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Microbial and Biochemical Aspects of Antibiotic Producing Microorganisms from Soil Samples of Certain Industrial Area of India- An Overview

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Abstract: Antimicrobial agents produced by the soil borne microorganisms like (*Actinomycetales, Pseudomonas auregenosa, staphyllo coccous spp etc)* community in the industrial areas as well as in agricultural soil have been reported to amend with diverse amounts of municipal solid waste compost (MSWC) or farmyard manure (FM). It was shown, by using the 16S rDNA polymerase chain reaction—restriction fragment length polymorphism and sequencing methods for *Actinomycetales* isolates, tow major clusters. The cluster SI is composed of two related families: *Streptomycetaceae*, dominant family and *Pseudonocardiaceae*. However, SII is composed of *Nocardioidaceae* family. The actinomycetes yielded about 70 % of these, and the remaining 30 % are products of filamentous fungi and non-actinomycete bacteria. Most of the bioactive compounds from actinomycete sort into several major structural classes such as amino glycosides (e.g., streptomycin and kanamycin), ansamycins (e.g., rifampin), anthracyclines (e.g., doxorubicin), β-lactam (cephalosporins), macrolides (e.g., erythromycin), and tetracycline. The present paper is the compilation of studies aimed at isolating and characterizing the nutraceutically as well as medicinally significant compounds, mainly antibiotics from the soil of vicinity of certain industrial areas of India, capable of acting on clinically resistant strains of infectious organisms.

Keywords: Actinomycetes, Amino glycosides, Antimicrobial agents, β-lactam, Cephalosporins, Erythromycin.

INTRODUCTION

Soil as a living system inhabits assorted cluster of living organisms, both micro flora (fungi, bacteria, algae and actinomycetes) and micro-fauna (protozoa, nematodes, earthworms, moles, ants). The density of living organisms in soil is exceptionally high i.e. as much as billions / gm of soil, usually density of organisms is less in cultivated soil than uncultivated / virgin land and population decreases with soil acidity [1]. While exploring the studies on microbial isolation in view of screening nutraceutically significant, the activities of metal ions and metalloids are also taken into consideration. The logical reason behind it is that metal ions such as iron and copper are among the key nutrients that must be provided by dietary sources. In developing countries, there is an enormous contribution of human activities to the release of toxic chemicals, metals and metalloids into the atmosphere [2]. These toxic metals are accumulated in the dietary articles of man. Numerous foodstuffs have been evaluated for their contributions to the recommended daily allowance both to guide for satisfactory intake and also to prevent over exposure. Further, food chain polluted with toxic metals and metalloids is an important route of human exposure and may cause several dangerous effects on human [2]. Autotrophs use inorganic carbon from CO2 and are "primary producers" of organic matter, whereas heterotrophs employ organic carbon and are decomposers/consumers [2]. Organic compounds include cellulose, lignins and proteins (in cell wall of plants), glycogen (animal tissues), proteins and fats (plants, animals). Cellulose is degraded by certain specific [3]. Antibiotics are one of the most significant commercially exploited secondary metabolites produced by the bacteria and employed in a wide range. Most of the antibiotic producers used today are the soil microbes. Fungal strains and *Streptomyces* members are extensively used in industrial antibiotic production [4]. Bacterium is easy to isolate, culture, maintain and to improve their strains. Microbes are omni present and exist in a competitive environment. Bacillus species being the predominant soil bacteria because of their resistant endospore formation and production of vital antibiotics like bacitracin etc. are always found inhibiting the growth of the other organisms [3]. An enhancement in the antibiotic production has been studied under various parameters like temperature, pH, carbon source concentration, and Sodium nitrate concentration, which may help in the industrial production. The extracted substance was found effective against the gram positive endospore forming bacilli and gram positive cocci. Though a large number of antibiotics are commercially available [5], the search for the most potential one is still on, and the present compiled work may provide some potential information on the antibiotic production and the control of microbial strains.

A preliminary investigation was performed about the presence of industrially important thermophilic bacteria in

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the hot springs and in its vicinity to the site of industrial area reflecting the bioactive status of the two randomly picked up clones of bacterium samples [5, 6]. The staining, morphological, biochemical, growth parameters, antibiotic studies proved that the bacteria are of gram negative and rod shaped nearer to the *Escherichia coli* cell type. Antibiotics are the best known products of actinomycete. Over 5,000 antibiotics have been identified from the cultures of Grampositive and Gram-negative organisms, and filamentous fungi, but only about 100 antibiotics have been commercially used to treat human, animal and plant diseases [6]. Known so far, the actinomycetes produce an enormous variety of bioactive molecules, e.g., antimicrobial compounds [7]. One of the first antibiotics used is streptomycin produced by *Streptomycin grisius* [8]. On the whole, the last 55 years have seen the discovery of more than 12,000 antibiotics. The actinomycetes yielded about 70 % of these, and the remaining 30 % are products of filamentous fungi and non-actinomycete bacteria [9]. Most of the bioactive compounds from actinomycete sort into several major structural classes such as amino glycosides (e.g., streptomycin and kanamycin) [10], ansamycins (e.g., rifampin) [11], anthracyclines (e.g., doxorubicin) [12], β -lactam (cephalosporins) [13], macrolides (e.g., erythromycin) [14] and tetracycline [15]. In the past decade, pharmaceutical as well as nutraceutically important antibiotics were recognized as emerging soil pollutants. Compounds such as tetracyclines and sulfonamides reach agricultural land mostly through contaminated manure from medicated livestock used as fertilizer [16, 17]. Pharmaceutical antibiotics are a large group of structurally diverse compound classes that comprise mostly polar and ionizable compounds [18]. Hence, their soil sorption behavior deviates from that of well-studied hydrophobic organic pollutants. In addition to hydrophobic interactions, antibiotics may sorb to soils *via* hydrogen bonding, van der Waals forces, cation exchange, cation bridging, and surface complexes [19, 20]. The present scientific essay is the compilation of studies aimed at isolation and characterization of the antibiotics from the soil of vicinity of certain industrial areas of India under following sections:

Industrialization, Biostimulation and Bioaugmentation

As industrialization expands, petroleum hydrocarbons become a greater potential source of contaminants in the soil and water environments [1-3]. Recently, more and more oil spill accidents have been reported. Chang and Lin [2] reviewed 242 accidents with storage tanks in industrial facilities over the last 40 years. They reported that 74% of accidents occurred in petroleum refineries, oil terminals, or storage facilities and other industries [21]. To remediate oil terminals contaminants in these environments, biostimulation and bioaugmentation are generally considered as eco-friendly techniques. However, the use of extrinsic micro-organisms is unlikely to be globally acceptable. Further, biostimulation is a technique that relies on increasing the activity of the resident bacteria by adding up the factors that are shown to exhibit the limitation of activity, such as nutrients or air. This technique is projected to be rather more effective because the indigenous bacteria are likely to be better adapted to the soil environment requiring treatment [2]. However, biostimulation sometimes does not work well and may take longer because bacteria with the ability to degrade xenobiotics may be scarce at contaminated sites or because the high-concentration of antibiotics reduces the activity of degrading microorganisms.

Assimilation of Phosphates by Microorganisms

Phosphorous is essential for growth and productivity of plants. It plays a pivotal role in plants in certain specific physiological and biochemical activities such as cell division, photosynthesis, and development of good root system and proper utilization of carbohydrates [22]. Phosphorous deficiency leads to the leaves turning brown accompanied by small leaves, weak stem and slow development. In ancient times the use of animal manures to provide phosphorous for plant growth was common agricultural practice. Organically bound phosphorous enters in soil during the decay of natural vegetation, dead animals and from animal excretions. At that time role of micro flora on soil fertility was hardly understood. Assimilation of phosphate from organic compounds by plants and microorganisms take place through the enzyme, phosphatase that is present in a wide variety of soil microorganisms. The medium [23] used to monitor the population density of phosphate solubilizers exhibit a clear zone around the colonies reflecting the well demarcated phosphate solubilization. Phosphate cleaved as a consequence of the specific action of the enzyme phosphatase secreated by phosphate solubilising bacteria was performed according to the protocol described by Alanis [24].

Antimicrobial Agents

Antimicrobial agents are natural or synthetic chemical substances which have the capacity of inhibiting or terminating total metabolic cell activity. These chemical molecules are classified depending on their targets. They can also be referred to as broad or narrow spectrum depending on its strength of action towards their targets, principally including (a) cell wall synthesis; (b) protein synthesis and (c) DNA replication. The major classes of antimicrobial agents are as (a) lactams including penicillins, cephalosporins, monobactams, carbapenems; (b) aminoglycosides; (c) tetracyclines; (d) sulfonamides; (e) macrolides such as erythromycin; (f) quinines; and (g) glycopeptides such as vancomycin. These secondary metabolites can affect many metabolic reactions in a cell in order to render effect. Penicillins and cephalosporins mode of action is the biosynthesis of the peptidoglycan present in the bacterial cell wall [25]. They affect specifically the transpeptidase that forms the peptide cross-linking. Beta-lactams also affect peptidoglycan synthesis by forming covalent bonds with a specific group of proteins known as the penicillin binding proteins [26]. Both gram positive and gram negative micro-organisms posses these proteins and other antimicrobials such as the quinolones and noviobiocin inhibit DNA replication by affecting enzymes, namely, DNA gyrase and the eukaryotic topoisomerase II [27]. Antimicrobials, known as 6-Aniniluracil, inhibits DNA polymerase III. Classes of antimicrobials like tetracyclines, chloramphenicol and macrolides inhibit protein synthesis [28- 31].

Ecological Interactions

In nature, multiple ecological interactions take place; which can be negative or positive for the organisms involved. The organisms and the physical-chemical conditions present in an ecological niche will delimit the type of interactions that can be observed. Competition is an interaction encountered in all habitats since the prevailing organisms need to do so in order to survive. Also, it is known that when various communities in an ecological niche utilize the same type of substrates they must compete [31]. Both theoretical and empirical studies suggest that in plant and animal communities, spirited interaction is the key determinant of species abundance and diversity [32]. As part of physiological and metabolic processes, communitiest, which are found colonizing definite areas provide the production of intracellular or extra-cellular low molecular weight components such as alcohols, fatty acids, secondary metabolites and some antimicrobial agents [32-34]. The substances secreted to the environment can be harmful or toxic to the surrounding organisms acting as a competitive advantage for the secretor. Amensalism or antagonism is the term used in the classification of ecological interactions where one component has the competitive advantage of producing and secreting substances that have inhibitory effects on other populations [35, 36]. The substances must alter the habitat in a disadvantageous style so that the interaction may be categorized as antagonism or amensalism.

Soil Microbial Interactions

An ecological niche is composed of many microhabitats; each microhabitat is composed of a microscopic diversity which includes bacteria, protozoa, fungi, and a macroscopic diversity that includes plants and insects. Soil is a complex medium in which one can encounter many kinds of microbial communities. Application of nucleic acid-based techniques to analyze soil microbial communities has revealed high prokaryote diversity [37]. The microbial diversity or communities present in soil principally depend on the composition of the soil and many physical chemical properties that the medium possesses. Also, the flora and decomposing organic mater on the surface of the soil will influence vastly with the microbial diversity present. For example, the fallen trees, barks and flowers provide nutrients both to the microbes and plants present, through microbial degradation of carbohydrates, lipids and proteins to sugars, fatty acids, glycerol and amino acids and respectively to mineralization. Besides providing these nutrients, plant secondary metabolites that are generally toxic to micro-organisms will need to be degraded or detoxified by certain microbes. These degraders (microbes) are selectively pressured and ultimately evolve to produce novel secondary metabolites possibly to counteract the toxic plant secondary metabolites [38]. There are approximately 10^6-10^9 colony forming unites per gram of soil. Microbes present in medium posses advantages that will permit or facilitate their survival in that medium. For example, Skujins research has demon-strated that in desert crusts or in soil that has low water availability, gram-positive and spore forming microbes are most abundant. The gram-positive bacteria posses a thicker layer of murein in their cell wall which makes the cell less vulnerable to the limiting conditions present in these habitats. Also, spore forming bacteria can resist long periods of desiccation and limiting nutrient conditions since they compact and protect their genomic material in the bacterial spore, until conditions are favorable for sporulation to occur [38].

The Soil Rhizosphere

Interactions that take place in the rhizosphere can be beneficial for the plant and also for the microbial community present. The effect that takes place in the soil rhizosphere is due to the influx of mineral nutrients to the plants roots through diffusion alongside the efflux and accumulation of plant root exudates [37, 38]. Every metabolically active system has the capability of secreting molecules as by-products. Exudates released by plants have various effects in the surrounding ecosystem. For example, they can alter the physical-chemical properties of soil by inhibiting the growth of other plants, enhancing symbiotic relationships, and selecting the type of microbiota that can colonize the area. Plant exudates can attract plant pathogens such as nematodes [37, 38].

Soil Antimicrobial Agent Producing Microbes

Many groups of microorganisms like gram-positive, gram-negative bacteria and fungi have the ability of synthesizing antimicrobial agents. Pandey *et al.* [39] state that the top cultivable antimicrobial agent producers present in soils are the actinomycetes. The actinomycetes are a group of gram-positive bacteria that exhibit characteristics of both bacteria and fungi. These microbes produce filamentous structures which agglomerate forming pseudo-mycelia. Actinomycetes are also spore forming microbes, characteristic shared with fungi. Some of the characteristics that they share with bacteria is the formation and composition of the cell wall, the flagella and the ribosome. About 10% - 33% of the total bacterial community present in soil is comprised by these bacteria, being the genera Streptomyces and Nocardia the most abundant actinomycetes found in soil. The genus Streptomyces is the responsible of the synthesis of the majority of antimicrobial agents with clinical importance amphotericin, erythromycin, streptomycin, tetracycline, and rifamycin. Protein synthesis is the mode of action of all the above except for amphotericin which attacks the cell membrane. Also the majority of these antibiotics are of broad spectrum. These microbes exhibit a vast metabolic versatility. They can complete many physiological cycles that produce intermediate molecules such as enzymes or secondary metabolites with antibacterial, antifungal and antiviral capabilities [40]. Another group of gram-positive bacteria present in soil and responsible for the production of anti-microbial agents with clinical and agricultural importance is the genus Bacillus. This genera is characterized by being gram-positive, spore forming rods [41]. It has been demonstrated that these microbes produce antimicrobial agents in various stages of their growth curve. For example, *B. subtilis* can produce non ribosomal oligopeptides with antifungal and antimicrobial properties such as surfactins, inturinics and bacilysin. Ribosomal antibiotics are also synthesized by this strain which include subalancin and subtilosin [41]. Cyanobacteria are known to be colonized by various heterotrophic bacteria. With a view to understand the associated organisms from cyanobacterial products especially 6 different *Spirulina* products such as two forms of *Spirulina* powder, one crunchy form and three different tablets were selected. A total of 30 bacterial strains were isolated and their biochemical and cultural characteristics were studied. The isolates were subjected for silver nano-particle production. Microscopic results revealed that most of them were gram positive and non-motile. Among the 30 isolates most of them can able to synthesize nanoparticles with silver. It indicates that the colonial characteristics alone cannot serve as a tool to characterize the bacterial isolates. Hence these isolates were carefully examined and selected for further analysis [41].

Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil [39]. They have provided many important bioactive compounds 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 [40]. Almost 80% of the world's antibiotics are known to come from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora* [41, 42]. According to the World Health Organization, over-prescription and the improper use of antibiotics has led to the generation of antibiotic resistance in many bacterial pathogens. Nowadays, the drug resistant strains of pathogen emerge more quickly than the rate of discovery of new drugs and antibiotics. Because of this, many scientists and pharmaceutical industry have actively involved in isolation and screening of actinomycetes from different untouched habitats, for their production of antibiotics [39-41]. Serious infections caused by bacteria have become resistant to commonly used antibiotics and become a major global healthcare problem in the 21st century [40]. *Staphylococcus aureus*, for instance, a virulent pathogen that is responsible for a wide range of infections, has developed resistance to most classes of antibiotics [38, 39]. Clinicians and public health officials have faced hospital acquired drug resistant *S. aureus*, which also bears resistance to many antibiotics. Hence there is need to rediscover new drugs active against these drug resistance pathogens. Majority of the actinomycetes in soil that are potential drug sources remain uncultivable, and therefore inaccessible for novel antibiotic discovery. Enright [28] reviewed the literature on isolation of actinomycetes and suggested that only 10% of the actinomycetes are isolated from nature. Most of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi [29, 30]. *Actinomycetes* can be isolated from soil and marine sediments. Although soils have been screened by the pharmaceutical industry for about 50 years, only a small fraction of the surface of the globe has been sampled, and only a small fraction of *Actinomycetes taxa* has been discovered. This study was undertaken to isolate *Actinomycetes* from the soil samples of wasteland and garden of Ghaziabad, India and to assess their anti-bacterial properties. The resistance problem demands that to discover new antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. So we need to screen more and more actinomycetes from different habitats for anti-microbial activity in hope of getting some actinomycetes strains that produce antibiotics that have not been discovered yet and active against drug resistant pathogens.

A total of 117 actinomycetes strains were isolated from the wasteland alkaline and garden soil samples of the Ghaziabad and screened for their anti-bacterial activity. They were evaluated for their inhibitory activities on four test microorganisms. Fifteen actinomycetes isolate which exhibited antimicrobial activity against at least two of the test organisms and were characterized by conventional methods [42-44]. The cultural characteristics of isolates were also studies in different culture media [41]. The results indicated that six isolates were highly active against *Staphylococcus aureus* strains. Seven isolates were highly active with an inhibition zone more than 20 mm in diameter. Most of the isolates inhibited growth of the Gram negative bacteria tested. All the antibiotic producing actinomycetes were isolated at different temperatures from non agricultural wasteland alkaline soil and compost rich garden soil. Fifteen isolates showed activity against bacteria in which most of them from wasteland alkaline soil where the less interference by human for agriculture or other purpose. These micro-organisms may have capability to produce some of the most important antibiotics ever developed [32, 45-52] provided a bioremediation concept [53] is taken into consideration prior to isolation and purification of antibiotics. Further, the sorption of sulfonamides is strongly influenced by pH and ionic strength [54-56]. Relevant sorption occurs to both soil organic matter (SOM) and soil minerals, that is, clay minerals and pedogenic oxides [55, 57]. However, as for other compounds sorption is dominated by SOM, and first studies were published that further elucidated the relevant sorption sites and mechanisms [57-59]. It was reported that sulfonamide antibiotics exhibit strong nonlinear sorption to functional groups of SOM through preferred site-specific sorption *via* hydrogen bonds and van der Waals interactions. In part strong desorption hysteresis and rapid formation of non-extractable residues were reported [59, 60] that indicates reaching of an energetically favorable status and inclusion of sulfonamides in voids of soil sorbents. Correlation with the abundance of SOM structural features was used to assign preferred binding sites of sulfonamides to SOM [57-59].

CONFLICT OF INTERESTS

Declared none.

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