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An Analysis upon Isolation and Antibacterial Activities of Actinomycetes from Air

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Abstract – The main focus of this study was to isolate some antibiotic producing actinomycetes strains from air. The air sample used was dark brown and sandy, with no vegetation covering (bare). Isolation of air actinomycetes was done by culture-dependent methods and isolates were tested for antibiotic production on selected indicator bacteria plates. The results indicated that a total of 3.56 × 105 actinomycetes colonies were isolated per gram of dry air. Furthermore, microscopic examination of the isolates indicated 6 major colony types (CTs) in the air. Only 3 CTs were found to be active against one or more indicator bacteria, with inhibition zones that ranged from 7 mm to 12.5 mm in diameter. From the results, it was suggested that the low yield of antibiotic producing actinomycetes isolates obtained in this study, could be improved by employing a combination of several molecular analysis methods.

Actinomycetes are one of the most attractive sources of antibiotics. In the present studies, total of 15 strains were isolated from Dr. Ram Manohar Lohia Hospital and RML Park in Lucknow, U.P India. Isolated strains were identified for their antibacterial activity but only six isolate showed good result, they were evaluated 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 showing best result which was 30 mm zone of inhibition against S. aureus and MIC was found to be 0.0009 mg/ml.

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INTRODUCTION

Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the air (Oskay *et al*, 2004). 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 (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). Present time most of the disaeases caused by bacteria have become resistance to most of the antibiotic (Alanis 2005). *Staphylococcus aureus* is commonly known pathogen that is responsible for infections like pneumonia diabetes, cancer, vascular disease, and lung disease have developed resistance to most classes of antibiotics (Enright 2003), Physicians acquired methicillin-resistant *S. aureus* (MRSA), which also bears resistance too many antibiotics. During this time, vancomycin has been the therapeutic answer to MRSA, Vancomycin resistant strains have emerged clinically.

Vancomycin-resistant *S. aureus* (VRSA) challenges clinicians, not only because of vancomycin and methicillin resistance, but also because of resistance to many other antibiotics, including aminoglycosides, macrolides, and fluoroquinolones.certain undesirable side effects and the spread of pathogens with this new antimicrobial drug resistance emphasize the need for the development of other newer antimicrobial agents with activity against such gram positive bacteria (Nathwani 2005). Also the other cause is gram negative antibiotic-resistant opportunistic pathogens. Gram negative environmental and enteric organisms currently threaten patients in hospitals and communities with multi-drug resistance, including broad resistance to first, second, and third generations of penicillin's and cephalosporin's. These bacteria, like *Pseudomonas aeruginosa*, are common organisms which were

found in environment, which act as opportunistic pathogens in clinical cases where the defense system of the patient is compromised (Lyczak et al. 2000). Also other intrinsically antibiotic resistant organisms such as *Stenotrophomonas maltophilia* are emerging as opportunistic pathogens. The end result of this phenomenon is that many strains of bacteria have become resistant, and in many cases multi-resistant to these therapeutic agents, to overcome this problem a new antibiotic needed hich will show positive result.

Microbial diversity is a vast frontier and potential goldmine for the biotechnology industry because it offers countless new genes and biochemical pathways to probe for enzymes, antibiotics and other useful molecules (Singh & Agrawal 2002). The diversity of terrestrial Actinomycetes are of extraordinary significance in several areas of science and medicine, particularly in antibiotic production (Magarvey *et al.* 2004). Actinomycetes are diverse group of Grampositive bacteria that usually grow by filament formation. They belong to the order Actinomycetales (Superkingdom: Bacteria, Phylum: Firmicutes, Class: Actinobacteria, Subclass: Actinobacteridae). They have high G+C (>55%) content in their DNA. They are the best common source of antibiotics, and provide approximately two-third of naturally occurring antibiotics, including many of medical importance.

Need of new antimicrobial agents is greater than ever because of emergence of multidrug resistance in common pathogens, the rapid emergence of new infections and the use of multidrug resistant pathogens in bioterrorism Resistance of bacteria to the effects of antibiotics has been a major problem in the treatment of diseases. Infectious diseases are still the second leading cause of death worldwide (Luzhetskyy *et al.* 2007).

Though the recent quests for novel antibiotics have employed more recently established approach of target-based discovery using bacterial genomics, combinatorial chemistry, and high-throughput screening, these powerful tools have not yet yielded any antibiotics approved for clinical use, and the prospects for their success are not encouraging. On the other hand, programs aimed at the discovery of antibiotics from microbial sources have yielded an impressive number of compounds over the past 50 years, many of which have application in human medicine and agriculture. Hence, the traditional method of screening antibiotics from microorganisms is no longer considered glitzy science (Baltz 2007).

Choice of natural materials like airs in researches is based on the assumption that samples from widely diverse locations are more likely to yield novel microorganisms and therefore hopefully, novel metabolites as a result of the geographical variation. Besides, the important approaches helpful in discovering new microbial species or unknown bioactive substances include isolation and characterization of microorganisms from the most extreme habitations and relatively unknown or unstudied areas.

In this regard, Kalapatthar (5545m), Mount Everest region of Nepal is of significant interest. Its high altitude and seasonal snow create extreme habitation which is likely to harbor unusual types of microorganisms while poorly studied habitation increases chance of finding novel microorganisms. Keeping these points in view, the present study was undertaken to isolate and characterize antibacterial Actinomycetes from air samples of this area.

Actinobacteria, high guanine and cytosine containing bacteria is one of the dominant phyla of the bacteria found on almost natural substrates. They play an important role in decomposition of organic materials and carbon cycle. The taxonomy of the actinomycetes has been subject to unending controversy because of its filamentous, branching growth which resembles with a fungal type of morphology.

Actinomycetes represent a high proportion of the air microbial biomass and have the capacity to produce a wide variety of antibiotics and extracellular enzymes. Most of the known natural antibiotics are produced from actinomycetes. Moreover, these are important source for novel antibiotics and hence having a high pharmacological and commercial interest including control of infectious diseases. Medical or economic significant Actinobacteria mainly lies in subclass Actinobacteridae, order Actinomycetales. The order Actinomycetales is composed of approximately 80 genera, nearly all from terrestrial airs, where they live primarily as saprophytes.

Actinomycetes are important sources of new bioactive compounds such as antibiotics and enzymes which have diverse clinical effects and are active against many pathogenic organisms Actinomycetes and their bioactive compound show antibacterial and anti microbial against various pathogens and multi drug resistant pathogens e.g. Vancomycin-Resistant Enterococci, Methicillin-Resistant Staphylococcus aureus (S. aureus), Shigella dysenteriae (S. dysenteriae), Klebsiella sp. and Pseudomonas aeruginosa (P. aeruginosa) etc. The need for new, safe and effective antimicrobial agent is the major challenge to the pharmaceutical industry now a days, especially with the obvious increase in opportunistic infections in the immune compromised host via and multiple drug resistant strains.

Among all the known microbes, members of the actinomycetes genus especially Streptomyces species bioactive metabolite with broad spectrum of activities which has antibacterial, antifungal, antibiotic, antiphrastic, antitumor, antiviral, insecticide, herbicide, immunemodulators, antithrombatic agents. Thus screening and isolation of promising strains of actinomycetes with potential antibiotics is a thrust area of search since many years. Streptomycetes are widely used in industries due to their ability to

produce numrous chemical compounds including antibiotics, enzymes and anti-tumor agents.

Actinomycetes are ecological diverse group of bacteria, constitute the major microbial population in air producing active secondary metabolites. As there is geographic variation in Indian air type and their contents, hence it is quite likely that the distribution of antibiotic producing actinomycetes is also variable. Therefore, exploration of unexplored ecosystems for actinomycetes is necessary for the identification of novel antibacterial metabolites. The objective of present study was isolation of actinomycetes from different air samples (Gwalior) for screening of antibacterial compounds against multidrug resistant bacteria of clinically relevance.

ISOLATION AND SCREENING OF *ACTINOMYCETES* FROM MANGROVE AIR

Mangroves are the unique woody plants of intertidal coasts situated in tropical and subtropical zones. Mangrove forests are among the world's most productive ecosystems. They are adapted to survive in harsh conditions such as high salinity, high temperature, low oxygen, extreme tides, muddy and anaerobic air, and forceful winds. The mangroves biomass is greater than any other aquatic systems. Mangroves prevent air erosion and deposition of silt. Global mangroves have an estimated cover of 15.2 million hectares (FAO, 2007). Marine environments are largely untapped source for the isolation of newmicroorganisms with potentiality to produce active secondary metabolites. Among such microorganisms, *Actinomycetes* are of special interest, since they are known to produce chemically diverse compounds with a wide range of biological activities. Multiple antibiotic resistant pathogens are rapidly emerging and hence there is a constant demand for new antibiotics in market. The name "*Actinomycetes*" was derived from Greek word "atkis" (a ray), and "mykes" (fungus) and has the features of both bacteria and fungi.

Taxonomically, *Actinomycetes* are clubbed with bacteria in same class of schizomycetes but confined to order Actinomycetales. *Actinomycetes* are Gram positive with high G+C content in their DNA. *Actinomycetes* are numerous and widely distributed in air. They are heterotrophic, aerobic and mesophilic (25-30°C) and some species are thermophilic growing at temperature 55-65°C e.g. *Thermoactinomycetes*, *Streptomyces. Actinomycetes* possess various unique and interesting features. They are of great importance as antibiotic producers and also other therapeutically significant compounds. Recently, the marine derived *Actinomycetes* are recognized as a source of novel antibiotic and anticancer agent with unusual structure and properties. In the present study, an attempt was made to isolate and screen the *Actinomycetes* from the mangrove air of Dumas-Bhimpore region, which can be valuable resource of various enzymes and antibiotic production.

METHODOLOGY

Collection and Preparation of Air Sample The air used was dark brown and sandy, with no vegetation covering (bare; air 3). Air sample collection was from a location between the North and East wings of Haworth building, University of Birmingham, United Kingdom, in an area that was relatively steep and not quite in the shade.

Actinomycetes Enrichment This process was done aseptically. First, the air slurry was made by suspending 0.1 g of the collected dry air in 10 ml distilled water. The slurry was mixed by vortexing for 2 min and four 1 in 10 fold serial dilutions were made from the slurry. These dilutions were done in duplicates (A and B). Next, 3 ml volumes of the provided top agar were poured onto the bottom agar plates. The plates were allowed to set, after which 0.1 ml portions of each dilution, were then plated by spreading on the set chitin agar plates. All spread plates where labelled and incubated at 25℃ for a period of 14 days.

Examination of Plates

On day 14, the number of colonies formed in each dilution of both groups of plates (A and B), were counted and recorded. All plates were carefully observed under the microscope to detect diversity in colonies formed. The richness, evenness and diversity index, where also calculated and recorded. Eight different colony types were then picked out with sterile forceps and streaked out on glucose yeast extract agar plates, to obtain pure single colonies. The streaked plates were then incubated.

Test for Antibiotic Production Single colonies of the grown actinomycetes cultures were examined with the microscope, to detect diversity in appearance. Four indicator microorganisms (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus epidermis*), were spread plated as broth cultures on 8 separate plates. A total of 8 actinomycetes were then deposited for each indicator organism, as described in the lab manual. All processes were performed aseptically, to avoid contamination.

Examination of Inhibition of Indicator Organisms Inhibition of indicator bacteria was accessed by measuring the diameter of clear zones formed around actinomycetes cultures, in each plate. All observations and data were recorded.

RESULTS

Colony Number and Colony Diversity - After about 14 days of incubation, the chitin agar (top agar) was able to support the growth of only the actinomycetes and prevented growth of other air bacteria and fungi. Cycloheximide used in the bottom agar, further prevented fungi interference by inhibiting mRNA translation, hence causing cell growth arrest and cell death of opportunistic fungi. Six major colony types (richness) were observed.

Inhibition of Indicator Bacteria Only 3 actinomycetes isolates (CT 4, CT 5 and CT 6) inhibited one or more indicator bacteria. Some of the zones of inhibition were present but not very obvious. The widest inhibition zone (diameter = 12.5 mm) was recorded for CT 4 which inhibited only the growth of P. aeruginosa. The narrowest inhibition zone (diameter = 7mm) was observed in CT 6, which also inhibited two other indicator organisms (B. subtilis and S. epidermis). Meanwhile CT 1, CT 2 and CT 3 were not seen to inhibit any of the indicator organisms.

DISCUSSION

Air actinomycetes isolated from bare sandy air (air type 3) in this study, were of six colony types. On initial microscopic examination, the clear zones observed in some grown actinomycetes colonies (CT 5 and CT 6), indicate that the organisms broke down the chitin for growth and development. Colony types (CT 1, CT 2 and CT 3) that did not develop clear zones, may have utilized other nutrients in the medium such as nitrates, phosphates and sulphates, in order to grow. It has been reported that 1g of air when plated, harbours up to 10 billion microorganisms, of which about 4.2 × 106 CFU/g (dry weight) are accounted for by bacteria species. The number of actinomycetes (3.5 × 105 CFU/g dry air) obtained in this study was however lower than expectation. This may be partly attributed to the geographical location of the air. As indicated in the methodology, the air was collected from an area that was relatively steep and not quite in the shade. The steepness of the terrain, exposure to the sun rays and lack of vegetation covering of the air (bare) may have caused leaching of actinomycetes and therefore have partly contributed to the observed low actinomycetes CFU/g of dry air..

CONCLUSION

The present study reported the antimicrobial activities exhibited by different isolates of actinomycetes isolated from different biotopes of Punjab, India. Out of the many isolates, isolate A5 showed maximal antagonistic activity against the microorganisms used. It showed broad spectrum of antimicrobial activity as it inhibited both Gram positive and Gram negative bacteria and also some fungi. Therefore, this isolate proves to be a promising isolate which can be further studied for its applications in producing important pharmaceutical compounds and also as a biocontrol agent against plant pathogenic fungi. Further work on optimization of this isolate's antagonistic activity, purification of the important bioactive compound and study of its other properties like anti-tumorigenic and angiogenic activities are underway.

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