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## ORIGINAL ARTICLE

# Studies on Antimicrobial Activity of Fresh Water Actinomycetes against Gram Negative Bacteria

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**ABSTRACT**
*Microbial strain is developing resistance power against existing antibiotics, stressing the urgency for discovery of new therapeutic compounds. Actinomycetes only produce 70-80% of the available antibiotics. The chances of isolating undiscovered strains from the terrestrial habitats have diminished so that the search for novel antibiotics has switched to actinomycetes from normal habitats or to discovery of strains/species found in unusual habitats. The present study reports screening, identification, and antimicrobial activity of actinomycetes of fresh water environment against Gram negative bacteria. Totally 44 morphologically different actinomycetes colonies were isolated from fresh water ecosystem. More number of powdery colonies was observed on starch casein agar. Antibacterial activity of actinomycetes strains was tested by agar well method. From These 44 isolated actinomycetes majority of actinomycetes contain the antibacterial substances. The isolates were tested for antagonistic activity against E.coli, Pseudomonas aeruginosa, Proteus vulgaris Klebsiella pneumonia, Salmonella typhy.*
***KEYWORDS:*** *Gram's Negative Bacteria, Actinomycetes, Antibiotics, Fermentation.*

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## INTRODUCTION

The search for new antibiotics effective against multi-drug resistant pathogenic bacteria is an important area of antibiotic research in the contemporary scenario. Natural products having novel structures have been observed to perform inherent biological activities.

Microorganisms are virtually an unlimited source of novel compound with many medicinal applications and consequently their secondary metabolite screening for pharmaceutically significant novel antibiotic and other compounds and drug lead molecules has assumed greater attention in recent times. Actinomycetes are the most widely distributed groups of microorganisms in nature. They are attractive, bodacious and charming filamentous Gram-positive bacteria with true aerial hyphae, belonging to the phylum *Actinobateria* and order actinomycetales, that represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain Bacteria [1]. The majority of actinomycetes are free living, spore forming and saprophytic bacteria. *Actinobacteria* are widely distributed in terrestrial and aquatic ecosystems, especially in soil, where they play a crucial role in the recycling of refractory biomaterials by decomposing complex mixtures of polymers in dead plant, animal and fungal tissues.

Around secondary metabolites produced by microorganisms have been reported and over 10,000 of these compounds are produced by actinomycetes, representing 45% of all bioactive microbial metabolites discovered [2]. The secondary metabolites obtained from actinomycetes are of special interest because of their diverse biological activities such as antibacterial, antifungal, antioxidant, antitumor and antiviral.

The search for antibiotics was conducted in many environments and with different approaches but fresh water was neglected from this research until now [3]. Therefore, the natural background of antibiotic

concentrations or antibiotic producers in this environment is known so far. Important questions are if new antibacterial substances can be found in fresh water. Hence the present work was to isolate and screen antibiotic producing actinomycetes from fresh water samples in Beed district. The outcome of this finding may be important to give direction for researchers and for future treatment of multidrug resistant human pathogens.

## MATERIAL AND METHODS

### Isolation of Actinomycetes

Five water samples were collected from different freshwater systems of Beed. Starch casein agar [4] medium was prepared and sterilized at 121°C in 15 lbs pressure for 15 minutes. Then it was added with glycerol 50mg/ml and cyclohexamine 20 mg/ml to prevent the bacterial and fungal growth. The collected water samples were spread over on the agar plates. The inoculated plates were incubated at 28 ° C for 7 days.

### Characterization of actinomycetes

The isolates were observed under the microscope by using cover slip culture method .Cover slip method was used for the identification [5]. After the suitable incubation, cover slip in the medium facilitates the distinction between substrate mycelium and aerial mycelium. Then the cover slip observed under microscope and identified according to Bergey's Manual of Determinative Bacteriology, Ninth edition (2000)[6].

### Fermentation and extraction of secondary metabolites

Starch casein broth was used as the fermentation media. Inoculate the fresh water isolated actinomycetes in this medium incubated at $37^0$C for 5 to 6 days on rotary shaker in 150 ml
conical flask. Then the culture broth was centrifuged at 4000 rpm for 20 min and filtrate used to antimicrobial activity. Antimicrobial activities were assayed by using well diffusion method [7,8] against the test organisms.

**Test organisms: -** ***E.coli, Pseudomonas aeruginosa, Proteus vulgaris Klebsiella pneumonia, Salmonella typhy.***

### Antibacterial activities of isolated.

The grams negative bacterial strains were pre-grown in nutrient broth at 37°C for 24hrs.Sterilized nutrient agar medium was poured into petri plates and after solidification the bacterial culture were evenly spread over appropriate media by using sterile spreader . Then the wells were punched in the bacterial spread medium with 3mm diameter by using a sterile cork borer, wells. . A 100µl of actinomycetes broth cultures were are pipette into separate wells. Inoculated plates were incubated at 37°C for 24-48 hr. After incubation, the results were observed and measured the diameter of inhibition zone around the each well [9].

## RESULT AND DISCUSSION

**Antibiotic Sensitivity with gram negative bacteria:** The bioactive compounds producing Actinomycetes were selected and tested its antibiotic sensitivity against gram negative bacteria shown in table. The organism showed both positive and negative interactions. In the negative interaction the organism produced inhibition zones which were measured and recorded. The microbial interactions were analyzed by the determination of the size of the inhibition zone. [9].

## CONCLUSION

This study shows that the isolated actinomycetes isolates have the potential to act as sources of new antibacterial compounds against microorganisms to humans. Here, we found that fresh water is a good source of biodiversity and has been adequately acceptable due to its vast floral diversity and also microbial diversity. The results showed that all the isolates were able to inhibit the extracellular growth of filaments in the test organism. Although From these isolated actinomycetes **P2, P3, D3, D4, D7,A1 ,A2, A7, S2, S10, V1,**and **V2** more effective against other . The research of discover a novel compound of pharmaceutical interest requires the isolation of a large number of isolates and will be more promising if diverse fresh water actinomycetes are sampled and screened [10]. Such an endeavour can help to discoveries and novel secondary metabolites which can be used in different uses which will undoubtedly help to mankind.

**Table 1:** Result of secondary screening of antibiotics activity of actinomycetes by agar well method.

| | **Zone of inhibition in mm** | | | | | |
|---|---|---|---|---|---|---|
| **Sr no,** | Actinomyctes no | *E.coli,* | *Pseudomonas aeruginosa,* | *Proteus vulgaris* | *Klebsiella pneumonia,* | *Salmonella typhy.* |
| 1. | P1 | 01 | 05 | 02 | 01 | 05 |
| 2. | P2 | 10 | 05 | 07 | 05 | 05 |
| 3. | P3 | 07 | 10 | 15 | 10 | 10 |
| 4. | P4 | 04 | 01 | 05 | 05 | 00 |
| 5. | P5 | 02 | 01 | 10 | 00 | 03 |
| 6. | P6 | 07 | 01 | 10 | 01 | 02 |
| 7. | P7 | 03 | 03 | 02 | 01 | 00 |
| 8. | P8 | 06 | 03 | 05 | 05 | 03 |
| 9. | D1 | 05 | 04 | 06 | 05 | 03 |
| 10. | D2 | 05 | 05 | 05 | 03 | 04 |
| 11. | D3 | 07 | 07 | 06 | 07 | 04 |
| 12. | D4 | 07 | 05 | 06 | 07 | 03 |
| 13. | D5 | 10 | 07 | 01 | 01 | 02 |
| 14. | D6 | 05 | 07 | 01 | 00 | 01 |
| 15. | D7 | 10 | 10 | 10 | 05 | 10 |
| 16. | D8 | 05 | 02 | 05 | 05 | 13 |
| 17. | D9 | 10 | 10 | 07 | 07 | 00 |
| 18. | D10 | 03 | 03 | 05 | 05 | 02 |
| 19. | D11 | 07 | 05 | 02 | 05 | 05 |
| 20. | A1 | 07 | 10 | 10 | 05 | 10 |
| 21. | A2 | 10 | 05 | 05 | 05 | 05 |
| 22. | A3 | 10 | 10 | 10 | 00 | 00 |
| 23. | A4 | 03 | 05 | 03 | 01 | 03 |
| 24. | A5 | 05 | 02 | 06 | 01 | 05 |
| 25. | A6 | 04 | 10 | 02 | 10 | 10 |
| 26. | A7 | 04 | 05 | 10 | 10 | 04 |
| 27. | A8 | 05 | 02 | 07 | 03 | 07 |
| 28. | A9 | 05 | 03 | 05 | 03 | 03 |
| 29. | A10 | 07 | 02 | 10 | 05 | 07 |
| 30. | A11 | 02 | 01 | 06 | 03 | 03 |
| 31. | S1 | 05 | 10 | 10 | 06 | 02 |
| 32. | S2 | 15 | 07 | 10 | 04 | 14 |
| 33. | S3 | 05 | 10 | 10 | 07 | 00 |
| 34. | S4 | 05 | 10 | 02 | 10 | 03 |
| 35. | S5 | 01 | 02 | 03 | 01 | 01 |
| 36. | S6 | 07 | 03 | 03 | 07 | 05 |
| 37. | S7 | 04 | 05 | 02 | 01 | 06 |
| 38. | S8 | 06 | 02 | 02 | 00 | 06 |
| 39. | S9 | 05 | 05 | 05 | 10 | 02 |
| 40. | S10 | 10 | 07 | 05 | 06 | 07 |
| 41. | S11 | 05 | 10 | 10 | 05 | 00 |
| 42. | S12 | 04 | 04 | 05 | 03 | 05 |
| 43. | V1 | 10 | 09 | 10 | 08 | 10 |
| 44. | V2 | 09 | 10 | 10 | 09 | 05 |

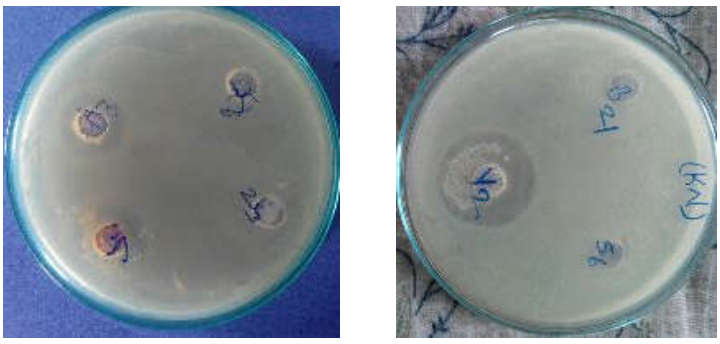

***Figure 1***: Antibiotics activity by agar well method.

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