GENERAL CHARACTERISTICS OF *M. tuberculosis*

*Mycobacterium tuberculosis* is a slow-growing, aerobic rod-shaped acid-fast bacterium. This bacterium is a highly contagious facultative intra-cellular parasite, usually of macrophages and has a slow generation time of 15-20 hours (Goldrick, 2004). *M. tuberculosis* strains are very weakly Gram-positive. Members of mycobacterial species contain a unique lipid-rich cell wall composition that allows them to take basic dyes and resist decolourization with acid-alcohol and so are called acid fast bacilli. Acid-fast bacilli appear pink against a blue background when stained by Ziehl-Neelson staining. An agar based Middlebrook medium and egg based Lowenstein-Jensen (LJ) medium are the two important solid media commonly used to grow *M. tuberculosis*. The colonies are small and buff colored when grown on either medium. It takes 2-8 weeks to get visual colonies on either type of media. The cell wall structure of *M. tuberculosis* is unique among prokaryotes and it is a major determinant of virulence for the bacterium. The cell wall has high lipid content and allows the bacteria to survive within macrophages. It also provides the organism with a resistant barrier to many common drugs (Katzung, 2001; Williams and Lemke, 2002). The cell wall mycolic acids are thought to be a significant determinant of virulence in *M. tuberculosis*. More complete understanding of the biosynthetic pathways and gene functions and the development of antibiotics to prevent formation of the cell wall are areas of great interest (Nanci and Knechel, 2009).

PATHOGENESIS OF TUBERCULOSIS

Human beings are the primary host for *M. tuberculosis*. Infection is spread via airborne dissemination of aerosolised bacteria containing droplet
nuclei of 1–5 μm in diameter that carry *M. tuberculosis* from an individual with infectious TB disease to an uninfected individual. The infectious droplet nuclei are inhaled and get lodged in the alveoli in the distal airways. *M. tuberculosis* is then taken up by alveolar macrophages, initiating a cascade of events that result in either successful containment of the infection or progression to active disease (primary progressive TB). Risk of development of active disease varies according to time since infection, age, and host immunity (Comstock *et al.*, 1974; Sutherland, 1976). However, the life-time risk of disease for a newly infected young child has been estimated at 10%.

Latent tuberculosis infection (LTBI) is defined as a clinical condition without clinical or radiological signs of active disease and is manifested only by a positive tuberculin skin test (Nuermberger *et al.*, 2004). Approximately 2 billion people or one third of the world’s population, have LTBI, and approximately 10% of them will develop active TB during their life time. There is plenty of evidence that the basis for LTBI in humans is persistence of tubercle bacilli *in vivo* for long periods of time. This status is currently defined as dormancy or non-replicating persistence (NRP) (Nuermberger, 2004).

**Tuberculosis chemotherapy**

Since the control measures for TB such as Bacillus Calmette Guérin (BCG) vaccination and chemoprophylaxis appear to be unsatisfactory, treatment by anti-TB drugs becomes the only option available. The therapy of mycobacterial infections, in particular tuberculosis is challenging for a number of reasons. This bacterium is not susceptible to many classes of antibacterial agents. As a result, tuberculosis often requires treatment with drugs that are not commonly used for other microbial infections and often have small therapeutic widows.
The beginning of TB-chemotherapy illustrated well the importance of nature in the fight against diseases. The first drug discovered to treat TB – streptomycin (SM) - an aminoglycoside isolated from the actinomycete, *Streptomyces griseus*, had unlocked the door to the antibiotic treatment of TB (Schatz *et al.*, 1944). While monotherapy with streptomycin was able to cure lethal forms of acute paucibacillary TB such as meningitis and miliary disease, it was soon evident that it resulted in the emergence of resistant mutants and treatment failure among patients with multibacillary forms like cavitary pulmonary TB. After the discovery of streptomycin, several synthetic drugs were introduced into the market. In 1946, Lehman from Sweden discovered para-aminosalicylic acid (PAS) as an effective TB drug. This was quickly followed in 1952 by the discovery of highly active TB drug isoniazid (INH). Both, PAS and INH ushered in the era of combination therapy. INH represented a major milestone in the tuberculosis chemotherapy because of it’s highly active, inexpensive nature with no significant side effect. Therapy with SM, PAS and INH prevented the selection of SM-resistant mutants and resulted in the cure of patients with 18 months of treatment. For more than 20 years this combination was the standard treatment for TB (Nuermberger *et al.*, 2004).

Remarkably, the nicotinamide lead also led to the discovery of pyrazinamide (PZA) in 1952 and ethionamide (ETH)/prothionamide (PTH) in 1956. Further, screening of extracts from soil microbes led to discovery of many other antituberculosis drugs viz. cycloserine, kanamycin and its derivatives such as amikacin, viomycin, capreomycin and rifamycins. Rifamycins and their derivatives are the drugs of choice for treatment of TB since the 1970s (Zhang, 2005).
The goals of tuberculosis treatment are to ensure cure without relapse, to prevent death, to impede transmission, and to prevent the emergence of drug resistance (Fox et al., 1999). Numerous antibiotics with anti-TB activity have long been classified as ‘first line’ or ‘second line’ drugs on the basis of their anti-TB activity and toxicity. First line drugs are with promising anti-TB activity and limited toxicity whereas the drugs with lesser activity and/or greater toxicity are considered as second line drugs (Table 1). Second-line drugs are used primarily in the treatment of patients harbouring bacilli resistant to the first-line drugs.

As suggested by WHO, the current standard chemotherapeutic regimen for treating new pulmonary TB patients consists of a multidrug combination of the first-line drugs comprising an initial intensive phase of rifampicin (RIF), isoniazid (INH), pyrazinamide (PYZ or PZA), and ethambutol (ETB) daily for 2 months and a continuation phase of RIF and INH for a further 4 months, either daily or 3 times per week. INH and RIF are the two most potent anti-TB drugs that kill more than 99% of tubercular bacilli within 2 months of initiation of therapy (Mitchison, 1985; Iseman and Madsen, 1989). Using these drugs in conjunction with each other reduced the duration of anti-TB therapy from 18 months to 6 months (Rattan et al., 2005).

**Limitations of current tuberculosis therapy**

When administered appropriately, combination anti-TB therapy can be highly effective anywhere in the world. Regimens employing first-line drugs are orally bio-available, relatively cheap, and generally well tolerated. But this regimen is lengthy and complex, inviting nonadherence, drug interactions, drug toxicity, and treatment programs require substantial supervision to monitor adherence and tolerability (Boogaard et al., 2009). New regimens for TB that
could be administered for a shorter duration of therapy or more intermittently without sacrificing efficacy would reduce the burden of supervising drug administration and make treatment more widely available. Unfortunately, it is difficult to see how existing first-line drugs could be used more effectively in this regard, and there are no new agents in the later stages of the drug development pipeline (Nuernberger et al., 2004). In addition, the emergence of *M. tuberculosis* strains to the available drugs causes major concern which results in higher death rates, especially among HIV-infected persons (Rattan et al., 2005).

Further, pediatric patients constitute a high risk population by *M. tuberculosis* infection. According to WHO statistics, 250,000 children develop the disease and approximately 100,000 die every year. Pharmacokinetics of several anti-TB drugs has shown poor efficacy in children. Also, there are a very limited number of anti-TB liquid formulations. Most of the first-line drugs are not available in pediatric form and they are produced only extemporaneously. For example, a liquid suspension of RIF (Rifaldin®, Sanofi-Aventis) is available in Spain. This not only results in less compliant regimen but also makes dose-per-body weight adjustments difficult. Manipulation of solid forms (e.g. crushing and mixing with food or beverages) may lead to unpredictable changes in the stability of active compounds and their bioavailability (Sosnik et al., 2010).

**Anti-TB drug resistance**

The history of anti-TB drug resistance is fairly recent, emerging just over 60 years ago with the development of anti-TB drugs. For decades the problem was identified in local areas among patients treated at reference centres in industrialized countries. With the discovery of rifampicin (RIF) in 1966, and the
expansion of its use between 1970 and 1990, patients who were already carriers of isoniazid resistant to *M. tuberculosis*

strains became resistant to rifampicin. This was the start of a progressively growing problem which has reached epidemic proportion in some countries (Caminero, 2010). An individual may develop the drug resistant form of TB via inadequate therapy that enable the selection of drug resistance (acquired resistance) or infection with a drug-resistant TB strain (primary resistance).

The emergence of MDR-TB and XDR-TB pose serious threat to the public and it also further complicates the TB global emergency (Haydel, 2010). They are resistant to our best antibiotics and are associated with greater morbidity and mortality than antibiotic susceptible TB. While infection with an exogenous drug resistant TB strain is related to infection control measures, the development of acquired *M. tuberculosis* resistance is multi-faceted and can be attributed to various social, political, economic, epidemiological and pathophysiological factors (Kim *et al.*, 2005). Efforts to understand the molecular basis of *M. tuberculosis* antibiotic resistance have advanced significantly and investigations of potentially unique genetic traits in MDR- and XDR-TB strains are ongoing. Unlike other bacterial pathogens, there is no evidence that gene acquisition contributes to antibiotic resistance in *M. tuberculosis* (Nachega and Chaisson, 2003).

While MDR-TB can be effectively treated with a long-term regimen of second-line antibiotics (Nathanson *et al.*, 2006), XDR-TB is often considered very difficult to treat, or is even untreatable, with existing chemotherapeutic agents (Haydel, 2010). The diagnosis of MDR-TB or XDR-TB further subjects the patients to as many as 20 pills per day, as well as antibiotic intramuscular
injections for 18-24 months. This lengthy treatment is not only more expensive than first-line antibiotics, but also comes with devastating, toxic side effects, emotional and social anxieties and psychological stresses (Rajbhandary et al., 2004; Jakubowiak et al., 2008). A large retrospective study revealed that XDR-TB cases have a worse clinical outcome than MDR-TB cases resistant to all first-line antibiotics (39% vs 54% treatment success, respectively) (Migliori et al., 2007). Therapy for LTBI is also protracted and comes in various regimens that may contain any combination of isoniazid, rifamycin, pyrazinamide and an approved fluoroquinolone, in the case of drug resistant LTBI and is fraught with additional adverse affects (Kaneko et al., 2011). In order to combat MDR and XDR-TB and the overall spread of antibiotic resistant TB strains, the need for new anti-TB antibiotics is imminent.

**Global portfolio of candidate anti-TB drugs in clinical development**

After the discovery and development of new anti-TB drugs flourished in the mid-1900s, the TB drug pipeline was reduced to a mere leaky faucet with the new classes of antibiotics virtually nonexistent. It has been more than 40 years since the last novel TB-specific antibiotics were introduced into clinical practice. Given the challenge of treating MDR and XDR-TB, there are some new classes of antibiotics in the current anti-TB pipeline. There are atleast 13 drugs in various stages of clinical evaluation for TB till 2010 (Connell et al., 2011). These can be divided into several categories: i) novel drugs being developed for TB treatment, ii) current first line TB drugs being re-evaluated to optimize their efficacy and iii) currently licensed drugs for other indications and next /generation compounds of the same chemical class being re-proposed for TB (Table 2).
Clearly, there is an urgent need for new anti-TB antibiotics with novel mechanism of action with all the following required properties (Table 3).

**Anti-TB natural products**

Although different types of anti-TB agents are available in world market, there is a growing interest in natural products for novel anti-TB drug discovery, due to non-specific side effects associated with synthetic therapeutics agents and unusual chemical diversity present in natural products. Natural products have been recognised as the source of most active ingredients of medicine. More than 80% of drug available in world market were natural products or inspired by them (Newman and Cragg, 2007; Butler, 2008). Natural products derived scaffolds are therapeutic templates for the design of new therapeutic drugs using medicinal chemistry and computer-assisted design techniques. Thus, they have a remarkable impact on the treatment of TB in comparison with classical FDA-approved drugs such as rifampicin, kanamycin and cycloserine. Anti-TB compounds isolated from natural sources such as plants, microbes and marine organisms have been found with different skeleton chemical forms and conformations (Pauli *et al.*, 2005).

Plants have been used worldwide in traditional medicines for the treatment of various diseases and it is estimated that even today approximately 65-75% of the world’s population rely on medicinal plants as the primary source of medicines. The phytochemical study of some of these plants has yielded a number of active natural products. Next to microorganisms, plants are the important source for anti-TB compounds. Several recent review and research articles have highlighted the underutilized potential of plant species as sources of antimycobacterial extracts and chemicals (Gautam *et al.*, 2007; Green *et al.*, 2011; Sivakumar and Jayaraman, 2011; Molly *et al.*, 2012). Among the plant-
derived antimycobacterial compounds belonging to an exceptionally wide diversity of classes, alkaloids, terpenoids, coumarins, peptides and phenolics are more dominant (Mmushi et al., 2010). Of 17,500 higher plant species occurring in India only about 365 species have been evaluated so far for antimycobacterial activity (Gautam et al., 2007).

The potential of marine organisms is well documented in the recent past. Yet, their utility for anti-TB drug discovery is still in its infancy. Till 2000, there are only two reports of in-vitro anti-TB activity from marine origin. Massetolide A and viscosin are cyclic depsipeptides isolated from cultures of Pseudomonas species isolated from a marine alga and tube worm, respectively. There are very few anti-TB compounds isolated from marine macro organisms such as molluscs (kahalalides A and F), sponges (heteronemin), corals (litosterol) (El-Sayed et al., 2000; Souza, 2006). Bioactive substances from natural sources are available in extremely low quantities leading to limitations in using the reservoir of marine organisms for bioassay and therapy. To overcome these problems, few methodologies such as mariculture, bioreactors, sponge cell culture, genetic modification and most importantly chemical and semi-synthetic approach can be pursued. Certain anti-TB compounds produced by marine sponges (agelasine) and corals (litosterol) have been synthesized by chemical methods (Mancini et al., 2007). Unfortunately, none of the several hundreds of non-microbial natural products with antimycobacterial activity have moved forward in drug development (Pauli et al., 2005).

Microorganisms that live together in the environment develop long-lasting methods to keep each other at bay. As a result, many of our most effective bactericidal agents have come from environmental organisms (Ymele-Leki et al., 2012). Microbes are the most exploited sources for bioactive natural
products including anti-TB compounds. Till date, more than 1000 antimycobacterial compounds have been reported from microbial sources among which actinomycetes are the best reported microbial source. All the commercially available natural product based-anti-TB drug in current practice is only from actinomycete origin. There are very few reports on anti-TB compounds from other bacteria such as *Janthinobacterium sp.* Ant5-2 (J-PVP) and *Flavobacterium sp.* Ant342 (F-Y OP) isolated from land-locked freshwater lakes of Schirmacher Oasis, East Antarctica (Mojib *et al.*, 2010) and *Bacillus subtilis* isolated from leaf of egg plant, China (Xiaoxi and Jun, 2010). Antimycobacterial activity of the fungal species such as *Fusarium, Penicillium* and *Aspergillus* isolated from Sudarban mangrove forest was reported by Radhakrishnan *et al.*, (2011a).

**Actinomycetes**

Actinomycetes are aerobic, gram positive filamentous bacteria with high G+C (Guanine+ Cytosine) containing DNA. Actinomycete was first discovered by Ferdinand Cohn in 1875 and it was first named by Actinomyces (ray fungus) by Harz in 1877. Actinomycete was first recognized by Gasperini in 1890 as potential destroyers of bacteria and fungi (Waksman, 1959). Actinomycetes grow well on simple laboratory media with different chemical composition but their growth is much slower than that of other bacterial groups. In solid medium, most actinomycetes form leathery, smooth surfaced, cottony colonies with varying sizes. Most actinomycete genera form mycelial growth called substrate/vegetative or primary mycelium. In addition, from the primary mycelium, the secondary/aerial or reproductive mycelium grows on the surface of the medium which form asexual spores. The temperature ranging between 20°C and 30°C and pH between 5.5 and 8.0 are conducive for the growth of
most actinomycetes. Nearly one month of incubation is needed for the primary isolation of actinomycetes (Balagurunathan and Radhakrishnan, 2007).

Actinomycetes are the most successful group of bacteria that occur in multiplicity of natural and man-made environments due to their ability to utilize all the available substrates in the environment. They are present in both terrestrial and aquatic ecosystems. Most of the actinomycetes are free living saprophytes but some are parasitic or symbiotic to plants and animals. Soil is the single most reservoir for actinomycetes. Among the total microbial population in soil, actinomycetes group occupies 10 to 50%. A single gram of rich agriculture soil can contain $10^5$ colony forming units (CFU) of Streptomyces and $10^4$-$10^5$ CFU of *Micromonospora* and other genera. The number and types of actinomycetes are highly affected by the physico-chemical properties of soil, climatic condition of that particular ecosystem, etc., (Labeda and Shearer, 1990).

**Actinomycetes characterization and identification**

The characterization of a strain is a key element in prokaryote systematics. Although various new methodologies have been developed over the past 100 years, both the newer methodologies and those considered to be ‘traditional’ remain key elements in determining whether a strain belongs to a known taxon or constitutes a novel one (Tindall *et al*., 2010). It should be noted that the term Actinobacteria refers to all members of the phylum whereas the designation actinomycetes refers only to strains belonging to the order Actinomycetales. The class actinobacteria comprises of 5 subclasses, 9 orders, 55 families, more than 240 genera and 3000 species (Goodfellow and Fiedler, 2010). Actinomycetes are quite different from other eubacteria with respect to cellular morphology and cell wall chemistry. A number of different methods
have been used to classify actinomycetes. Actinomycete taxonomy was traditionally based on morphological characteristics such as size, shape and colour of the colonies on specific media. Gram staining and acid fastness and pigment production are the other parameters used while classifying using morphology. Other morphological features that are taxonomically important include the colour, colony morphology and surface arrangement of conidiospores (Shirling and Gottlieb, 1966). Physiological attributes such as nutritional requirements, fermentation products and growth conditions such as oxygen, temperature and inhibitory products are also important when classifying actinomycetes. The antibiotic resistance pattern within the genus *Streptomyces* have been used for speciation (Wellington and Cross, 1983) and selective isolation (Wellington et al., 1987).

Cell wall constituents are major characteristic used in chemotaxonomy. The composition of cell walls varies greatly among different group of actinomycetes. The presence of diaminopimelic acid (DAP) isomers is one of the most important cell wall properties of the gram positive bacteria including the actinomycetes. 2,6 DAP is widely distributed as a key amino acid and it has optical isomers (Sasaki et al., 1998). Chemotaxonomy also involves the analysis of other macromolecules such as the isoprenoid quinines (Menaquinones and ubiquinones), lipids (lipopolysaccharides and fatty acids including mycolic acids), polysaccharides and related polymers (methanochondroitin and wall sugars) and proteins. Although chemotaxonomy is still considered useful in actinomycete taxonomy, it is not always reliable as several genera may exhibit similar chemical properties. In addition, the techniques are cumbersome and time consuming.
The comparison of the DNA nucleotide sequences of two strains provides a rapid and accurate method of establishing relatedness (Priest and Austin, 1995). 16s rRNA is a major component of the small (30S) ribosomal subunit which is important for subunit association and translational accuracy. The 16S rRNA gene consisting of 1542 bases, is highly conserved among the microorganisms and is therefore an excellent tool for studying phylogenetic relationships. The 16s rRNA genes of many phylogenetic groups have characteristic nucleotide sequences called oligonucleotide signatures. Oligonucleotide signatures are sequences which occur in most or all members of a phylogenetic group and can be used when designing primers which are genus or species specific. In sequence based techniques, primers to the extremities of the gene are used to amplify the DNA. The amplified DNA can either be sequenced directly or cloned into a phage or plasmid vector prior to sequencing. After the sequences have been generated they are compared by aligning the corresponding nucleotide sites. These types of simple comparisons of sequence positions will provide an estimate of how closely related the organisms are. Analysis of the 16S rRNA gene offers a time saving alternative to the classical methods of identification summarised above (Konstantinidis and Dittiedje, 2005). 16s rRNA sequencing has been used to reclassify actinomycetes species that were incorrectly classified using classical identification methods. In India, there are some novel actinomycetes isolated from different ecosystems and identified based on conventional and molecular methods (Table 4). But their bioprospecting potential is less explored.

**Bioproducts from actinomycetes**

Amongst prokaryotes, members of the order Actinomycetales, notably the genus *Streptomyces*, remain the richest source of natural products,
including clinically useful antibiotics, antimetabolites, antiparasitic, antiviral and antitumor agents (Berdy 2005; Newman and Cragg, 2007). Actinomycete sources account for about 45% of all microbial bioactive secondary metabolites with 7600 of these compounds (80%) being produced by *Streptomyces*. About 74% of all actinomycete products and 70-75% of various bacterial products exhibit antibacterial and/or antifungal activities. In contrast, only 40-45% of all fungal products have some kinds of antimicrobial activity against fungi. The antitumor activity is displayed by 30%, 24% and 27% of actinomycetes, bacterial and fungal products, respectively (Berdy, 2005). Despite this astonishing productivity, it has been predicted that only about 10% of the total number of natural products that can be synthesized by these organisms have been discovered (Watve et al., 2001). The application of genomic technologies which showed that the whole genomes of *Rhodococcus sp.* RHA1, *Saccharopolyspora erythraea* NRRL 23338, *Salinispora tropica*, *Streptomyces avermetilis* MA-4680 and *Streptomyces coelicolor* A(3)2 contained around 20 or more natural product biosynthetic gene clusters for the production of known or predicted secondary metabolites (Balagurunathan and Radhakrishnan, 2010).

The power of actinomycetes in the competitive world of chemical synthesis can be appreciated by the fact that even simple molecules are made by fermentation rather than by complex chemical synthesis. Most of the actinomycete natural products are so complex and contain many centres of asymmetry that they will probably never be made commercially by total organic synthesis. There are five major groups of bioproducts produced by actinomycetes (Balagurunathan and Radhakrishnan, 2010). This includes primary metabolites, secondary metabolites, bioconversion products, microbial cell products and recombinant products (Table 5). Actinomycete genera such
as *Streptomyces*, *Rhodococcus* and *Thermomonospora* are recognized as a new source for the biosynthesis of gold and silver nanoparticles (Balagurunathan *et al.*, 2011). Actinomycetes are also employed in the biodegradation of complex environmental pollutants (Sharma and Pant, 2001; Jayabarath *et al.*, 2010).

Bioactive metabolites, in particular antibiotics, production by actinomycetes is strain-specific and conditional. It has long been known that there are actinomycete strains belonging to the same species that produce antibiotics different from one another and also that there are strains belonging to different species that produce the same antibiotic. Antibiotic production is therefore, not species specific, but strain specific (Hotta and Okami, 1996).

**Bioactive pigments from actinomycetes**

A number of actinomycete species produces a wide variety of pigments that are important to cellular physiology and survival. It still remains uncertain why these pigmented secondary metabolites from actinomycetes have antibiotic, anticancer, immunosuppressive and/or antioxidant activities. Due to such diverse and promising activities against different kinds of diseases, these compounds also play an important role in both pharmaceutical and agricultural research (Soliev *et al.*, 2011). Streptomycetes produce many intensely pigmented molecules that can be isolated in pure form. Each Streptomyces strain produces its own distinct cocktail of pigmented natural products that determines the strain’s characteristics. Various biologically active pigmented compounds were isolated from actinomycetes especially from *Streptomyces* (*Table 6*).
Medium consistency, morphology and metabolite production in actinomycetes

Actinomycetes, in particular Streptomycetes, have a filamentous growth habit adopted for surface growth in solid cultures in nature (Shahab et al., 1994). However, our understanding of the physiology of these organisms is largely based on their growth in submerged liquid cultures. According to the literature, submerged fermentation followed by extraction of the metabolites from the filtrate is the mostly used current procedure, probably due to the easy accessibility of solubilized compounds in the culture filtrate. However, some disadvantages are apparent. Actinomycetes are often found to produce antibiotics only on solid media (Shomura et al., 1979; Pickup et al., 1993; Salamoni et al., 2010; Radhika et al., 2011; Zhong et al., 2011). Very little is known as to why such activity is restricted and not detected in submerged cultures (Shomura et al., 1979; Pickup et al., 1993). In addition, antibiotics like fumaridamycin were detected with much difficulty in submerged cultures because the mycelium of the producing strain inactivated the antibiotic more readily than in agar culture (Mayurama, 1975). Certain antibiotics like tetracycline, rifamycin and neomycin are produced from actinomycetes by solid state fermentation using different agriculture substrates (Barrios-Gonzalez et al., 2003; 2005).

Production of a majority of industrially important secondary metabolites from actinomycetes is carried out using submerged fermentation processes where they exhibit diverse morphological forms (Braun and Vechtlifshitz, 1991; Treskatis et al., 1997). Morphology is influenced by environmental conditions such as medium composition and shear stress (O’Cleirigh et al., 2005; Whitaker, 1991). Further, morphology and product formation have been
observed to be closely related (Manteca et al., 2008). For eg. morphology and avermectin production by *Streptomyces avermetilis* were influenced by factors such as nitrogen source, dissolved oxygen level and inoculum volume (Yin et al., 2008). Pellets of small size and high density were found to promote avermectin production. However, the relationship between morphology and product formation is not well understood.

**Anti-TB compounds from actinomycetes**

The first drug discovered to treat TB was streptomycin, an aminoglycoside isolated from the actinomycete *Streptomyces griseus*. Another outstanding example is rifampicin, an ansamycin antibiotic isolated from *Streptomyces mediterranei* renamed as *Amycolatopsis mediterranei*. Following the discovery of streptomycin, in the period known as the golden era of TB research (1940-70), several synthetic drugs were introduced in the market. However, actinomycetes still played a crucial role in drug discovery against TB. For example, other aminoglycosides such as kanamycin from *Streptomyces kanamyceticus*, the semi-synthetic amikacin produced from kanamycin A and capreomycin from *Streptomyces capreolus*, as well as D-cycloserine from *Streptomyces sp.*, are being used in TB treatment as second line drugs.

More compounds from actinomycetes of terrestrial and marine origin are still in different stages of investigation to be developed as potential anti-TB drugs. In India, there are very few works on the anti-TB activity of microbes, especially actinomycetes. Raja and Prabakaran (2011) reported the antimycobacterial effect of psychrophilic actinomycetes such as *Streptomyces, Micromonospora* and *Micropolyspora* isolated from Manali Ice point: Himachal Pradesh. Antimycobacterial activity of actinomycetes isolated from marine and terrestrial ecosystems were reported by Radhakrishnan et al., (2010; 2011b).
Some important anti-TB compounds isolated from actinomycetes (Waksman and Lachevalier, 1962; Pogell, 1998; Iwatsuki et al., 2007; Xie et al., 2007) are given in table 7.

**Actinomycetes from rare ecosystems**

Now-a-days, it is becoming increasingly difficult to find novel metabolites from common actinomycetes as regular screening leads to the rediscovery of mostly known compounds. However, it is not the end of an era but an endless frontier. Using standard procedures for the isolation of novel actinomycetes from poorly studied habitats is an alternative (Okoro et al., 2009) and by applying new methods, rare or uncommon actinomycetes can be isolated. Novel species may contain unique compounds as the evolution of secondary metabolites (Jenson et al., 1996).

Actinomycetes are traditionally considered as organisms that cannot occupy natural ecological niches that are characterized by extreme conditions. Actinomycetes have specific environmental needs differing from those of other mycelial bacteria. Now-a-days, great amount of data on the isolation of actinomycetes resistant to extreme environmental factors like acidity, salinity, temperature and pressure has been accumulated. Actinomycetes are isolated from various rare ecosystems such as forests, mountains, deep sea, desert and alkaline soils all over the world (Zenova et al., 2011) (Table 8). There are certain reports available on bioactive metabolites of actinomycetes isolated from rare ecosystems (Table 9). But there are no considerable reports on antitubercular compounds from actinomycetes of rare ecosystems.
Actinomycetes from desert ecosystem

The physicochemical properties of desert soils greatly influenced the metabolic activities of actinomycetes. Desert soil actinomycetes are also a potential source for novel bioactive metabolites. For example, the metabolic profile of *Streptomyces sp.* strain C34 isolated from the Chilean hyper-arid Atacama Desert soil is dependent on the culture media used for its growth. The application of an OSMAC (one strain-many compound) approach on this strain using a range of cultivation media resulted in the isolation and identification of three new compounds from the rare class of 22-membered macrolactone polyketides, named chaxalactins A-C. All the three compounds showed strong activity against Gram positive bacteria but weak activity against the Gram negative strains tested. In addition, the known compounds desferoxamine E, hygromycin and 5′ dihydro hygromycin were detected (Rateb et al., 2011a). Nachtigall et al., (2011) isolated 22-membered macrolactone antibiotics named Atacamycins A-C from *Streptomyces sp* C38 isolated from hyper-arid soil of Atacama Desert in the North of Chile. Schulz et al., (2011) isolated aminoquinone derivatives, Abenquines A-D from *Streptomyces sp.* strain DB634 isolated from Atacama Desert. This showed antibacterial and antifungal and enzyme inhibitory activity.

The microbial diversity of Thar Desert, India was investigated three decades ago. The authors reported that among the total microbial population, actinomycetes are dominant in Thar Desert soil. Diraviyam et al., (2010) studied the antioxidant activity of melanin pigment extracted from a Thar Desert soil actinomycete *Streptomyces griseorubiginosus* D5.
Screening methods for anti-TB products

With the increasing need for drugs to combat TB, there is an urgent need for rapid, low cost, high throughput assays for screening of new drug candidates. Due to the slow growth of *M. tuberculosis*, incubation times for drug susceptibility assays which rely on the development of colonies or turbidity are excessively long (Collins and Fransblau, 1997). Moreover, conventional method using LJ medium cannot be used for testing novel extracts and compounds as their heat stability is not known: it requires mixing these novel compounds with the egg medium and inspissating it. There has been a number of mycobacterial drug susceptibility assays described over the period of time and also used for the screening of natural products for anti-TB activity (Sanchez and Kouznetsov, 2010). These include the classical disc diffusion method (Pauli et al., 2005), agar dilution methods, and broth dilution assay, radiometric (BACTEC) (Collins and Franzblau, 1997), dye based (Franzblau et al., 1998; Mohammadzadeh et al., 2006) and fluorescent (Collins et al., 1998) / luminescent reporter assays (Shawar et al., 1997). Most of these assays, however, lack one or more of the attributes of a mass screening assay: rapidity, high throughput and low cost of the supplies and equipment.

Luciferase Reporter Phage (LRP) Assay

Assay strategies employing reporter phages are attractive alternative to cumbersome conventional method. Drug susceptibility was assessed based on efficient production of photons by viable mycobacteria infected with specific reporter phages expressing firefly luciferase gene. In the presence of drugs, resistant organisms continue to produce light while susceptible organisms do not, as they get killed. Jacobs et al., (1993) first reported the feasibility of
luciferase reporter phage (LRP) assay. The first LRP constructed was TM4 derived phage that is capable of infecting both fast and slow growing mycobacteria. LRP assay is a rapid, less laborious and less time consuming method for high throughput screening of a large number of compounds for antmycobacterial activity. Shawar et al., (1997) investigated the ability of LRP assay to detect antmycobacterial activity of plant extracts. Over all 480 extracts were tested, and about 99% agreement was observed between luciferase assay and microplate alamar blue assay. Investigators also found drug activities in LRP assay was parallel to MIC determined by conventional and BACTEC 460 (Banaiee, 2001).

Natural products from various sources and synthetic compounds are being screened for antmycobacterial activity by adopting LRP assay. Prabu Seenivasan et al., (2006) screened certain plant essential oils against M. tuberculosis by LRP assay. Antimycobacterial activity of chalcone derivatives (Sivakumar et al., 2007; 2010a), novel 1,3,5-triphenyl 1-2-pyrazolines (Sivakumar et al., 2010b), quinoline coupled 1,2,3-triazoles (Karthik Kumar et al., 2011) and novel 4-(morpholin-4-yl)-N’-(arylidene)benzohydrazides (Raparti et al., 2009) were reported by using LRP assay. Radhakrishnan et al., (2010; 2011b) screened the crude extracts prepared from terrestrial and marine actinomycetes against standard and clinical isolates of M. tuberculosis using LRP assay. Crude solvent extracts prepared from selected marine fungal isolates were also screened for M. tuberculosis H37Rv using LRP assay (Radhakrishnan et al., 2011a). Molly et al., (2012) reported the antmycobacterial activity the extracts of medicinal plant, Astonia scholaris.

In many of the anti-TB drug discovery programme, either slow growing mycobacterial species or avirulent strain of M. tuberculosis H37Ra was used as
a surrogate host, instead of using clinical *M. tuberculosis* isolates. In place of the highly infectious and slow growing pathogen, various members of less pathogenic, fast growing mycobacteria were used such as *M. phlei*, *M. marinum*, and *M. fortuitum*. The most widely used fast grower is *M. smegmatis*, which has proven amenable to genetic manipulation including target screening, and has also served as a model for rapid screening of compound libraries. The recent discovery of TMC207 from HTS against this organism has firmly established its usefulness for this purpose (Kaneko *et al.*, 2011). Chaturvedi *et al.*, (2007) stated that the *M. smegmatis* based screening showed 100% specificity and 78% sensitivity vis-a-vis MDR *M. tuberculosis*. Recently, Gupta and Bhakta (2012) reported a fast growing, non-pathogenic *M. aurum* as an integrated surrogate model for screening drugs against *M. tuberculosis*.

Although the review clearly illustrates the number of works on actinomycete bioactive compounds from rare ecosystems, there is no clear evidence for potential use of the compounds in practice particularly against tuberculosis. So the present study is aimed to highlight the isolation of antibiotics against *M. tuberculosis* from actinomycetes of rare/unexplored ecosystems.