1.0 INTRODUCTION

1.1 Solar saltwork environment

One-third of the worldwide sodium chloride production (about 200 million tons per year) is manufactured through solar saltworks. India is the fourth largest salt producing country in the world with an average annual production of about 145 lakh tonnes. Salt, the common name for the compound of sodium (\(\text{Na}^+\)) and chloride (\(\text{Cl}^-\)), is the first substance after water to have attracted humans' attention in their evolution from wilderness to civilisation. Both its significance in the creation of life itself on the planet and its importance as a commodity are paramount (Herrmann et al., 1973; Young, 1977). Life exists over the whole range of salt concentrations encountered in natural habitats from freshwater environments to hypersaline lakes such as the dead sea, saltern crystallizer ponds, and other places saturated with respect to sodium chloride (Cayol et al., 1994).

Multi-pond solar saltworks provide a wide range of environments characterized by an increasing salinity, along the salt production circuit, that varies from seawater levels up to sodium chloride saturation and sometimes even above saturation (Rodriguez-Valera, 1988; Javor, 1989). As water evaporates and salinity increases, the remaining water is pumped or flown by gravity to the following pond, so that salinity in each specific pond, along the circuit, is kept within narrow limits, i.e., basically constant. Each pond can thus be considered in chemical equilibrium, meaning that it is characterized by a specific habitat, to which a well-adapted and established community can be associated (Pedros-Alio et al., 2000).
Saltwaters are a specific type of coastal wetland’s characterized by the permanent flooding of 90% of the area with brine for salt production. This integrated coastal ecosystem is specially designed according to characteristics of the area (e.g., geomorphology, climate, tides flux); they are unique in terms of their architecture, and by combining their production process with the conservation of the coastal biodiversity (e.g. phyto and zooplankton, fishes, birds) (Korovessis and Lekkas, 2009; Lopez et al., 2010).

Worldwide saltworks can vary greatly in terms of the concentrations of inorganic nutrients and of the brines (the solution of salts). These variations might depend on geographical location, season and management practices, among other factors (Oren, 2000). Although these systems have been extensively studied in many geographic locations, Brasiliensia are organizing developing methods and techniques able to assist on brine management (Davis, 2000, 2009). The nutrient concentrations within a single pond can also vary considerably even within a few days, as shown in the study by Joint et al. (2002) in the saltworks of Santa Pola, (Alicante, Spain). Nutrients (nitrogen and/or phosphorus) are sometimes added as fertilizers to enhance the development of benthic microbial mats or planktonic communities of light-absorbing microorganisms (Oren, 2009).

Attached seaweeds and seagrasses, small animals, and microscopic organisms inevitably develop a biological system composed of planktonic benthic communities in the ponds of every solar saltworks. The plants, algae, and bacteria with photosynthetic pigments use sunlight energy and inorganic nutrients (e.g., combined nitrogen and phosphate) to manufacture organic matter, but the entire group of organisms consumes
and oxidizes these substances. The biological system can help or harm salt production (Davis, 1993; Sammy, 1983).

1.2 Extremophilic microbes

Study of extremophilic microbes and identification of their metabolic properties are most important tasks in biotechnology (Vonothini et al., 2008). The discovery of extremophiles has drastically changed our understanding towards the diversity of life itself and the conditions under which it can be sustained (Atomi, 2005). Extremophiles are organisms that are able to live and reproduce in extreme environments. They are adapted to live at high temperatures in volcanic springs, at low temperatures in the polar regions, under high pressure in the deep sea, at very low and high pH values (pH 0-3; acidophiles or pH 10-12; alkaliphiles), temperature (-2°C to 15°C; psychrophiles; and 60°C to 115°C; thermophiles) or at very high salt concentrations (5 – 30 %) (Fujiwara, 2002; Van den Burg, 2003).

Saline and hypersaline environments are widely distributed throughout the world either salt lakes or salt mines. These hypersaline environments are too harsh for normal life to exist, but a variety of microbes, such as Bacteria and Archaea, can survive. These organisms have evolved to exist in these extreme environments which can fall into a number of different categories, including halotolerant, moderately, borderline and extremely halophilic have adapted to thrive in ecological niches in saline and hypersaline environments (Kushner and Kamekura, 1988). As a result, these microorganisms can produce unique enzymes and metabolite and are able to develop biological activity in conditions in which their counterparts could not be functional. These properties could be exploited for the development of additional bio-industrial
processes designed basically on the optimal conditions of these biomolecules (Kamekura, 2002).

Two fundamentally different approaches are involved in order to cope with osmotic challenges associated with life in saline environments (Madigan and Oren, 1999). In the case of halophilic Bacteria, the cytoplasm contains low concentrations of salt compared with surrounding environment, but it has high organic solute levels. The halophilic Archaea (halobacteria), on the other hand, have developed an entire biochemistry that functions at saturating salt concentrations (Oren, 2002). The increasing numbers of available data on haloarchaeal genomes promise a major increase in the use of halophilic extremozymes in a variety of applied fields in the near future. These data will help to reveal novel halophilic enzymes with biotechnological potential in various economical and technological fields.

There is a general argument that less than 10 % of the living organisms in a defined environment are cultivable and further improvement of gene expression technologies will help to increase the exploration of microbial diversity (Schloter et al., 2000). It could be possible to construct gene expression libraries from the most diverse sources, which might lead to the discovery of many new extremozymes in the near future. These extremozymes will be used to design a wide range of novel biocatalytic processes that are faster, more accurate, specific and environmentally friendly. In order to increase the role of extremozymes in various areas of industries, it is necessary to develop concurrent protein engineering and convergence of bio/nanotechnologies (Eichler, 2000). The great economic potential of extremozymes will bear plenty of fruits in various industrial processes, agricultural, food, chemical and pharmaceutical from further exploration of extremozymes (van den Burg, 2003).
1.3 Haloalkaliphilics

Haloalkaliphilic are the group of organisms with twin extremities of pH and salinity, which have been investigated after isolation from a variety of habitats (Jeon et al., 2005; Kalyuzhnaya et al., 2008; Singh et al., 2012; Sorokin et al., 2005). The limited studies on the unique applications of extremophiles indicate that the unique “survival strategies” available for these organisms. Alkaline adapted microorganisms can be classified into two main groupings, alkaliphiles (also called alkalophiles) and alkalitolerants. The term alkaliphiles (alcali from arabic, soda ash, phile, loving) is generally restricted to those micro-organisms that actually require alkaline media for growth. The optimum growth rate of these microorganisms is observed in at least two pH units above neutrality. Organisms capable of growing at pH values more than 9 or 10, but with optimum growth rates at around neutrality or less, are referred to as alkalitolerant (Jones et al., 1994; Grant and Tindall, 1980; Kroll, 1991). In the media used to isolate alkalophilic bacteria, a sample could be enriched with different substrates such as peptones, glucose, ox bile, casamino acids and caseine (Duckworth et al., 1996). The pH of the small scale cultures grown in media is controlled by Na₂CO₃ or an equivalent amount of Na₂CO₃.10H₂O (maintaining the pH values at 10-11), and/or Borax/NaOH, Na₂HPO₄/NaOH buffer systems (buffering capacity over the range of pH 9-12 in various media) (Grant and Tindall, 1980).

Naturally occurring environments with extreme pH values which support microbial growth are widely distributed. Often, organisms growing in these environments experience far more neutral pH values than the average value of their ecosystem owing to the nature of their microenvironment (Grant, 1992). The soda lakes in the Rift Valley of Kenya and similar lakes found in other places on Earth are highly
alkaline with pH values of 11 to 12. The lakes are usually saline to varying degrees (5 % to 30 % w/v NaCl) (Tindall, 1988). One of the most striking features of many alkaline, saline lakes is the coloration of the waters. Depending on a variety of conditions related to water chemistry, dense populations of microorganisms may colour the lakes green, orange, purple or claret. In many cases it has been possible to show that this overt indication of microbiological activity is due to blooms of specific algae, cyanobacteria, eubacteria or archaebacteria (Tindall, 1988).

Microorganisms are highly efficient in their ability to produce many kinds of bioactive compounds. A large number of antibiotics have been shown to produce by various types of bacteria, such as actinomycetes. Screening bacteria from alkaline habitats or those grown under extreme cultural conditions remains a profitable area for investigation. Some new antibiotics were produced by certain bacteria when an alkaline medium with high alkalinity (pH 9 to 10.5) was used (Sato et al., 1983). The alkaliphilic actinomycete Nocardiopsis strain, a producer of phenazine, successfully grew at pH 10.0 in culture medium (Tsai et al., 1995). In a recent research study, microorganisms isolated from the alkaline saline Lake Acigol in Turkey were screened for their activity against other microorganisms (Eltem and Ucar, 1998). The discovery of these bioactive compounds provides evidence that organisms from such environments are also capable of producing antibiotic type compounds.

1.4 Actinomycetes

Actinomycetes are the dominant group of soil population together with bacteria and fungi. They are Gram-positive bacteria having high G+C (>55 %) content in their DNA and are originally considered as an intermediate group between bacteria and
fungi (Naikpatil and Rathod, 2011) filamentous nature found in most environments including terrestrial and aquatic habitats (Strzelczyk et al., 1969). Actinomycetes which exhibit considerable physiological and biochemical diversity and the order are so diverse in terms of morphology, phylogeny and chemotaxonomy (Kekuda et al., 2010). This group was initially classified based on their branching of filamentous morphology which occurs during the growth cycle (Willey et al., 2010). Thus, due to the presence of the filamentous forms which branches out, these organisms were wrongly classified as fungi for many years before they were rightly placed in the bacteria kingdom (Madigan et al., 2009). Actinomycetes is an important class of microbial resources, they are important producers of antibiotics and other important bioactive substances. So far, about two-thirds of the world's antibiotics were secreted by actinomycetes (Liu, 2002). Recent reports have revealed that salt requiring marine actinomycetes are a vigorous source of new natural products and serve as model systems in drug discovery (Marderosian, 1969; Fencial, 1997).

Actinomycetes are an important group of microorganisms due to their ability to produce a wide array of secondary metabolites. Almost 80 % of the world’s antibiotics are known to come from actinomycetes, mostly from the genera Streptomycyes, Micromonospora and Nocardiopsis (Pandey et al., 2004). A wide taxonomic range of actinomycetes have the ability to produce secondary metabolites with biological activities such as antibiotic, antifungal, antiviral, anticancer, enzyme, immunosuppressant and other industrially useful compounds (Baltz, 2007; Olano et al., 2009; Demain and Sanchez, 2009; Kekuda et al., 2010; Naine et al., 2011, Newman and Cragg, 2007).
Streptomyces are gram positive (Lechevalier and Lechevalier, 1970a, Lechevalier and Lechevalier, 1970b) free living, saprophytic bacteria which comprise a group of branching unicellular microorganisms. They produce branching mycelium which may be of two kinds (substrate mycelium and aerial mycelium). Among actinomycetes, the Streptomyces group is one of the dominant genera. Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and over 10,000 of these compounds are produced by actinomycetes, representing 45% of all bioactive microbial metabolites discovered. Around 7,600 compounds are produced by Streptomyces species. Many of these secondary metabolites are potent antibiotics, vitamins, alkaloids, plant growth factors, enzymes and enzyme inhibitors which has made Streptomyces the primary antibiotic-producing organisms exploited by the pharmaceutical industry (Bonjar et al., 2004; Berdy, 2005).

The genus Nocardiopsis was proposed by Meyer (1976) on the basis of chemotaxonomic and morphological characteristics. At the time of writing, the taxon encompasses 24 recognized species (Li et al., 2006), which form a distinct clade within the evolutionary radiation occupied by members of the family Nocardiopsaceae (Rainey et al., 1996; Cui et al., 2001; Kroppenstedt and Evtushenko, 2006). Nocardiopsis strains are frequently isolated from saline and alkaline soils (Li et al., 2004, 2006; Hozzein et al., 2004). Nocardiopsis strains are distributed ubiquitously in the environment (Kroppenstedt and Evtushenko, 2002). Recent reports have shown that Nocardiopsis strains are frequently isolated from alkaline soils with high salt concentrations (Mikami et al., 1982; Al-Tai and Ruan, 1994; Yassin et al., 1993). Many of the Nocardiopsis species prefer moderately alkaline conditions (pH 8.5) (Kroppenstedt, 1992) and some grow better on media supplemented with sodium chloride.
1.5 Bioactive compounds from actinomycetes

Microbial natural products are an important source of both existing and new drugs. Among the producers of commercially important metabolites, bacteria have proven to be a prolific source with a surprisingly small group of taxa accounting for the vast majority of compounds discovered till date (Bull, 2004). Among these, Actinomycetes are the most economically and biotechnologically priceless prokaryotes. Representative genera of actinomycetes include Streptomyces, Actinomyces, Arthrobacter, Corynebacterium, Frankia, Micrococcus, Micromonospora and several others. Secondary metabolites produced by actinomycetes possess a wide range of biological activities (Berdy, 2005; Mann, 2001; Pecznska Czoch and Mordaski, 1988). The genus Streptomyces alone produces a large number of bioactive molecules (Reeveset et al., 1998).

Progress has been made recently on drug discovery from actinomycetes by using high throughput screening and fermentation, mining genomes for cryptic pathways, and combinatorial biosynthesis to generate new secondary metabolites related to existing pharmacophores (Baltz, 2008). Actinomycetes comprise about 10% of the bacteria colonizing marine aggregates and can be isolated from marine sediments (Ward and Bora, 2006). Many actinomycete isolates from deep oceans contain non-ribosomal polyketide synthetase (NRPS) and polyketide synthetase (PKS) pathways, the hallmarks of secondary metabolite production (Salmon et al., 2003). There is an occurrence of distinct rare genera in the marine ecosystem as evidenced by the taxonomic description of the first marine actinomycete *Rhodococcus marinonascens* (Helmke and Weyland, 1983).
Secondary metabolites produced from marine actinomycetes have distinct
c hemical structures, which may form the basis for the synthesis of new drugs (Mincer,
2005). Enrichment and selective isolation methods can also be used to isolate rare
actinomycetes from marine ecological niches having the potential to biosynthesize
novel bioactive compounds (William et al., 2007). A great hurdle however, in the
search of these actinomycetes is that more than 90 % of the organisms remain
uncultivable under laboratory conditions. To explore the genomic diversity of the
marine ecosystem and estimate their biosynthetic capability, the techniques of
metagenomics can be used. But large insert metagenomic libraries can be prepared
from marine samples with ease. By designing a suitable vector, which can
accommodate large size inserts, it is possible to isolate novel bioactive compounds
from marine unculturable actinomycetes (Schmeisser et al., 2007; Sharma et al., 2005).

1.6 Application of bioactive compounds

Halophilic organisms, during the evolution, acquired the capability to produce
secondary metabolites with unique biological activity (Imhoff et al., 2011). These
compounds have found a wide range of applications as antibacterial (Teasdale et al.,
2009; Plaza et al., 2010), antifungal (Nishimura et al., 2010), antimalarial, antiprotozoa
(Dos Santos et al., 2011), and antiviral (Cheng et al., 2010), as well as being active in
diseases related to the cardiovascular, immune, and nervous systems (Asolkar et al.,
2009; Sakurada et al., 2010; Mayer et al., 2013). Metagenomics revealed to be a very
powerful tool also for the exploitation of bioactive compounds from marine bacterial
communities, since it is extremely hard to isolate and cultivate symbiotic bacteria of
marine macroorganisms, e.g., sponges that has been recently indicated as promising
source of novel compounds, in particular as anticancer, by a large body of literature
(Schirmer et al., 2005; Kennedy et al., 2007).
Actinomycetes have been described as the greatest source of antibiotics since Waksman introduced Streptomyces into his systemic screening program for new antibiotics in the early 1940s. Actinomycetes have provided about two-thirds (more than 4,000) of the naturally occurring antibiotics that have been discovered, including many of those important in medicine, such as aminoglycosides, anthracyclines, chloramphenicol, macrolides, b-lactams, and tetracyclins (Nisbet, 1982; Iwai and Takahashi, 1992). In addition to antibiotics, a variety of different approaches using fermentation broths of actinomycetes have been utilized for the development of novel agents for cancer therapies. Until now, cytotoxic substances, such as actinomycin D, mitomycin C, bleomycin and doxorubicin, originating from streptomycetes have been mainly used in cancer therapies (Nakae et al., 2000). However, to overcome the secondary effects of these compounds, intensive searches have been aimed at identifying more selective and novel structural agents.

Actinomycetes are of special interest, since they are known to produce chemically diverse compounds with a wide range of biological activities (Bredholt et al., 2008). The demand for new antibiotics continues to grow due to the rapid emerging of multiple antibiotic resistant pathogens causing life threatening infection. Although, considerable progress is being made within the fields of chemical synthesis and engineered biosynthesis of antibacterial compounds, nature still remains the richest and the most versatile source for new antibiotics (Baltz, 2006; Pelaez, 2006). Traditionally, Actinomycetes have been isolated from terrestrial sources although, the first report of mycelium forming Actinomycetes being recovered from marine sediments appeared several decades ago (Weyland, 1969). Recently, the marine derived Actinomycetes have become recognized as a source of novel antibiotic and anticancer agent with
unusual structure and properties (Jensen et al., 2005). Actinomycetes represent a ubiquitous group of microbes widely distributed in natural ecosystems around the world and especially significant for their role on the recycling of organic matter (Srinivasan et al., 1991). The literatures suggested that, marine sediment sources are voluble for the isolation of novel Actinomycetes with the potential to yield useful new products (Goodfellow and Haynes, 1984). However, it has been resolved whether Actinomycetes form part of the marine microbial community of sediment samples originated from terrestrial habitats and were simply carried out to sea in the form of resistant spores (Weyland, 1981; Goodfellow and Williams, 1983). Microorganisms found in marine environments have attracted a great deal of attention, due to the production of various natural compounds and their specialized mechanisms for adaptation to extreme environment (Solingen et al., 2001).

1.7 Halophilic enzymes

Enzymes are increasingly being used in the chemical industry as catalysts for numerous reactions. The global market of enzymes is estimated at around US$1.5 billion and is expected to grow by 5–10 % annually (Lievonen, 1999). Compared with conventional chemical catalysts, enzyme catalysis is highly specific (Scheper, 1999; Bommarius, 2004) and it functions under temperatures, pressures and pH that are compatible with life (Abramovicz, 1990). About 75 % of the enzyme used by value is accounted for by the detergent, food and starch processing industries. These are mostly hydrolytic enzymes such as proteases, amylases, lipases and cellulases. Specialty enzymes account for around 10 % of the enzyme market and are finding increasing uses in the development of new drugs, medical diagnostics and numerous other analytical uses (Roberts et al., 1999).
Extremophilic enzymes or extremozymes, are finding increasing industrial use because of their ability to withstand extremes of temperatures and other conditions (Eichler, 2001). Enzyme catalysis in nonaqueous media has created new possibilities for producing useful chemicals such as modified fats and oils, structured lipids and flavor esters (Sharma et al., 2001; Krishna, 2002). These enzymes are already being used in many biotechnological applications providing economic benefits and energy savings. As a result of their high activity at mild temperatures or fast heat inactivation, a lower concentration of the enzyme is required to reach a given activity reducing the costs of enzyme preparation. Also, they can minimize undesirable chemical reactions that can occur at higher temperatures (Cipolla et al., 2012). These properties are of particular relevance for the food and feed industry to avoid spoilage and change in nutritional value and flavor of the original heat-sensitive substrates and products (Cavicchioli et al., 2011; Florczak et al., 2013).

1.8 Non ribosomal peptide synthetase (NRPS)

A broad range of biologically active polyketide and peptide compounds with applications in medicine, agriculture, and biochemical research are synthesized by type-I polyketide synthases (PKS-I) and nonribosomal peptide synthetases (NRPS) (Beyer et al., 1999). They possess a large range of biological activities, with functions ranging from chemical defence against other microorganisms (vancomycin), to iron sequestration (yersiniabactin) or components of the cell wall (glycopeptidolipids). Moreover, some of these peptides, such as the antibiotic vancomycin, the immunosuppressor cyclosporin or the antitumour agent bleomycin have proved to be of great therapeutic value. A large number of therapeutically useful cyclic and linear peptides of bacterial or fungal origin are synthesized via a template directed, nucleic
acid independent nonribosomal mechanism. This process is carried out by mega-
enzymes called nonribosomal peptide synthetases (NRPS) (Bingle and Lazarus, 1999;
Nicholson et al., 2001; Sauser et al., 2002).

Polyketides are a large family of medicinally important natural products, which
are formed through the condensation of acylthioester units such as malonyl-CoA and
methylmalonyl-CoA to yield metabolites with diverse structures and biological
activities. Broadly speaking, there are three separate types of polyketide synthases
(PKSs) recognized in bacteria. Multimodular PKSs consist of one or more large
multidomain polypeptides where the growing polyketide chain is sequentially passed
from one active site to the next. Depending on the nature of their constituent catalytic
domains, these megasynthases generate chemical variety and complexity in a stepwise
fashion (Fischbach and Walsh, 2006). In contrast, iterative PKSs are comprised of a
single set of catalysts that assemble a polyketide of controlled chain length through
repetitive use of active sites (Hertweck et al., 2007). In both cases, the nascent
polyketide product is frequently acted on by further tailoring enzymes to generate the
antibiotic. A third type of PKS (called type III PKSs) is fundamentally different in that
the growing polyketide chain is never directly attached to a protein (Austin and Noel,
2003).

Earlier phylogenetic studies have suggested that PKSs share a complex
evolutionary history among themselves and with prokaryotic and eukaryotic fatty acid
synthases (Jenke Kodama et al., 2005; Kroken et al., 2003). The most widely studied
family of iterative PKSs is one that is responsible for the biosynthesis of several
polyfunctional aromatic antibiotics such as actinorhodin, tetracycline, doxorubicin, and
frenolicin. Its core set of enzymes includes a heterodimeric ketosynthase (KS) and chain length factor (CLF), an acyl carrier protein (ACP), and a malonyl-CoA:ACP transacylase (MAT) usually recruited from fatty acid synthases. Together these four proteins comprise the minimal PKS necessary to generate a polyketide (Reeves, 2003). The presence or absence of these accessory enzymes therefore plays an important role in the diversity of aromatic polyketides found in nature.

The synthesis of many complex polyketide antibiotics in bacteria is catalyzed by multimodular PKSs. The core of each module consists of a KS, acyltransferase (AT), and ACP domain. Together they extend the growing polyketide chain by two carbon atoms and also generate an ACP bound β-ketoacyl intermediate. The β-keto group can be modified by optional accessory domains, such as KR, dehydratase (DH), and enoyl reductase (ER) domains, which are typically attached to the core module. It has been suggested multimodal PKSs arose from gene and intragene module duplication, and that the prototypical PKS module shares ancestry with a vertebrate fatty acid synthase (Cortes et al., 1990; Donadio et al., 1991). By understanding the nature’s strategies for evolving novel multimodular PKSs, it may be possible to obtain useful clues for biosynthetic engineering in the laboratory.

NRPS are organized as iterative modules, one for each amino acid to be built into the peptide product. Usually the order of such modules is colinear to the sequence of the synthesized peptide, presenting an assembly line comparable to a linear workflow (Stein et al., 1996, Marahiel et al., 1997). A typical module comprises 1000 residues and is responsible for one reaction cycle of selective substrate recognition and activation as adenylate, covalent intermediate fixation as an enzyme-bound thioester,
peptide-bond formation. The reaction cycle, is accomplished by the division of the working steps to specialized semiautonomous domains. A minimal elongation module consists of a 55 kDa adenylation (A) domain, responsible for substrate selection and activation through ATP hydrolysis (Turgay et al., 1992, Stachelhaus, and Marahiel, 1995).

Nonribosomal peptide synthetases are multimodular enzymes that make nonribosomal peptides through a thiotemplate mechanism independent of ribosomes. Nonribosomal peptides can be composed of D- and L- amino acids, protein and nonprotein amino acids, hydroxy acids, ornithine, amino acids, and other unusual constituents (Schwarzer et al., 2003). Nonribosomal peptides can be linear, cyclic, or branched cyclic and may be modified by glycosylation, N-methylation, or acylation. In addition to structural diversity, nonribosomal peptides have a broad spectrum of biological activities, some of which have been useful in medicine, agriculture, and biological research (Kleinkauf and Von Doehren, 1996; Von Dohren et al., 1997; Smith et al., 1990; Schwarzer and Marahiel, 2001). Products made by nonribosomal peptide synthetases or nonribosomal peptide synthetase / polyketide synthase hybrid enzymes include well-known antibiotics (penicillin, erythromycin, and vancomycin), immunosuppressants (cyclosporine and rapamycin), antitumor agents (actinomycin, bleomycin, and epothilone) (Shen et al., 2001; Von Dohren et al., 1997) and toxins involved in pathogenesis (HC-toxin, enniatin, AM-toxin, and probably victorin) (Haese et al., 1993; Johnson et al., 2000; Panaccione et al., 1992; Scott-Craig et al., 1992; Walton, 1996, Walton et al., 2004).
The progress that has been made in the past decade toward understanding the molecular principles of nonribosomal peptide synthesis has been recently extended to the structural level. With structural information now available for a prototype specificity-conferring A domain of an NRPS (Conti et al., 1997) and for a PCP domain (Weber et al., 2000), we have in hand structural data on a minimal initiation module. In addition, the crystal structure of the enzyme that converts the NRPS surfactin synthetase from its inactive apo form to the active holo form, the 4’PP-transerase Sfp, has been solved (Reuter et al., 1999). This information will help us to understand the structure/ function relationship of modules, the elementary building blocks of an NRPS.

Several gene clusters encoding nonribosomal peptide biosynthesis, such as those for enterobactin in *E. coli* (Coderre and Earhart, 1989), surfactin in *B. subtilis* (Nakano et al., 1992), and gramicidin in *B. brevis* (Borchert, et al., 1994), and polyketide biosynthesis, such as those for nystatin in *Streptomyces noursei* (Brautaset et al., 2000) and possibly landomycin in *Streptomyces cyanogenus* (Westrich et al., 1999). The complete 9 Mb genome of *Streptomyces avermitilis* was published in 2003 (Ikeda et al., 2003). The strain was known for the production of the polyketide macrolides avermectin and oligomycin, and the genome sequence revealed a total of 37 secondary metabolite gene clusters, including 13 PKS (polyketide synthase), 8 NRPS (non-ribosomal synthetase), various terpenoid, siderophores and bacteriocin encoding clusters.

The present study intends to isolate haloalkaliphilic actinomycetes from solar salt works, identify the strains by various methods including molecular approaches; screening and sequencing of NRPS gene clusters, optimization and characterization of secondary metabolites and finally looking into the positive isolates for the presence of antimicrobial and anticancer activities.
The objectives of the present study are

- Identification of antagonistic haloalkaliphilic actinomycetes from solar salt works in Tamilnadu
- Identification of highly antagonistic haloalkaliphilic actinomycetes by genomic level.
- PCR based screening and sequencing of NRPS gene clusters from the antagonistic haloalkaliphilic actinomycetes
- Functional based screening of NRPS positive isolates for the presence of antimicrobial and anticancer activities
- To optimize the growth of NRPS positive strains with different parameters
- Characterization of secondary metabolites from the antagonistic haloalkaliphilic actinomycetes