CHAPTER I

INTRODUCTION
Nature’s problem solvers

In a handful of soil there are around 5,000 different microorganisms. They are nature’s workhorses, recycling garbage and releasing essential products back into the environment; they also produce the enzymes and other proteins that can be used to solve problems in industries and homes all over the world.

Growing better all the time

Using the oldest known biotechnology – fermentation grows microorganisms in large tanks. In 24 hours, one microorganism turns into trillions of microorganisms that all produce the enzymes and other proteins we require.

Better and more sustainable industrial products

Enzymes

Enzymes produced by the microorganisms in the tank to industries within for example Bioenergy, Food & Beverages, and Household Care.

One example is the textile industry in which the enzymes reduce the use of harsh chemicals, energy, and water, which means reduced costs and a cleaner environment.
Most of the reactions in living organisms are catalyzed by protein molecules called enzymes. Enzymes can rightly be called as the catalytic machinery of living systems. They have been used since the dawn of mankind in cheese manufacturing and indirectly via yeasts and bacteria in food manufacturing. The practical application and industrial use of enzymes has really taken off. It is the basis of the new industry called biotechnology. Biologically active enzymes may be extracted from any living organism. A very wide range of sources are used for commercial enzyme production. Of the hundred or so enzymes being used industrially, over a half is from fungi and yeast and over a third is from bacteria with the remainder divided between animal (8%) and plant (4%) sources (Burhan et al., 2003). The reason for the preferential use of microorganism as sources of industrial enzymes are; (i) their rapid growth (ii) limited space required for their cultivation (iii) easy availability of raw materials with constant composition for their cultivation (iv) low production cost (v) more predictable and controllable enzyme contents of microbes (vi) the ease with which they can be genetically manipulated to generate new enzymes with altered properties that are desirable for their various applications. In addition to protein engineering, there is always a chance of finding microorganisms producing novel enzymes with better properties and suitable for commercial exploitation. Only those microbes that produce substantial amounts of extracellular enzymes are of industrial importance. Thus, although enzymes are widespread in nature, microbes serve as a preferred source of these enzymes.

Extremophiles are microorganisms that can grow and thrive in extreme environments, like high or low temperature, high or low pH, high salinity, very low water activity, high pressure, low oxygen etc. They may be psychrophiles or may be thermophiles but
psychrophiles are true extremophiles as they are adapted not only to low temperatures, but frequently also to further environmental constraints. In the ocean depths and sediments they are faced with extremely high pressures, and therefore called as piezo-psychrophiles or baro-psychrophiles (Yayanos, 1995). The microbial communities that are found in sea ice, which comprise bacteria, algae, fungi and protozoa, are exposed to salt concentrations of several molar in brine veins, and are therefore termed as halo-psychrophiles (Staley and Gosink, 1999). Carpenter et al. (2000) reported that on the snow surface of glaciers and polar caps, psychrophiles are exposed to strong ultraviolet radiation. According to Friedmann (1982), the endolithic microbial communities that are found in rocks of the Antarctic dry deserts, which comprise lichens, yeasts, cyanobacteria and heterotrophic bacteria, survive low water and nutrient availability and in alpine caves and cracks, microorganisms also evolve in a poor environment in the absence of light called as troglo-psychrophiles.

These extremophiles are structurally adapted at the molecular level to withstand these harsh conditions and among them biocatalysts play a major role which are called as extremozymes produced by these microorganisms that function under extreme conditions. There are various extremozymes like 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 (Gome and Steiner, 2004). These psychrophilic enzymes are not only of extraordinary interest at the fundamental level to investigate the thermodynamic stability of proteins, but also to understand the relationship between stability, flexibility and their catalytic efficiency. In the field of biotechnology, cold-adapted
microorganisms contributed to much extent level but little is known about these microorganisms and optimum conditions for their use need to be carefully evaluated.

Amylase enzymes are ubiquitous in occurrence, being found in all living organisms and produced by various sources such as plants, animals and by variety of microorganism. Microbial amylase produced by various bacteria and fungi are among the most important hydrolytic enzymes and have been studied extensively since the advent of enzymology. Alpha-amylases (endo-1,4-α-D-glucan glucohydrolase, EC 3.2.1.1) belongs to the enzyme class of hydrolases which randomly cleaves the 1,4-α-D-glucosidic linkages between the adjacent glucose units in linear amylose chain of starch. Amylases have emerged as one of the leading biocatalyst with proven potential to find usage in a wide array of industrial applications. These enzymes account for about 30% of the world’s enzyme production (Maarel et al., 2002).

Microorganisms intricate a large array of amylases which are intracellular and/or extracellular. Intracellular amylases are important for various cellular and metabolic processes. Extracellular amylases are important as they hydrolyzed the insoluble starch into soluble end products like glucose and maltose which are later absorbed by cells for their utilization. The extracellular amylases are of commercial value and find multiple applications in various industrial sectors ranging from conversion of starch to sugar syrups, to the production of cyclodextrins for the pharmaceutical industry, etc. (Maarel et al., 2002). Moreover production of these extracellular amylases are advantageous if done through microbial sources, such as cost effectiveness, consistency, less time and space required for production and ease of process modification and optimization (Burhan et al., 2003).
Psychrophilic microorganisms living in polar deep-sea or any constantly low temperature environments such as glaciers and mountain regions produce enzymes adapted to function at low temperature. The two distinguished properties of cold-active enzymes with most obvious biotechnological applications are high catalytic activity at low temperatures and low thermo-stability at high temperatures. Cold-active enzymes offer economic benefits through energy savings by (i) overcoming the requirements for expensive heating, (ii) functioning in cold environments, (iii) providing increased reaction yields, (iv) accommodating high levels of stereo-specificity, (v) minimizing undesirable chemical reactions that could occur at high temperatures, and (vi) by facilitating rapid and easy inactivation of the enzyme when required. The ability to heat-inactivate cold-active enzymes has particular relevance to the food industry where it is important to prevent any modification of the original heat-sensitive substrates and products (Ramteke and Bhatt, 2007).

Psychrophilic enzymes can be very useful for industrial and domestic processes as amylases; especially cold-active alkaline amylases;

1. They can be used in detergents since washing clothes at low temperatures protect the colors of fabrics and reduce energy consumption.
2. In food industry cold active $\alpha$-amylase can be used for the reduction of haze formation in juices and retardation of staling in baking industry.
3. They are also very useful for paper industry as it reduces the viscosity of starch for appropriate coating of paper.
4. In Pharmaceutical Industry they can be used as a digestive aid.
5. Psychrophilic microorganisms have also been proposed for the biopulping, bioremediation of polluted soils and waste waters during the winter in temperate
countries, when the degradative capacity of the endogenous microflora is impaired by low temperatures.

6. Using enzymes with high activity below 20°C in food processing, limits the growth of other contaminating microorganisms, shorten the process times, and avoid designing expensive heating steps.

7. Cold-active α-amylases could be used in the brewing industry to speed the mashing phase at low temperatures.

8. In addition, reporter genes making cold-active microorganisms as valuable additions to the arsenal of molecular tools.

Although cold adapted microbes and their enzymes have great potential in biotechnological application, both from the national and international scenario, but it is apparent that very little is done. Novozymes-2005 report suggests that the estimated global cold-adapted industrial enzyme market has increased substantially, to a current value of US$ 2.1 billion and is expected to grow by 10-15% annually (Margesin, 2008). However, the taxonomy, ecology, biochemical and physiological characteristics of microorganisms producing cold-adapted enzymes have just started to be thoroughly investigated. Keeping this view it will be most fitting and appropriate to launch a programme on cold-active enzymes from cold-adapted microorganisms and their biotechnological application in developing countries like India. Therefore, the aim of the present study was to characterize cold-adapted extracellular amylase and explore the biotechnological potential of cold-adapted microorganisms and enzymes along with the upcoming approaches for discovering and developing novel amylases.
Objectives of the study:

1. Isolation and identification of cold-active α-amylase producing microorganisms.

2. Optimization of enzyme production by fermentation technology.

3. Extraction and purification of cold-active α-amylase.


5. Applications of enzyme as a detergent additive for cold washing.