Secondary metabolites of actinomycetes as potential source of antibiotics

Sajad Ahmad Bhat*, Ruqeya Nazir, Tauseef Ahmad Malik, Fayaz Ahmad Shah

Centre of Research for Development, University of Kashmir, Srinagar-190006
Email: sajadku3567@gmail.com

Abstract: The actinobacteria produce an enormous variety of bioactive molecules of commercial importance. One of the first antibiotics used was Streptomycin, produced by *S. griseus*. For decades, microbial natural products have been one of the major sources of novel drugs for pharmaceutical companies, and today all evidence suggests that novel molecules with potential therapeutic applications are still waiting to be discovered from these natural sources, especially from actinomycetes. Any appropriate exploitation of the chemical diversity of these microbial sources relies on proper understanding of their biological diversity and other related key factors that maximize the possibility of successful identification of novel molecules. This review focuses about bioactive metabolites produced by actinobacteria.

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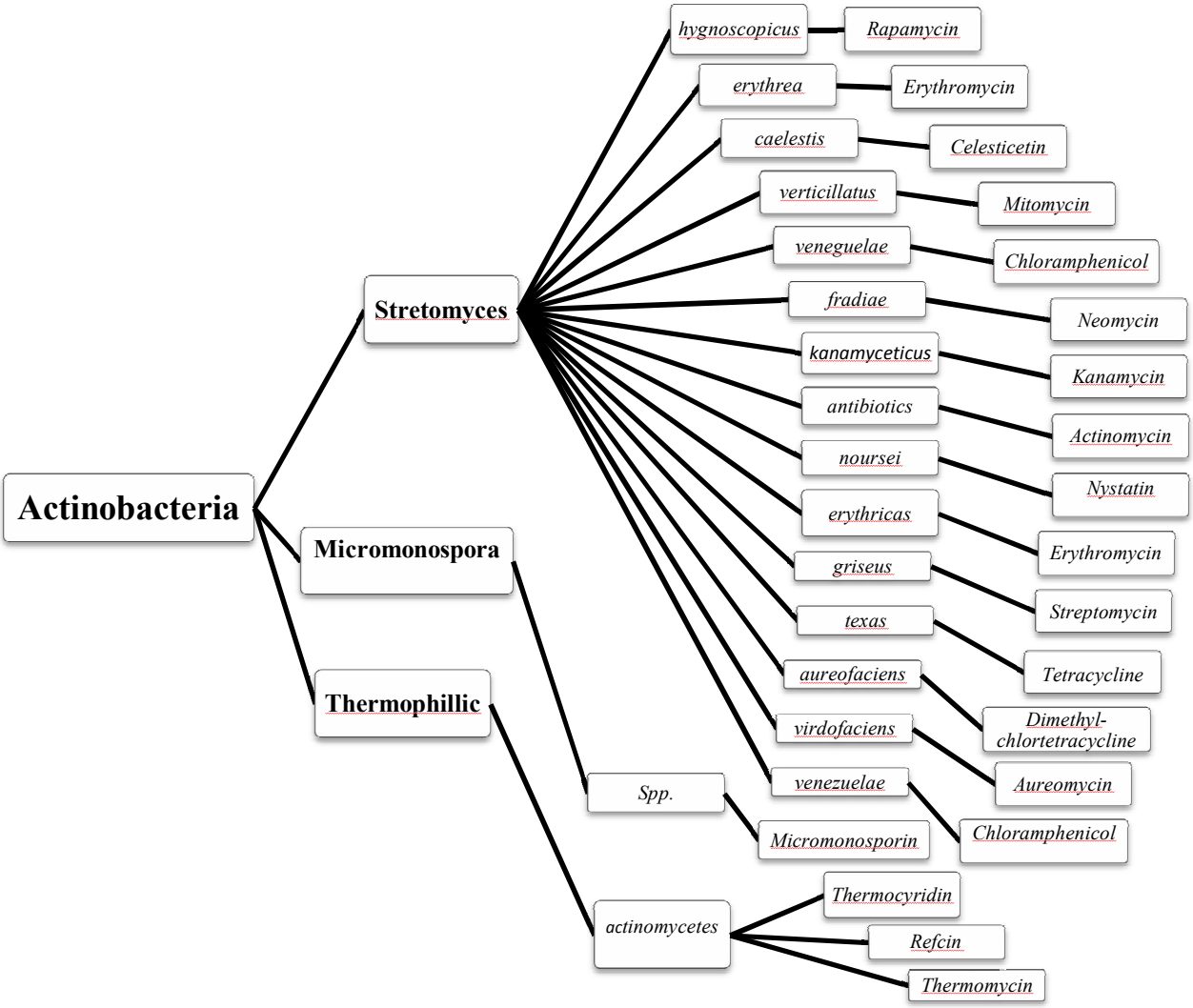
Introduction

Actinobacteria are filamentous bacteria belonging to phyla actinobacteria and the order actinomycetales (Waksman, 1959). Actinobacteria are the most widely distributed group of microorganisms in nature which primarily inhabit the soil (Oskay et al., 2004). They are aerobic, gram-positive bacteria, very widely distributed and form major group of soil population (Kuster, 1968). They were distinguished as gram-positive bacteria with a high GC content in their DNA to more than 70%. Soil as their main natural habitat is nutritionally, biologically and physically complex and variable; as a result, they are able to perform a broad range of metabolic processes and to produce an immense diversity of bioactive secondary metabolites (Ventura *et al., 2007*) The number and types of actinomycetes present in a particular soil is greatly influenced by geographical location such as soil temperature, type of soil, soil pH, amount of organic matter, cultivation, aeration etc. Actinomycetes have provided useful secondary metabolites of high commercial value and continue to be routinely screened for new bioactive compounds. These searches have been remarkably successful and approximately two thirds of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes (Okami et al., 1988). Almost 80% of the world's antibiotics are known to come from Actinomycetes, mostly from the genera Streptomyces and Micromonospora (Pandey et al., 2004). Actinomycetes are the most economical and biotechnologically valuable class of prokaryotes producing bioactive secondary metabolites notably antibiotics (Blunt and Prinsep, 2006) anti tumor agents, immunosuppressive agents and enzymes (Berdy, 2005; Cragg and Newman, 2005; Strohl, 2004).

Actinomycetes are the main source of clinically important antibiotics, most of which are too complex to be synthesized by combinatorial chemistry, making three quarters of all known products; the Streptomyces are especially prolific producing around 80% of total antibiotic products (Bull and Stach, 2005; Imada and Okami, 1998; Garson, 2005). Micromonospora is the runner up with less than one-tenth as many as Streptomyces (Lam, 2006). In addition to antibacterial compounds they also produce secondary metabolites with other biological activities of which the Streptomyces spp. amounts for 80% of the total production by actinomycetes (Cragg and Newman, 2005). Majority of the actinomycetes in soil that are potential drug sources remain uncultivable, therefore inaccessible for novel antibiotic discovery. Goodfellow and Haynes reviewed the literature on isolation of actinomycetes and suggested that only 10 % of actinomycetes are isolated from nature. Most of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi. The selective antimicrobial activities of actinomycetes differ greatly, both quantitatively and qualitatively, as could easily be demonstrated by their respective antibiotic spectra. The nature of the active agents or the antibiotics produced by these organisms depends upon the species; frequently upon the strain; the composition of the medium in which it is grown, and the conditions of cultivation (Waksman and Schatz, 1945). Although soils have been screened by pharmaceutical industry for about 50 years, only a small fraction of actinomycetes taxa have been discovered. Hence more

focus needs to be shifted in exploring unexplored habitats.



A SYSTEMIC OVERVIEW OF LITERATURE

Microbial metabolites account for a majority of drugs available in the market. Among bacteria, Actinobacteria are known for the production of different classes of antibiotics including aminoglycosides, anthracyclins, glycopeptides, β-lactams, macrolides, nucleosides, peptides, polyenes, polyethers, terpenes and tetracyclines which possess wide range of biological activities.

Emergence of multidrug resistant pathogens has triggered the need for discovery of new antibiotics with unique modes of action as exemplified by the following case studies. Emergence of methicillin-resistant *Staphylococcus aureus* causes serious infections. Vancomycin was earlier used to circumvent the problem of methicillin resistant *Staphylococcus aureus,* but unfortunately vancomycin resistant *S. aureus* have been reported in hospitals. Penicillin, ampicillin, streptomycin or gentamicin are used in various combinations for the treatment of infections caused by enterococci. Some strains have developed resistance to aminoglycosides, β lactam antibiotics and it leads to the failure of combination therapy. Occurrence of Penicillin resistant Streptococcus pneumonia (PRSP) is on rise worldwide. MDR tuberculosis strains exist owing to development of resistance by the causative agent towards important anti-TB drugs, including isoniazid and rifampicin. Effective treatment of the infections caused by these organisms is yet to be established.

The term antibiotic appeared as early as 1928 in the French microbiological literature as antibiosis. The phenomenon of antagonism between living organisms was frequently observed even since 1877, when Pasteur and Joubert noticed that aerobic bacteria antagonized the growth of *Bacillus anthracis*. In 1940 Waksman had forecasted, "We are finally approaching a new field of domestication of microorganisms for combating the microbial enemies of man and his domesticated plants and animals. Surely microbiology is entering a new phase of development. During the 1960s, the phase of discovery of antibacterial compounds slackened, but efforts were then made to search also for antifungal, antimycoplasmal, antispirochetal, antiprotozoal, antitumor, antiviral and antiphage compounds, as well as for antibiotics for non-medical uses such as antioxidants.

By far the most extensively studies of the actinomycetes suborders is Streptomycineae, mostly because the genus Streptomyces has been the source of vast numbers of useful antibiotics. Streptomyces are widespread in soil and are the most frequently isolated actinomycetes. Hundreds of Streptomyces spp. have been described, and more than 70 have phylogenetically sorted. Stapley *et al.,* (1972) isolated a number of actinomycetes from soil which were found to produce one or more number of a new family of antibiotics, the cephamycins, which are structurally related to cephalosporin. Yasuji *et al.,* (1975) demonstrated a new antibiotic, fumaramidmycin which has been isolated from a Streptomycete NR-7GG1 which was characterized and named *Streptomyces kurssanovii*. The strain produced the antibiotic only when grown on agar plates but not in the submerged culture broth, where the contact with the vegetative mycelia appears to cause the inactivation of the antibiotic. The antibiotic showed antimicrobial activity against both gram-positive and gram-negative bacteria.

Then Waksman intentionally employed *Mycobacterium tuberculosis* as a test organism in seeking anti-tuberculosis drugs and found streptomycin and many antibiotics with antibacterial, anti-fungal, anti-protozoan, anti-parasitic, anti-viral and anticancerous activities by employing various detection methods. Though the detection method in screening is important, new substances can be found by combining the detection method with the improved methods of culture selection and cultivation. Lechevalier *et al.,* (1989) isolated a novel family of antitumor antibiotics, the calicheamicins from the fermentation broth of *Micromonospora echinospora* subspecies calichensis. These antibiotics exhibited significant activity against Gram-positive and gram-negative bacteria *In-vitro*. Calicheamicin demonstrated antitumor activity against P388 leukemia and B16 melanoma *in vivo.*

The two methods given by Hayakawa *et al*., (1991) for the isolation of the rare actinomycetes Streptosporangium and Dactylosporangium from soil use the ability of both these sporangiospores of Streptosporangium and the globose bodies (aleuriospore) of Dactylosporangium to withstand heating and treatment with benzethonium chloride (BC). Li *et al.,* (1992) mutagenized natural non-antibiotic producing Streptomyces sp. 1254 by UV irradiation and two active mutants were isolated. Mutant 113 produced novel anthracycline compounds designed mutactimycins. Mutactimycin A was active against the bacteriophage of *B. subtilis* and some viruses in tissue culture. The mutant 2-6 synthesized a basic water-soluble antimicrobial antibiotic. Williams *et al.,* (1993**)** described new methods for the detection and identification of novel actinomycetes from a range of environment and also approached to the detection of actinomycetes ranged from investigation of neglected habitats and extreme environment (e.g. alkali soils and oil drills) to the analysis of DNA extracted from the environment and use of specific phages.

The continuing problems of the identification of actinomycete were also considered. The macroscopic and microscopic studies of an actinomycete growing on agar can provide useful and rapid clues for identification of their respective genus. Macroscopic characters include colony characteristics such as size, shape and color of spore formation. Cultures are observed for microscopic features including fragmentation or non fragmentation of substrate and aerial mycelium, presence of sclerotia, spore chain morphology and spore surface ornamentation. On the basis of spore chains, the strains can be placed into groups. Takizawa *et al*., (1993) isolated actinomycetes from sediment samples collected in Chesapeake Bay with an isolation medium containing nalidixic acid, which proved to be more effective than heat treatment of samples. Moncheva *et al*., (2002) reported that forty-seven actinomycete strains were isolated from Antarctic soils. Nineteen of them showed antagonistic activity against Gram-positive and Gram negative bacteria. Kozo Ochi *et al.,* (2003) showed certain rpsL mutations (which encodes the ribosomal protein S12), mutations that confer resistance to Streptomycin, markedly activate the production of antibiotics in Streptomyces spp. These rpsL mutations are known to be located in the two conserved regions within the S12 protein.

Acinobacteria also possess the ability to synthesize compounds having antifungal activities as shown by a study carried out by Jain *et al.*, (2004). He isolated a strain of *Streptomyces purpeofuscus* CM 1261, from a sample of compost collected locally,

which was found to possess strong antagonistic activity against 4 human pathogenic fungi i.e. *Candida albicans, Aspergillus niger, Microsporum gypseum and Trichophoton sp*. The active antifungal compound produced by it was found to be a heptane group of polyene antifungal antibiotic. Usha *et al.,*(2005) used cell free supernatant of the selected actinomycete isolate which were able to inhibit the growth of all human pathogens (*S. aureus, Proteus vulgaris, P. aeroginosa, B. megaterium, K. pneumoniae, C. albicans, A. niger, S. cervisiae*) used in this study. Out of the 5 actinomycetes isolates Streptomyces sp (S1) exhibited high antibacterial activity. The remaining isolates also showed very promising activity against human pathogen. Gurung *et al.,* (2009) isolated 79 actinomycetes from soils of Kalapatthar Mount Everest region. Twenty seven (34.18%) of the isolates showed an antibacterial activity against at least one test-bacteria among two Gram positive and nine Gram negative bacteria in primary screening by perpendicular streak method. Thirteen (48.15%) showed antibacterial activity in secondary screening. The active isolates from primary screening were heterogeneous in their overall macroscopic, biochemical, and physiological characteristics through unweighted pair group method using average (UPGMA) cluster analysis. Delineation of the three active isolates showing potent broad spectrum antibacterial activity revealed that they belonged to distinct taxonomic groups.

Further in 2009 Kekuda *et al.,* evaluated antioxidant and antihelminthic activity of two Streptomyces species isolated from Western Ghat soils of Agumbe, Karnataka. The isolates were identified as Streptomycetes based on colony appearance, spore arrangement, biochemical characteristics. The antioxidant activity of butanol extract of the culture broth of Streptomyces sp. No 1 and 2 was assessed using DPPH free radical assay and Fe+3 reducing assay. The solvent extracts of the isolates showed a dose dependent antioxidant activity. Antihelminthic activity was determined in adult Indian earthworm model. Among isolates, Streptomyces sp. No. 2 was found to possess marked antioxidant and antihelminthic efficacy than Streptomyces sp. No. 1.

Recently Gayathri *et al.,* (2011) isolated and identified actinomycetes strains from marine habitat possessing antimicrobial activity against the common human pathogens. Out of 20 isolated actinomycetes 10 were identified and selected for antibacterial activity. Out the 10 dominant actinomycetes species *Streptoverticillium album* was highly dominant and showed the best level of antibacterial activity against three human pathogens, *Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli*. Pandey *et al.,* (2011) evaluated Actinomycetes for their inhibitory activity on 3 strains of microorganism (*E. coli*, *P. aeruginosa* and *S. aureus*). Isolation of Actinomycetes strain was obtained by serial dilution method and grown on actinomycetes isolation agar. Antibacterial compounds were produced by submerged fermentation and activity of compounds were checked against bacterial culture by antibiogram analysis where intracellular and extracellular compounds showed positive result, compare to intracellular compounds, extracellular compounds was showed best result which was 30 mm zone of inhibition against *S. aureus* and MIC was found to be 0.0009 mg/ml.

Rakshanya *et al.,* (2011) isolated actinomycetes and evaluated their antimicrobial activity against different pathogenic bacteria. Actinomycetes isolates were identified as Streptomyces sp, Micromonospora sp, Nocadia sp. The cell free supernatant of the selected isolate were able to inhibit the growth of all human pathogens (*S. aureus, Proteus vulgaris, P. aeroginosa, E. coli*, *B. subtilis, B. megaterium, K. pneumoniae, C. albicans, A. niger, S. cervisiae*) used in this study. Out of the 5 actinomycetes isolates Streptomyces sp (S1) exhibited high antibacterial activity against Staphylococcus aureus. The remaining isolates also showed very promising activity against human pathogens. Sharma *et al.,* (2011) evaluated antimicrobial activity of actinomycetes against multidrug resistant S*taphylococcus aureus* and various other pathogens. Out of 134 isolates, 51 showed antimicrobial activity against one or more test organisms and six exhibited promising broad-spectrum activity against all the tested organisms. The observed cultural, morphological, physiological and biochemical characteristics confirmed that these isolates are species of the genus Streptomyces.

More recently, Nakade (2012) collected and analyzed hyper saline soil samples from different sites in Kolhapur district of Maharashtra over a period of one year for actinomycetes population. Members of genus Streptomyces were identified by MICRO-IS software. Isolates other than Streptomyces genus were identified using Bergeys manual of systematic bacteriology. Isolates were screened for antibacterial and anti-fungal activity. Results indicated that hyper saline soils are rich in biodiversity of actinomycetes, four species showed antifungal activity and ten showed anti-bacterial activity.

Antibiotic resistance is a form of drug resistance whereby some sub-microorganism, usually a bacterial species are able to survive exposure to one or more antibiotics. Infections caused by resistant microorganisms often fail to respond to the standard treatment, resulting in prolonged illness and greater risk of death. Antimicrobial resistance is not a recent phenomenon, but it is a critical health issue today.

Over several decades, to varying degrees, bacteria causing common infections have developed resistance to each new antibiotic, and AMR has evolved to become a worldwide health threat. With a dearth of new antibiotics coming to market, the need for action to avert a developing global crisis in health care is increasingly urgent. Antibiotic resistant bacteria cannot be controlled or killed by antibiotics. Methicillin resistant Staphylococcus aureus, vancomycin resistant Enterococcus and multidrug resistant Mycobacterium tuberculosis are serious public health problems. This situation compels researchers to search for new anti-microbial substances, particularly from micro-organisms.

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