CHAPTER 1

INTRODUCTION

Marine diversity constitutes an infinite pool of novel chemistry, making up a valuable source for innovative Biotechnology. Marine microbes represent a potential source for commercially important bioactive compounds. Although the diversity of life in the terrestrial environment is extraordinary, the greatest biodiversity is localized in Oceans (Donia and Hamann, 2003). More than 70% of our planet’s surface is covered by Oceans, and life on Earth is believed to be originated from the sea. The experts estimate, that in some marine ecosystems, such as deep sea floor and coral reefs, the biological diversity is higher than in tropical rainforests (Haefner, 2003).

Actinomycetes are filamentous Gram positive bacteria with high GC content. They are found in various parts of the ecological systems. Actinomycetes are a group of bacteria that live in soil and decompose organic matter such as cellulose. Actinomycetes are one of the most diverse groups of filamentous bacteria capable of surviving in a number of ecological niches due to their bioactive potential. Some types of Actinobacteria are responsible for the peculiar odor (Petrichor) emanating from the soil after rain. The chemical that produces this odour is known as Geosmin. Actinomycetes are well recognized for their metabolic versatility that is frequently accompanied by the production of primary and secondary metabolites of economic importance. Most of the actinomycetes were isolated from the terrestrial source. As marine environmental conditions are extremely different from terrestrial ones, it is surmised that marine actinomycetes have different characteristics from those of terrestrial counterparts and, therefore, might produce different types of bioactive compounds.

Astonishingly the actinomycetes from the marine environment are much novel than the terrestrial environment. The marine environment is proving to be a
major source of new natural products, including antimicrobial and anticancer compounds, most notably being expressed by actinomycete (Okoro et al., 2009). This may be due to the challenge offered by the marine environment. The marine environment is a challenging environment for the organisms to survive. The survival difficulty includes the climatic and environmental extremities (pH, wind and tide actions). Due to these conditions the organisms produce certain compounds that help them endure their survival. Furthermore, these organisms produce such enduring compounds which serve the benefit of man.

The need for new metabolites is due the increasing demand for new antibiotics and drug resources. Though secondary metabolites are produced only by microorganisms not all of them are capable of producing efficient secondary metabolites. The propensity to form these compounds is unevenly distributed in microbial community and seems more closely associated with existence in a competitive environment such as the marine environment than with phylogeny. It has always pronounced that the actinomycetes are excellent producers of secondary metabolites.

In the beginning of drug discovery, actinomycetes are widely recognized for their ability to produce secondary metabolites. Their ability to produce secondary metabolites is preferred because of its uniqueness. The microbial products, especially those from actinomycetes have been a phenomenal success for the past seven decades. Bio-prospecting for new leads are often compounded by the recurrence of known antibiotics in newer microbial isolates. In the historical line the actinomycetes have helped in the developments of novel drugs which are still in use. For example the discovery of Penicillin in 1928 and that of Streptomycin in 1943, has been pivotal to the exploration of nature as a source of new lead molecules. Streptomycin, the first treatment for tuberculosis, was derived from the largest genus of these bacteria, *Streptomyces*. Erythromycin and tetracycline are two other examples of common medicines derived originally from actinomycetes. Despite all these deterrents, actinomycetes have proved to
be a mine of novel antibiotics, which selectively destroys the pathogens without affecting the host.

The living conditions to which marine actinomycetes had to adapt during the evolution range from extremely high pressure (with a maximum of ~1100 atmospheres) and anaerobic conditions at temperatures just below 0 °C on the deep sea floor, to high acidic conditions (pH as low as 2.8) and temperatures of over 100 °C near hydrothermal vents at the mid-Ocean ridges. It is likely that this is reflected in the genetic and metabolic diversity of marine actinomycetes, which remains largely unknown. Indeed, the marine environment is a virtually untapped source of novel actinomycete diversity (Bull et al., 2005) and, therefore, of new metabolites as well (Magarvey et al., 2004; Jensen, et al., 2005; Fiedler et al., 2005). However, the distribution of actinomycetes in the sea is largely unexplored and the presence of indigenous marine actinomycetes in Oceans remains elusive. This is partly due to the lack of effort spent in exploring marine actinomycetes, whereas terrestrial actinomycetes has been, until recently, a successful source of novel bioactive metabolites. Among marine microorganisms, actinomycetes have gained special importance as the most potent source of antibiotics and other bioactive secondary metabolites (Kim 2006).

Recently, the rate of discovery of new compounds from existing genera obtained from common soil has decreased therefore it is critical that novel actinomycetes from unexplored habitats such as marine environment be pursued as sources of novel antibiotics and others bioactive compounds such as enzymes. While most of the studies on actinomycetes have focused on antibiotic production, only few reports have dwelt on their enzymatic potential. The majority of actinomycetes are free living and found widely distributed in many natural environments including various soil, freshwater habitat, marine habitat, organic matter habitats and colonizing plants. They are a promising source for novel actinomycetes for the production of wide range of important enzymes, some of which are produced on an industrial scale, but many other remained to
be harnessed. They have the ability to degrade a wide range of hydrocarbons, pesticides, aliphatic and aromatic compounds. They perform microbial transformations of organic compounds, a field of great commercial value. Members of many genera of actinomycetes have potential use in the bioconversion of underutilized agricultural and urban wastes into high-value chemical products, because of their ability to produce diverse class of enzymes.

Early evidence supporting the existence of marine actinomycetes came from the description of *Rhodococcus marinonascene*, the first marine actinomycete species to be characterized. Further support has come from the discovery that some strains display specific marine adaptations (Jensen et al., 1991) whereas others appear to be metabolically active in marine sediments (Moran et al., 1995). However, these early findings did not generate enough excitement to stimulate the search for novel actinomycetes in the marine environment. Recent data from culture-dependent studies have shown that indigenous marine actinomycetes indeed exist in the Oceans (Bull et al., 2005). These include members of the genera *Rhodococcus* (Jensen et al., 2005), *Streptomyces*, *Salinispora*, *Marinophilus*, *Solwaraspora*, *Salinibacterium*, *Aeromicrobium marinum*, *Williamsia maris* and *Verrucosispora* (Stach et al., 2004; Jensen et al., 2005).

Among these, the most exciting finding is the discovery of the first obligate new marine actinomycete genus, *Salinispora* (formerly known as *Salinospora*), and the demonstration of the widespread populations of this genus in Ocean sediments (Mincer et al., 2002). *Salinispora* strains were also isolated from the Great Barrier Reef marine sponge *Pseudoceratina clavata* (Lam, 2006). Furthermore, Mincer et al. (2002) have demonstrated that *Salinispora* strains are actively growing in some sediment samples indicating that these bacteria are metabolically active in the natural marine environment. In this context, Grossart et al. (2005) have illustrated that actinomycetes account for approximately 10% of the bacteria colonizing marine organic aggregates and that their antagonistic activity might be highly significant in maintaining their presence, which affects
the degradation and mineralization of organic matter. Therefore, actinomycetes are active components of marine microbial communities. They form a stable, persistent population in various marine ecosystems. The discovery of numerous new marine actinomycete taxa, their demonstrated metabolic activity in their natural environments and their ability to form stable populations in different habitats clearly illustrate that indigenous marine actinomycetes indeed exist in the Oceans (Magarvey et al., 2004).

The information about assortment of the gram positive, filamentous secondary metabolite producing actinomycetes in the rhizosphere soil of Coastal sand dune (CSD) is scanty. Coastal sand dunes (CSD) are common in different parts of the world. Coastal sand dune of the marine environment is virtually untapped ecosystem. Often the sand dunes in the coastal areas are likely to have drastic conditions (Kurtbo ¨ke et al., 1993). The coastal sand dunes itself, are formed due to continuous tide and wind action. The coastal sand dunes are inhabited by a variety of plants, animals and microorganisms. The plants in this particular ecosystem have to undergo an intricate environmental stress to survive. Henceforth they are different from the other plants, through their adaptations. Not only supported by their adaptations but the plants are also supported by the symbiotic microorganisms in and around the rhizosphere soil. The coastal sand dunes constitute a variety of microenvironments due to substrate mobility and physiochemical processes (Arun et al., 1999). Plants establishing on coastal sand dunes are subjected to several environmental fluctuations such as high solar radiation, nutrient deficiency, drought, salt spray and high winds which affect their growth, survival and community structure (Arun et al., 1999). These in turn induce the microbes in association with the plants to adapt mutually to different habitats (Rosa et al., 1995).

Though the vast marine microbial diversity and their potential are realized (Sponga et al., 1999), the diversity and potential applications of actinomycetes from coastal sand dunes are not yet studied comprehensively. Evidently the focus on the coastal sand dunes is not adequate compared to the other environment
(Arun et al., 1999). It has been proven many times that most of the microorganisms from neglected ecosystem contribute novel drugs rather than the ecosystems that are frequently explored. The possibility of rhizosphere of coastal sand dunes harbouring diverse and beneficial marine actinomycetes, which remains unexplored, is immense. The coastal sand dune actinomycetes may alter their growth characteristics in response to salinity. These coastal sand dune actinomycetes may contain some plant protective and plant productive activities which can be explored to use them as bioinoculants in coastal saline soils for crop improvements.

A large fraction of antibiotics in the market are obtained from actinomycetes. They produce enzyme inhibitors useful for cancer treatment and immunomodifiers that enhance immune response. Actinomycetes are also important in Plant Biotechnology as strains with antagonistic activity against plant pathogens are useful in biocontrol. Actinomycetes represent a high proportion of the soil microbial biomass and have the capacity to produce a wide variety of antibiotics and of extracellular enzymes. Several strains of actinomycetes have been found to protect plants against plant diseases. Actinomycetes have been found to play an important role in rhizosphere soil (Suzuki et al., 2000; El-Tarabilya and Sivasithamparam, 2006; Norovsuren et al., 2007; El-Tarabily et al., 2008). Attention has been paid to the possibility that these microorganisms can protect plant roots from plant pathogen and promote plant growth. For plant root protection, the modes of action of actinomycetes include antibiosis, parasitism, the production of extracellular hydrolytic enzymes and competition for iron (Goodfellow and Williams, 1983; El-Tarabily et al., 2008; Getha et al., 2005; Errakhi et al., 2007).

Further, India is an agricultural country. About seventy percent of our population depends on agriculture. One-third of our National income comes from agriculture. Our economy is based on agriculture. The development of agriculture has much to do with the economic welfare of our country. The need of native solutions to improve the standards of agriculture is required more than
ever. The indulgence of Biotechnology in agriculture is significant for the improvement of farming in India.

Tomato occupies second position amongst all the vegetables in terms of production in India. The estimated area and production of tomato for India are about 3, 50,000 hectares and 53, 00,000 tons respectively (planningcommission.nic.in/aboutus/committee/). It contributes significantly to the nutrition of the people as a source of vitamins and minerals. Tomato is protective supplementary food. As it is short duration crop and gives high yield, it is important from the economic point of view and hence area under its cultivation is increasing day by day. Several studies have shown that constraints such as biotic factors (pests and diseases) restrain the production of tomato (Vincent et al., 2012).

Tomato is attacked by vast range of fungus, bacteria and viruses. The most common diseases are anthracnose, bacterial spot, bacterial blight, bacterial canker, buck eye rot, damping off, early blight, fusarium blight, late blight, leaf curl, mosaic, powdery mildew,rots, and septorial leaf blight Harrison and Beckman 1987. Fusarium Wilt is a worst disease of tomato occurring all over India caused by *Fusarium oxysporum* a fungal phytopathogen (http://agricoop.nic.in/Agristatistics.htm.). The fungus is soil borne and makes its way into the plant through the roots. Once inside, it clogs and blocks the xylem, the tissue that moves water and some nutrients through the plant, preventing water from traveling up the stem and out into the branches and leaves. There are not many pesticides that effectively control the fusarial wilt of tomato.

The use of agrochemical is an easy option for the farmers due to their economic stand. Consequently, the accumulation of agrochemicals in the soil diminishing the very nativity and the prosperity of the soil comes in hand. Additionally, resistance is gained by the very pathogen leading to the search of new agrochemical. On the other hand the use of alternative, biocontrol is under practice. Use of Biocontrol agents is accepted as a suitable and environmentally friendly alternative or a supplemental way of reducing the use of chemicals in agriculture against plant disease management (Stephane et al., 2005). Formulated
preparation of both fungi and bacteria as Biocontrol agent have been applied to seeds, seedlings and planting media to reduced tomato wilt disease (Anitha and Rabeeth, 2009).

Various studies have indicated that actinomycetes from different sources have potential for the biological control of Fusarium wilt. Only few studies have suggested that actinomycetes from the marine environment have potential for biological control. Many of the phytopathogens are inhibited by actinomycetes such as *Streptomyces violaceusniger*, *Streptomyces olivaceus*, *Streptomyces griseus*, *Streptomyces viridodiasticus* and *Streptomyces hygroscopicus*. These are some of the actinomycetes showing antagonistic activity against *Fusarium oxysporum* in green house conditions (Getha *et al.*, 2002; Anitha and Rabeeth 2009; Bafti *et al.*, 2005; Degtyareva *et al.*, 2009).

Many scientific publications and reviews indicate that actinomycete species are capable of effectively controlling fungal and bacterial plant pathogens. All these studies suggest that actinomycetes are able to compete and replace the chemical pesticides. Therefore, a few plant pathogens have been controlled successfully by actinomycetes species, but many attempts to develop biocontrol formulations have met with problems in practice. In order to develop actinomycete as biocontrol agents for commercial use, the consistency of their performance must be improved (Doumbou *et al.*, 2001). Accomplishing this will require research in many diverse areas, because biological control is the culmination of complex interactions among host, pathogen, antagonist, and environment. Selection of diverse environment not only leads to unique organism but also to unique and novel metabolites.

The diversity and beneficial bioactivities of marine actinomycetes from the sand dunes of coastal area along Bay of Bengal is not yet attempted. Hence this study which comprises of isolation and characterization of actinomycetes from the rhizosphere of coastal sand dunes and application potential antagonistic marine actinomycetes for the control of fusarial wilt in tomato has been undertaken.