GENERAL INTRODUCTION

Living entities are found almost everywhere on the planet earth, including various extreme environments of harsh conditions. Extremophilic microorganisms are important with respect to biological adaptation, biotechnology and ecology. Among the extreme habitats, sea with the average salinity of 3.5 % (35 g/L, 0.6 M) accounts for one of the largest extreme habitats. Marine microorganisms represent a potential source of the biocatalysts and bioactive compounds with potential biomedical, environmental and other applications (Singh et al., 2013; Gohel et al., 2015; Romano et al., 2017; Sathiyanarayanan et al., 2017).

Microorganisms capable to grow under the high salinity possess extremozymes, proteins and adaptive strategies to withstand such harsh environmental conditions (Ventosa et al., 1998; Oren, 2002, 2008, 2013; Singh et al., 2013; Sinha and Khare, 2013, 2014a; Gohel et al., 2015; Raval et al., 2014; Barroca et al., 2017; Siddiqui et al., 2017; Zweerink et al., 2017).

In comparison to the extensive literature available on the physiology, halo-adaptation, biochemistry, enzymes, ecology and diversity of the halophilic/halo-alkaliphilic archaea and bacteria, the aerobic haloalkaliphilic marine actinobacteria (commonly known as actinomycetes) have not been studied at greater length. During the early investigations on the hyper-saline/saline environments, the halophilic actinobacteria of marine habitats were often neglected, while there are evidence of their occurrence in a wide variety of saline ecosystem; such as saline lakes, saltern ponds/lakes, desert, oceans, extreme arid environments, hypersaline soils/sediments, and salted artificial man-made environments (Bull, 2011; Subramani and Aalbersberg, 2012; Singh et al., 2013; Bennur et al., 2015; Gohel et al., 2015; Hozzein, 2015; Martin and McMinn, 2017; Pearce, 2017; Poli et al., 2017).

The halophilic microorganisms are classified according to their behaviour toward salt and several different classification schemes have been proposed (Trupener and Galinski, 1986; Ramos-Cormenzana, 1989; Vreeland, 1992), the most extensively used classification being the one proposed by Kushner, (1978) (Ventosa et al., 1998, 2008).
This classification is proposed on the basis of the salt requirement for growth. According to the classification, the microorganisms which require 15-30 % (2.5-5.2 M) NaCl are termed as extreme halophiles, whereas moderate halophiles, require 3-15 % (0.5-2.5 M) NaCl and slight/marginal halophiles are able to grow optimally at 1-3 % (0.2-0.5 M) NaCl concentrations (Ventosa et al., 1988; Oren, 2002, 2006, 2011, 2013; Hozzein, 2015). The salt requirement and tolerance varies according to the growth conditions, mainly temperature and medium compositions (Oren, 2006).

The halophiles are salt-loving microorganisms living in diverse environments with the capacity to balance the osmotic pressure in high salinity and extreme alkalinity (Sorokin and Kuenen, 2005; Sorokin, 2005; Hozzein, 2015). These microorganisms are affiliated to all the three domains of life: Archaea, Bacteria, and Eukarya. They are aerobic as well as anaerobic in nature (Oren, 2002, 2008).

The halophilic and halotolerant Actinobacteria are Gram-positive bacteria with high G+C content and muramic acid in the cell wall. They have striking metabolic diversity and play a significant role in the ecosystem (Hozzein, 2015). It is well known that few members of the phylum Actinobacteria are the richest source of the natural products amongst the prokaryotes (Berdy, 2005) and account for about 45% of all microbial bioactive secondary metabolites (Berdy, 2005; Hozzein, 2015).

The genus Streptomyces of the actinomycetes have a large number of species and are known to produce a diverse variety of antibiotics, novel bioactive secondary metabolites and other valuable substances of high commercial value (Anderson and Wellington, 2001; Vijayakumar et al., 2007; Kiruthika et al., 2013; Younis et al., 2016; Kamjam et al., 2017). The culture-dependent studies have included cultural and morphological studies, sediment pre-treatment, use of taxon-selective medium and biochemical fingerprinting (Sun et al., 2010; Singh et al., 2013; Gohel et al., 2015; Wang et al., 2017). With the advent of new tools, microorganisms have been classified and clustered and many ambiguities related to the taxonomic positions of many organisms addressed (McKelvey, 1982; Franz, 2005; Quast et al., 2012; Barka et al., 2016). These advanced tools and approaches have been successfully employed to identify and study the diversity of the cultivable and uncultivable microorganisms in a
particular ecosystem. A large number of the microbial population remains unidentified and inaccessible and hence metagenomics has come into existence (Purohit and Singh, 2009; Cloney, 2017; Kikani et al., 2017; Zotchev et al., 2017).

Enzymes are obtained from different sources, including plants, animals and microorganism. The enzymes from haloalkaliphilic microorganisms have certain properties that make them suitable for various applications under harsh conditions (Ventosa et al., 1998; Oren, 2008; Singh et al., 2013; Amoozegar et al., 2017; Harding et al., 2017; Popinako et al., 2017). *Bacillus* and *Streptomyces* have been the conventional sources of the enzymes, particularly the proteases. The other genera of the actinomycetes, particularly those from the extreme environment are not explored for their enzymatic potential. Moreover, due to the limited available information on haloalkaliphilic actinomycetes, it is would be interesting to investigate their extremozymes. In view of these facts, the present work has focused on the diversity of the actinomycetes of the Arabian sea of the Coastal Gujarat and characterization of their extracellular proteases.
Research Objectives

- Physico-chemical analysis of the seawater samples collected from the Arabian Sea water off Okha Port (22.46 °N and 69.08 °E), Dwarka Beach (22.23°N and 68.97°E) and Somnath Beach (20.88° N and 70.40° E)

- Isolation, distribution and maintenance of the cultivable sea water actinomycetes using various microbiological techniques and differential media

- Studies on the phenotypic, biochemical fingerprinting and chemotaxonomic characteristics, antibiotic susceptibility, multiple antibiotic resistance (MAR) indexes, antimicrobial activities

- Cluster analysis and diversity evaluation by phenograms based on phenotypic characters

- Assessment of the actinobacterial diversity by a combination of the conventional microbiological techniques and molecular characterization by 16S rRNA gene sequencing and amplified 16S rDNA restriction analysis (ARDRA)

- Phylogenetic analysis using various bioinformatics tools to establish the phylogenetic relatedness

- Assessment of the ecological diversity indices to evaluate species richness and evenness in the marine ecosystem

- To examine the relationships of the biological or response variables to their environment by Canonical Correlation Analysis (CCA)

- Exploration of the biocatalytic potential of the sea actinomycetes for the extracellular enzymes on selective media plates, mainly proteases, amylases, and cellulases
• Optimization of various physico-chemical parameters, affecting the production of extracellular alkaline proteases

• To investigate the effect of various amino acids on the repression of alkaline protease synthesis in marine actinomycetes

• Protease production in response to various organic solvents

• Purification and characterization of alkaline proteases with respect to enzyme stability, enzyme kinetics, solvent tolerance and protein denaturation and thermodynamics

• Investigation of the structural attributes of the protease by the Circular Dichromism (CD) and Fourier Transfer Infrared spectroscopy (FT-IR)

• Assessment of the suitability of the enzyme in deharing, feather degradation, gelatin hydrolysis and silver recovery in used X-ray films and deproteinization in the waste crab shell
A GLIMPSE ON THE RESEARCH

Marine Actinomycetes

Bio-catalytic Potential
- Purification, Biochemical characterization
  - Biochemical properties, Kinetic parameters, Thermodynamics, Structural elucidation, Applications
- Production, Repression, Partial purification
  - Industrial applications

Actinobacterial Diversity
- Isolation, Biochemical fingerprinting, Multiple antibiotic resistance indexes, Diversity indices, Chemotaxonomic analysis
- 16SrRNA gene sequencing, Bioinformatics analysis, Amplified rDNA restriction analysis