Isolation and Screening of Actinomycetes from Soil Sample of Dal Lake (Kashmir) Against Selected Pathogens

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***Abstract*-Actinomycetes are considered as one of the most diverse groups of filamentous bacteria capable of thriving into different types of ecological niches due to their bioactive potential. The main objective of the present study was Isolation and Screening of Actinomycetes from soil of Dal Lake of Kashmir region and characterized for morphological identification and evaluated for antibacterial activity against human pathogens. Total 50 Actinomycetes were isolated and characterized for morphological identification and evaluated for antibacterial activity. Out of these isolated, five actinomycetes showed antimicrobial activity against selected bacterial pathogens. Screening using agar diffusion assay was carried out and total five actinomycetes isolated were found active against MTCC cultures Including *Bacillus subtilis (*MTCC 441), *Pseudomonas aeruginosa (*MTCC 4673), *Staphylococcus aureus* (MTCC 3160), *Pseudomonas fluorescens (MTCC 103),Mycobacterium smegmatis (MTCC 994), E. coli (*MTCC 443) and *Streptococcus mutans* (MTCC 890). The study indicated that Dal Lake of Kashmir had diverse group of actinomycetes with broad spectrum antimicrobial activity.**

Key words: Dal Lake, actinomycetes, antibacterial activity, pathogens

I. INTRODUCTION

Actinomycetes are gram positive bacteria frequently filamentous and sporulating with DNA rich in G+C from 55-75 % [1]. Actinomycetes have provided many important bioactive compounds of high commercial value and are being routinely screened for new bioactive substances [2]. Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil [3]. They are one of the major groups of soil population [4]. The number and types of actinomycetes present in a particular soil would be greatly influenced by geographical location such as soil temperature, soil type, soil pH, organic matter content, cultivation, aeration and moisture content. The diversity of terrestrial Actinomycetes are of extraordinary significance in several areas of science and medicine, particularly in antibiotic production [5]. Actinomycetes are prolific producers of novel antimicrobial agents [6]. Vast numbers of these antimicrobial agents are discovered from Actinomycetes by screening natural habitat such as soils and water bodies [7, 8, and 9]. Actinomycetes have the ability to produce secondary metabolites with biological activities such as antibiotic, antifungal, antiviral, anticancer, enzyme, immunosuppressant and other industrially useful compounds [10, 11, 12, 13 and 14]. The 80% of the world's antibiotics are known to come from *Actinomycetes*, mostly from the genera *Streptomyces* and *Micromonospora* [15]. The genus, Streptomycetes, is responsible for the formation of more than 60 % of known antibiotics while a further 15 % are made by a number of related Actinomycetes, Micromonospora, Actinomadura, Streptoverticillium and Thermoactinomycetes [16]. 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 and rare Actinomycetes. The non‐Streptomycetes are called rare actinomycetes, comprising approximately 100 genera.

Over the last decade, it has become clear that antimicrobial drugs are losing their effectiveness due to the evolution of pathogen resistance. The emergence and spread of multidrug resistance pathogens has increased substantially over the 20 years [17]. Nowadays, the drug resistant strains of pathogen emerge more quickly than the rate of discovery of new drugs and antibiotics. This resistance increasingly limits the effectiveness of current antimicrobial drugs. The problem is not just antibiotic resistance but also multidrug resistance. In 2004, more than 70% of pathogenic bacteria were estimated to be resistant to at least one of the currently available antibiotics [18]. The so-called *superbugs* (organism that are resistant to most of the clinically used antibiotics) are emerging at rapid rate [19]. The WHO has predicted that between 2000 and 2020, nearly 1 billion peoples will become infected with *Mycobacterium tuberculosis* and this disease will cost the lives of 35 million peoples. To overcome this problem a new antibiotic needed which will show positive result.

Dal Lake is a lake in Srinagar, the summer capital of Jammu and Kashmir. The present study is aimed to isolate and identify Actinomycetes obtained from soil sample of Dal Lake of Kashmir and to assess their antimicrobial properties against pathogens.

II. MATERIALS AND METHODS

- **Collection and preparation of soil sample**

Soil samples were aseptically collected from Dal Lake of Kashmir. Soil sample (approx. 500 g) were collected using some clean, dry and sterile polythene bags along with sterile spatula, marking pen rubber band and other accessories. These samples were air-dried for 1 week, crushed and sieved. The Sieved soils were then used for Actinomycetes isolation.

- **Isolation of Actinomycetes**

From collected sample, 5g of the soil were suspended in 50ml of Normal saline (NaCl0·85g/L).The soil suspension were incubated in an orbital shaker incubator at 28 ^{0}C with shaking at 200 rpm for 3 min. Actinomycetes were isolated by spread plate techniques following the serial dilution of soil. The following mediums were used for the isolation of Actinomycetes Starch casein agar medium, Water-yeast extract-agar (WYE), Actinomycetes Isolation Agar media. Isolated plates were incubated at 28°C for 7-15 days for fast growing Actinomycetes. Plates were checked for the growth of typical Actinomycetes colonies up to 7 days.

- **Morphological Identification**

Identification of Actinomycetes to genus level was conducted by first using morphological and chemical criteria according to Bergey's Manual of Determinative Bacteriology [20]. The micro-morphology of Actinomycetes strain was carried out for gram straining, type, shape and size by under light microscope. Microscopic characterization was done by cover slip culture method. The mycelium structure, color and arrangement of conidiophores and Arthrospore on the mycelium were observed through the oil immersion (1000×) microscope. Colonies were identified on the basis of their colony morphology and color. Color of aerial mycelium was determined from mature, sporulating aerial mycelia of the Actinomycetes colonies on Actinomycetes agar media.

- **Screening of Antimicrobial Actinomycetes**

For the screening of antimicrobial Actinomycetes, we took the isolated Actinomycetes culture and inoculated them in the required broth. After inoculation in the broth, we gave them incubation in the orbital shaker and we kept checked the antimicrobial activity of inoculated cultures against MTCC pathogenic bacterial strains randomly using by using agar well diffusion assay [21].

- **Study of antibacterial activity**

In diffusion assay a substance with biological activity is allowed to diffuse through an agar gel previously seeded with a test organism. After incubation the micro colonies, which form wherever growth is possible, produce a haze in the agar. Therefore the test organism serves as an indicator to make visible some low concentration of a chemical that limits its growth. For antibiotic assay, a clear zone of inhibition appears near the point of application.

Levofloxacin (10 units per Disc) was taken as positive control for each test organism. The plates were incubated overnight at 37^{0}C and observed for the zone of inhibition around the wells. The Actinomycetes isolates which showed the zone of inhibition i.e. antibacterial activity against test organisms were confirmed for the results.

III. RESULTS AND DISCUSSION

Actinomycetes Isolation agar medium is best as compared to the Starch casein agar medium, Water-yeast extract-agar (WYE), in terms of yield. Colonies having characteristic features such as powdery appearance with convex, concave or flat surface and color ranging from white, gray to pinkish and yellowish were selected. Furthermore, bacterial configuration same as Actinomycetes were accepted from gram staining. Fifty selected isolates were examined microscopically and identified by their morphological and culture characteristics. The Fifty isolates found come under three genera such as Actinomycetes, Micromonospora and Streptomyces, out of which 34 belong to Actinomycetes, 15 from Streptomyces and 1 from Micromonospora.

Morphologically distinct Actinomycetes isolates were selected for anti-bacterial activity screening against the pathogenic test organisms. Out of Fifty isolates screened for antibacterial activity, only 4 showed positive results. B1, B4 showed the antibacterial activity against *E.coli*. 33 and 34 showed antibacterial activity against *Pseudomonas fluorescens*. Their zone of inhibition was measured and the results were noted. Further study will be needed to find out the active molecules from the actinomycetes.

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Actinomy	Zone of Inhibition (mm)		
	E. coli	*Bacillus subtil*	*Pseudomonas*
B1	6	Nil	8
B4	5	6	Nil
10	4	Nil	6
33	Nil	7	7
34	5	Nil	8

Table 4: **Zone of inhibition shown by actinomycetes isolates**

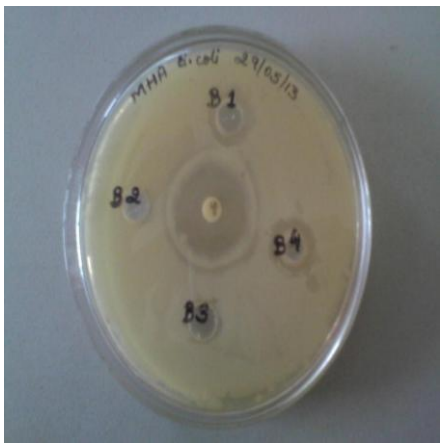

Fig1

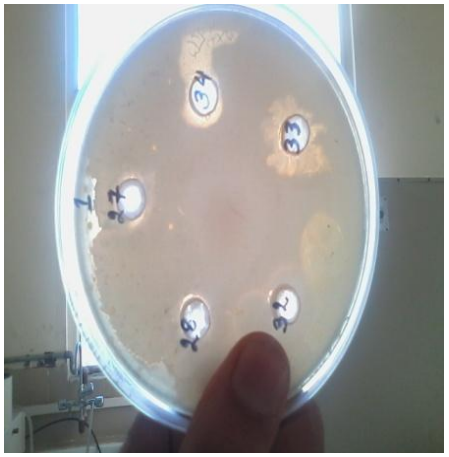

Fig 2

Fig 1: *Antibacterial activity shown by B1& B4 against E.coli*

Fig 2: *Antibacterial activity shown by 33 & 34 against Pseudomonas fluorescens.*

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