CHAPTER 1

GENERAL INTRODUCTION
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Extremophiles, able to live in unusual habitats, can potentially serve in a variety of industrial applications (Horikoshi, 2008). As a result of adaptation to extreme environments, extremophiles have evolved unique properties, which can provide significant commercial opportunities. The groups of bacteria that can grow under alkaline conditions in the presence of salt are referred as haloalkaliphiles. The dual extremity of halophiles and alkaliophiles make them interesting models for fundamental research and exploration of biotechnological potential (Dodia et al., 2008a and b; Joshi et al., 2008; Bominadhan et al., 2009; Purohit and Singh, 2011). Haloalkaliphilic bacteria have largely been studied from the concentrated hyper saline environments; Soda Lake, Solar Saltern, Salt brines, Carbonate springs and Dead Sea. The exploration of the natural saline and alkaline environments beyond the above boundaries is just the beginning (Patel et al., 2006; Thumar and Singh, 2007a and b; Dodia et al., 2008a and b; Joshi et al., 2008; Thumar and Singh, 2009; Purohit and Singh, 2011).

The enzymes from extremophilic organisms, particularly halophilic and haloalkaliphilic bacteria and archaeabacteria are relatively less explored. Among the enzymes, proteases are among the most important groups of industrial enzymes and account for about 60% of the total worldwide commercial enzymes (Horikoshi, 2008), two-third of them being obtained from microbial sources (Carvalho et al., 2008; Carolina et al., 2009). Several microbes have been investigated for these enzymes and over the years, Bacillus species have emerged as the key producers of extracellular proteases having potential applications in detergent, food, pharmaceutical, leather and chemical industries (Boominadhan et al., 2009; Carvalho et al., 2009; Joshi et al., 2008; Raj et al., 2010; Purohit and Singh, 2011). Most of the studies on haloalkaliphilic bacteria; however, have focused on diversity and phylogenetic analysis of the organisms and only limited information is available on their enzymatic and other biotechnological potential. With the advancement in molecular tools, it would be possible to expand the horizons of biocatalysis (Purohit and Singh, 2009; Siddhupura et al., 2010; Purohit and Singh, 2011; Horikoshi et al., 2011a and b; Karan et al., 2011a and b; Sudhir et al., 2011).
Maintenance of stability and activity in high salt is a challenge for halophilic and
d haloalkaliphilic proteins (Dodia et al., 2008; Wang et al., 2009). The enzymes from
extremely halophilic archaea and bacteria require high concentrations of salt for
activity and stability and inactivated in Escherichia coli unless refolded in the
presence of salts under in-vitro conditions. Recombinant DNA technology in
conjunction with other molecular techniques is being used to improve and evolve
enzymes leading to new opportunities for biocatalysts (Zhang et al., 2009;
Ni et al., 2009; Singh et al., 2010a). Therefore, cloning of the potential genes coding
for different enzymes would be an attractive preposition to begin with. The
developments related to cloning and expression of genes and solubilization of
expressed proteins from halophilic and other extremophilic organisms in heterologous
hosts will facilitate enzyme-driven catalysis (Machida et al., 1998; Kim et al., 1998;
Machida et al., 2000; Singh et al., 2002; Singh et al., 2009; Kumar et al., 2011).

Since 95-99% of microorganisms in majority of the habitats are not cultivable,
significantly limited information is available about their genomes, genes and encoded
enzymatic activities. Metagenomics is the large-scale study of the DNA of naturally
existing microbial communities rather than laboratory-cultivable organisms. It’s an
emerging approach to explore diversity and harness microbial potential based on the
analysis of the total genomic DNA of microbial communities in their natural
environments, followed by cloning and expression of the genes into a cultivable host
organism.

In the direction of metagenomics studies, isolation and analysis of environmental
data are the key steps, which would allow to mine microbial diversity and help
understanding the dynamics, properties and functions of these organisms (Desai and
Madamwar, 2007; Purohit and Singh, 2009; Siddhpura et al., 2010). For extreme
habitats, the metagenomics is least explored and understood. The advances in
metagenomics would revolutionize the investigations in microbial ecology and
biotechnology, leading to exploration of uncultured microbial population and
discovery of new enzymes for various applications (Risenfield, 2000). Diversity of
the organisms from a given habitat would certainly play significant role in commercial
successes, particularly in applications for which bulk enzyme or product quantities
have to be produced. The phylogenetic complexity of the environments can range over
orders of magnitude. Therefore, the potential for applications of haloalkaliphilic organisms in biotechnology seems endless.

India has enormous and unique coastline of the two oceans, a couple of gulfs and a bay. Among the various parts, Gujarat (Western India) accounts for 1600 km long coast, with industrial activities of mega projects, representing the remarkable diversity within the natural microbial flora. Of this, the Saurashtra region under Kathiawar peninsula occupies a total stretch of 865 km. Existence of halotolerant, haloalkalitolerant and haloalkaliphilic bacteria clearly indicated the wide spread distribution of such organisms in natural saline environment (Joshi et al., 2008).

Although, the field of microbial biotechnology is diverse, only limited attention has been paid to haloalkaliphiles particularly from moderately saline habitats. In realization of these facts, the present work in this thesis aims at the investigation of microbes from saline habitats of the Coastal Gujarat. The work was majorly focused on the microbial diversity, phylogeny, secretion of proteases and enzymatic characteristics. Further, cloning, over-expression and characterization of recombinant enzymes was undertaken. Metagenomics to find novel sequences of alkaline proteases from the saline habitat has also been attempted. Comparative studies of native, recombinant and metagenomic derived alkaline proteases for solvent tolerance would be significant for non-aqueous enzymology.
OBJECTIVES

- Isolation of Halophilic/ Haloalkaliphilic bacteria from saline habitats

- Detection, screening and optimization of extracellular enzymes among the Haloalkaliphilic bacteria obtained from saline environment

- Purification and characterization of alkaline proteases from potential strains with respect to enzyme catalysis and stability

- Cloning and sequencing of alkaline protease genes from potential strains of Haloalkaliphilic bacteria and characterization of recombinant enzymes

- To explore the diversity and novel alkaline protease genes from the uncultured organisms by sequence and function based approaches

- To elucidate structure and function relationship of alkaline proteases through bioinformatics tools