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ISOLATION, CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT ACTIVITIES OF SECONDARY METABOLITES PRODUCING ACTINOMYCETES OF TERRESTRIAL ORIGIN

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Abstract - Microbial secondary metabolites are important sources of natural compounds when compared toothers with potential, beneficial the rapeutic applications. There are chances of discovery of new Streptomyces species and new compounds from there spective genus. Due to ever and over increasing resistance of pathogenic bacteria tour currentarsenal of antibiotics , a great need exists for the isolation and discovery of new antibiotics and other drug agents. Based on the above concept actinomy cetesaremainly targeted for secondary metabolites production and evaluation of compounds therapeutically. Methods.

***Key Words*:** Actinomycetes,Dot plotassay,DPP Hassay,GC-MS, Phosphomolybdenu massay, TLC

1.INTRODUCTION *(Size 11, Times New roman)*

Natural products" is an indistinct term used to define substances isolated from predominant and alluring sources like plants, insects, mammals and microorganisms etc. They play a pivotal role in the development of new therapeutic agents. The word "natural" used to indicate that they are not of synthetic origin, which does not include the compounds present in living cell like DNA, amino acids, sugars, fatty acids that are crucial for metabolism in the living cell. Such compounds are vital for the growth, development and survival of the cell, irrespective of the source i.e. plants, animals or even microorganisms. The processes leading to the formation of such important and prevalent compounds as a result of end products of catabolism are known as "primary metabolism". The second word "product" refers to the outputs of the enzyme-mediated chemical reaction in the cell called metabolic pathways. But, they have broader meaning, so "compounds" or "metabolites" are more precise than "natural products". These products have no role to play in the fundamental life processes of the living cell and so are not ubiquitous. Their absence does not lead to the immediate death of the organism, but survival is hindered to a larger extent. The process of biochemical synthesis of such products is known as "secondary metabolism".

1.1. The microbial natural products research continues to remain a vital route for the development of therapeutic agents from lesser known or new bacterial taxa (Alderson et al., 1985). The identification of lead molecules of significant importance through high-throughput screening of microbial secondary metabolite is becoming increasingly fertile. It is a well known fact microorganisms are untapped source of biologically active compounds with many therapeutic applications. Among them, actinomycetes hold a prominent position because of their structural diversity and established ability to produce new compounds (Antal et al., 2005). In particular, Streptomyces are widely acknowledged and notable genus of actinomycetes that has remarkable potential for its offering in drug discovery task (Arasu et al., 2008). They are known to produce bioactive metabolites of different classes. The species of Streptomyces have contributed more than 50% of the metabolites obtained from natural sources and continues to be a potent source of metabolites of useful applications (Augustine et al., 2005). Actinomycetes species are known to produce wide range of metabolites that possess bioactivities such as antibacterial, antifungal, antitumor, immunosuppressive and enzyme inhibition

Actinomycetes are a heterogenous and most extensively distributed group of gram- positive bacteria with unique chemical and morphological diversity (Baskaran et al., 2011). They primarily inhabit the soil although their presence in extreme and unexplored environments has also been reported (Baur et al., 2006). Actinomycetes are aerobic, filamentous, saprophytic, heterotrophic and have high G+C content. They possess filamentous structure which can be branched to form a stable mycelium (Berd, 1973). Later, the mycelium may break into pleomorphic, rod or coccus shaped fragments. When grown on suitable medium, the colony of actinomyctes branch profusely on the surface of agar and form network of mycelia. The mycelia formed on the surface are called aerial mycelia, and those formed beneath agar surface are known as substrate mycelia (Bonev et al., 2008). The spores formed by these free-living saprophytic bacteria become airborne when dispersed. Even though true hyphae and spores are regarded as characteristic feature of fungi, they form distinct evolutionary line of organisms. They are differentiated from other group of bacteria by morphological features, nucleic acid and pairing studies.

Table 1 - An example of a table.

1.2 Distribution of actinomycetes

Actinomycetes, the most favourable and pervasive group of bacteria are found in a broad range of natural and man-made environments. These spore bearing

bacteria are categorized mostly on the basis of morphological characteristics. Most of these filamentous and multiracial bacteria due to their bioactive potential are capable of surviving in almost all types of ecological niches such as soil, air, water, marshy places, plant residues, compost etc,. They are invaluable prokaryotes and comprise a significant constituent of microbial population in soil habitats. They form extensive branching substrates, aerial mycelia and widely distributed in soil. Although they are primarily recognized as soil organisms, actinomycetes are dispersed in almost every natural substrate and are the most eminent producers of biologically important compounds. Though majority of them are free living but pathogenic species like Actinomyces israelli are also found as infectious agents in plants, animals and humans. The marine environments are also traversed for the novel actinomyctes isolates and large numbers of actinomycetes have been revealed from habitats like marine sediments, sponges, deep sea, river mud, hot springs and lake bottoms. They are also omnipresent in riverine environment and extensive studies also suggested worldwide dissemination of these bacteria in oceans. Section headings should be left justified, bold, with the first letter capitalized and numbered consecutively, starting with the Introduction. Sub-section headings should be in capital and lower-case italic letters, numbered etc, and left justified, with second and subsequent lines indented. All headings should have a minimum of three text lines after them before a page or column break. Ensure the text area is not blank except for the last page.

1.2.1 General guidelines for the preparation of your text

Air drying: Simple drying of samples at room temperature suppresses the growth of bacterial colonies while actinomycetes growth is enhanced. Slow-growing actinomycetes spores are resistant to dessication as compared to other bacterial species, therefore it air drying of samples decreases the number of bacterial contaminant and enhances the number of slow-growing actinomycetes. Differential centrifugation: Motile zoospore forming actinomycetes can be selected by differential centrifugation. This method can be incorporated in isolation procedure. Some of the motile zoospores forming genera are Actinokineospora, Actinosynnema, Dactylosporangium, Plamonospora, Sporichthya, Catenuloplanes etc. Other physical and chemical pre-treatment methods are explained

1.2.3 Background to the research gap

The emergence of infectious disease and multidrug resistant pathogens represents a global threat and has increased many folds over the last few decades. The accumulation of resistant modules encoded by genes existed both within the genome and plasmids have greatly reduced the efficacy of the existing therapeutic options against the pathogens (Ekwenye and Kazi, 2007). Thus, for the betterment of public health, there is a substantial need to search for the novel biologically important compounds that effectively target the

1.3 Aim and objectives of the study evolving pathogens and cease spreading of life threatening diseases. Keeping this in mind, we have designed a research problem of isolating actinomcyetes strains from various niches which would be screened for biological activities. The positive strains would be identified and cultured for the production of bioactive compounds. Subsequently, the characterization of bioactive compounds would be carried by chromatographic and spectral applications.

II. LITERATURE REVIEW

(Ravikumar et al., 2011). Bio-discovery of microorganisms can be defined as the exploration of microbial secondary metabolites to detect and evaluate their bioactive potential for medicinal, agricultural and industrial applications (Horikoshi, 1999). The microbial resource centres collect compounds from microorganisms isolated from different habitats, under different conditions and parameters and increase the metabolite production to evaluate their potential in different bioassays only to define target activity. The variety of microbial lead molecules developed into therapeutically important compounds have significantly contributed to the improvement of human health. However, recent reports suggest that only a small fraction of approximately 5% of fungal species and 0.1% of bacteria have been explored for metabolites production (Ilic et al., 2007). The microbial metabolites are brought in use most commonly by: the fermentation broth which can produce bioactive molecules directly, fermentation product can act as raw material, or products are used as lead compound for a chemical synthesis (Liang et al., 2005). The metabolites of microbial origin not only possess potent therapeutic activities but desirable pharmacokinetic properties also require for chemical development.

Apart from plants, microorganisms that have made utmost contribution in production of about 22,500 biologically active compounds are actinomycetes (45%), fungi (38%) and

unicellular bacteria (17%) (Igarashi, 2004). The diversity among microorganisms plays a major role by providing unique structural diversity. More than 10,000 biologically active molecules of microbial origin are currently in use as a lead molecules or derivatives (Jonsbu et al., 2002). Among the

proliferous source of natural products, actinomycetes hold a distinguished position and are known as reservoir of chemically diverse metabolites of industrial and pharmaceutical interest. The actinomycetes have edged out other microorganisms in recent decades and continuous efforts are made to explore their use in therapeutic applications and innovative research.

Actinomycetes are ubiquitous group of prokaryotic microbes widely spread in a varied range of natural and man-made ecosystems all over the world (Ludwig et al., 1994). They are abundantly soil inhabitants but are found distributed in different ecological niches, including plant residues, food products, manures, compost, agricultural lands, contaminated lands and marine environments (Adegboye and Babalola, 2012; Ai-Min et al., 2009; Alexander, 1977). They are found to colonize in various extreme and unexplored environments viz. hot springs, salt pans, deep sea ecosystem, soils at higher altitudes, frozen soils of polar regions, arid soil of deserts, saline and alkaline lakes (Pathirana et al., 1992; Pathom-aree et al., 2006). The basic elements that allow microorganisms to survive and regulate in any environment includes carbon and energy sources, growth factors, mineral nutrients, temperature, pH, ionic composition and different forms of interaction between organisms (You et al., 2007). The occurrence of actinomycetes in diverse ecosystem and their isolation from unique natural habitat have always fascinated researchers for the discovery of novel species, novel genera and even novel families with varying community structures of actinomycetes that produce novel bioactive compounds of significant interest.

Antibiotics are produced by wide range of microorganisms, especially fungi, bacteria and actinomycetes and inhibit or kill other potential competitors at low concentration (Lazzarini et al., 2000). The slow growing actinomycetes are well recognized to be a thriving source of antibiotics, especially the genus Streptomyces, whose ability to produce industrially important compounds, particularly antibiotics, remains unrivalled possibly because of extra- large DNA complement of these bacteria and the occurrence of plasmids (Madigan and Martinko, 2007). Nearly 80% of known medical and commercial antibiotics are produced by genus Streptomyces and its varying species. Streptomycin, one of first antibiotic found was produced by Streptomyces griseus (Schatz et al., 2005). Reportedly, over 200 antibiotics from Streptomyces griseus and over 40 antibiotics from Streptomyces hygroscopicus are known, making them the major contributors to the antibiotic market (Demain, 1999).

The antibiotics produced by Streptomyces species are used in the field of veterinary, human medicine, agriculture and industry (Kurtboke, 2012). Antibiotics reported from actinomycetes are placed together in major classes such as ansamycins (ritamycin), macrolides (erythromycin, azithromycin and clarithromycin), aminoglycosides (streptomycin, tobramycin, kanamycin, gentamycin and neomycin), teracyclines and β-lactum (penicllin, cephalosporin and carbapenems) (Ikeda et al., 2003).

(Valderrama et al., 2003) owing to their low cost, huge productivity, vast availability, stability as well as environmental protection. Among diverse actinomycetes genera, Streptomyces, Thermomonospora and Cellulomonas have been extensively utilized for the production of important extracellular enzymes. Some industrially important enzymes of actinomycetes are pronase and kerase which are obtained from Streptomyces griseus and Streptomyces fradiae respectively. These enzymes are used in commercial production of hydrolysate proteins from different protein sources (Hiramatsu and Ouchi, 1963). The proteases of Streptomyces are known for easy elimination of the mycelium by filtration or centrifugation (Phadatare et al., 1993). The amylases of Streptomyces are used as softening agent in baking industry (Dejong, 1972). Similarly, Streptomyces griseus, Streptomyces karnatakensis, Streptomyces albidoflavus, and Nocardia spp. have been revealed as potential source of L-asparginase (Narayana et al., 2008).

III. MATERIAL AND METHOD

The frequency of uncovering novel bioactive compounds has depleted to a larger extent over the years. As a consequence, the exploration of biodiversity to expand the range of novel and effective biomolecules is critical to address this reduction. For this purpose, exploitation of rare genera from common habitats or under-investigated species from unexplored habitats is considered important approaches (Barka et al., 2015). Actinomycetes from unexplored ecosystem can be a potential source of useful bioactive metabolites especially antibacterial and antifungal compounds (Indraningrat et al., 2016). Western Ghats of India is considered as one of the global biodiversity hotspots and among the most plenteous ecosystems with regard to the existence of new bacterial species (Gautham et al., 2012).

The forest regions in the Western Ghats are home to several actinomycetes species and largely unexplored. In this regard, few reports are available pertaining to the isolation, screening and

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characterization of microbial diversity for the potential to produce novel and effective bioactive molecules. The Kashmir valley in India is naturally blessed with indigenous diversity of microbes but, actinomycetes strains from high altitude cold regions of Apparwath glacier, Gulmarg, Kashmir are yet to be explored for their bioactive potential. Marine ecosystem is the world's most dynamic and unexploited environments that inhabit various flora and fauna of therapeutic importance (Jose and Jebakumar, 2014). Marine microbial communities are the richest and diversified source of low molecular weight bioactive compounds, though the nature of the microbial inhabitants and their metabolites are not well understood (Butler, 2008).

Therefore, in the present chapter various ecological habitats were explored to find out the existence of diverse population of actinomycetes producing bioactive secondary metabolites. The prevalence and existence of actinomycetes in various habitats selected in this study were reported before, but the sampling sites mentioned are never before been explored to such extent. In the present work, soil and marine sediment samples were collected for effective isolation of different actinomycetes. The rationale behind this approach was that actinomycetes present in different ecological niches may exhibit variable metabolic activity and may produce diverse bioactive metabolites (Tiwari and Gupta, 2012). While exploring

different habitats, the maximum number of presumptive actinomycetes were isolated from soil samples collected from forest regions of Bisle Ghat (91), Virajpet (48), Gundya (39) and Subramanya (33) situated in Western Ghats, Karnataka; Gibbon Wildlife Sanctuary, Jorhat, Assam (62), Dodital Lake, Uttarkashi (56) and Apparwath glacier, Gulmarg, Kashmir (54). Forty marine derived actinomycetes were isolated from Havelock Island, Andaman and Nicobar Islands (24) and Gulf of Mannar National Park, Rameshwaram, Tamil Nadu, India (16).

IV. RESULTS AND DISCUSSION

Secondary metabolites were extracted from the filtrate by the solvent extraction method. Extraction of secondary metabolites was carried out by culturing the selected actinomycetes isolates in 2 ltr Erlenmeyer flasks (Borosil, India) (45 nos. for each strain) each containing 750 ml of production medium (ISP-2 broth) and incubated at 28±2oC for 21 days at an agitation rate of 140 rpm under shaking condition (Ahsan et al., 2017). After incubation, culture broth was harvested to separate mycelium using double-layer detached cheese cloth. The culture filtrate (broth) was centrifuged at 8000 rpm for 15 min in order to obtain culture supernatant free from cells and debris. The cell-free culture supernatant was filtered through 0.45 µm Whatman filter paper (Sigma, Mumbai, India) and subsequently extracted thrice with an equal volume of ethyl acetate (Himedia, India). The solvent and broth mixture was shaken vigorously for complete extraction. After removing the lower aqueous phase (culture broth), the upper organic phase (ethyl acetate) was then concentrated under reduced pressure at 50oC using a rotary flash evaporator (Hahn-Shin, Bucheon, South Korea) to obtain the crude extract. The extracts were transferred to pre-weighed vials, evaporated under vacuum and dissolved in dimethyl sulfoxide (DMSO) (Qualigens Fine Chemicals Pvt. Ltd., San Diego, USA) and stored at 4oC until used further. The obtained extract was used to determine antimicrobial, antioxidant, enzyme inhibitory and cytotoxic activities.

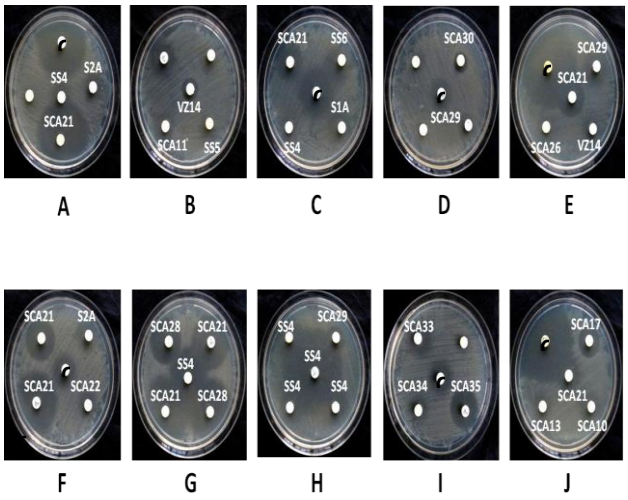

Figure 4.1: Antibacterial activity of crude extracts from selected actinomycetes isolates

Figure 4.2: Colony morphology of selected actinomycetes strains grown in SCA plates

V. CONCLUSION

Microbial communities comprise interminable and unique pool of chemical entities, thus making up a notable source for biotechnology and industries. A vast number of valuable antibiotics and bioactive metabolites have been derived from actinomycetes isolated from diverse niches. In this regard, scientific communities have switched over to new environments for bio-prospecting in search of novel biologically active compounds. Actinomycetes are the most economically and commercially important prokaryotes as they are the richest source of industrially important enzymes, antibiotics, enzyme inhibitors and other significant pharmaceutical products with high commercial values. What makes actinomycetes a preferable source of novel bioactive compounds over other sources is their versatility and diversity. Although there are plenty of reports on bioactive compounds from actinomycetes, we are still in early stages of the study. Future success in natural product discovery not only depends upon the extensive research on actinomycetes from diverse source but also relies on the new and improved technologies. Screening strategies, whole genome sequencing and scanning approaches along with polyphasic taxonomy provide a coherent way to study actinomycetes biology, taxonomy and ecology. Advances in metabolic engineering techniques will help in understanding the chemical biology of rare and uncultivable actinomycetes and exploration of novel pharmaceutical compounds with improved therapeutic applications. Therefore, it needs a combined effort among biologists, chemists, bioengineers, taxonomists and ecologists to exploit the biodiversity and bioactive potential of actinomycetes.

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