

Actinomycetes as Antibiotic Agents: Future generation Bio-factory

Nalinee Kumari¹, Ekta Menghani² and Rekha Mithal³

Department of Microbiology¹ and Biotechnology², JECRC University, Jaipur, Rajasthan, India

Department of Chemistry, JECRC Foundation³, Jaipur, Rajasthan, India

E.mail: nalineek9@gmail.com

Abstract: Actinomycetes are renowned producers of various types of secondary metabolites like Antibiotics Antibacterial, Antiparasitic, Antitumor, Pesticides, and Herbicides etc. Major Antimicrobial Agents with clinical significance produced from Streptomyces sp. and Micromonospora sp. Streptomyces represents 45% of all bio actives compounds. Many secondary metabolites are used in Agriculture industries, Farming etc. Increase in development of antibiotics lead to increase in bacterial resistance towards antibiotics. Many antibiotics have diverse side effects as well. There is a urgent need to discover new antibiotics with its clinical applications to fight against all resistant antibiotics

Keywords- Actinomycetes, Antibiotics, Antimicrobial Agents, Multi-drug Resistance (MDR), Penicillins, Secondary metabolites,



1. INTRODUCTION TO ACTINOMYCETES

In the clinical and agriculture scenario there are many antibiotics that have been obtained from the bacteria, fungi and actinomycetes. Many of which have commercial importance as 75% of which is produced by gram positive actinomycetes like Streptomyces sp.[1] Actinomycetes are potential producers of many antimicrobial compounds[2] and research has been confirmed that a large number of antimicrobial agents have been produced from the soil and aquatic systems[3],[4],[5]. In nature all organisms have to fight with other microorganisms to survive and for this they have to produce some of the substances like antimicrobial agents, enzymes, antibiotics etc. some of the compounds (synthetic or chemical) act by inhibiting growth of microorganisms or by killing the microorganisms[6],[7]. These compounds have different mechanism of action as some obstruct with their biological activity viz; transcription, replication, protein synthesis cell wall synthesis etc [8]. Most of the actinomycetes are free living and abundantly scattered in natural environment

.They are found in both terrestrial and aquatic environment and have high acceptance in adverse environmental conditions [9]. For the study of microbial diversity use of molecular techniques has been brought a great attainment in the microbial ecology like in the soil [10], [11]. A series of bioactive secondary metabolites produced by actinomycetes with various biological activities such as; antibiotics, antifungal, antibacterial, anti-parasitic, immunosuppressive agents, enzymes etc [12]. Now a day almost all organisms have acquired resistance to most of the antibiotics but the contribution of antimicrobial agents still continued isolated from the actinomycetes. Actinomycetes species are well known saprophytic organisms that decompose the organic matter; especially they degrade the biopolymers that includes starch, chitin soil, and lignocelluloses [13]. Various antimicrobial compounds have been isolated from the actinomycetes such as, aminoglycosides, anthracyclines, macrolides b-lactams, peptides, polyene, actinomycin, tetracyclines etc [14]. 60% of the herbicides and insecticides have been reported in the last 5 years that were discovered

from the *Streptomyces* [15]. For the screening of novel antibiotics taxonomic characterization like very morphological, physiological, ecological and molecular characterization is very important and has been used for the identification and classification of many microorganisms [16]. Scientists have been used various types of techniques for the characterization of secondary metabolites discovered from the actinomycetes [17]. There are various multidrug resistant organisms that include: *Streptomyces*, *pseudomonas*, *Staphylococcus* etc. are resistant to almost all types of antibiotics. Strains of tuberculosis have been reported to be resistant to drug treatment, in some countries like United States. Organisms develop resistance due to incorporation of foreign substance in their genome resulting into mutation in their genome.

2. SOIL ACTINOMYCETES

Soil is a medium in which is free living organisms are present like bacteria, fungi, protozoa, nematodes and also with plant and insecticides. Among all organisms, bacteria are easy to isolate from soil and also easy to handle culture and maintain. Among all organisms bacillus is predominant bacterial species because they have resistant endospore and produce vigorous antibiotics like bacitracin and it always inhibits growth of other organisms [18].

In a study, it is found that the rhizospheric soil on the treatment of CaCO_3 ; promote the isolation of the actinomycetes from the soil. The rhizospheric soil is medium which rich source of clinically significant microorganisms is. Many actinomycetes have been reported for antibacterial and antioxidant activity [19]. Actinomycetes especially *Streptomyces sp.* have antifungal and antibacterial activity against various microorganisms [20]. In the recent research studies were made to isolate Novel actinomycetes with antifungal activity against some clinical fungal pathogen [21]. Soil *Streptomyces* have

higher antimicrobial activity against multidrug resistant microorganisms like *Staphylococcus aureus*, *E. coli* and many other Pathogens [22]. Many of the antimicrobial agents are produced by a variety of microorganisms in which gram positive, gram negative and fungi are included and the key foundation of antimicrobial agents are soil actinomycetes [23]. *Streptomyces* produces a huge number of antimicrobial agents which all have clinically importance such as amphotericin, rifamycin, erythromycin, streptomycin tetracycline etc [24]. So the soil has a mass of diversity in which many microorganisms are present which is cultivable and uncultivable and compose most of the part of the soil. Actinomycetes play an essential ecological role in the recycling and mineralization of nutrients in the soil. They support to recycle nutrients by degrading enormous numbers of organic matter in the soil and are found most common in compost. They perform as plant growth promoters via helping in nitrogen fixation, solubilization of nutrients, immobilization of nutrients, siderophores production, biological control and soil structure protection Actinomycetes are of pronounced practical importance in nature and seem to be ultimately involved in soil ecology.

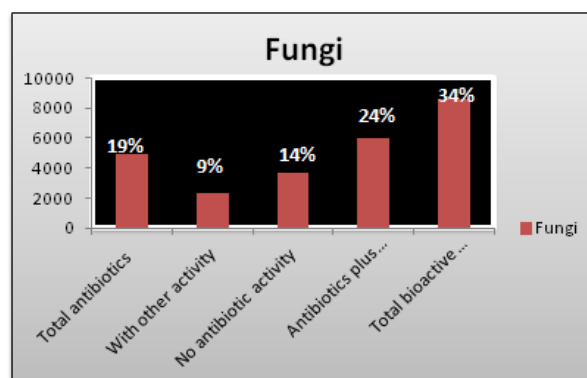
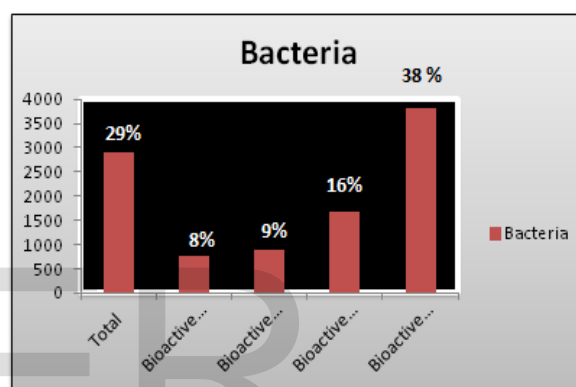
2.1 SOIL ANTIMICROBIAL AGENTS PRODUCING MICROBES

Many gram positive and gram negative bacteria and fungi have the capability to synthesize a variety of antimicrobial agents but the major source of antimicrobial agents is the actinomycetes and they are cultivable producers that are present in the soil [23]. The actinomycetes are a group of gram-positive bacteria that show characteristics of both bacteria and fungi and produces filamentous structures which forms pseudo-mycelia. They are spore forming microorganisms and have cell wall, flagella and ribosome like in bacteria. 10% - 33% of the total bacteria present in

soil are of bacteria, especially the genera *Streptomyces* and *Nocardia* present in a great number [25]. There are a number of antimicrobial agents that have been produced by *Streptomyces* and their clinical and agricultural importance included as amphotericin, erythromycin, streptomycin, tetracycline, and rifamycin [26]. *Bacillus* is another group of organism that have the ability to produce antimicrobial agents having clinical and agricultural significance. They also are gram positive, spore forming rods.

Any chemical or natural origin, which inhibits the growth of other organism having low molecular weight molecules produced from microorganisms are secondary metabolites. 80% of the world's antibiotic comes from the actinomycetes especially from the genera *Streptomyces* and *Micromonospora* species [27], [28]. Around 7600 compounds produced from *Streptomyces* species and out of 12000 antibiotics that are discovered from the last 5 decades 70% are from actinomycetes and 30% from fungi and other bacteria [29]. The antibiotics obtained from actinomycetes sort into several major structural classes such as amino glycosides (e.g., streptomycin and kanamycin) [30], ansamycins (e.g., rifampin) [31], anthracyclines (e.g., doxorubicin) [32], β -lactam (cephalosporins) [33], macrolides (e.g., erythromycin) and tetracycline [34]. There are a variety of antibiotics which is produced by *Streptomyces* for example- amphotericin, erythromycin, streptomycin, tetracycline, and rifamycin [35] having clinical importances like antibacterial, antifungal, anti-parasitic, antiviral actions. Many of antibiotics coming from *Streptomyces* strains that compete with other soil organisms [36]. It has been investigated that 75% of antibiotics coming from *Streptomyces* species have commercial and medical significance. The streptomycin antibiotic was discovered from *Streptomyces griseus* [37]. Actinomycetes vary in the amount of purification like

some are crude preparations, where as others have been crystallized such as: actinomycetes lysozyme, actinomycin, micromonosporin, streptothricin, streptomycin, and mycetin. Out of many actinomycetes some have capacity to produce more than one antibiotic (eg. *Streptomyces griseus*) as well as the same antibiotic may be produced by different species of actinomycetes (e.g.: Actinomycin, streptothricin). As a consequence, antibiotic may be the same, even when produced by dissimilar actinomycetes [38].



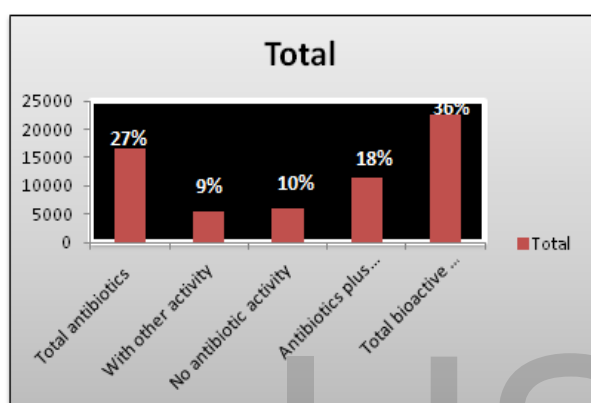
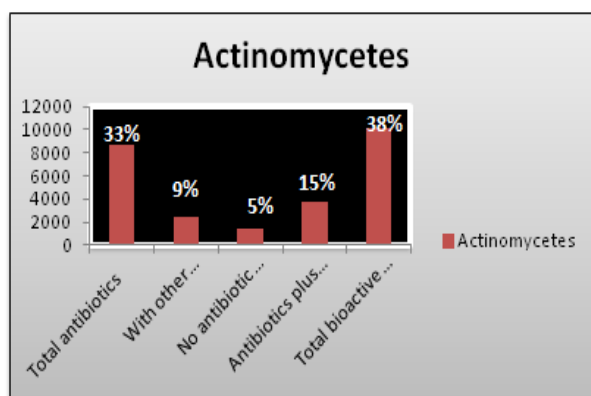


Fig:1 Distribution of antibiotics and bioactive metabolites in various genera :Bacteria, Fungi, Actinomycetes and Total [39]

2.2 SECONDARY METABOLITES PRODUCED BY ACTINOMYCETES

Microbial metabolites have been measured always as enormous apprehension in the discovery of antimicrobial agents. Now day's novel compounds with therapeutic applications are waiting to be discovered from the secondary metabolites, specifically produced by actinomycetes. The best identified secondary metabolites produced by Actinomycetes are the antibiotics and they are referred as the 'wonder drugs' for their effectiveness against many pathogenic microorganisms [40].

Secondary metabolites are not crucial for vegetative growth of the producing organisms but they play as, functioning as defense compounds or signaling molecules in biological interactions. They are produced at the end of the exponential growth phase and their syntheses extensively depend on the growth conditions provided them. Production is generally affected by the source of nutrient like carbon or nitrogen [41], [42]. They are structurally varied and most of them have biological activities, such as antimicrobial agents, toxins, pesticides, ionophores, bio regulators, and quorum signaling. These bioactive metabolites are used as antimicrobial agents for the treatment of various diseases [43]. Secondary metabolites isolated by actinomycetes exhibit a great number of miscellaneous and multipurpose biological effects.

3. MARINE ACTINOMYCETES

It has been reported that actinomycetes are circulated in the diverse marine ecosystems and culture dependent and independent methods have confirmed that native marine actinomycetes exist in the oceans and show diversity among other actinomycetes. Many of which have potential to expand therapeutic agents with novel activity [44]. In the recent study the actinomycetes were isolated from Caspian Sea sediments at a depth of 5-10 m. Out of them some strains were used for the antibacterial activity, Hydrolytic exo enzymatic (amylase and protease) activity and antibiotic susceptibility tests were performed against some test organisms. As a result marine actinomycetes were found potent source of bioactive compounds and antibiotics [45]. Actinomycetes have been found to produce a great amount of L-glutaminasen enzyme at different favourable conditions for the maximum production was at temperature 30° c, pH 7, and salinity 3.5% and time 96 hrs at 480 nm [46].

4. INTRODUCTION TO ANTIMICROBIAL AGENTS

The first antibacterial agent was prontosil derived in 1935 by Gerhard Domagk [47],[48],[49] and was the first of the "sulfa" drugs discovered and this discovery was escorted in the antibiotic era. Bacteriostatic agents like tetracycline, are those agents which restrain the growth and multiplication of bacteria. Bactericidal agents are those agents which not only inhibit the growth but also trigger pathways within the cell that causes death of microorganisms for example fluoroquinolones. The bactericidal drugs acts by killing the cell and are irreversible to a bacteriostatic agent [50].

Antimicrobial agents are those chemical compounds which prevents the growth of the microorganisms. They are natural or synthetic chemical compounds which can act by interfering in the metabolic activity of the organisms. They can be divided on the basis of their activity as broad spectrum and narrow spectrum antibiotics. Secondary metabolites produced by certain microorganisms are frequently large, elaborated organic molecules that require complex enzymatic synthesis [51]. Several antimicrobial agents are present but they all cannot be used because of their antimicrobial activity as they can be toxic to human beings [52]. Antimicrobials should have some characteristics like: to be non-toxic, non-allergenic, effective and selective, chemically stable, active against more than one bacterium and should be low-priced [53]. The ratio of therapeutic effect and the toxic effect in the human body is shown by the drugs therapeutic index (TI) [54]. Antibiotics are not only natural compounds, but also they can be chemically modified or man-made synthetic molecules.

They can also be classified based upon their mechanism of action. The four classes are:-1) Protein inhibitors, 2) DNA RNA inhibitors, 3) cell wall inhibitors, 4) folate inhibitors [55].

4.1 RESISTANCE TO ANTIMICROBIAL AGENTS

Antimicrobial resistance is a rising global risk to human and animal health [56]. Researchers have been warned that misuse could lead to selection and propagation of mutant resistant forms of bacteria within 2 decades from the discovery of penicillin antibiotic [57], [58]. Actinomycetes have resistant towards these antibiotics like penicillin, chloramphenicol and tetracycline. Cultivated when actinomycetes cultivated under *Curcuma longa L* antibiotic produced by actinomycetes may be used against human and plant bacterial and fungal diseases [59]. The overuse of these antibiotics has caused an gradually more multiple drug resistant strains (MDR) and they includes streptococcus, pseudomonas and staphylococcus. The search for novel antimicrobial agents with their clinical importance is very necessary because of increase in the number of resistant pathogens like *Mycobacterium tuberculosis*, *Enterococcus*, *Pseudomonas sp.*, *Streptococcus pneumoniae* and *Staphylococcus aureus*⁶⁰. Most of the Bacteria are reported to be resistant to presently available antibiotics according to a report of New England journal of medicine. Microorganisms have acquired resistant through a number of ways [60] as discussed below:-

- 1) Bacteria produces enzyme which destroys the antimicrobial agents or modify them before they reaches its target cell. So that they no longer predictable by their target.
- 2) Cell wall becomes impermeable to the antimicrobial agents.
- 3) Mutation causes alteration at target site so that it no longer binds with antimicrobial agents.
- 4) An efflux pump possess by bacteria which expel the antimicrobial agent from the cell before it reaches to its target.

5) Specific metabolic pathways present in the bacteria.

Bacteria can also acquire resistant to antimicrobial agents by genetic events like: mutation, conjugation, transformation, transduction [60],[61].

4.1.1 Mutation- Mutation is an altered form of the target site and spontaneous mutation occurs at low frequency as a effect chromosomal resistance develops when the bacteria exposed to the antibiotic only the mutant cell survives [50].

Spontaneous mutations

Spontaneous mutations are those mutations; occur randomly as replication errors or an incorrect repair of a damaged DNA in vigorously dividing cells. They present a significant mode of generating antibiotic resistance. They are also known as growth dependent mutations. E.coli exhibits resistance against Quinolone due to the mutations in at least seven positions in the *gyrA* gene or three positions in the *parC* gene [62].

Hypermutators

During a long term non-lethal antibiotic selective pressure, bacterial population enters a transient state of a high alteration rate this is recognized as hyper mutable state. This condition is found in many bacterial species includes *E. coli*, *S. enterica*, *Neisseria meningitides* (*N. meningitides*), *H. influenzae*, *S. aureus* *Helicobacter pylori* (*H. pylori*), *Streptococcus pneumoniae* (*S. pneumoniae*), and *P. aeruginosa* [63]. Various investigations have been suggested that hyper- mutations play a noteworthy function in acquisition of antibiotic resistance in many pathogens [64],[65],[66].

Horizontal gene transfer

For the transfer of genetic material, most general way to spread antibiotic resistance are transduction (via bacteriophages), conjugation (via plasmids and conjugative transposons), and transformation (via incorporation into the chromosome of chromosomal DNA, plasmids, and other DNAs) [67]. Horizontal gene transfer occurs between closely related bacteria and phylogenetically related bacteria.

4.1.2 Transformation - A process in which the host cell receives DNA from the environment is called as Transformation and this is mostly occurred in gram positive organisms. This process occurs naturally when host cell is competent. For this phenomenon cell should be in a specific growth phase in which cell has the capability to take foreign DNA by breakage of the cell wall that can recognize the DNA that is present in the environment.

4.1.3 Conjugation- An extra chromosomal structure known as plasmid present in bacterial cell and many plasmids contain antimicrobial resistant gene. Conjugation is the process in which a bridge like structure forms that is known as pilus. From this structure the copy of genetic material occurs, produced by replication process. As an outcome the resistant bacteria show resistant toward the antibiotics.

4.1.4 Transduction – When a section of DNA or plasmid packaged in the viral coat and enters into the host cell, that host cell becomes resistant due to the existence of resistant gene in the plasmid [50].

A “post-antibiotic” era in which no antimicrobials will be able to fight against simple infections is the ultimate terror driving effort to recognize the complexities of antimicrobial resistance [68].

Conclusion

There are thousands of antimicrobial metabolites that are recognized until now and the number of all known natural products is around one million. Most of them derived from Actinomycetes. It is an obvious questions where is the border in the diversity of natural products ? Where is the limit or is there any limit at all, in the continuous increase in the number of Novel microbial compounds. The reinvestigation of the known natural or microbial compounds and specially the whole microbial population with at extensive range, more selective, sensitive and specific methods, especially in the light of the expanding knowledge of microbial genetics and acquired knowledge about various genomes, would be fruitful. In all means, in the future we will discover more and more Novel functions; new activities of the microbial metabolites will understand their real role and function and will develop the area of their practical utilization.

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