorganisms from Gujarat coast for their extracellular hydrolytic properties pertaining to xylanase, amylase and lipase, optimization of physiochemical parameters for maximum enzyme production and studies related to enzyme kinetics.

The study also encompasses evaluation of a xylanolytic and moderately halo-alkaliphilic marine bacterium for its potential in enzymatic hydrolysis of lignocellulosic agricultural wastes for procuring significant value-added intermediates which could be taken as starting material for biofuel production and other biotechnologically important finished products. The current work also determines
hydrolytic activity of amylase on raw starch from different agricultural sources and its potential applicability in the starch industry.