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ANTIBACTERIAL SCREENING OF ACTINOMYCETES FROM SOILS OF WULAR LAKE KASHMIR, INDIA

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ABSTRACT

A total of 30 Actinomycetes were isolated from soil sample of Wular Lake of Kashmir region and characterized for morphological identification and evaluated for antibacterial activity. Out of these actinomycetes isolated, four actinomycetes showed antimicrobial activity against selected bacterial pathogens. Furthermore during screening using agar diffusion assay a total four actinomycetes isolates 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 Wular Lake of Kashmir had diverse group of actinomycetes with broad spectrum antimicrobial activity.

KEYWORDS : *Wular Lake, actinomycetes, isolates, antibacterial activity, identification.*

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INTRODUCTION

Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil [1]. They have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new antimicrobials. These approaches have been remarkably successful and approximately two thirds of naturally occurring antibiotics have been isolated from actinomycetes [2]. Almost 80% of the world's antibiotics came from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora.* One of the first antibiotics used is streptomycin produced by *Streptomyces griseus* [3].

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 emerge more quickly because of this, many scientists and pharmaceutical industry have actively involved in isolation and screening of actinomycetes from different untouched habitats, for the production of novel antimicrobial agents [4]. Infections caused by bacteria have become resistant to commonly used antibiotics and

become a major global healthcare problem in the 21st century [5]. Physicians acquired methicillin-resistant *S. aureus* (MRSA), which also bears resistance too many antibiotics. During this time, Vancomycin the only therapeutic answers to MRSA infections, whereas resistant to Vancomycin had already been reported in clinical setting [6].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. The emergence of drug resistance in all important human pathogens emphasizes the need for the development of new antimicrobial agents with activity against such resistant bacterial pathogen [7]. Also Gram negative bacteria like *Pseudomonas aeruginosa*, which act as opportunistic pathogens in clinical cases where the defense system of the patient is compromised currently threaten patients in hospitals and communities with multi-drug resistance, [8, 9, 10, 11]. Also other intrinsically antibiotic resistant organisms such as *Stenotrophomonasmaltophilia*are emerging as opportunistic pathogens. Most of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi [12, 13, 14, 15].

Wular Lake, India's largest fresh water lake and one of the largest in Asia, is in Bandipora district of state Jammu and Kashmir in the Indian. The lake basin was formed as a result of tectonic activity and is fed by the Jhelum River. The lake's size varies seasonally from 12 to 100 square miles (30 to 260 square kilometers). 34°20′N 74°36′E / 34.333°N Maximum 14.m The present study is undertaken to isolate actinomycetes from the soil sample of Wular Lake Kashmir and to assess their anti-bacterial properties against selected group of human pathogens.

MATERIALS AND METHODS

Collection of soil sample

Soil samples were collected from Wular Lake of Kashmir. Soil sample (approx. 500 g) will be collected using some clean, dry and sterile polythene bags along with sterile spatula. These samples were air-dried for 1 week, crushed and sieved. The Sieved soils were then used for actinomycetes isolation.

Isolation of actinomycetes

For each 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. Serial dilutions were made at 10^{-4}, 10^{-6} and 10^{-8} and plated out on Starch casein agar medium, Water-yeast extract-agar (WYE), Actinomycetes Isolation Agar media. A small portion of typical isolated colonies were streaked on tryptone-soya agar media incubated at 25°C for 2-7 days. Plates were checked for the growth of typical actinomycetes colonies up to 7 days.

Morphological Identification

The micro-morphology of actinomycete 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 conidiophore 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 starch-casein agar media.

Collection of test microorganism

The pathogenic bacterial strains were obtained from the MTCC (Microbial type culture collection) Chandigarh and maintained by subculturing on Muller Hilton agar (Himedia Mumbai India) at 37°C. The test organisms with their MTCC numbers are described in the table 1

Study of antibacterial activity

Screening of isolated actinomycetes was performed by Agar Diffusion assay. 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. Penicillin was taken as positive control for each test organism. The plates were incubated overnight at 37℃ 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.

RESULTS

Isolation, Morphological and cultural characteristics of selected isolates

The three medium that were we used for isolation of given isolates, starch casein agar medium is best as compared to the other two in the means of yield. Isolation plates developed various types of bacterial actinomycetes and fungal colonies. 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. Thirty selected isolates were examined microscopically and identified by their morphological and culture characteristics. The thirty isolates found come under three genera such as Actinomycetes, Micromonospora and Streptomyces, out of which 18 belong to Actinomycetes, 11 from Streptomyces and 1 from Micromonospora.

Morphologically distinct actinomycetes isolates were selected for anti-bacterial activity screening against the pathogenic test organisms. In the initial process of screening against pathogenic test organisms, the results then showed very less antibacterial activity but after optimizing the fermentation conditions, the antibacterial activity of isolates will enhance. The cultures after fermentation conditions start showing activity against the resistant cultures (MRSA). Among thirty isolates, four isolates showed appropriate antibacterial activity against test organisms. The antibacterial activity of thirty isolates used against eight test organisms is described in table 2.

Out of thirty isolates screened for antibacterial activity, only four of them showed positive results. These are A6 which belongs to Streptomyces. A11 and A15 belongs to Actinomycetes and A12 belongs to Micromonospora. A6 showed the antibacterial activity against *Pseudomonas aeruginosa, Streptococcus mutans, and Pseudomonas fluorescens.* A11 showed the antibacterial activity against *Mycobacterium smegmatics,* and *Pseudomonas fluorescens.* A12 showed the antibacterial activity against *Mycobacterium smegmatics, Pseudomonas aeruginosa, Streptococcus mutants, Bacillus Subtilis*. A15 showed the antibacterial activity against *Pseudomonas aeruginosa, Pseudomonas fluorescens.* The results yielded from all the above tests showed that A6 and A12 may relate to the broad spectrum antibiotics because it showed the antibacterial activity against more than 4 test organisms. Their zone of inhibition was measured and the results were noted (Table 3). *The four isolated actinomycetes which showed antimicrobial activity against some test organisms were then again screened with respect to positive control penicillin for comparison.*

DISCUSSION

Actinomycetes are gram positive bacteria which belong to the order Actinomycetales [16]. Survey on actinomycetes have been Carried intensively in various different environments which possess different living conditions. The study survey has been extended to un- and underexplored environments, niche or extreme habitats in various parts of the world in the last few decades[17].Most actinomycetes dwell in soil and also in aquatic environment [18, 19]. Studies on freshwater sources have been done far less as compared to that of marine environment. Bioactive actinomycetes have been reported from Nile River in Egypt [20, 21] Krishna River in Andhra Pradesh, India [22].In the course of screening for novel antimicrobial antibiotics from soil samples,

antibiotic-producing actinomycetes cultures were recorded from soil samples taken from Wular Lake of Kashmir. Wular Lake is 40 kms from Srinagar and it happens to be the largest fresh water lake in India. As stated earlier, actinomycetes have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances. The survival of the microorganisms in such harsh and challenging habitation might be due to their adaptation to the environment and ability to produce resistant structures like spores. Isolates grow mostly on tryptone soya agar, water yeast extract and starch casein agar. Colonies were chalky granular and heaped rudimentary to extensively branched vegetative hyphae, growing on agar surface. The hyphae fragment into bacteriods, rod shaped to coccoid elements which stained Gram positive.

A total of thirty actinomycetes isolates were screened for their antibacterial against eight species of pathogenic bacteria (Table 1)Among the thirty isolates tested four showed antibacterial activities against more than one genus of test pathogen (Table 2). *Isolate A11 & A12 showed active antimicrobial activity against Mycobacterium smegmatis (Figure 1a). A6, A12 &A15 showed active antimicrobial activity against Pseudomonas aeruginosa(Figure 1b).A6 &A12 showed active antimicrobial results against Streptococcus mutans(Figure 1c).A12 was active against Bacillus subtilus(Figure 1d).A6, A12 & A15 showed active antimicrobial activity against Pseudomonas fluorescens(Figure 1e).*

The above results indicate that Wular Lake of Kashmir region is rich with Actinomycetes. Further intensive studies on the actinobacterial diversity of unique biotopes in this region of Kashmir should form an important input into Indian biotech industry. A wide array of bacteria is becoming resistant to drugs that are used to treat infections. Broad spectrum antibiotics are designed to work against a broad spectrum of bacteria [23]. Broad spectrum antibiotics act against both gram positive and gram negative bacteria. A broad spectrum antibiotic is only useful as long as it kills most bacteria and organisms which can quickly adapt to resist antibiotics present a significant challenge. The resistance, which is called Antimicrobial resistance (AMR), is a major obstacle to the treatment of infectious diseases worldwide. Faced with the extent of AMR, and the dwindling number of effective antimicrobial drugs, the World Health Organization (WHO) has stated that it considers AMR to be one of the greatest threats to human health [24]. There is a huge variation in the time for emergence of resistance, which varies among organisms and antibiotics. For example, penicillin resistance in *Staphylococcus* species emerged rapidly, whereas penicillin resistance among *Streptococcus pneumoniae* took several decades. Eventually, resistance rises to such a high level that it reduces the efficacy of the drug in a human population. At this point, a new antibiotic is required, which is active against resistant bacteria. Today with the increase in opportunistic infection in human the need for effective, safe and novel antibacterial is a big challenge to the pharmaceutical industries [25]. The bioactive potential of fresh water actinobacterial in terms of production of antibacterial metabolites is noteworthy [26, 27, 28]

CONCLUSION

This endeavor was undertaken with an objective of identifying Cultural new actinomycetes having novel antibacterial activity against the resistant pathogenic bacteria from virgin soils of Wular Lake Kashmir. In conclusion, this study has shown that freshwater environments could serve as potential reservoirs for actinomycetes of antimicrobial importance with varying spectra of activities.The widest activity spectrum and the largest inhibition zones were shown by strains A6, A11 and A12 and A15.Thus there is definite scope for bio prospecting of antagonistic actinomycetes from Wular Lake

ecosystem once appropriate further studies are undertaken.

Thus the aim of the project was to obtain new antimicrobials from a previously inaccessible source - uncultured microorganisms that make up 99% of the total microbial diversity. Bacteria that cause infections in the community and in hospitals are becoming increasingly resistant to currently available antibiotics; therefore there is an urgent need for new antibiotics that will ensure public health in the years to come. The purpose of this study is to employ a new technology to discover the next generation of effective antibiotics. These antibiotics will also provide the public with a stronger defense against the threat of bioterrorism

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Figures & Tables

Figure 1: *Actinomycetes isolates showing zone of inhibition against selected pathogens*

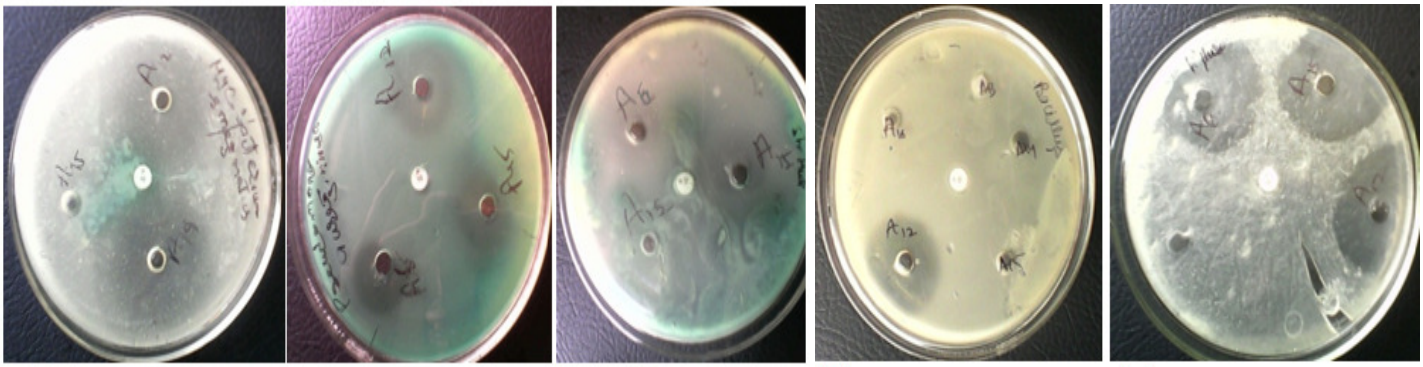

Table 1.Test organisms taken for antibacterial screening

S. No	Test organism
1.	*Bacillus subtilus (MTCC 441)*
2.	*Pseudomonas aeruginosa (MTCC 4673)*
3.	*Staphylococcus aureus* (MTCC 3160)
4.	*Pseudomonas fluorescens (MTCC 103)*
5.	*Mycobacterium smegmatis (MTCC 994)*
6.	*E. coli (*MTCC 443)
7	*Streptococcus mutans* (MTCC 890)

Table 2: Antibacterial activity of isolates against eight test organisms

Actinomycetes Isolates	Test Microbes							
	Mycobacterium Smegmatis	*Pseudomonas aeruginosa*	*E. coli*	*Staphylococcus aureus*	*Streptococcus mutans*	*Bacillus subtilus*	*Pseudomonas fluorescens*	*MRSA*
A1	-	-	-	-	-	-	-	-
A2	-	-	-	-	-	-	-	-
A3	-	-	-	-	-	-	-	-
A4	-	-	-	-	-	-	-	-
A5	-	-	-	-	-	-	-	-
A6	-	+	-	-	+	-	+	+
A7	-	-	-	-	-	-	-	-
A8	-	-	-	-	-	-	-	-
A9	-	-	-	-	-	-	-	-
A10	-	-	-	-	-	-	-	-
A11	+	-	-	-	-	-	+	+
A12	+	+	-	-	+	+	-	+
A13	-	-	-	-	-	-	-	-
A14	-	-	-	-	-	-	-	-
A15	-	+	-	-	-	-	+	-
A16	-	-	-	-	-	-	-	-
A17	-	-	-	-	-	-	-	-
A18	-	-	-	-	-	-	-	-
A19	-	-	-	-	-	-	-	-
A20	-	-	-	-	-	-	-	-
A21	-	-	-	-	-	-	-	-
A22	-	-	-	-	-	-	-	-
A23	-	-	-	-	-	-	-	-
A24	-	-	-	-	-	-	-	-
A25	-	-	-	-	-	-	-	-
A26	-	-	-	-	-	-	-	-
A27	-	-	-	-	-	-	-	-
A28	-	-	-	-	-	-	-	-
A29	-	-	-	-	-	-	-	-
A30	-	-	-	-	-	-	-	-

Table.3: **Zone of inhibition shown by actinomycetes isolates**

Actinomycete Isolates	Zone Of Inhibition (mm)							
	Mycobacterium smegmatics	***Pseudomonas aeruginosa***	***E. coli***	***Staphylococcus aureus***	***Streptococcus mutans***	***Bacillus subtilus***	***Pseudomonas fluorescens***	***MRSA***
A6	Nil	25	Nil	Nil	28	Nil	24	24
A11	15	Nil	Nil	Nil	Nil	Nil	16	12
A12	14	24	Nil	Nil	23	18	Nil	19
A15	Nil	26	Nil	Nil	Nil	Nil	24	21

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