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Research Article

# Isolation and Screening of Potent Antibiotic-Producing Actinomycete

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**ABSTRACT**

This study delves into the realm of actinomycetes, with a specific focus on their ability to produce antibiotics. Actinomycetes, a subset of gram-positive bacteria, are well-known for generating various secondary metabolites, including antibiotics. Indeed, a majority of today's antibiotics trace their origins back to actinomycetes, notably the *Streptomyces* genus. The research aims to isolate actinomycetes from soil samples and assess their effectiveness against different pathogenic bacteria and fungi, with the goal of extracting natural compounds with potential medical uses. 23 actinomycete morphotypes were isolated from diverse soil sources such as agricultural fields, garbage dumps, and gardens. These isolates were then screened for their antimicrobial activity against various test microorganisms, including *Escherichia coli* (MTCC 2939), *Bacillus subtilis* (MTCC 1305), *Candida albicans* (MTCC 183), and *Aspergillus flavus* (MTCC 873). Notably, isolate BT 22 demonstrated superior antibacterial and antifungal properties compared to other isolates. Further analysis confirmed the isolate's identity as belonging to the *Streptomyces* genus, known for its prolific production of bioactive compounds. These findings highlight the potential of actinomycetes, particularly *Streptomyces*, as a valuable source of natural products with a wide range of applications in medicine and industry. The discovery of novel antimicrobial compounds from these organisms offers promise in combating antibiotic resistance and advancing therapeutic strategies

**Keywords:** Actinomycetes, *Streptomyces*, Secondary metabolites, Antibiotics, Antimicrobial.

## INTRODUCTION

The pursuit of novel antimicrobial agents has been a cornerstone of medical research in recent decades, marked by significant accomplishments in combating infectious diseases.[1] Nonetheless, the persistence of infectious diseases, the swift emergence of new drugs, and the development of multidrug resistance by pathogenic microorganisms undermine the effectiveness of current antibiotics. This has spurred the necessity to explore microorganisms from natural sources for new antibiotics containing novel biomolecules, which exhibit activity against a broad spectrum of pathogens.[2,3]

Within the realm of natural antimicrobial discovery, actinomycetes have emerged as a particularly promising avenue of investigation. These gram-positive bacteria, characterized by their high GC content in their DNA,[4] are renowned for their prolific production of bioactive secondary metabolites, including antibiotics.[5,6] Actinobacteria are widely acknowledged as prolific producers of a diverse array of secondary metabolites. Recent genomic revelations have significantly influenced the utilization of these metabolically versatile bacteria across various domains.[7] Displaying a filamentous nature, actinomycetes generate two distinct types of branching mycelium known as aerial and substrate mycelium. Various factors, such as geographical location, soil type, temperature, organic matter content, moisture levels, cultivation, and aeration, influence the quantity and diversity of actinomycetes in a given soil. Actinomycetes play a crucial role as a predominant component in the microbial population of most soils, with *Streptomyces* species comprising around 90% of the total actinomycetes population.[8] These soil-dwelling actinomycetes possess the capability to produce a diverse array of secondary metabolites, with approximately 70% of clinically used naturally derived antibiotics originating from them.[9] Notably, about 80% of the world's antibiotics are sourced from actinomycetes, predominantly from the genera *Streptomyces* and *Micromonospora*.[10]

In the 21st century, a significant global healthcare challenge has emerged due to the escalating resistance of microorganisms to commonly employed antibiotics. This has prompted a renewed exploration for novel antimicrobial agents from *Streptomyces*, driven by the rapid emergence of antimicrobial resistance among pathogenic microorganisms.[11] Pathogens such as methicillin/oxacillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin-resistant *Enterococcus* (VRE), extended-spectrum beta-lactamase (ESBL) producing bacteria like *E. coli* and *Klebsiella* sp., and penicillin-resistant *Streptococcus pneumoniae* (PRSP) represent formidable adversaries due to their ability to evade traditional antibiotic treatments.[12]

In response to this pressing need, the present study embarks

on an ambitious quest to explore the antimicrobial potential of actinomycetes. Drawing upon soil samples sourced from diverse ecological niches, ranging from garbage dumps to agricultural fields and gardens, this research endeavors to unearth novel bioactive compounds capable of combating both bacterial and fungal pathogens. By harnessing the biochemical prowess of actinomycetes, this endeavor seeks to identify promising candidates for the development of next-generation antibiotics, thus addressing the critical healthcare challenges posed by antimicrobial resistance.

## MATERIAL AND METHODS

### Sampling site and Soil sample collection

In a systematic screening initiative aimed at isolating actinomycetes, 08 samples were systematically gathered from 3 various terrestrial ecosystems renowned for their actinomycete presence, viz., garbage dumps, agricultural fields, and gardens. To prevent any potential contamination, soil and sediment samples were aseptically collected from a depth of 2 to 3 cm within the soil, utilizing a sterile trowel, and placed in autoclaved, dry, clean, and sterile insulated containers. These samples were stored under aseptic conditions at 4℃ until they were ready for isolation.

### Isolation of actinomycetes

The soil samples were dried at room temperature and weighed. Serial dilution up to $10^{-5}$ dilution was done prior to spreading. After serial dilution, 100 µL suspension each from vials of $10^{-3}$, $10^{-4}$ and $10^{-5}$ was spread plated on the starch casein agar plates (SCA composition: soluble starch: 10 g, $K_2HPO_4$: 2 g, $KNO_3$: 2 g, casein: 0.3 g, $MgSO_4.7H_2O$: 0.05 g, $CaCO_3$: 0.02 g, $FeSO_4.7H_2O$: 0.01 g, agar: 15 g, distilled water: 1000 mL and pH: 7.0 ± 0.1). The plates were incubated at 37 ± 0.5°C for 7 days. Repeated subculture was performed by streaking on the SCA to obtain a pure culture. Isolated colonies with different colors, hard textures and powdery forms were identified based on the macroscopic observation. Following the incubation period, slants housing the pure actinomycete isolates were preserved at 4°C for subsequent investigations.

### Primary screening of actinomycetes

Two bacterial pathogens viz., *Escherichia coli* (MTCC 2939) and *Bacillus subtilis* (MTCC 1305), and two fungal pathogens viz., *Candida albicans* (MTCC 183), and *Aspergillus flavus* (MTCC 873) were used as test organisms to find the antimicrobial potential of actinomycetes. The actinomycetes isolates were screened for antibacterial and antifungal activity using Starch casein nutrient agar (SCNA) media by cross streak method.[13] Each SCNA plate was streaked with each actinomycete isolate at the centre of the plate and incubated 35 ± 2°C for 7 days. Then, 24-hour subcultures of bacteria were streaked perpendicular to the actinomycete isolates and the plates were incubated at 35 ± 2°C for 24 hours. After incubation, the zone of inhibition of the test organisms indicates that the actinomycete isolate has antibacterial activity, whereas the full growth of the test organism indicates that the actinomycete isolate has no antibacterial activity. The actinomycete isolate showing maximum inhibition was further selected for secondary screening.

### Secondary screening of actinomycetes

The selected isolate was inoculated in starch casein nutrient broth and incubated on a rotary shaker incubator at 35 ± 2°C for 10 days. The broth culture obtained was filtered through Whatman no .1 filter paper and then centrifuged and the clear supernatant broth was obtained. The crude extract was tested for its antimicrobial activity against the selected pathogens by agar well diffusion method.[14] The test organisms were swabbed onto solidified Muller Hinton agar plates, and wells with a diameter of 6 mm were punched into the plate. Subsequently, 100 µL of a crude extract containing antimicrobial secondary metabolites was dispensed into individual wells. The plates were then incubated at 35 ± 2°C for 24 hours. Following incubation, the diameter of the inhibition zone surrounding the wells was measured and documented.

### Characterization and Identification of Actinomycetes

Morphological, biochemical and physiological studies carried out characterization of the selected potent actinomycete isolate.

#### *Morphological identification*

Gram staining was performed in order to determine the nature of the selected actinomycete and the cover slip technique was performed by microscopic observation to examine the spore chain morphology.

#### *Biochemical identification*

Biochemical tests, including oxidase and catalase activity, Methyl Red test (MR), Voges-Proskauer Test (VP), $H_2S$ production, Indole test, casein hydrolysis test and amylase test, were performed. The selected isolate was identified by following Bergey's Manual of Determinative Bacteriology.[15]

#### *Physiological identification*

The colony morphology of the selected actinomycete isolate was studied under a high-power magnifying lens by examining the color of the colony, nature of the mycelium, spore elevation and margin and pigmentation.

## RESULTS AND DISCUSSION

### Isolation of actinomycetes

Twenty-three distinct colonies of actinomycetes were isolated from soil samples collected at eight different sites. The identification of these colonies as actinomycetes was based on observable traits, including a powdery texture, branching filaments, spore formation, dry appearance, presence of diffusible pigment, mature aerial mycelium, and substrate mycelium.[16] These colonies were isolated into pure cultures using the streak plate method.

### Primary screening

In the present study, on primary screening, 11 out of 23 isolates showed significant inhibition against at least one test pathogen. Out of 23 actinomycetes isolates, the isolated actinomycetes (BT 22) showed antibacterial activity against both Gram-negative and Gram-positive test bacteria along with antifungal activity by inhibiting *E.*

*coli* MTCC 2939, *B. subtilis* MTCC 1305, *C. albicans* MTCC 183 and *A. flavus* MTCC 873.

Comparable outcomes to those described above were observed in a study involving soil-isolated strains of actinomycetes bacteria, especially those influencing the growth of the aflatoxin-producing mold *A. flavus* TISTR 3041. In the dual culture assay, the SP-O2 isolate displayed significant inhibitory activity.[17]

The isolate showing maximum antimicrobial potency was selected for further secondary screening.

## Secondary screening

Secondary screening of the selected isolate was performed by the Agar well diffusion method. The antimicrobial metabolites of BT 22 were found to inhibit all the test organisms viz., *E. coli* MTCC 2939, *B. subtilis* MTCC 1305, *C. albicans* MTCC 183 and *A. flavus* MTCC 873. The maximum zone of inhibition, i.e., 36 mm was found to be against *C. albicans.* The zone of inhibition formed showed the antimicrobial potency of the selected strain against different test organisms (Figs 1 and 2). The findings of the antibacterial activity tests in the present study revealed that the isolated actinomycetes isolates were equally effective against both gram-positive and gram-negative bacteria. These results are in contrast with a previous study, where it was found that the actinomycetes isolates were more effective against gram-positive bacteria than gram-negative bacteria.[18] The results of the present study are also in contrast to many other studies where the actinomycetes isolated from the mangrove soil of Jenu, Tuban belonging to *Streptomyces* and *Nocardia* genera were only able to inhibit the growth of *E. coli*, but not *S. aureus* and *C. albicans*.[19]

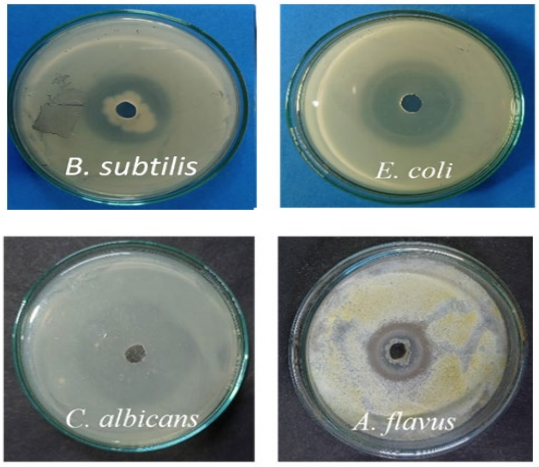


Fig. 1: Secondary screening against *B. subtilis, E. coli, C. albicans* and *A. flavus*

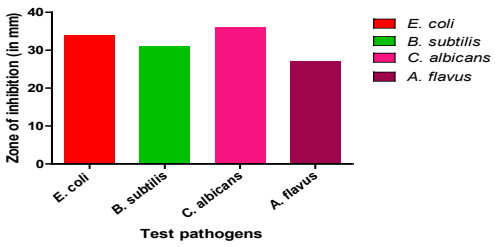


Fig. 2: Secondary screening of BT 22 against bacterial and fungal test organisms

However, previous studies reported that actinomycetes could inhibit the growth of species of *Bacillus, Staphylococcus, E. coli, Klebsiella,* and *Pseudomonas*.[20,21]

## Characterization and identification of actinomycetes

### *Morphological identification*

The actinobacterial strain grew well on SCNA media with spore formation (Table 1).

### *Biochemical characterization*

The biochemical tests of BT 22 revealed that the actinomycete strain was showing positive results for catalase test, oxidase test, urease test, citrate test, and casein hydrolysis and were also able to produce hydrogen sulfide and the strain was showing negative results for nitrate reduction test, methyl red test as well as Voges-Proskauer test (Table 1).

### *Physiological characterization*

Microscopic studies by the cover slip technique showed the formation of branched mycelium (Fig 3). Gram's staining was performed and the strain was found to be gram-positive with spherical cells. The

Table 1: Morphological and biochemical characterization of BT 22

| *Morphological features* | *Results* |
|---|---|
| Spore mass | Grey |
| Spore chain | Straight |
| Spore shape | Cylindrical |
| Substratum mycelium | Grey |
| Aerial mycelium | White |
| Diffusible pigment | Absent |
| *Biochemical tests* | *Results* |
| Catalase | + |
| Oxidase | + |
| Urease | + |
| $H_2S$ production | + |
| Nitrate reduction | - |
| Methyl red (MR) | - |
| Voges-Proskauer (VP) | - |
| Citrate utilization | + |
| Casein hydrolysis | + |
| *Utilization of Nitrogen source* | *Results* |
| D-alanine | + |
| L-arginine | + |
| L-tyrosine | + |
| *Utilization of Carbon source* | *Results* |
| D-glucose | + |
| Fructose | + |
| Sucrose | + |

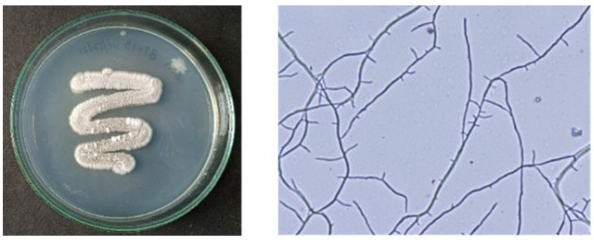

Fig. 3: (a) Aerial mycelium of BT 22 (b) Aerial hyphae bearing straight spore chains under light microscope

isolate BT 22 was found to utilize various Carbon and Nitrogen sources (Table 1).

Based on its morphological, microscopic, and biochemical features, isolate BT 22 was classified as *Streptomyces* sp.. Poopal's investigation in 2009 highlighted the ongoing significance of *Streptomyces* species as a major source of natural products. Among the 589 new compounds reported from Actinobacteria during the reviewed period, an impressive 65% were attributed to *Streptomyces* species.[7]

## CONCLUSION

The recently isolated strain *Streptomyces* sp. (BT 22) showcased a remarkable antimicrobial activity, demonstrating potency against a diverse array of pathogens, including gram-negative bacteria, gram-positive bacteria, and fungal pathogens. This broad-spectrum efficacy underscores its potential utility in combatting various infectious agents. Such noteworthy results not only signify its immediate significance but also advocate for its thorough exploration and exploitation in pharmaceutical realms. With its multifaceted antimicrobial properties, *Streptomyces* sp. (BT 22) presents an enticing opportunity for in-depth investigation, potentially paving the way for the development of novel therapeutic agents to address a spectrum of infectious diseases.

## CONFLICTS OF INTEREST

All the authors declare that they have no conflict of interest.

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