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CONTRIBUTION OF SOIL MICROORGANISMS IN ANTIBIOTIC PRODUCTION

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Abstract:
Antibiotics are the metabolic by products of complex biosynthetic pathways in the microorganisms. They are often touted as one of the most important discoveries of the modern medicine. It contributes to save millions of lives and to control the majority of bacterial infectious diseases. Intensive research on antibiotics has been carried out globally for approximately thirty five to forty years whereas the search for new antibiotics still continues in a rather over looked hunting grounds. Recent studies dealt with the search for new antibiotics and their interest stemmed in the soil microbials due to its large biodiversity in search for new useful compounds. There are also reports presented by different researchers on the secondary metabolites potential of different Bacterial, Fungal and Antinomycetes species. This article compiles the historical background, factors, needs, source and future prospects of secondary metabolites and the role and bioactivity of microorganisms' in habitat in soil for the development of new antibiotics as a life saving agents for everyone. To determine microbial diversity within the soil is crucial in the past but with the new methods it is now possible to detect both culturable and un-culturable microbial species. It is a brief overview of the antibiotic development through years and its emergence. Thus in this review contribution of soil microbials for the development of novel antibiotics and designing more effective preventive measures in the future have been discussed.

Keywords: *Antibiotics, Soil microbials, Life saving agents.*

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INTRODUCTION:

Secondary metabolites are the important candidate for the development of drugs or other technological development. Secondary metabolites may be produced from natural sources i.e., from fungi, bacteria, plants and many of the marine-organisms (tunicates, corals, snails, etc). Secondary metabolites were produced from the organisms that were able to inhibit the growth of other organism's competing for the same ecological niche. Broadly antibiotics can be defined as a chemically heterogeneous group consisting of organic molecules having microbial origin. At low concentration, antibiotics were deleterious to metabolic activities and it inhibits the growth of many other microorganisms [1]. The term "antimicrobials" includes all the agents that act against all other types of microorganisms that includes bacteria (anti-bacterial), fungi (anti-fungal), viruses (anti-viral), protozoa (anti-protozoal) and antitumor. Nowadays microorganisms present in the soil became an important source for the intensive search of important molecules used in industries [2]. Many of the microorganisms were diversely distributed in the nature and were still unknown, yet many more important molecules or useful products were to be identified from soil microbial having the potential to produce new antibiotics. Today both industrial and academic interest in soil microorganisms is on rise in search of novel commercially important products and unique biologically active metabolites from them. The diversity of the microorganisms present in soil was of great significance due to its importance in the early discoveries of useful antibiotics [3]. Microorganisms in the soil such as bacteria, protozoa, algae and moulds were competing under limited nutrients have to devise strategies for their survival. Although the first commercially produced antibiotic penicillin, was discovered by the chance where most of the today's produced antibiotics were discovered by systematic searching. Thus attention is always turned to the soil whenever the new antibiotic producers are being sought [4]. Filamentous microorganisms such as *Actinomycetes* & Fungi were main source of secondary metabolites that were able to produce antibiotics. The filamentous microorganisms freshly isolated from soil are the important source of secondary metabolites. The nature has immense potential to provide structurally diverse broad spectrum secondary metabolites [5].

Definition of Antibiotics

Antibiotics are low molecular weight and biological active compounds [6]. The word antibiotic was proposed by Selman Waksman in 1945, he defined antibiotic as a microbial origin chemical substance. Further in 1981 Webster's Third International Dictionary termed antibiotic as a natural substance produced by a microorganism that inhibit or kill other microorganisms. Furthermore, Brock's define antibiotic as a chemical agent released by a microorganism that is harmful for another organisms [7]. Gottlieb and Shaw (1967) defined antibiotics as an organic substance that was produced from many microorganisms that at low concentration was harmful to the growth and metabolic activities of other microorganisms [8]. Lancini and Parenti (1982) defined antibiotics as low molecular weight special inhibitory products that exclude lactic acid, enzymes, ethanol and other substances that prevents the growth of other microorganisms [9]. Though in the middle of the last century, in clinical practices antibiotics was introduced for the management of microbial infections. In ancient Greece, China, Egypt and other places of the world the use of microorganisms was well documented. The modern era of the antibiotics was started when Alexander Fleming in 1928 discover penicillin from fungus, *Penicillium notatum* [10].

Discovery and Origin of Antibiotics

Antibiotics have been used for more than fifty years to cure infectious diseases [7]. Prior to the 1940s, infectious diseases were either treated with antiseptics, silver compounds, surgical drainage, tincture and with arsenicals of the time. Over the latter half of the 20th century the rapid succession of antibiotics was miraculous which provide clinicians to treat the bacterial infections [11]. Endocarditis was almost fatal and diagnosis of meningitis or pneumonia was a death sentence. During the half of the 19th century, Robert Koch was working to identify microorganisms that cause diseases such as typhoid, cholera and tuberculosis. Scientist of time studied different methods such as vaccination for fighting infectious diseases. Besides their fatal effect scientists were also carrying out research to find out effective antibiotics and antibacterial agents from these microorganisms. By the 1910, Ehrlich developed first synthetic antimicrobial drug which contains the arsenic compound Salvarsan. Although it was not much effective against wide range of bacterial infections but it did effective against the sleeping sickness (Trypanosomiasis), Spirochaete disease of syphilis and Protozoal disease. Until 1945 this drug was used then by the discovery of Penicillin it was replaced by it. In the middle of last century when antibiotics was first introduced it was hailed as a wonder drug. The first antibiotic Penicillin, a Beta-lactam antibiotic was discovered by Alexander Fleming. Later Ernst Chain and Howard Florey led penicillin to next level to treat infections. Selman

Waksman find out the ability of other microorganisms to produce anti-bacterial substances by using screening method and this technique led to successful discovery of Streptomycin. After the Second World War, the effort was still continued to find out other novel antibiotic structures which led to the discovery of many peptide antibiotics i.e., (Bacitracin 1945, Chloramphenicol 1947), a Beta-lactam antibiotics (Cephalosporin C 1955), the Tetracycline antibiotics (Chlortetracycline 1948), the Cyclic peptide antibiotics (Cycloserine 1955), and the Macrolide antibiotics (Erythromycin 1952).

Table 1: Evolution of antibiotics/chemotherapeutics: Discovery of the first important preparations till 1950.

antibiotic	natural source	first description as anti-infective drug	discoverer
sulfanilamide (prontosil)	-	1932	G.Domagk
penicillin	Penicillium notatum	1941[1]	A.Fleming, Florey, Chain
streptomycin	Streptomyces griseus	1944	S.A.Waksman
cephalosporin	Cephalosporium acremonium	1945	G.Brotzu
bacitracin	Bacillus subtilis	1945	B.A.Johnson
chloramphenicol	Streptomyces venezuellae	1947	I.Ehrlich
polymyxin	Bacillus polymyxa	1947	C.G.Ainsworth
chlortetracyclin	Streptomyces aureofaciens	1948	B.M.Duggar
neomycin	Streptomyces fradiae	1949	S.A.Waksman
oxytetracyclin	Streptomyces rimosus	1950	A.C.Finlay
colimycine	Bacillus colistinus	1950	Y.Koyama

[1] Penicillin was discovered by A.Fleming in 1928 but the first therapeutic usage was realized by Florey only in 1941. :

Adopted from Review of antibiotics http://old.lf3.cuni.cz/studium/materialy/infekce/en_atb.pdf

In the last fifty years among twenty three thousand bioactive metabolites more than seventeen thousand antibiotics were discovered from soil microorganisms [12].

Factors affecting Antibiotic production

Factors affecting antibiotic production

Medium Composition	Fermentation Conditions
Carbon source	pH
Nitrogen source	Temperature
Inorganic phosphates	Oxygen
Inorganic salts	
Trace metals	
Precursors	
Inhibitors	
Inducers	

Adopted from Chandrashekhara. S, 2010 Vinayaka Mission University, Salem [13]

Need of New Antibiotics

Several new approaches were used to fulfill the need of antibiotics. Brute force approach assay bacteria to produce antimicrobial substances. Combinatorial biosynthesis techniques introduce each microorganism in niches and introduce metabolic pathways in it resulting in antimicrobial compounds. DNA sequencing searches certain DNA from entire genome to synthesize anti-microbial products. Recently 10,000 *Actinomycetes* produces antibiotic products (phosphoric acid products) using screening procedure [7]. *Actinomycetes* were also grown on a medium to check the ability of amino acid arginine utilization, used as a nitrogen source [14].

Classification of Antibiotics

Antibiotics were most important discovery of humankind in modern world. Several important microorganisms naturally produced antibiotics. Antibiotics produced by Bacterial species include Bacitacin from *B. licheniformis*, Gentamycin from *Micromonospora purpurea*, Gramicidin from *Bacillus brevis*, Polymyin B from *B. polymyxal*. [15]. Antibiotics produced by *Streptomyces* include Streptomycin from *S. griseous*, Oxytetracycline from *Srimosus*, Erythromycin from *S. erythraeus*, Nyasin from *S. noursei*, Chlortetracycline from *S. aurefaciens*, Cycloheximide from *S. griseous*, Kanamycin from *S. knsnsmyceticus*, Amphotericin B from *S. nodus*, Chloramphenicol from *S. venezuelal*, Tetracycline from *Streptomyces* sp [15, 16]. Potent antibiotics by fungi were Penicillin from *P. notatum*, Cephalosporin from *Cephalosporium* sp, Fusidic acid from *Fusidium coccineeum* and Griseofulvin from *Penicillium griseofulvium* [15, 17].

Soil as a source of Antimicrobial agents

Soil is a complex and very diverse environment providing versatile source of antibiotic producing organisms [18, 19]. Soil contains more organisms than there are humans in the planet. Billions of the microorganisms live in the soil. It is a lively habitat comprised of many living organisms (bacteria, fungi, algae, and protozoa, micro-arthropods to the more complex nematodes and, insects, earthworms and small vertebrates. These organisms grow, eat and move in the soil. They decompose organic matter and mix humus in the soil layers and organisms live in the soil differ in moisture, pH, food available and pore size. In the soil the density of living organisms is exceptionally high whereas microorganisms were less in the cultivated than virgin or uncultivated land soil. Soil acidity decreases the population of microorganisms [20]. Many soil bacteria that can suppress or inhibit other microorganisms competing under the same niche were able to produce secondary metabolites [21]. Secondary metabolites were produced by many microorganisms in soil habitat such as bacteria, fungi, *Actinomycetes*, plants and so forth. Among the various groups of organisms, the *Actinomycetes* occupy a prominent place that has the capacity to produce such metabolites [22]. Identification of antibiotic producing soil bacteria is ongoing process nowadays. Several microbiological techniques were utilized to examine these microorganisms. Approximately 85% of important antibiotics were produced by genus bacteria whereas filamentous organisms produce 75% of medically and commercially useful antibiotics [23]. Number of important antibiotics were produced from soil fungi has several applications in medical for development of pharmaceutical products [24]. Microbial population plays an important role in pharmaceutical and biotechnological industries as it offers many new biochemical pathways and countless new genes to probe for enzymes, antibiotics, and other useful biological compounds. There are number of important microorganisms present in the soil that were able to produce industrially and medically useful compounds.

Contribution of Microorganisms as a source of Secondary metabolites

History of the microbial technology shows that many commercial materials like antibiotics, pesticides foods and beverages were produced using many microorganisms. During last three decades, for the production of new antibiotics with the emergence of new diseases screening of microorganisms has rapidly increased. The focus of studies was on the exploitation of useful antibiotics from *Actinomycetes* & fungi. Studies on the secondary metabolites revealed that the secondary metabolites produced from different microorganisms have unique molecular skeleton [25]. The filamentous microorganisms such as fungi and *Actinomycetes*, freshly isolated from soil habitat were the main source of secondary metabolites. More than 180 different secondary metabolites were produced by *Streptomyces* species [26].

The groups of bacterial species have the ability to produce secondary metabolites. The most common species includes the genus of *Bacillus* which produces number of secondary metabolites. Many important antibiotics such Bacillaenes, Moenomycins, Bacillomycins and Difficidins were isolated from different strains of the *Bacillus* sp [27].

The most important sources of antibiotics having microbial origin are the following groups of microorganisms i.e., Bacteria, Fungi and *Actinomycetes*.

Bacteria:

Bacteria are single celled, tiny microorganisms and somewhat longer in length. They were present in numbers. A teaspoon of soil contains 100 million to 1 billion bacterial species which were active in each acre of the soil. Bacteria were easy to isolate and culture. They are naturally antibiotic producer in the soil for their survival and availability of nutrients. Broad spectrum antibiotics were secreted by gram negative bacteria. Identification of antibiotic producing soil bacteria is ongoing process nowadays. Several microbiological techniques were utilized to examine these microorganisms. The main antibiotic producer includes *Bacillus* sp and *Pseudomonas* sp. Members of these genera produce several important useful antibiotics which includes Polymyxin B, Colistin, Gramicidin, Bacitracin and Tyrothricin from *Bacillus* species whereas *Pseudomonas* species produces Pyrrolnitrin and Pyocyanin [28]. Bacillus antibiotic production accounts for 70% whereas 13% for *Pseudomonas* [29]. Apart from these two, another important genus includes *Myxobacterium*. Approximately 80% of the isolated *Myxobacteria* produce secondary metabolites with antibiotic activity, and has the ability to exhibit antifungal activity.

Fungi:

Fungi are the naturally antibiotics producer. Penicillin is one of the most important antibiotics produced by fungi. Members of *Aspergillus*, *Cephalosporium* also have the ability to produce secondary metabolites. Among these about ten have been commercialized i.e., Cephalosporin, Penicillin G, V, 0, Griseofulvin, Variotin, Fumagillin, Fusidic acid, Xanthocillin and Siccanin [28]. The most commonly used fungal antibiotics include Fusidic acid, Penicillins and Cephalosporin C which was found among the metabolites of different fungal species [28].

The discovery of new drugs was increasing to identify structurally novel compound that possesses useful biological activity. Fungus species such as *Tolypocladium inflatum* , *T. geodes* and some strains of *Verticillum* sp, *Acremonium* sp, *Beauvaria* sp, *Fusarium* sp and *Paecilomyces* sp [30, 31] were able to produce Multipliolides-A, Cyclosporine-A whereas some important lactose compounds were produced by genus *Xylaria mutiplex* [32]. *Aspergillus* sp produce most of the Polyene antibiotics. β-lactam antibiotics such as Cephalosporin, Pencillin and their relatives were produced from *Cephalosporium* and *Penicillium* group [33] and were used in current chemotherapy [34]. *Acromonium fusidioides* produces Fucidin and Fusidic acid having anti bacterial activity against gram negative bacteria [35]. Napthaquinones were produced by *Fusarium solani* and *Fusarium oxysporum* with antibacterial properties [36]. *Cryptosporiopsis guercina* have the ability to produce Crytocandin. A Lipopeptide antibiotic shows strong inhibitory activity against fungi, pathogenic to humans i.e., *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Candida albicans*, and against plants pathogenic fungi i.e., *Botrytis cinerea* and *S. sclerotium* [37]. Chaetomin and Epidithiadiketo-piperazine was produced by specie *Chaetomium globosum* having strong antifungal activity [38].Chaetomin was active against gram positive bacteria whereas *Chaetomium cochlioides* produces Cochliodinol exhibits both anti-bacterial and anti-fungal activity [39]. A Botrydiplodin antibiotic produced by *Botryosphaeria rhodina* sp was active against both Gram positive & negative bacteria. A compound, Gliovirin produced by *Glioclabium virens* shows antibiotic activity against *Pythium ultimum* [40].

Actinomycetes:

Actinomycetes were widely distributed and most important group of microorganisms in soil. *Actinomycetes* inhabit the soil [41]. *Actinomycetes*, a large group of bacteria are filamentous, gram-positive, long, unicellular possess both fungal and bacterial properties. They grow as hyphae like fungi. *Actinomycetes* are slow growers, active at high Ph, decompose chitin and cellulose. They received tremendous attention, both scientifically and commercially [42]. Major source of *Actinomycetes* was soil [43, 44, 45 and 46] and were the main antibacterial producer. The most important *Actinomycetes* genera include *Streptomyces*, *Streptosporangium*, *Microbispora*, *Micromonosporaactinoplanes*, *Thermoactinomyces*, *Dermatophilus*, *Nocardia* (*Farcinica asteroides*), *Nocardia* (*madurae*), *Actinomyces* (*bovis*), *Actinomyces* (*israeli*). The most important soil based *Actinomycetes* were the main source of drugs includes Streptomycin, Erythromycin, Actinomycin, and Vancomycin. Actinomycin was first of all isolated in 1940. 70% of known antibiotics were produced by *Actinomycetes* [47, 48].

Streptomyces was a genus of *Actinomycetes*, gram positive bacteria, having complex multicellular development produce useful antibiotics of natural origin i.e., of soil used as pharmaceuticals and agrochemicals [49], display anti-bacterial, anti-fungal, anti-viral, anti-protozoic and antitumor properties [23]. Most antibiotics were species specific. *Streptomyces* species accounts for 90 to 95% of *Actinomycetes* sps isolated from soil samples [42, 50]. Approximately about 92% of the antibiotics of *Actinomycetes* origin discovered till date have been isolated from *Streptomyces* sp. *Actinomycetales* have the greatest number of antibiotics used commercially and about 72 of them being utilized for various purposes [28].

Bioactivity of Secondary metabolites

Biological activities were exhibited by several microorganisms that were able to produce secondary metabolites. Microorganisms having incredible array of chemical structures were able to produce secondary metabolites and results in versatile biological activity. An important new feature in the exploitation of microorganisms was utilization of microbial metabolites in pharmacological fields (Statins, Cyclosporin) [51]. Secondary metabolites produced by several microorganisms were utilized directly in medicine, pharmaceuticals and agriculture and also in the biological and chemical derivitization to design rational drugs.

Biodiversity of Microorganisms

Biodiversity refers to the taxonomical entities or number of species in a given geographic area or ecosystem and were studied specifically for their value as organic compounds producers which is so called secondary metabolites. These compounds were studied, isolated and characterized by biologists & chemists for the evaluation of their pharmacological, biological and chemotaxonomic potential in order to explore the chemical diversity. Till date approximately six thousand species of the prokaryotes especially bacteria were known. It is estimated that about 106 to 109 bacterial strains exist in the nature and about seven thousand fungal species were known and they were expected to increase in the future [52]. The above calculation of microorganisms provides an estimated data for the researchers to exploit secondary metabolites. Filamentous species i.e. fungi and *Actinomycetes* were tremendous source of secondary metabolites representing 60% of the total bioactive microbial secondary metabolites [12]. Certain genera of fungi such as *Trichoderma*, *Penicillium*, *Fusarium* & *Aspergillus* sps were the tremendous producers of the secondary metabolites [53]. Every day new species of fungi and *Actinomycetes* are being discovered which opens tremendous opportunities for the scientists and it also evidences that our knowledge of these microorganisms is far from exhaustive.

FUTURE PERSPECTIVE OF SECONDARY METABOLITES:

In 21st Century improvement in technologies advances the production & synthesis of natural products. Natural products and related structures become even more important and it focuses the study around itself for the development of improved new medicines due to its important discoveries of functionally relevant microbial origin secondary metabolites. In the treatment of resistant pathogens currently available antibiotics might remain useful but it is possible that in the future physicians will run out of options. New approaches are required to combat the spread of drug resistant bacterial pathogens and the emerging infections globally. One of the most important issues is the pattern or increase in the death rates from infectious diseases. In the 20th century from 1900-1980 the rate dropped from 797 per 100,000 people to 36 per 100,000 people. A reduction is made by a factor of more than 20 and a testament in part to the efficacy of the antibiotics [55].

In spite of availability of many antibiotics, the need for the discovery and development of new ones still exists in order to solve the therapeutic problems in future, which includes the continuing problem of drug resistance amongst the pathogenic species. Organisms which were previously seem to be commensals are now becoming the dangerous pathogens due to the abuse of the antibiotics. Example includes the species of *Staphylococcus*, *Proteus* and *Yeasts*. Currently few satisfactory systemic antifungal antibiotics exist outside the Amphotericin-B but unfortunately even Amphotericin-B is not always effective. The need for new antibiotics is growing to be used in the agriculture for combating plant diseases and antiviral agents must also need to be developed [54].

These were the some of the major problems that cannot be solved by chemical synthesis of the antibiotics alone so need for new antibiotics exhibiting new profiles of mechanism and activity may be found mainly by further systematic screenings of soil microorganisms and this needs to be performed according to the new fundamental principles.

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