

Isolation and Characterization of Bioactive Actinomycetes from Forest Soils of Kinnerasani Wildlife Sanctuary, Telangana

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ABSTRACT

Forest ecosystems represent rich reservoirs of microbial diversity with enormous biotechnological potential. Among soil microorganisms, actinomycetes are particularly important due to their ability to produce a wide range of bioactive metabolites including antibiotics, enzymes, antifungal compounds, and plant growth-promoting substances. The present study aimed to isolate and characterize bioactive actinomycetes from soils of the Kinnerasani Wildlife Sanctuary located in Bhadrachalam District, Telangana. Soil samples were collected from multiple ecological niches including rhizosphere zones, leaf-litter-rich soils, shaded forest floors, and moist regions near water bodies. Samples were processed using the serial dilution technique and plated on selective media such as Glycerol Yeast Extract Agar and Starch Casein Agar to facilitate actinomycetes isolation. Distinct colonies showing typical actinomycetes morphology were purified and preserved for further analysis. Morphological observation, Gram staining, and biochemical tests including catalase activity, oxidase reaction, and starch hydrolysis were performed for preliminary characterization. Most isolates exhibited Gram-positive filamentous structures and demonstrated strong catalase activity, confirming their actinomycetal nature. Starch hydrolysis results indicated significant extracellular enzyme activity among several isolates. Enumeration studies revealed a diverse microbial population dominated by Gram-positive rods, cocci, and filamentous organisms across different soil samples. The diversity observed in forest soils indicates the presence of metabolically versatile microorganisms capable of producing valuable bioactive compounds.

Keywords: Actinomycetes, Soil Microbiology, Forest Ecosystem, Bioactive Compounds, Antimicrobial Activity

INTRODUCTION

Soil ecosystems represent one of the most biologically complex environments on Earth. They harbour a vast diversity of microorganisms including bacteria, fungi, actinomycetes, algae, and

protozoa, which play essential roles in nutrient cycling and organic matter decomposition. These microorganisms contribute significantly to soil fertility, ecosystem stability, and environmental sustainability.

Among soil microorganisms, actinomycetes are particularly significant due to their filamentous morphology and remarkable metabolic capabilities. They are Gram-positive bacteria belonging to the phylum Actinobacteria and are characterized by high guanine-cytosine content in their genomic DNA. These microorganisms are widely distributed in terrestrial environments and play an important role in decomposing complex organic compounds such as cellulose, lignin, and chitin. Actinomycetes are also recognized as one of the most important sources of natural bioactive compounds. Approximately 60–70% of clinically useful antibiotics are derived from actinomycetes, particularly from the genus *Streptomyces*. In addition to antibiotics, they produce enzymes, antifungal compounds, immunosuppressive agents, and plant growth-promoting substances.

Forest ecosystems provide ideal environments for exploring microbial diversity because they contain abundant organic matter, diverse vegetation, and minimal anthropogenic disturbance. The Kinnerasani Wildlife Sanctuary located in Bhadrachalam District of Telangana is characterized by dry deciduous forests, diverse plant species, and rich soil organic matter. Such ecological conditions support the growth of diverse microbial communities including actinomycetes. Despite the ecological richness of forest ecosystems, microbial diversity in many wildlife sanctuaries remains largely unexplored. Therefore, the present study was undertaken to isolate and characterize actinomycetes from forest soils of the Kinnerasani Wildlife Sanctuary.

MATERIALS AND METHODS

The study was conducted in the Kinnerasani Wildlife Sanctuary located in Bhadrachalam District, Telangana, India. The sanctuary represents a protected forest ecosystem with diverse flora and fauna. Soil samples were collected from different ecological niches including areas near water bodies, shaded forest floors, and leaf-litter rich zones.

Soil samples were collected from undisturbed forest locations using sterile spatulas after removing surface debris. Samples were collected from a depth of 5–15 cm and transferred into sterile polyethylene bags for transportation to the laboratory. The samples were air-dried at room temperature before further processing to suppress fast-growing bacteria and fungi. Isolation of actinomycetes was carried out using the serial dilution plate technique. One gram of air-dried soil was suspended in sterile distilled water to obtain a soil suspension. Serial dilutions were prepared and plated onto selective media such as Glycerol Yeast Extract Agar Starch Casein Agar. The plates were supplemented with antifungal agents to suppress fungal growth and incubated at 27°C for five days. Colonies exhibiting typical actinomycetes characteristics such as chalky texture, powdery appearance, and aerial mycelium were selected and purified through repeated sub culturing.

Gram Staining

Gram staining was performed to determine the Gram reaction and cellular morphology of the isolates. Microscopic examination revealed Gram-positive filamentous bacteria with branched hyphae characteristic of actinomycetes.

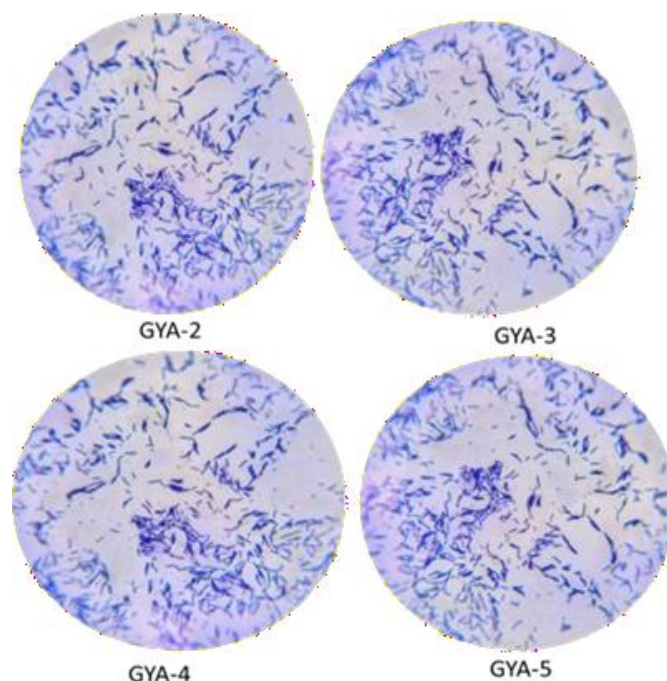


Figure 1: Gram staining and microscopic characterization of actinomycetes isolate.

Catalase Test

Catalase activity was tested by adding hydrogen peroxide to the bacterial culture. Immediate bubble formation indicated a positive reaction.

Oxidase Test

The oxidase test was performed using oxidase reagent. Most isolates showed negative oxidase reactions, which is typical for soil-derived actinomycetes.

Starch Hydrolysis

The ability of isolates to hydrolyse starch was tested using starch agar plates. After incubation, iodine solution was added to detect starch degradation. Clear zones around colonies indicated positive starch hydrolysis and extracellular enzyme production.

RESULTS AND DISCUSSION

Selective isolation techniques resulted in the growth of several colonies exhibiting typical actinomycetes morphology. Colonies appeared dry, chalky, and powdery with well-developed aerial mycelium. Such morphological characteristics are consistent with members of the genus *Streptomyces*, which are commonly found in soil environments.

Enumeration studies revealed a diverse microbial population across all soil samples. Microscopic

examination showed the presence of Gram-positive rods, cocci, and filamentous bacteria in different samples. The distribution of these microorganisms varied depending on soil microhabitats. According to the enumeration table in the progress report, each soil sample contained multiple morphologically distinct microorganisms, indicating rich microbial diversity within the forest soils of the sanctuary.

Biochemical tests revealed that the majority of actinomycetes isolates were catalase positive and oxidase negative. The catalase positive reaction indicates the ability of these microorganisms to detoxify hydrogen peroxide produced during metabolic processes. Starch hydrolysis tests showed that several isolates were capable of producing extracellular amylase enzymes. This enzymatic capability demonstrates the ability of actinomycetes to degrade complex organic compounds present in forest soils. The results of this study highlight the ecological importance of forest soils as reservoirs of diverse microorganisms. Actinomycetes isolated from such environments possess significant metabolic capabilities and may serve as potential sources of bioactive compounds. Previous studies have demonstrated that actinomycetes produce antibiotics, antifungal compounds, anticancer agents, and industrial enzymes. Therefore, exploring actinomycetes diversity in forest ecosystems may contribute to the discovery of novel bioactive compounds useful in medicine, agriculture, and biotechnology.

Table 1 Enumeration of organisms observed in each soil sample based on Gram staining and microscopic examination.

Sample No.	Organisms Identified in samples			
S-1	Gram-positive rod	Gram- positive rod	Gram-positive cocci	Gram-positive filamentous
S-2	Gram-positive rod	Gram- positive rod	Gram-positive cocci	Gram-positive filamentous
S-3	Gram-positive filamentous	Gram- positive rod	Gram-positive rod	
S-4	Gram-positive rod	Gram- positive rod	Gram-positive cocci	Gram-positive filamentous
S-5	Gram-positive cocci	Gram- positive rod	Gram-positive rod	
S-6	Gram-positive rod	Gram- positive rod	Gram-positive cocci	
S-7	Gram-positive filamentous	Gram- positive rod	Gram-positive cocci	Gram-positive rod
S-8	Gram-positive rod	Gram- positive rod	Gram-positive cocci	
S-9	Gram-positive cocci	Gram- positive rod	Gram-positive rod	Gram-positive filamentous
S-10	Gram-positive rod	Gram- positive rod	Gram-positive cocci	Gram-positive filamentous
S-11	Gram-positive filamentous	Gram- positive rod	Gram-positive cocci	Gram-positive rod
S-12	Gram-positive rod	Gram- positive rod	Gram-positive cocci	

CONCLUSION

The present investigation demonstrated that forest soils of the Kinnerasani Wildlife Sanctuary harbouring diverse populations of actinomycetes and other Gram-positive microorganisms. Isolation and characterization studies confirmed the presence of filamentous actinomycetes isolates exhibiting typical morphological and biochemical characteristics. The results indicate that these microorganisms possess significant metabolic and enzymatic capabilities. Forest ecosystems therefore represent valuable sources of bioactive microorganisms with potential applications in pharmaceutical, agricultural, and industrial biotechnology. Further studies involving molecular identification and antimicrobial screening of these isolates may lead to the discovery of novel bioactive compounds.

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