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"Isolation and Characterization of Actinomycetes from Soil and Screening Their Antifungal Activities"

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Abstract: **Actinomycetes are one of the most attractive sources of antibiotics. In the present studies, total of 11 Actinomycetes strains were isolated from the Noida, U.P. India. Isolated strains were identified for their antifungal activity these isolates showed good result, they were evaluated for their inhibitory activity on 4 strains of pathogenic microorganism such as (Asp. niger, Alternaria alternata, Alternaria solani & Alternaria pori). Isolation of Actinomycetes strain was obtained by Serial dilution method and grown on actinomycetes isolation agar. Antifungal compounds were checked against fungal culture. The culture characteristics of isolates were also studies in different culture media. The results indicated that 11 Actinomycetes isolates were highly active against Alternaria pori and Aspergillus niger, Alternaria alternate, Alternaria solani strains. Out of 11 Actinomycetes isolates 5 isolates showed a inhibition higher than 50% for the above mentioned plant pathogenic fungi. Thus, these isolates could be developed into formulations for non toxic, non chemical fungicide for use on crops.**

Keywords: **Antifungal activity, Actinomycetes, Alternaria sp., inhibition percentage.**

I. INTRODUCTION

Fungi are eukaryotes and thus have protein and nucleic acid synthesis machinery similar to that of higher animals. It is, therefore, difficult to find compounds that selectively inhibit only fungal metabolism and not the growth and metabolism, of humans or plants. There is lack of effective and safe antifungal drugs and fungicides. Therefore, there is a pressing need of non-toxic and effective antifungal compounds. The pioneering work of Waksman showed that actinomycetes are capable of producing medically useful antibiotics. *Actinomycetes* are the most widely distributed group of micro organisms in nature which primarily inhabit the soil (Oskey *et al*, 2004). They have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. Approaches to search for and discover new antibiotics are generally based on screening of naturally occurring actinomycetes. The main objective of the present study was to isolate, screen and characterize naturally occurring antifungal actinomycetes.

Plant diseases caused by fungi include rusts, smuts, rots, and may cause severe damage to crops. For crops excessive use of chemical fungicides in agriculture has led to deteriorating human health, environmental pollution, and development of pathogen resistance to fungicide. Because of these problems in fungal disease control, a serious search is needed to identify alternative methods for plant protection, which are less dependent on chemicals and are more environmentally friendly.

Aspergillus niger, *Alternaria pori* , *Alternaria solani* and *Alternaria alternata* are virulent pathogen that is responsible for a wide range of infections and has developed resistance of most classes of fugicides. Hence there is need to rediscover new formulations active against these drugs resistance pathogens. Soil a*ctinomycetes* particularly *Streptomyces sp* enhances soil fertility and have antagonistic activity against wide range of soil-borne plant pathogens (Aghighi et al.,

2004). *Actinomycetes* constitute a diverse group of microorganisms that are widely distributed in terrestrial, freshwater and marine habitats (Radhika *et al.*, 2011).

The present study was undertaken to isolate antifungal compound producing *Actinomycetes sp*. The production and its antifungal activity was to be tested for identification of one or more Antifungal compound produced by the various strains.

II. MATERIALS AND METHODS

1. Soil Sample Collection

Soil Samples were collected from different places of NCR. In this investigation 21 samples were taken from different localities of NCR. 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 soils were used for actinomycetes isolation (Boroujeni *et al.*, 2012)

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 (Liu et al (2011). The media are added to the tetracycline and ampicillin to inhibit bacterial 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 fungal 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.

Test Organisms- In vitro antifungal activities were performed against the common plant pathogenic fungi : *Aspergillus niger, Alternaria alternata, Alternaria pori, Alternaria solani* .

4. Characterization and Identification of Actinomycetes

I. Microscopic Observation

Morphological examination of the Actinomycetes was done by using cellophane tape and cover slip-buried methods, Gram staining, Lactophenol blue staining was performed to check the morphology of the cells and spore chain morphology was identified. Colour of aerial mycelia was determined from the mature, sporulating aerial mycelia of the actinomycete colonies on SCN agar (Hamedani *et al.*, 2012). Colour of the substrate mycelia (reverse of the plate) was also observed along with diffusible pigments, if any (Padmadhas and Ragunathan 2010). 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.

II. 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, and Glucose, Sucrose and Lactose fermentation test.

5. Screening of Isolates for Antifungal activity

Actinomycetes isolates were selected for antifungal activity screening against the pathogenic test organism by inoculation methods and well diffusion method on potato dextrose medium. The Actinomycetes isolates often encounted show

antifungal activity on potato dextrose agar medium. Most of the active isolates were active against different pathogen. Antifungal activity was tested in agar well diffusion technique against *Aspergillus niger*, *Alternaria alternata*, *Alternaria pori* and *Alternaria solani* etc.

III. RESULTS AND DISCUSSION

The 11 Actinomycetes were isolated at two different temperatures 28°C or 37°C. (Table: 1) All the 11 Isolates were screened against fungi. All the 11 Isolates showed the antifungal 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 11 soil samples that were collected from soil of Noida region. The soil sample from Sec.-55 and Sec. - 72 Noida gives the higher number of actinomycetes isolates. (Table: 1).

All Actinomycetes isolates were Gram's positive. The cultural characteristics (pigment production), morphological characteristics of the different actinomycetes isolates are presented in (Table-2). Different isolates showed varying results in the Biochemical test as shown in (Table: 3). 11 isolates was showed positive antifungal 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 Antifungal activity isolated at different temperature.

Origin	Isolation temperature	Total strains isolated	No. of active Isolates against fungi
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: 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	+
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	+

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Table 3: 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 4: Antifungal activity of isolates (% inhibition of fungal growth)

	In vitro Inhibition of Fungal pathogens in percentage *(%)*			
Fungal pathogen	***Alternaria pori***	***Alternaria solani***	***Alternaria alternata***	***Aspergillus niger***
***Actinomycetes* Culture Code**				
A1	30	59.2	52.2	62
A2	47.5	48.1	47.7	58.6
A3	37.5	59.2	54.5	58.6
A4	35	74.0	70.4	31
A5	30	59.2	52.2	55.1
A6	30	42.5	45.4	31
A7	57.5	53.7	34	51.7
A8	57.5	44.4	22.7	55.1
A9	30	37	45.4	41.3
A10	30	50	47.7	38
A11	42.5	50	40.9	31

IV. CONCLUSION

11 isolates showed activity against fungi in which most of them from Noida soil. 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. In the present study , the A4, A3, A5, A6 and A7 isolates of *Actinomycetes* which have been isolated show more than 50% inhibition for all the four plant pathogenic fungi used as test organism. This demonstrates that these isolates could be used as the much need antifungal formulations for crops in the fields. The spraying of these isolates would decrease the use of toxic chemical fungicide. Our study has established the rich actinomycetes diversity of the region, especially the various niche habitats of NCR and these studies also helps conserve and utilize them in bio industry.

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