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ACTINOBACTERIA AS POTENTIAL SOURCE OF ANTIMICROBIALS OF PHARMACEUTICAL IMPORTANCE

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Abstract: Actinomycetes are filamentous bacteria belonging to phyla actinobacteria and the order actinomycetale being aerobic, gram-positive bacteria with high GC content in DNA and show wide distribution in nature. The actinomycetes produce an enormous variety of bioactive molecules of commercial importance. One of the first antimicrobial used was Streptomycin, produced by *Streptomyces 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. 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 many naturally occurring antibiotics have been isolated from actinomycetes. Almost 80% of the world's antibiotics are known to come from Actinomycetes, mostly from the genera Streptomyces and Micromonospora. Actinomycetes are the most economical and biotechnologically valuable class of bacteria producing antimicrobials notably antibiotics anti tumor agents, immunosuppressive agents and enzymes. The most recent estimates suggest that by now we know only approximately as little as 0.1% of the bacteria. And among the ones already described, only a small fraction has been examined for metabolite profile. Also, recent techniques like metagenomics presents immense potential for exploiting yet unknown microbial species particularly actinomycetes form unexplored niches.

Keywords: Antimicrobials, actinobacteria, drug resistance, Metagenomics, rDNA

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INTRODUCTION

Bioactive substances are low molecular compounds exhibiting various activities and microorganisms and plants have been important sources of natural medicinal substances[1]. There is a fast emergence of newer infections and the organisms developing resistance which render already existing antibiotics less effective. Therefore a constant search for new antibiotics to overcome these problems is need of hour. Most of the antibiotics in clinical use are direct natural products or semi synthetic derivatives from actinomycetes or fungi. Unexpected functions of known secondary metabolites are being unraveled and they have interesting applications in many life threatening diseases such as prion diseases, Alzheimer's disease, cancer (hepatoma, breast, hematopoietic, colorectal, gastric, pancreatic, leukemia, renal cell and other carcinomas, and fibrosarcoma) pulmonary disease, cardiovascular disease, parasitic diseases and viral diseases such as AIDS. Since most of the compounds have been successfully used in humans, the relatively low degree of toxicity gives great hope that these unexpected activities will be exploitable as future cures for the terrible diseases confronting humans today, e.g., Alzheimer's disease, multiple sclerosis, cancer, parasitic diseases, cystic fibrosis, viral diseases and many others. Actinomycetes are the most economically and biotechnologically valuable prokaryotes and are responsible for the production of about half of the discovered secondary metabolites. Because of the excellent track record of actinomycetes in regard a significant amount of effort has been focused on the successful isolation of novel actinomycetes from terrestrial sources for drug screening efforts in the past fifty years. Recently the rate of discovery of new compounds from terrestrial actinomycetes has decreased whereas the rate of reisolation of known compounds has increased. Thus, it is excited that new groups of actinomycetes from unexplored or under exploited habitats be persued as sources of novel bioactive secondary metabolites[2]. In terms of lifestyle, these bacteria range from pathogens (e.g. *Mycobacterium* spp., *Nocardia* spp., *Rhodococcus* spp., *Corynebacterium* spp., *Propionibacterium* spp.) to soil dwellers (e.g. *Streptomyces*) to plant commensals (*Leifsonia* spp.) to nitrogen-fixing symbionts[3] (*Frankia*) . This diversity is reflected in the genome sequences of these bacteria[4] of which 96 have so far been deposited into the public database.

Why actinomycetes

Drug development is the frontier area of research. Microbial diversity constitutes an infinite pool of novel chemistry, making up a valuable source for innovative biotechnology. Actinomycetes are one of the most important groups of secondary metabolite producers. Among various genera, Streptomyces, Saccharopolyspora, Amycolatopsis, Micromonospora and Actinoplanes are the major producers of commercially important biomolecules. Actinomycetes have the proven ability to produce secondary metabolites with biological activities ranging from antibiotic, antifungal, antiviral, anticancer, enzyme, immunosuppressant to other industrially useful compounds [5-9]

Over 25000 bioactive secondary metabolites (including antibiotics) were published in the scientific and patent literature, and about a half of them were produced by actinomycetes. However, the development of new drugs from actinomycetes and other microbes and plants is more and more difficult in the whole world due to too many known compounds and microbes. In order to overcome these challenges, some new concepts based on genome were described, including new habitats, new methods, new species, new gene cluster, new products and new use[10]. In other words, novel microbial species should contain new gene cluster synthesizing new secondary metabolites, as long as getting new species is an important premise for obtaining new compounds. Many companies and laboratories focused on new actinomycete resources from new habitats, such as oceans, extreme environment, plants and animals, to develop new drugs. In recent years, research works on uncultivable microorganisms has been carried out in many laboratories[11-14]. But in our view, obtaining pure cultural actinomycetes is still an important premise and new hope for discovery of novel compounds for drug development.

Actinobacteria status

Actinomycetes comprises an important group of Gram-positive bacteria[14] , characterized by the formation of substrate and aerial mycelium on solid media, presence of spores and a high GC content of the DNA (60-70 mol %). The attention paid to this group raised notably after the discovery of streptomycin by Waksman and Schatz in 1943[15]. Actinomycetes are physiologically diverse bacteria as evidenced by their production of numerous extracellular enzymes and by the thousands of metabolic products, they synthesize and excrete. Many of these products are antibiotics. Actinomycetes are the major antibiotic producers in the pharmaceutical industries. In nature, biodegradation by actinomycetes plays an extremely useful role in waste removal and is an integral part of recycling of materials in nature [16-17]. As a result of their great metabolic diversity, actinomycetes have great biotechnological potential for the production of pharmaceuticals and for converting waste materials into useful chemicals. Species of actinomycetes produce cellulases, xylanases, amylases, lipases, collagenases, proteases, Chitinases, lignases etc. The ability of actinomycetes to degrade lignin and cellulose could be very important in the production of liquid fuels and chemicals from lignocellulose. Other beneficial activities of actinomycetes related to agriculture and forestry include their activity as biological control agents in the regulation of fungal diseases and the capacity of *Frankia* species to carry out symbiotic nitrogen fixation[17].

The actinomycetes are a group of bacteria which possess many important and interesting features. They are of considerable value as producers of antibiotics and of other therapeutically useful compounds. They exhibit a range of life cycles which are unique among the prokaryotes and appear to play a major role in the cycling of organic matter in the soil ecosystem[18]. Therefore, actinomycetes hold a prominent position due to their diversity and proven ability to

produce new compounds, because the discovery of novel antibiotic and non-antibiotic lead molecules through microbial secondary metabolite screening is becoming increasingly important.

Actinobacteria as untapped source of antimicrobials –Global scenario

Actinomycetes are unparalleled sources of bio-active metabolites including antibiotics, plant growth factors, and other substances[19-21]. *Streptomyces* and other actinomycetes are major contributors to biological buffering of soils and have roles in organic matter decomposition conducive to crop production[22]. Biological control is slow but can be long lasting, inexpensive, and harmless to living organisms and the ecosystem; it neither eliminates the pathogen nor the disease, but brings them into natural balance [23]. Intensive research on plant growth promoting bacteria (PGPB) is underway worldwide for developing biofertilizers and biocontrol agents (BCAs) as better alternatives to agrochemicals, as the latter harm the environment and human health besides demanding high costs. As most PGPBs show inconsistent performance in the field conditions, there is urgent need for survey of indigenous strains suited to local conditions. Actinomycetes are known to produce bioactive substances, especially antibiotics that are effective against phytopathogenic fungi[24].

Indian scenario

Terrestrial actinomycetes have been of great global interest to scientists for the past 55 years, pertaining to the discovery of novel genera and various bioactive metabolites. In India, a significant amount of effort in discovering antimicrobial compounds against clinically important pathogens from terrestrial actinomycetes has been made. The terrestrial habitats of India, for the survey of actinomycetes includes cold deserts of Himalayas, Rothang Hill of Himachal Pradesh, Gangetic belt soil of Kanpur, lateritic and sandy soils of different states such as Pune, Maharashtra, Karnataka, Andhra Pradesh etc. Duraipandiyan et al. isolated twelve actinomycete strains from the Himalayan soil with anti-microbial activity. Isolate, ERIH-44 exhibited antibacterial and antifungal activity [25]. Mukhopadhyay et al. reported an antifungal macrocyclic lactone, "Maclafungin" from a soil actinomycete from Billimora, Gujarat and active against filamentous fungi (Human and Phytopathogens) and yeast like Candida albicans[26].

A study was carried out on the utilization of carbon and nitrogen sources by Streptomyces *kanamyceticus* M 27 for the production of antibiotic by Pandey *et al*[27]. Dextrose as carbon source (2%) and NH4 H2 PO4 [0.68%] as a nitrogen source gave the optimal kanamycin yield. No direct correlation between the growth and antibiotic production was observed. Gopalakrishnan *et al*. (2011) isolated 137 actinomycetes from 25 herbal vermicompost for the biological control of Fusarium wilt of chickpea plants. The active isolates were identified as Streptomyces tsusimaensis, Streptomyces caviscabis, Streptomyces setonii, Streptomyces africanus and an unidentified Streptomyces sp[28]. Kumar *et al*., (2011) has screened 117

actinomycete isolates from alkaline wasteland and garden soil against bacterial pathogens. Among all the isolates, six have shown promising activity against Staphylococcus aureus[29]. Thus this wasteland appears to be a convenient microbial niche to be explored.

Microbial metabolites are the sources of life saving drugs e.g. against bacterial and fungal infections (penicillins, erythromycins, streptomycin, tetracyclines, vancomycin, and amphotericin), Cancer (doxorubicins, daunorubicin, mitomycin and bleomycin), transplant rejection (cyclosporine and rapamycin) etc. Microbial natural products are notable not only for their potent therapeutic activities but also for the fact that they frequently possess the desirable pharmacokinetic properties required for clinical development

Screening and Evaluation for antimicrobial activity

Large numbers of isolated differential isolates are subjected to several types of screening procedures with the aim to obtain potentially valuable isolates. Different methods of screening procedures are available in hand**.** Screening of Actinomycetes is done by the antimicrobial activity, preliminarily studied by cross streak method. After observing a good ribbon- like growth of the actinomycetes on the petri-plates, the pathogen was streaked at right angles to the original streak of actinomycetes and incubated at 28^{o}C±2^{o}C.The inhibition zones are measured after 24 and 48 hrs. A control plate maintained without inoculating the actinomycetes, to assess the normal growth of the bacteria. Based on the results of antagonistic activity the strains are selected for further studies.

In vitro screening of isolates for anti-bacterial activity

Morphologically distinct actinomycete isolates are also subjected to anti-bacterial activity screening against the pathogenic test organisms by conventional spot inoculation method[30] (Shomurat et al., 1979) and single line streak method[25] (Duraipandiyan et al., 2010) on agar medium. In spot inoculation method, pure actinomycetes isolates are spot inoculated on starch casein agar medium. The plates were incubated for few days, and then inverted for over chloroform in fume hood. Colonies are then covered with a 0.6% agar layer of nutrient agar medium, previously seeded with one of the test organisms. The antimicrobial activity is observed after suitable incubation period. In single line streak method, pure actinomycetes isolates are inoculated in a single streak down the middle of a plate of screening media and incubated for the production of any antibiotics. A single streak of each test organism is added perpendicular to the actinomycetes streak. Test organisms are placed perpendicular to culture streak.

Metagenomics as a potential tool

Metagenomics is the study of metagenomes (genetic material) recovered directly from environmental samples. Conventional culture dependent methods are inadequate for

analysis of natural environments because of their small size, the absence of distinguishing phenotypic characters. The fact that most of the organisms cannot be cultured is the most important factor that limits the evaluation of biodiversity[31]. Only a small fraction of microorganisms can currently be cultured from environmental samples, and even if a microorganism is cultured, its role in a community and contribution to ecosystem function are not necessarily revealed[32]. Characterization of natural microbial communities by molecular methods has been widely used to reveal intrinsic genetic diversity. Because of its power to reveal the previously hidden diversity of microscopic life, metagenomics offers a powerful lens for viewing the microbial world that has the potential to revolutionize understanding of the entire living world.

The opportunities for the discovery of new organisms and the development of resources based on microbial diversity are greater than ever before. Molecular sequences have given microbiologists a way of defining microbial phylogeny. The sequences are the basis of tools that will allow microbiologists to explore the distribution and function of microbes in the environment.

Little is known about the microbial world especially poorly understood actinobacteria, because so few microbes have been grown under laboratory conditions and also because there are immense numbers of low abundance ribotypes that have not been detected. Metagenomic studies have shown that there are large groups of microorganisms in many environments that cannot be cultured and thus cannot be sequenced. Much of the interest in metagenomics comes from the discoveries that the vast majority of microorganisms had previously gone unnoticed. Metagenomics is a young and exciting technique that has broad application in biology and biotechnology. The potential benefit of exploration of microbial genetic resource derives from future biotechnological exploitation of the gene pool and from new insights into the biological mechanisms of adaptation. The microbial genetic resource is hiding within it the as yet undiscovered microorganisms that may be cure for many diseases, means to clean the polluted environments, as food sources and as the means to manufacture the products of our daily use. Metagenomic approach to actinobacteria might bring novel and yet unknown microbes to the fore front.

CONCLUDING REMARKS

Actinobacteria specially Streptomyces continues to be one of the best factories among actinomycetes and can deliver novel scaffolds if appropriate tools are put in place to reveal them in a cost-effective manner. The true value of the microbial genetic resources is not known, a single property of one organism may be worth millions, yet that property if undiscovered, the organism may be considered of little economic use. It is essential that the vast majority of microbial diversity is made available for screening of useful properties and

research and investigations. The technological advances that are becoming available are bringing together all disciplines involved in natural products research and shaping new ways in which microbial natural products can be mined and exploited. Without doubt, there are strong indications of renewed interest from many sectors in exploring natural product libraries for novel scaffolds. The challenge today is to be able to translate current developments into industrial-scale processes, and this remains the major hurdle that will have to be overcome if scientific community wants to revitalize natural products discovery.

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