Chapter – 1
General Introduction & Objectives

At the heart of science is an essential balance between two seemingly contradictory attitudes—an openness to new ideas, no matter how bizarre or counterintuitive they may be, and the most ruthless skeptical scrutiny of all ideas, old and new. This is how deep truths are winnowed from deep nonsense. -Carl Sagan
General Introduction

As per estimates, fish processing industry generates enormous amounts of wastes which stand at 63.6 MMT globally and 3.3 MMT in India (FAO, 2010). The major non-edible by-products arising out of fish processing include viscera, skin, scales, bones and bone frames. One of the major causes of large scale waste accumulation is that most of the fisheries sector is highly unorganised and the wastes generated after domestic consumption are not properly disposed. These wastes are a rich source of proteins and lipids and efforts all over the world are being made by researchers to recover these bioactive components (Rustad, 2003). Fermentation is a biological method wherein micro-organisms in the form lactic acid bacteria (LAB) are used to generate acid in situ for preservation of waste or recovery of biomolecules (Rai et al., 2010). In addition, the interest of numerous research groups lies in the utilization of waste residues generated by industries as inexpensive substrates for microbial growth and metabolite production (Horn et al., 2005). Furthermore, fish waste is rich in microflora like LAB that could be exploited for numerous industrial applications.

LAB, frequently termed the “Lactics”, are beneficial microorganisms that occur naturally in several raw materials like milk, meat and flour used to produce foods (Rodriguez et al., 2000). They have traditionally been used in the food processing industry because of their ability to improve the organoleptic characteristics as well as the overall quality of foodstuffs (Aymerich et al., 2000). Several species of LAB have been explored for their potential as probiotics in human health such as inhibition of tumour cell line growth (Park et al., 1998), improvement of immune system (Kimura et al., 2006), treatment of diarrhoea and reduction of hypercholesterolemia (Reuter, 1997). Some of the metabolites produced by them, termed “bacteriocins”, are capable of interfering with the growth of
other microorganisms and can be applied to food systems (Vandenberg, 1993). Apart from antimicrobial peptides, LAB are also capable of producing proteolytic (Arizcun et al., 1997) and lipolytic enzymes (Lopes et al., 1999).

Lipases (triacylglycerol acylhydrolases; EC 3.1.1.3) are a class of serine hydrolases which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids over oil-water interface (Liu et al., 2006). They are of utmost importance to the food industry as they play a major role in flavor development which is an important organoleptic parameter. On evaluation of fermented meat products it was found that the hydrolytic and oxidative changes occurring in the lipid fraction during the maturation process are mainly due to bacterial lipases and esterases (Demeyer et al., 1974). Enzymes with lipolytic activity have been identified in LAB and their commercial applications in dairy foods have been well studied (Adams & Brawley, 1981; El Soda et al., 1986). Lipases also play a major role in the degradation of lipid-rich fish waste through enzymatic hydrolysis of lipids present in fish processing waste that could be exploited for in situ enrichment of PUFA (Rai et al., 2010).

Most of the industrial applications generally prefer microbial lipases over plant and animal lipases since they are diversified in their enzymatic properties and substrate specificity (Ghosh et al., 1996). Bacterial lipases of commercial importance are obtained from *Achromobacter*, *Alcaligenes*, *Bacillus*, *Bulholderia*, *Chromobacterium* and *Pseudomonas* (Saxena et al., 2003; Gupta et al., 2004). LAB are generally considered to be weakly lipolytic, as compared with other groups of microorganisms. However, it is believed that lipolytic activity by LAB plays an important role in the determination of the special aroma of many different cheeses suggesting their unique property and action. Certain LAB, which are used as bulk starter cultures (*Lactococcus* and *Lactobacillus*), together with some
other bacteria (Leuconostoc, Enterococcus, Pediococcus, and Micrococcus), which can survive pasteurization and/or contaminate cheese during maturation, are believed to significantly contribute to cheese fat hydrolysis (El Soda et al., 1995; Collins et al., 2003a). Furthermore, they are considered as GRAS and are used extensively as starter cultures in food and feed industries (Saito, 2004). Although there are reports on lactic acid bacterial lipase production (Lopes et al., 1999; Sarantinopoulos et al., 2001; Thapa et al., 2006), they are less in comparison to other microorganisms like Bacillus and Pseudomonas.

The major criteria for microbial lipase production include physico-chemical as well as nutritional factors since they are inducible enzymes. Optimization has become an indispensable tool in the designing of fermentation media as the medium composition significantly affects product concentration, yield and productivity (Gupta et al., 2004). Moreover, design of experiments and statistical analysis of the responses using Response Surface Methodology (RSM) has become one of the common practices in the field of Biotechnology. Optimization helps in increasing enzyme production facilitating easy extraction, purification and characterization. The growing demand for lipases has shifted the trend towards prospecting for novel lipases, improving the properties of existing lipases for established technical applications and producing new enzymes tailor-made for entirely new areas of application.

Against this background, the research work was planned and carried out. The major objectives of the study are outlined in the following section.
Objectives of the study

The research work was initiated with the following objectives-

1. Isolation of lipolytic LAB from fish processing waste
2. Optimization of conditions for enhanced lipase production
3. Characterization and properties of lipase
4. Application of lipase producing LAB isolates in selected food system