CHAPTER I

INTRODUCTION
CHAPTER I

INTRODUCTION

Mankind has been exploiting the functions of enzymes since the ancient civilization. However, the knowledge on the molecular nature of the enzymes begins in the year 1833 when the enzyme diastase (now known as amylase), pepsin, urease etc. were successfully isolated and purified. Today about 4000 enzymes are known, and of these about 200 are used commercially. Lipase is one of such prolific enzyme whose existence was discovered nearly 100 years ago by the microbiologist C.Eijkmann in bacterial strains (Jaeger and Eggert, 2002).

Lipases (Triacylglycerol acylhydrolase, EC 3.1.1.3) constitute a group of enzymes defined as carboxyesterases that hydrolyse long chain acylglycerols at the lipid water interface (Castro et al. 2005; Boekema et al. 2007). The natural function of lipases is to hydrolyze triglycerides into diglycerides, monoglycerides, fatty acids and glycerol. In addition to triacyl glycerol a number of other low and high molecular eight carboxylic-, thiol-, poly-acid esters and amides are also accepted as substrates by these hydrolase enzymes.

Among all enzymes, lipases are gaining more importance. This great interest in lipases is mainly, owing to their properties in terms of enantioselectivity, regioselectivity and broad substrate specificity. Lipases are omnipotent enzymes found in animal, plant and microorganisms. Although lipases are of widespread occurrence throughout the Earth’s flora and fauna, they are found more abundantly in microbial flora comprising bacteria, fungi and yeast (Pandey et al.1999). Relatively smaller numbers of microbial lipases are characterized in comparison to plant and animals. The microbial lipases are more popular among researchers, owing to its availability, easy handling, multifold property and abundant supply. In nature, the lipases obtained from different sources shows variation in reaction specificity, thus making it a versatile and interesting element of study. For instance
from fatty acid side some lipases attack short chain fatty acid (acetic, butyric, capric, caproic etc.) and some unsaturated fatty acid, while many other attacks nonspecifically and randomly split the fatty acid from triglycerides. Today lipases stand amongst the most important biocatalysts carrying out novel reactions both in aqueous and nonaqueous media. This is primarily due to their ability to utilize wide spectrum of substrates, high stability towards extremes of temperature, pH and organic solvents.

Lipases from the microbial sources are known to have many properties with respect to their specificities as their production could be controlled during fermentation, cultivation could be done in inexpensive media and could be extracted easily. Most lipases isolated and reported are found to be mesophilic in nature, which cannot hydrolyze a substrate that exists in solid form at room temperature (Bayoumi et al. 2007). To overcome this problem a search for thermophilic nature of enzyme is on as thermophilic lipases show higher thermostability, higher activity at elevated temperatures, and often shows more resistance to chemical denaturation. This makes them ideal tools in industrial and chemical processes where relatively high reaction temperatures and organic solvents are used. Thermostable enzymes are usually derived from thermophilic microbial strains, which may be expected to produce intrinsically more heat stable enzymes. The industrial demand for the thermostable enzymes continues to stimulate the search of novel thermophilic microorganisms from various unexploited regions of the earth, as small numbers of bacterial strains producing thermophilic lipases have been reported in the last decade (Kambourova et al. 2003).

Research has been stimulated worldwide for the isolation of novel lipase primarily by two strategies such as i) screening natural resources for the isolation of microbial strains bearing lipolytic property, ii) application of molecular biology tools to develop desired properties from existing lipase (Dharmsthiti et al. 1999). However, the most common approach is the screening of microorganisms from natural resources. Among the natural resources hot springs are considered as one of
the potential natural resource for isolation of novel microbial strains (Satyanarayana et al. 2005).

Hotsprings are manifestations of geological activity and represent extreme environments. The study of hotsprings provides information about microbial communities, especially those that are more heterogeneous and those in which greater microhabitat diversity may lead to greater microbial diversity (Tiedje et al. 1997). In thermal springs, the temperature is above 40°C, and it is kept constant by continual volcanic activity. Besides temperature, other environmental parameters such as pH, available energy sources, ionic strength and nutrients influence the diversity of thermophilic microbial population. The best known and well studied geothermal areas are in North America (Yellowstone National Park), Iceland, Newzealand, Japan, Italy and Soviet Union. Hotsprings are situated throughout the length and breadth of India, such as Manikaran in Himachal Pradesh, Soldhar and Ringigad in Uttranchal and Bukreshwar in West Bengal, India (Satnarayana et al. 2005). In North Eastern part of the country, the hotsprings are located mainly in Assam, Meghalaya and Arunachal Pradesh. The soil and water from these natural resources are mainly utilized for the isolation of microbial strains as they are nutrient-rich environments and provide high proliferation of the microorganisms. The microbial diversity of each soil depends on nutrient availability and several physicochemical properties related to the climate and type of soil. The major microbial population found throughout the world in hotsprings belongs to the genus Bacillus, Geobacillus, Thermoactinomyces, Clostridium, Thermus, Methanococcus, Pyrococcus and Thermomyces etc.

The studies of thermophilic and hyperthermophilic organisms and their enzymes have generated a considerable interest as they are thermostable and resistant to protein denaturants, as compared to other organisms. These enzymes are natural models of stable proteins and are remarkable tools for developing innovative biotechnological processes. The vast majority of lipases reported in the literature are extra cellular enzymes that are secreted through external membrane of the
microorganisms into the culture medium (Houde et al. 2003). The lipase activity from many species has been investigated with respect to optimal pH, temperature, nitrogen and carbon sources (Gupta et al. 2004). Optimization of fermentation conditions for microbial lipase is of great interest, since culture conditions influence the properties of the enzyme produced as well as ratio of extra cellular and intracellular lipases (Taipa et al. 1992). Therefore, optimization of the lipase production has been focused on improving fermentation conditions such as carbon or nitrogen source, temperature, pH, aeration, using inducers and source of inoculum etc. (Schmidt- Dannert 1999; Sharma et al. 2001; Gupta et al. 2004, Bayoumi et al. 2007; Kiran et al. 2008). The induction in lipase production in microorganisms is a process that results in change in the phenotype, which allows the production of energy, required for its metabolism and microbial growth. Edible oils such as vegetable oils, canola oil, and olive oil are used mainly as inducers. Microbial lipases that have been reported so far could be classified as acidic, neutral and alkaline lipases with respect to their optimum pH, with molecular weights ranging from 20- 80 kDa (Macrae, 1983; Gupta et al. 2004).

The industrial deployment of lipases can either be in situ by cultivating the desired microorganism in the medium with a suitable substrate (especially in the food and tanning industries), or by ex situ application by using purified commercial lipases. The biocatalytic potential of microbial lipases in both aqueous and non aqueous media in last one and half decades have shifted industrial fronts towards utilizing this enzyme for a variety of reactions of immense importance. Most of the commercial application of enzymes does not always need homogenous preparation of the enzyme. However a certain degree of purity is required depending upon the final applications in industries such as fine chemicals, pharmaceutical, cosmetics and detergent industry. For industrial purposes, the purification strategies employed should be inexpensive, rapid, high yielding and amenable to large scale operations. The successful exploitation of enzymes depends generally on the development of efficient and reliable methods for their separation and purification. The ammonium sulfate precipitation of lipase and other enzyme is the commonly used techniques
because of its low cost, high solubility and its protective nature on the enzymes. However application of some other techniques such as foam floatation and micellar extraction using surfactants are also gaining importance in recent years (Sarada, 1994; Hayes and Marchio, 1998; Schugerl, 2000; Gupta et al. 2004).

In the recent times lipases have found profound application in the field of detergents. About 32% of the bulk lipase produced worldwide are consumed in detergent industries itself due to its superior stain removing properties. In 1995, detergent enzymes represented 30% of the total enzyme market, estimated at US $30 million. In 2000, this market reached US $1.5 billion (Kirk et al. 2002). Removal of oil and fatty deposits by lipase is attractive owing to its suitability under washing conditions. To be a suitable additive in detergents, lipases should be both thermophilic as well as alkalophilic and capable of functioning in the presence of the various components of washing powder formulations (Jaeger et al. 1994). The stability and activity of detergent enzymes were crucial factors, which greatly improve surfactant properties of lipases (Pandey et al. 1999). Lipases which are stable and work at alkaline pH (8 to 11) are usually suitable for detergents powders and liquids and hold good potential for their use in detergent industry Besides this, due to their diversity with respect to enzymatic properties and substrate specificities, lipases are employed in the flavor industry, dairy industry include flavor enhancement of cheese, acceleration of cheese ripening, and manufacture and manufacture of cheese like product and lipolysis of butter, fat and cream and also in wood industry where hydrophobic component of wood, which creates problems in producing good quality paper, may be removed by using lipase. Potential application of lipase catalyzed reactions for the production of renewable biodiesel fuel from the vegetable oil has been recently inspiring the researcher’s worldwide (Sharma et al. 2002). Microbial lipases in recent years have been widely used for biotechnological applications in fat, food ingredients, detergents, dairy and textile industries, production of surfactants, and oil processing (Sharma et al. 2001; Sharma et al. 2002; Gupta et al. 2004; Ateslier and Metin, 2006).
The majority of current biocatalytic approaches rely on either using free enzyme in solution or living cells, which complicates product separation from the catalyst. The immobilization of lipase could reduce the cost factor of the process due to its easy regulation of activity, reusability; improve stability and possibility of continuous operation (Cheetham et al. 1979; Sharma et al. 2001; Nawani et al. 2006). Although a variety of approaches exist for the immobilization of enzymes, the enzyme immobilization is still in its infant stage. Several solid supports have been used for the immobilization (Aucoin et al. 2004; Hwang et al. 2004; Palamo et al. 2004). Among the various supports celite, silica, cellulose, ethyl cellulose, carbon and synthetic polymer as well as rice straw and alumina beads have been used for immobilization. Though various methods on immobilization have been reported, it is difficult to access the relative merits of different approaches in many studies the scope and limitation of stability and recyclability of immobilized enzymes are not clearly defined (Faber, 1997; Kazlauskas and Bomscheuer, 1998).

The overall aim of the research was to isolate novel lipase producing strains from the unexploited and endemic regions of the Arunachal Pradesh and their potential application in the field of detergents. Northeast India has been identified as Indo-Burma biodiversity hotspot (Myers et al. 2000) and till now a very little work has been done to explore its vast biodiversity. Hot springs are considered to be potential source of thermophiles, which contain environment friendly enzymes that are stable under high temperatures and could be used in the place of more hazardous chemicals that have been concocted by the modern industry (Haki and Rakshit, 2003). The study has been carried out as of till now no reports have been there about the occurrence of this hot springs in this part of the country. Isolation and screening of the microbial strains from the diverse ecological niches of this region may lead to the isolation of the novel lipase producing strains. Hence an effort has been made through this study to isolate and screen microbial strains from the soil samples collected from the hot springs of Arunachal Pradesh, India for lipolytic activity, Optimization of various parameters for production of lipase, purification, characterization and the application of the isolated lipase in detergent
formulation for the production of industrially useful compounds. In the present investigation attempts have been made to further improve the catalytic properties of the enzyme via the use of different immobilization techniques and reaction conditions. The specific objectives of the investigation are-

1. Collection of the samples from hot springs.
2. Chemical analysis of water and soil samples of the hot springs.
3. Isolation of the lipolytic organisms that can produce thermostable lipase.
4. Screening of the lipase-producing bacteria.
5. Optimization of media and culture conditions for lipase production.
6. Purification to homogeneity and characterization of lipase.
7. Characterization of the isolated lipase.
8. Evaluation of the detergent property of the purified lipase in comparison with different commercial detergents.
9. Immobilization studies.