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Isolation of *Actinomycetes* from Different Soil Sample (NCR Region) & Screening their Antibacterial Activity Against *E.Coli* and *Bacillus*

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Abstract: ***In the extant study, the soil samples were gathered up from NCR region. A total 9 Actinomycetes strains were isolated from the NCR. These Actinomycetes had been cloistered with respect to capability against Gram-positive and Gram-negative bacteria. Soil sample was isolated for their antibacterial activity. They were assess for their constraint activities on 2 test organism. The culture characteristics of screened bacteria were also studies in non-identical culture media. The results specify that 9 Actinomycetes isolates were highly active against E. Coli, and Bacillus strains. 9 Actinomycetes isolates were extremely vigorous with an inhibition zone more than 16 mm in diameter. All the antibiotics producing actinomycetes were isolated at 28 °C from soil sample. 9 Actinomycetes isolates showed activity against bacteria in which chiefly from alkaline soil. Where the less intervention by human for agriculture or other purpose. These Micro-organisms may have capability to produce powerful & essential medicines factories. Most of bacteria have elaborated resistance to almost all antibiotics. An infamous instance of multi-resistant bacteria is the 'hospital superbug', MRSA. Seeking of new antibiotics has become an aqueduct against time.***
Keywords: Antibacterial activity, Gram positive bacteria, Gram negative bacteria, Antibiotics drugs resistance, Non-identical.

I. INTRODUCTION

Actinomycetes are the most universally scatter groups of micro-organisms in soil. They have delievered many crucial bioactive enclosures of high commercial value and continue to be screened for new bioactive enclosures. Almost 80% of the World's antibiotics are known to exist from *actinomycetes*, mostly from the genera Streptomyces, Microbispora and Micromonospora (Pandey B., Ghimire P. nad Agrawal V.P. et al., 2004).

According to World Health Organization (WHO) over authorization and the inappropriate use of antibiotics has driven to the generation of antibiotics resistance in many bacterial pathogen (Oskey M., et al, 2004*). Streptococcus aureous, E.Coli, Staphylococcus aureus* and *Bacillus* strains are virulent pathogen that is liable for a massive range of infections that has developed resistance of most classes of antibiotics. Hence there is willing to retrieve new drugs active against these drugs resistance pathogens. Various antibiotics that have been use today are second hand of natural by-product of *actinomycetes* and fungi. The recent study was tackled to isolates *Actinomycetes* from the soil samples of NCR and to evaluate their antibacterial properties. The resistance problem command that to discover new antibacterial agents effective against pathogenic bacteria resistant to ongoing antibiotics. Hence, we need to isolate more and more *actinomycetes* from different terrain antibacterial activity in aim of getting some *actinomycetes* strains that give rise to antibiotics that have not been discovered yet and vital against drugs resistant pathogens. These organisms are liable for the characteristics musty or earthy odour of a freshly plugged field being attributable to temperamental substance that to be produced.

Actinomycetes are capable of demean many complex organic substances and accordingly play a crucial role constructed soil fertility. They have the potential to fused and excrete antibiotics. The isolation and characterization of *actinomycetes* were performed in different biochemical methods (Dhanasekaran et al.,2009). The mycelium morphology color and arrangement of conidiospores and arthrospore on the mycelium were inter-mediate group between bacteria and fungi. Various *actinomycetes* increases steadily as branching filament. many actinomycetes were grow on the common bacteriological media operate in the laboratories such as Nutrient agar, Isolation media. Antibiotics are the best-known product of actinomycetes till now.

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II. MATERIAL & METHODS

A. Soil Sample Collection

Soil sample were collected from different places of NCR Region. In this investigation 14 Soil samples were taken from different areas of NCR (National Capital Region). After that soil sample were gathered up in to polyethylene bags to reduce moisture losses during transportation. Soil collection was made from 10-15 cm shallowness of the soil (Sasdoun I. and Gharaibeh R. 2003). Did air dried for one week and crushed and sieved. The sieved Soil was used for *actinomycetes* screening.

B. Pretreatment

All Soil Sample had been mixed with calcium carbonate & after that pretreated for 2-5 days at 37°C. 1gm soil mixed with 0.1 gm Calcium carbonate & then incubates at 37°C for 2-5 days. This pretreatment process increases the population of *Streptomycetes spp.* or *Bacillus* in soil samples.

C. Isolation of Actinomycetes

Isolation of *actinomycetes* was accomplished by two ways that is serial dilution and spread plate technique by using isolation media and nutrient agar medium. One gram of soil sample was taken in 9ml of distilled water and mixed carefully. Serial dilution was made up of 10^{-5} ml of the dilution sample was inoculated in the isolation medium plates from individual dilution. The media are figure up to the tetracycline and ampicillin to overcome microbial contamination separately. Plates were incubated at both at 28°C and 37° C and noticing after 2-7 days. Streaking on isolation media plates brought to purify bacterial colonies that showed *actinomycetes* like presentation. The isolated strains are showed at 4°C during 2 process and maintained for longer interval by serial subculture.

Test Organisms- Antibacterial activities were performed against the *E.coli* & *Bacillus.*

D. Characterization and Identification of Actinomycetes

1) *Microscopic Observation:* Morphological examination of the *actinomycetes* was done by using Gram staining Lactophenol blue staining technique to check the morphology of the cells and spore chain morphology was recognize by cover slip culture technique. *Actinomycetes* form colonies that showed leathery and some produce pigments and have earthy smell.
2) *Gram Staining:* Take a glass slide, with the smear of culture in between the slide and heated gently over the burner. The smear was covered with a thin film of crystal violet for 1 min and washed gently in slow running tap water. Gram's iodine solution was immersed over the smear for 2 min and washed with tap water. Alcohol was used to decolorize the smear until the violet color quit to flow away. Then the slide was washed with water and counter stain safranine was immersed over the smear for 2 min, then the slide washed, drained, air, dried and observe under microscope. The culture confined the violet color indicates that it was Gram positive organisms.
3) *Morphological Identification:* Morphology of *Actinomycetes* were observe under a high-power magnifying lens and colony morphology was noticing with respect to color, aerial mycelium, size and nature of colony, slide color and felling the consistency with a sterile loop. Morphological characteristics of the strains were determined using the methods given by the International Streptomyces project (ISP) on ISP2-ISP7 media (Shirling et al., 1996)
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.
5) *Screening of Isolates for Antibacterial Activity: Actinomycetes* isolates were favoured for antibacterial activity screening against the pathogenic test organism by agar well diffusion methods on agar medium. The isolates often experience antibacterial activity on potato dextrose agar media. Various isolates were active against Gram positive and Gram-negative pathogen. Antibacterial activity was seen under agar well diffusion technique against *Bacillus* and *E. coli.*

III. RESULT AND DISCUSSION

Soil sample were collected from waste land soil from NCR Region. One gram of soil sample was dried for isolation of *Actinomycetes*. The 9 *Actinomycetes* were isolated from 14 soil sample at two non-identical temperature 28°C as well as 37°C. (Table:1) these cultures were isolated against bacteria but only the 9 isolates showed the antibacterial activity and were assigned as A1, A2, A3, A4, A5, A6, A7, A8, A9, (Table:2). Hence were also studied for culture aspects.

This study was retracted with a hope of isolation and screening of *Actinomycetes* in soil from NCR region and selective media and cultivation conditions clarified once.

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A total of 9 different *Actinomycetes* isolates were retrieve from 14 soil sample that were collected from NCR Region. The soil sample from Hapur road and Anand vihar gives the huge number of *Actinomycete*s isolates (Table:1).

All isolates cultivate on isolation agar media appear morphology typical of *Actinomycetes.* Since the colonies were slow growing, aerobic, folded and with aerial and substrate mycelia of different colors. *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 9 *Actinomycetes* put through for primary screening and laid open for purification methods by streak plate method. The potent antibiotics producing strains expose that all the strains belong to the genus Streptomyces. The isolated microorganism was Gram positive, having branching and were filamentous. Different isolates showed different results in the biochemical test shown in (Table:3).

Here, Out of 14 isolates the 9 isolates were expelled positive antibacterial results. These isolates were selected for their broad strech of activity and zone of inhibition in mm.

Table I- Total number of *Actinomycete*s Isolated with Antibacterial activity Isolated at different temperature.

Origin	Isolation temperature	Total strains isolated	No. of active Isolates against bacteria
Waste land near muradnagar	28°C, 37°C	1	1
Wasteland near Anand vihar	28°C, 37°C	7	4
Garden soil Hapur road	28°C, 37°C	5	3
Garden soil meerut road	28°C, 37°C	1	1
Total		14	9

Table II- Culture Characteristics of Selective isolates on Isolation agar medium.

Origin	Culture code	Color	Mycelium type	Pigment production	Gram's reaction
Waste land near Muradnagar	A1	Green	Aerial	Black	+
Wasteland near Anand vihar	A2 A3 A4 A5	White Dark Green White White	Aerial Aerial Substrate Aerial	Orange Black Yellow Orange	+ + + +
Garden soil Hapur road	A6 A7 A8	Green White White	Aerial Aerial Aerial	Yellow Orange Yellow	+ + +
Garden soil meerut road	A9	White	Aerial	Orange	+

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Table III- Biochemical characterization of *Actinomycetes* isolates

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

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

Culture code	*E.Coli*	*Bacillus*
Conc. Of antibiotic	25%	25%
A1	14	8
A2	13	10
A3	12	10
A4	11	18
A5	11	11
A6	12	10
A7	11	8
A8	12	9
A9	11	7

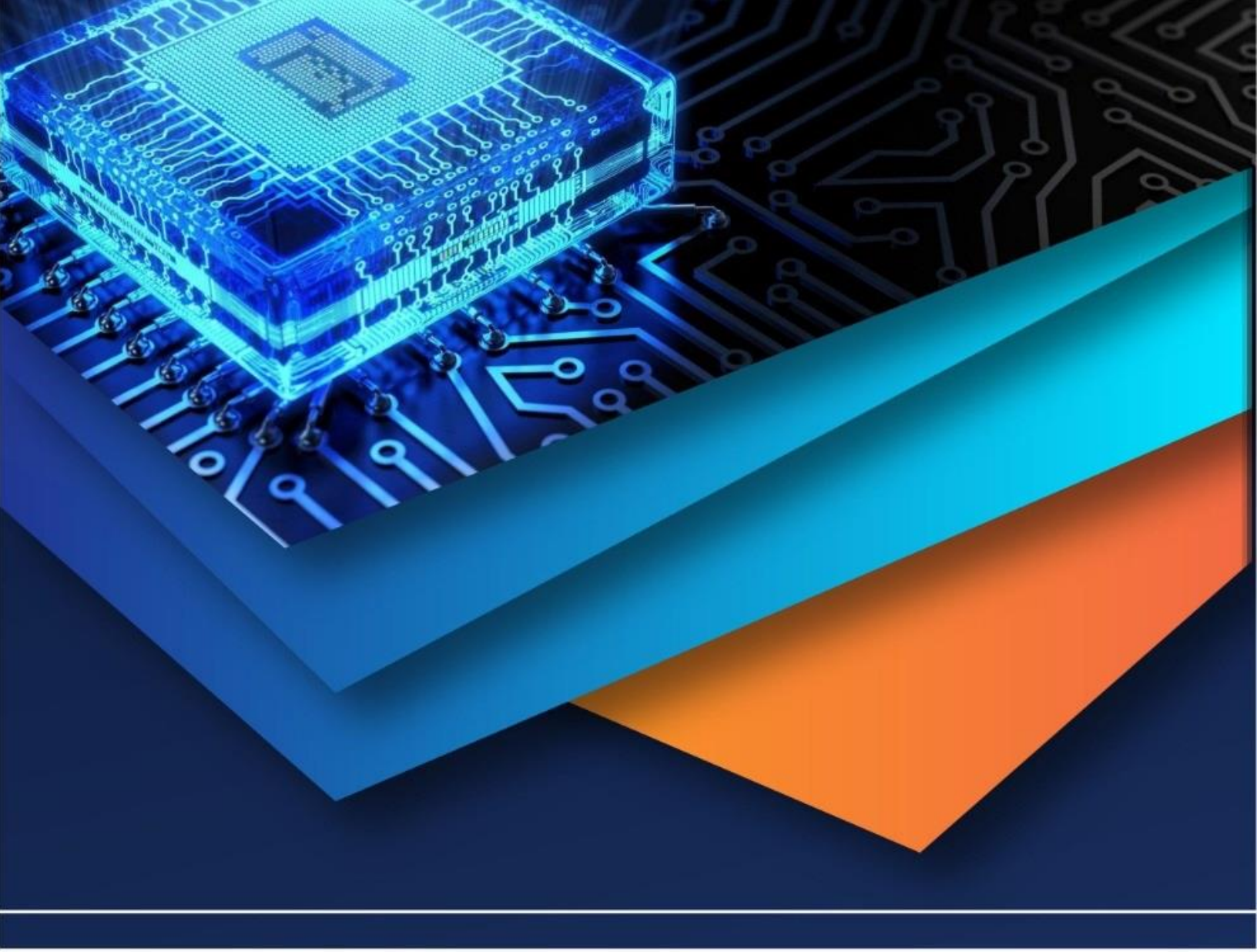
IV. CONCLUSION

9 Isolates designated activity against bacteria near NCR Region. These micro-organisms proved that they can function as the most important and powerful medicines factories ever developed. Also called as the roots of life saving treatments for bacterial infections. The number of terrestrial antibiotics exhibits recent approach to a saturation curve with an evident limit in the near future. The growing number of leading antibiotics in pharmacology for update treatment of drug resistant infectious pathogens has shortage of metabolites somehow in unspoiled territory where the human involvement is very few. Our studies will show the effectiveness and richness in actinomycetes assortment of the NCR region, also utilization in bioindustry. Actinomycetes which forms antibiotics are discovered and more to come in future.

REFERENCES

[1] Panday B., Ghimire P. and Agrawal V.P. (2004) International Conference on the Great Himalayas: Climate, Health, Ecology, Managemnet and Conservation, Kathmandu, Organized by Kathmandu University and the Aquatic Ecosystem Health and Management Society, Canada.
[2] Oskay M., Tamer A. U. and Azeri C. (2004) African J. Biotechnol , 3(9), pp441-446.
[3] D. Dhanasekaran 1 *, S. Selvamani 2 , A. Panneerselvam 3 and N. Thajuddin 4 1 School of Biotechnology, Chemical and Biomedical Engineering, VIT University, Vellore-632 014, India.African Journal of Biotechnology Vol. 8 (17), pp. 4159-4162, 1 September, 2009
[4] Saadoun, I. and Gharaibeh, R. (2003) The Streptomyces Flora of Badia Region of Jordan and Its Potential as a Source of Antibiotics Active against Antibiotic-Resistant Bacteria. Journal of Arid Environments, 53, 365-371.
[5] Shirling et al .,E. T.,& Gottlieb, D. (1966). Methods for characterization of Streptomyces Species1. International Journal of Systematic and Evolutionary Microbiology, 16(3), 313-340.

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