INTRODUCTION

The history of infectious diseases is as old as the human civilization, and need for protection against these infectious diseases always remains one of the major health concerns. Infectious diseases are leading causes of death worldwide which account for one-fourth to one third of total deaths worldwide (Asolkar et al., 2010). A literature survey identified 1,407 recognized species of human pathogens in which 177 are recognised as emerging and reemerging pathogens. Of all pathogenic species, 538 are bacteria including 54 (10%) regarded as emerging / reemerging. There are 60 bacterial families that contain human pathogens, wherein the enterobacteria and the mycobacteria account for most of the emerging and reemerging species (Woolhouse and Sequeria, 2005). In India, the range and burden of infectious diseases are enormous and it contributes to about 30% of total disease burden (John et al., 2011).

For more than a century, infectious diseases have been controlled by vaccination and by administration of antibiotics (Muzzi et al., 2007). Antibiotics have been identified by screening natural compounds from certain microbial sources and credited for their ability to inhibit microbial growth in vitro. Although such an approach has been effective, it is becoming interestingly difficult to produce sufficient coverage for all infectious diseases (McDevitt and Rosenberg, 2001). Antibiotic resistance has developed steadily as new agents have been introduced. There has been a dramatic increase in the occurrence of resistant organisms in both community and hospital settings for more than two decades (Hopwood, 2007).

Tuberculosis (TB) is an airborne and highly contagious chronic granulomatous bacterial disease caused by Mycobacterium tuberculosis (MTB).
The history of tuberculosis is very old and it has afflicted human beings from the time immemorial. Evidence of spinal tuberculosis has been found in Egyptian mummies several thousand years BC and references to TB are found in ancient Bobylonian and Chinese writings. Recent molecular genetic studies have shown that *M. tuberculosis* has a progenitor 3 million years old (Ahmad, 2011). In 1882, Robert Koch’s scientific brilliance led to the discovery of *M. tuberculosis* as the causative agent of the disease. Different means of TB curtailment were developed from time to time. In the 19th century, a French bacteriologist Calmette together with Guerin created the Bacilli Calmette-Guerin (BCG) vaccine for tuberculosis. Even though relatively ineffective, it is still in widespread use. The history of TB in India also dates back to 600 BC where in Sushruta Samhita, the disease is known as kshaya, “wasting disease,” or Raja Yakshmaa, “the king of diseases.” TB was rare until the second half of the 19th century. Concomitant with the growing population density caused by industrialization, its incidence has increased progressively since then (Ramachandran *et al.*, 1999; Mahadev and Kumar, 2003).

Tuberculosis is a communicable disease and patients with pulmonary TB are the most important source of infection. Infection is initiated by inhalation of droplet nuclei expectorated by patients with active pulmonary TB (open TB) typically when the patient coughs. The risk of infection is dependent on several factors such as the infectiousness of the source case, the doseness of contact, the bacillary load inhaled and immune status of the potential host (Ahmad, 2011).

According to WHO report, TB remains a leading cause of mortality worldwide in the 21st century. TB is a disease of poverty affecting mostly young adults in their most productive years. About 95% of TB deaths are in the
developing world. In 2009, there were 1.7 million people died from TB equal to 4700 deaths a day. In 2010, the number of TB cases who fell ill with TB dropped to 8.8 million. In 2010, there were 1.4 million people died from TB equal to 3800 deaths a day (WHO Report, 2011). There are 22 high-burden countries accounting for about 85% of the world’s TB cases. The highest number of TB cases occurred in Asia (55%) followed by Africa (31%). The six most popular countries of Asia such as China, India, Indonesia, Pakistan, Bangladesh and Philippines account for >50% of all TB cases worldwide. The estimated global incidence rate fell to 128 cases per 100000 population in 2010, after peaking in 2002 at 141 cases per 100 000 (WHO report, 2011). In 2009, India had 2 million new cases of tuberculosis, the highest for any country in the world, including 0.9 million smear-positive cases of pulmonary tuberculosis (WHO Report, 2010). In India alone, the number of TB deaths averages to about 1 person per every 1.5 minutes. The highest incident rate of TB was recorded for the African region which is mainly due to high prevalence of HIV infection. Nearly 80% of HIV infected TB patients were living in the African regions. Pulmonary TB accounts for >85% of active TB cases in high TB incidence countries due to the higher rates of active transmission, while extra pulmonary TB is also common in low TB incidence countries of the developed world, particularly among HIV infected individuals and immigrants (Golden and Vikram, 2005; Mokaddas et al., 2008).

The directly observed therapy strategy launched in 1993 has greatly contributed to the improvement of global TB control over the last 15 years. WHO-recommended ‘directly observed treatment, short course’ (DOTS) standard chemotherapeutic regimen for treating smear-positive, newly diagnosed pulmonary TB. Treatment of drug susceptible (DS)-TB involves an initial phase of four first-line TB drugs (isoniazid, rifampicin, pyrazinamide and
ethambutol), administered for an initial intensive phase of 2 months followed by a continuation phase of an additional 4 months of isoniazid and rifampicin (Koul et al., 2011). Since 1995, 41 million people have been successfully treated and up to 6 million lives saved through DOTS and the stop TB strategy. A total of 5.8 million TB cases were notified through DOTS programmes in 2009. Although capable of achieving a cure rate of 85% or more at a global level, this regimen is lengthy, cumbersome and requires considerable efforts to ensure patients’ adherence and treatment completion (Matteelli et al., 2010).

One worrying factor in the current TB problem is the prevalence of drug resistance among *M. tuberculosis* strains. *M. tuberculosis* strains resistant not only to first line drugs like isoniazid and rifampicin, but also increasingly resistant to second line drugs, are becoming more common (Lougeed et al., 2009). Tuberculosis caused by *M. tuberculosis* strains that are resistance to, at least two most powerful first line drugs, isoniazid and rifampicin is termed as Multi Drug Resistant (MDR)-TB. There were an estimated prevalence of 6,50,000 MDR-TB cases in 2010, and in 2008 it was estimated there were 150,000 deaths annually from MDR-TB (WHO Report, 2011). It was estimated that in 2009, 3.3% of all new TB cases had MDR-TB. In 2010, the largest WHO MDR-TB survey reported the highest rates ever, with 28% of new TB cases in some settings of the former Soviet Union. Many countries have developed plans to address MDR-TB, but the response globally is still insufficient (Jassal and Bishai, 2010). In this case, the second line drugs are used to treat MDR-TB. But, this has several disadvantages compared to use of first line drugs; the disadvantages include a far low efficacy, longer administration periods (18-24 months), higher rates of adverse affects, cost up to 100 times more compared to the available treatment and low cure rates (around 60%) (Souza, 2009).
Tuberculosis caused by strains of *M. tuberculosis* that are resistant to isoniazid and rifampicin, in addition to any one fluoroquinolone, and to at least one of the three injectable drugs namely capreomycin, kanamycin and amikacin is termed as Extensively Drug Resistant (XDR)-TB. XDR-TB cases have been confirmed in 58 countries (WHO report, 2010). It is virtually untreatable using current therapeutics. In 2009, the WHO estimated that only less than 2% of MDR-TB and XDR-TB cases were receiving appropriate treatment (Lougheed *et al.*, 2009).

The worldwide problem caused by TB and the lack of new drugs in the market make it imperative to develop novel drugs to fight efficiently for preventing the rapid spread of drug resistant TB strains. In this context, there is an urgent need for new anti TB drugs preferably with novel mechanism of action, less toxic side effects, improved pharmacokinetic properties with extensive and potent activity against resistant strains and also capable of reducing the total duration of treatment (Souza, 2006a).

Bioprospecting is the search and discovery of natural products that have a useful pharmacological or biological application (Ashforth *et al.*, 2010). Natural products occupy tremendous chemical structural space unmatched by any other small molecule families. They possess a range of biological activities thus remaining the best sources of drugs and drug leads (Liu *et al.*, 2012). By some accounts more than 70% of commercialized anti-infective drugs are based on natural products (Newman and Cragg, 2007). During the past 2 decades, the focus of drug-discovery efforts has shifted from natural products to synthetic compounds, but this shift has not led to the anticipated increase in drug productivity. For instance, 12 (26%) of the molecular entities approved by the Food and Drug Administration (FDA) in 2009-2010 are nature derived (Zhu
et al., 2011). The major advantage of natural products, especially in the case of microbial products, should be that they have gone through the evolutionary process over millennia to engender properties to enter and affect competing microorganisms.

Microorganisms of both terrestrial and marine origins have proven to be excellent sources of novel natural products. Programmes aimed at the discovery of antibiotics and other bioactive metabolites from microbial sources have yielded an impressive number of compounds over the past 50 years. (Busti et al., 2006). Of the 22,500 biologically active compounds that have been identified from microbes, 38% are from fungi, 17% are from unicellular bacteria and 45% are produced by actinomycetes, warranting actinomycetes reputation as one of the most prolific producers of bioactive natural products known to date (Berdy, 2005; Ashforth et al., 2010). Natural products are already among the most important anti-TB agents, with over 60% of approved and yet-to-be approved drug candidates either natural products or related to them. Anti-TB compounds were reported most commonly from microbes (Souza, 2009; Ashforth et al., 2010; Liu et al., 2012), followed by plants (Gautam et al., 2007) and in less from marine organisms (El-Sayed et al., 2000).

Actinomycetes are attractive, filamentous Gram-positive bacteria with true aerial hyphae. They are belonging to the phylum Actinobacteria (order actinomycetales) that represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain Bacteria (George et al., 2012). They have unprecedented ability to produce various bioactive metabolites. Actinomycetes are the well recognized as the richest source of bioactive compounds including clinically useful antibiotics, anti cancer agents and cell function modulators and hence of high pharmacological and
commercial interest (Butler, 2008). They are widely distributed in various normal and extreme ecosystems, due to their unparalleled ability to degrade wide range of complex substrates and withstand extreme physico-chemical conditions (Balagurunathan and Radhakrishnan, 2007; Balagurunathan and Radhakrishnan, 2010). According to some estimates, the top 10 cm of global soil contains $10^{25}$–$10^{26}$ actinomycetes, but only about $10^7$ have been screened for antibiotic production in the past 50 years, leaving plenty of room for further screening (Baltz, 2007). In the last decade itself, numerous novel actinomycete genera have been reported from different less/unexplored ecosystems. For example, the Actinopolymorpha singaporensis gen. nov. sp. nov., from forest soil (Wang et al., 2001), Salinispora arenicola gen. nov. sp. nov., (Maldonado et al., 2005) and Streptomyces nanhaiensis sp. nov., from marine sediments (Tian et al., 2012), halotolerant Nocardiopsis arabia sp. nov., (Hozzein and Goodfellow, 2008), thermotolerant Yuhushiella deserti gen. nov. sp. nov. from desert soil (Mao et al., 2011) and halophilic Salinactinospora qingdaonensis gen. nov., sp. nov., from salt pond (Chang et al., 2012).

Though more than 50 % of the microbial antibiotics discovered so far originate from actinomycetes, only a few soil derived genera such as Streptomyces and Micromonospora account for most of these compounds. Members of the genus Streptomyces are the most prolific producers of secondary metabolites accounting for up to 80% of the bioactive small molecules discovered from actinomycetes. The distribution of distinct chemical types of antibiotics according to their main producer types shows some outstanding features. The macrolides, polyene antibiotics, aminoglycosides and anthracyclines, each group covering about 400-500 compounds are all produced exclusively from actinomycetes species. All of the known (about 120) glycopeptide antibiotics are produced almost exclusively, and orthosomycins
mainly, by the rare actinomycetes species. The actinomycete compounds are usually too large to medium sized molecules with an average molecular weight of 400-800 covering almost 60% of these compounds (Berdy, 2005). Since 2000, 20 new antibiotics have been launched worldwide; of which 11 were natural product-derived and nine were synthetically derived. A majority of the natural product-derived antibiotics were belonging to the beta-lactam class and in particular 8 were synthesized based on the antibiotics isolated from actinomycetes as lead source (Butler and Cooper, 2011). Meanwhile, it is quite notable that further discovery of unknown metabolites from Streptomyces is predicted by the genome analysis: the number of metabolites actually isolated is far more below the number of secondary metabolite biosynthetic gene clusters identified in the whole genome of *Streptomyces avermetilis* and *Streptomyces coelicolor* (Igarashi *et al.*, 2012). In addition, most of the actinomycete-derived antibiotics are too complex to be synthesized by combinatorial chemistry (Baltz, 2007).

Secondary metabolites from actinomycetes have a long history in the treatment of TB. There are many clinically used anti-TB compounds with new structure and novel mechanism of action has been reported from actinomycetes (Butler and Buss, 2006). Since the discovery of streptomycin, the first antibiotic used for anti-TB therapy obtained from *Streptomyces griseus*, during 1944, numerous anti-TB antibiotics are reported from various actinomycete genera. Importantly, promising candidates such as rifamycin, erythromycin, pacidamycin, caprazamycin, capuramycin and thiolactomycin in clinical trials against MDR-TB are also of actinomycete origin (Souza, 2009b).

Because of this excellent track record of actinomycetes in this regard, a significant effort has been focused on the successful isolation of actinomycetes
from normal terrestrial sources for drug screening programs in the past 50 years. Recently, the rate of discovery of new compounds from normal terrestrial actinomycetes has decreased where as the rate of re-isolation of known compounds has increased (Berdy, 2005; Lam, 2006). New approaches have had to be developed to respond the lack of new molecules as novel privileged scaffolds and to improve the possibility of success of finding new chemical entities as leads for the development of drug candidates. Undiscovered species inhabiting unique environments with differing environmental constraints have been thought to be resources of novel compounds (Bull et al., 1992; Jensen and Fenical, 1996; Bull and Stach, 2007; George et al., 2012; Radhakrishnan et al., 2011b). The range of versatility of actinomycete metabolites is enormous and yields significant economic returns, yet, bio discovery from these sources depends on the detection and recovery of bioactive actinomycetes from previously unexplored environmental sources and further effective assessment of their metabolites in defined targets (Goodfellow, 2010; Kurtboke, 2010). Thus it is crucial that actinomycetes from unexplored or rare ecosystems be pursued as sources of novel bioactive secondary metabolites including antituberculcr antibiotics.

On the basis of this working hypothesis and the bioactive potential, the present work was planned targeting the exploration of large variety of rare ecosystems like desert, forest and hills for actinomycetes with special reference to the isolation of antituberculosis compounds.