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Isolation and Characterization of Actinomycetes from Soil and Screening their Antibacterial Activities against different Microbial Isolates

[1]JyotiTyagi, [2]Dr. Tripti Bhatnagar, [3]Dr. Fanish Kumar Pandey

School of Sciences, Noida International University, Gautam Buddh Nagar, Greater Noida (U.P), India

Abstract: **In this present study, the soil samples were collected from Noida field. A total 11 *Actinomycetes* strains were isolated from the Noida. These Actinomycetes were screened with regard to potential against Gram-positive and Gram –negative bacteria. Soil sample was screened for their antibacterial activity. They were evaluated for their inhibitory activities on 4 tests Organism. The culture characteristics of isolates were also studies in different culture media. The results indicated that 11 *Actinomycetes* isolates were highly active against *Streptococcus aureous, E.Coli, Staphylococcus aureus* and *Bacillus* strains. 11 *Actinomycetes* isolates were highly active with an inhibition zone more than 16 mm in diameter. All the antibiotic producing actinomycetes were isolated at 28°C from soil. 11 *Actinomycetes* isolates showed activity against bacteria in which most of them from alkaline soil. Where the less interference by human for agriculture or other purpose. These micro-organisms may have capability to produce some of the most important medicines ever developed.**

Keywords: **Antibacterial activity, Antibiotics drugs resistance, Soil sample.**

I. INTRODUCTION

Actinomycetes are the most widely distributed groups of micro-organisms in Soil. They have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. Almost 80% of the world's antibiotics are known to come from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora* (Panday B., Ghimire P. and Agrawal V.P. *et al.*, 2004).

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 (Oskey M., *et al*., 2004). *Streptococcus aureous, E.Coli*, *Staphylococcus aureus* and *Bacillus* strains are virulent pathogen that is responsible for a wide range of infections and has developed resistance of most classes of antibiotics. Hence there is need to rediscover new drugs active against these drugs resistance pathogens. Most of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi. The present study was under taken to isolate *Actinomycetes* from the soil samples of Noida and to assess their antibacterial properties. The resistance problem demands that to discover new antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. So we need to screen more and more actinomycetes from different habitat antibacterial activity in hope of getting some actinomycetes strains that produce antibiotics that have not been discovered yet and active against drugs resistant pathogens. These organisms are responsible for the characteristic musty or earthy odor of a freshly ploughed field being attributable to volatile substance which they produce.

Actinomycetes are capable of degrading many complex organic substances and consequently play an important role in building Soil fertility. They are also noted for their ability to synthesize and excrete antibiotics. The isolation and characterization of actinomycetes were performed in different biochemical methods (Dhanasekaran *et al.*, 2009). The mycelium structure color and arrangement of conidiospores and arthrospore on the mycelium were originally considered an intermediate group between bacteria and fungi. Most of actinomycetes grow slowly as branching filaments many

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actinomycetes will grow on the common bacteriological media used in the laboratory such as Nutrient agar, Isolation media. Antibiotics are the best known product of actinomycetes.

II. MATERIALS AND METHODS

1. Soil Sample Collection

Soil sample were collected from different places of Noida. In this investigation 21 Soil samples were taken from different localities of Noida. After taken soil sample it directly transferred in to polyethylene bags to minimize moisture losses during transportation. Each collection was made from 10-15cm depth of the soil (Sasdoun I. and Gharaibeh R. 2003). These were air dried for one week and crushed and sieved. The sieved Soil was used for actinomycetes isolation.

2. Pretreatment

All soil Sample had been mixed with calcium carbonate & pretreated for 2-5days at 37°C. 1gm soil mixed with 0.1g Calcium carbonate & incubates at 37°C for 2-5 days. This pretreatment enhances the population of *Streptomycetes spp*. in soil samples.

3. Isolation of Actinomycetes

Isolation of actinomycetes was performed by serial dilution and spread plate technique using isolation media and nutrient agar medium. One gram of soil sample was taken in 9ml of distilled water and mixed properly. Serial dilution was made up 10^{-5} ml of the dilution sample was inoculates in the isolation medium plates from each dilution. The media are added to the tetracycline and ampicillin to inhibit microbial contamination respectively. Plates were incubated at both at 28°C and 37°C and monitored after 2-7 days. Streaking on isolation media plates led to purify bacterial colonies that showed actinomycetes like appearance. The isolated strains are presented at 4°C during 2 methods and maintained for longer period by serial subculture.

4. Test Organisms

In vitro antibacterial activities were performed against the *Staphylococcus aureus, Bacillus sp, Streptococcus, E. coli.* etc.

III. CHARACTERIZATION AND IDENTIFICATION OF ACTINOMYCETES

1. Microscopic Observation

Morphological examination of the actinomycetes was done by using cellophane tape and cover slip-buried methods (Williams and Cross 1971).Gram staining, Lactophenol blue staining was performed to check the morphology of the cells and spore chain morphology was identified by cover slip culture technique.

2. Gram Staining

A smear of culture was taken in a clean glass slide and heated gently over a flame. The smear was covered with a thin film of crystal violet for 1 minute and washed gently in slow running tap water. Gram's iodine solution was flooded over the smear for 2 minute and washed with tap water. Alcohol was used to decolorize the smear until the violet color ceased to flow away**.** Then the slide was washed with water and counter stain safranine was flooded over the smear for 2 minute then the slide was washed, drained, air, dried and viewed under microscope. The culture retaining the violet color indicated that it was Gram positive organisms.

3. Morphological Identification

Isolates of Actinomycetes were observe under a high power magnifying lens and colony morphology was noted with respect to color, aerial mycelium, size and nature of colony, slide color and felling the consistency with a sterile loop.

4. Biochemical Characterization

Actinomycetes isolates were biochemically characterized by Catalase test, nitrate reduction test, IMVIC test, Starch hydrolysis test, Fermentation of citrate test, Triple sugar iron test, Citrate utilization test, Skim milk agar hydrolysis, Hydrogen sulphide test.

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IV. SCREENING OF ISOLATES FOR ANTIBACTERIAL ACTIVITY

Actinomycetes isolates were selected for antibacterial activity screening against the pathogenic test organism by agar well diffusion method on agar medium. The Actinomycetes isolates often encounted show antibacterial activity on potato dextrose agar media. Most of the active isolates were active against Gram positive and Gram negative pathogen. Antibacterial activity was tested in agar well diffusion technique against *Staphylococcus aureus, Streptococcus, Bacillus sp. and E. coli* etc.

V. RESULTS AND DISCUSSION

Soil samples were collected from waste land soil from Noida. One gram of soil sample was dried and taken for isolation of Actinomycetes. The 11 Actinomycetes were isolated from 21 Soil Samples at two different temperatures 28°C or 37°C. (Table: 1) All the cultures were screened against bacteria but only the 11 Isolates showed the antibacterial activity and were designated as A1,A2,A3,A4,A5,A6,A7,A8,A9,A10,A11 (Table:2). They were also studied for culture characteristics.

This study was undertaken with an aim of isolation and screening of Actinomycetes in soil from Noida region and selective media and cultivation conditions described previously a total of 11 different Actinomycetes isolates were recovered from 21 soil samples that were collected from Noida. The soil sample from Sec.-55 and Sec. - 62 Noida gives the higher number of Actinomycetes isolates (Table: 1).

All isolates grow on isolation agar media showing morphology typical of Actinomycetes. Since the colonies were slow growing, aerobic, folded and with aerial and substrate mycelia of different colors. All actinomycetes isolates were Gram's stain positive. The cultural characteristics (pigment production), morphological characteristics of the different actinomycetes isolates are presented in (table-2).

Out of 11 Actinomycetes subjected for primary screening and subjected for purification methods by streak plate method. The Identification of the potent antibiotic producing strains reveals that all the strains belong to the genus *Streptomyces.* The isolated microorganism were Gram positive, having branching and were filamentous. Different isolates showed varying results in the Biochemical test as shown in (Table: 3).

Out of 21 isolates the 11 isolates was showed positive antibacterial results. These isolates were selected for their broad spectrum of activity and zone of inhibition in mm.

Table 1- Total number of Actinomycetes Isolates with Antibacterial activity isolated at different temperature.

Origin	Isolation temperature	Total strains isolated	No. of active Isolates against bacteria
Waste land near Sec.-82	28°C, 37°C	1	1
Wasteland near Sec.-55	28°C, 37°C	9	4
Garden soil Sec.- 62	28°C, 37°C	10	5
Garden soil Sec,-110	28°C, 37°C	1	1
Total		21	11

Table 2.1- Culture Characteristic of Selective isolates on Isolation agar medium.

Origin	Culture Code	Color	Mycelium type	Pigment production	Gram's reaction
Waste land near Sec.-82	A1	Green	Aerial	Black	+
Wasteland near Sec.-55	A2	White	Aerial	Orange	+
	A3	Dark green	Aerial	Black	+
	A4	White	Substrate	Yellow	+
	A5	White	Aerial	Orange	+

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Garden soil Sec.- 62	A6	White	Aerial	Orange	+
	A7	Green	Aerial	Yellow	+
	A8	White	Aerial	Orange	+
	A9	White	Aerial	Yellow	+
	A10	White	Aerial	Orange	+
Garden soil Sec,-110	A11	White	Aerial	Orange	+

Table 2.2- Biochemical characterization of *Actinomycetes* isolates

Biochemical tests	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**
Nitrate reduction	-	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-
MR	-	-	-	-	-	-	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-	-	-
Citrate	+	-	+	+	-	-	+	-	-	-	-
TSI (Slant)	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	-	+	-	+	+	-	+	+	+	+
Skim milk agar hydrolysis	-	-	-	-	-	-	-	-	-	-	-
Hydrogen sulfide production	-	-	-	-	-	-	+	-	-	-	-
Catalase test	+	+	+	+	+	+	+	+	+	+	+

Table 3- Antibacterial activity of isolates with zone of inhibition in mm (Agar well diffusion methods).

Culture Code	***E. Coli***	***Bacillus***	***Step. aureus***	***Streptococcus sp.***
Conc. Of antibiotic	**25%**	**25%**	**25%**	**25%**
A1	15	10	14	13
A2	14	12	12	18
A3	14	11	11	20
A4	12	20	16	18
A5	12	12	16	24
A6	13	13	15	20
A7	12	15	12	28
A8	13	10	11	12
A9	13	8	11	28
A10	12	10	20	13
A11	12	21	10	22

VI. CONCLUSION

11 Isolates showed activity against bacteria in which most of them from Noida. These micro-organisms produce some of the most important medicines ever developed. They are the source of life saving treatments for bacterial and fungal infections. The number of terrestrial antibiotics seems currently to approach a saturation curve with an apparent limit in the near future. The increasing number of leading antibiotics in pharmacology for new treatment of drug resistant infectious pathogens has enforced the search for metabolites in so for untouched habitats where the human activities are very less. Our studies will establish the rich actinomycetes diversity of the region, especially the various niche habitats of Noida and also help conserve and utilize them in bioindustry.

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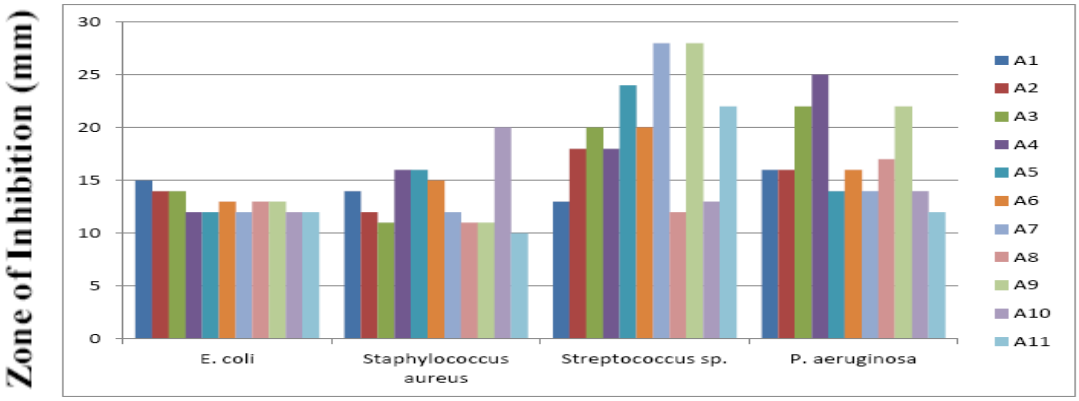

Figure- Antibacterial activity for Isolated Strains

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