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Actinobacterial Communities from Mid Karnataka Soils: Isolation, Characterization and 16S rRNA Based Identification

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ABSTRACT

Actinobacteria were isolated from diverse soil types of mid Karnataka, including black soil (Chitradurga), brown soil (Davangere) and termite mound soil (Dandeli), to explore culturable actinomycetes with potential biotechnological relevance. Air-drying pretreatment efficiently suppressed fast-growing bacteria and fungi and facilitated the selective recovery of filamentous, Gram-positive, powdery colonies on starch casein agar. A total of 14 morphologically distinct actinomycete isolates were obtained, showing variation in aerial and substrate mycelial colour as well as diffusible pigment production. Biochemical profiling revealed diverse physiological traits, with most isolates negative for common tests but showing selective positivity for catalase, citrate utilization and nitrate reduction. Partial 16S rRNA gene sequencing and phylogenetic analysis identified isolates mainly within the genus *Streptomyces*, along with representatives of *Micromonospora* and *Rhodococcus*, with sequence similarity values ranging from 97.78% to 99.99% to their closest type strains. These findings highlight mid Karnataka soils, including termite mound soils, as promising reservoirs of diverse actinomycetes that warrant further screening for novel bioactive metabolites and industrial enzymes.

***Key words*:** Actinomycetes; *Streptomyces*; Soil microbiota; 16S rRNA sequencing; Phylogenetic analysis.

INTRODUCTION

Actinomycetes, a diverse group of Gram-positive organisms, are notable in microbiology for their capacity to synthesize valuable bioactive compounds. They display a range of morphologies, including cocci, rods, mycelia, and various other forms. These organisms exhibit adaptability to extreme conditions, including desiccation, low temperatures, stress, and hyperosmotic stress, allowing them to inhabit both terrestrial and aquatic environments. The habitat range of these diverse actinomycetes is broadening, likely attributable to their capacity to endure extreme conditions (Shivaveerakumar *et al*., 2013). Actinomycetes are essential in the environment, facilitating carbon and nutrient turnover and contributing to humus formation, a critical component of soil. Humus plays a crucial role in the survival and efficiency of microorganisms, affecting processes such as respiration, photosynthesis, and nitrogen fixation (AbdElgawad *et al*., 2020).

Actinobacteria, characterized by a high guanine and cytosine content (≥55%), represents one of the predominant phyla of bacteria present in a variety of natural substrates. Their contribution is crucial to the decomposition of organic materials and the carbon cycle. Actinomycetes constitute a significant portion of the soil microbial biomass and possess the ability to synthesize a diverse range of antibiotics and extracellular enzymes (Barka *et al*., 2015). A significant portion of the recognized natural antibiotics is derived from

actinomycetes. Furthermore, these represent a significant source for new antibiotics, thus possessing considerable pharmacological and commercial value (Shivaveerakumar *et al*., 2014).

Among all known microbes, members of the actinomycetes genus, particularly Streptomyces species, are acknowledged for their prolific production of valuable bioactive metabolites. These metabolites exhibit a broad spectrum of activities, including antibacterial, antifungal, antibiotic, antiphrastic, antitumor, antiviral, insecticidal, herbicidal, immunomodulatory, and antithrombotic properties. Consequently, the screening and isolation of promising strains of actinomycetes with potential antibiotics has been a focal point of investigation for many years. Streptomycetes are extensively utilized in various industries because of their capacity to generate a multitude of chemical compounds, such as antibiotics, enzymes, and anti-tumor agents (Meenakshi *et al*., 2024).

Actinomycetes represent a highly diverse ecological group of bacteria, forming a significant portion of the microbial community in soil and are known for their production of active secondary metabolites. The geographic variation in Indian soil types and their contents suggests that the distribution of actinomycetes is likely to be variable as well (Siddharth *et al*., 2020). Consequently, investigating uncharted ecosystems for actinomycetes is essential for discovering new secondary metabolites. The aim of the current study was to isolate actinomycetes from various soil samples in mid Karnataka for the purpose of screening actinomycetes of valuable compounds.

MATERIALS AND METHODS

1.1 Collection of Soil samples

Soil samples were collected from various niche habitats in Davangere and Chitradurga districts.
The soil samples were transported to the laboratory in sterile zipper lock bags and maintained at room temperature until further analysis. Soil samples were air-dried for one week at room temperature, crushed using a mortar and pestle to obtain fine particles, sieved, and subsequently used for the isolation of actinomycetes (Gitari *et al*., 2023).

1.2 Pretreatment

The pre-treatment method is an approach to identify potentially valuable actinobacterial communities that are adaptable to various biological habitats. Pretreatment of soil samples enhances the proliferation of dormant actinobacteria while simultaneously eliminating undesirable fungi and bacterial propagules on the primary isolation plate. The pretreatment employed here was simply drying in the air which is proven to eliminate the population of bacteria and fungi (Jiang *et al*., 2016).

1.3 Isolation of actinobacteria

One gram of the soil sample was serially diluted tenfold in sterile distilled water and subsequently plated on starch casein agar (SCA, pH 7.2). The standard spread plate technique was used to evenly distribute a 0.1ml aliquot sample of each dilution from 10^{-2} to 10^{-6} over starch casein agar medium. The plates were cultured at 30°C for 7–14 days until actinomycete colonies appeared. Incubated colonies were kept on starch casein agar slants at 4°C for later use. The cultures were morphologically and biochemically examined (Baig *et al*., 2021).

1.4 Morphological and Microscopic Characterization

The variations in coloration of aerial and substrate mycelia, along with the diffusible pigments at the reverse side of the inoculated plates, were noted. The actinomycetes was confirmed based on the characteristics of mycelium coloration and the presence of diffusible pigments. The structure of the filaments or mycelium was analyzed using light microscopy. The Gram staining was performed to assess the Gram positive and negative nature of the actinomycetes at a magnification of 100X (Skladanowski *et al*., 2017).

1.5 Biochemical Characterization

The biochemical characterization of actinobacterial isolates is conducted to investigate their physiological properties. Actinomycetes employ a diverse range of organic substrates, particularly simple sugars, which they readily utilize without fermentation, leading to an alkaline shift in the media. The biochemical tests

conducted in this study included IMViC tests, catalase activity, H_2S production, citrate utilization, gelatin liquefaction, and carbohydrate utilization (Silavery *et al*., 2025).

1.6 Molecular Characterization

Morphological and biochemical traits of the isolate were studied. The isolate was identified by 16S rRNA gene sequencing. CTAB successfully isolated DNA (Balagurunathan *et al*., 2020). Primers were selected from reported conserved areas of the actinobacterial 16S rRNA gene. The forward and reverse primers (5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-GGTTACCTTGTTACGACTT-3′) were used for sequencing. The optimum PCR conditions were: The 35 cycles included a 30-second denaturation at 95°C and 1-minute elongation at 72°C. To complete DNA strand extension, 72°C was used for 10 minutes (Jiang *et al*., 2016). The 16S rRNA gene sequence was examined using an automated DNA sequencer.

Sequenced nucleotides were modified using MEGA 12 software version 12.0.14 and matched with GenBank 16S rRNA reference sequences using BLAST (NCBI, USA). The SMS-27 isolate and related species 16S rRNA sequences were acquired from NCBI GenBank and aligned using ClustalW. The phylogenetic tree was made using the Neighbour-joining method, and its reliability was determined using the bootstrap method with 1000 replications. MEGA 12 was used for phylogenetics (Larkin *et al*., 2007).

RESULTS

2.1 Collection of Soil Sample

A total of two different soil types were collected from the Davangere, Chitradurga and termite mound soil of Dandeli soil types.

2.2 Pretreatment

The pretreatment method was successful in hindering the growth of bacteria and fungi from the isolation plates. The air-drying pretreatment method employed here yielded actinobacterial colonies on ISP medium for the following soils of the current study as mentioned in the Table 1.

Place	Pretreatment Method	Soil type	No. of colonies	CFU/g
Chitradurga	Air Dry at room temperature	Black soil	06	0.6 x10^3
Davangere	Air Dry at room temperature	Brown soil	07	0.7 x10^3
Dandeli	Air Dry at room temperature	Termite mound soil	01	0.1 x10^3

Table 1: Pretreatment method employed, sampling sites and isolates obtained.

2.3 Isolation of actinobacteria

Actinobacterial isolates were obtained on serial dilution method on Starch casein agar plates and distinguished by their unique colony morphology and Gram staining characteristics. The characterization of these colonies as actinomycetes was confirmed by the presence of small to medium-sized, dry, powdery formations combined with filamentous structures that were heavily branched and Gram-positive. Table 1 provides details of sampling sites and number of colonies and CFU/ml of actinomycetes. The maximum actinomycetes colonies were found in the sample collected from Davangere (07 isolates) and least were in the sample collected from Dandeli soil type (1 isolates).

2.4 Morphological and Microscopic Characterization

The characterization of these colonies as actinomycetes was confirmed by the presence of small to medium-sized, dry, powdery colony types combined with filamentous structures that were heavily branched. The form of the colonies, the color of the aerial and substrate mycelium, and the synthesis of diffusible pigments were all observed. The morphology and color of the actinomycetes isolates varied, ranging from dark grey, light grey, white, light brown, to creamish white. In the starch casein agar medium, light brown, dark brown, pale pink, and purple pigments were seen to be noted.

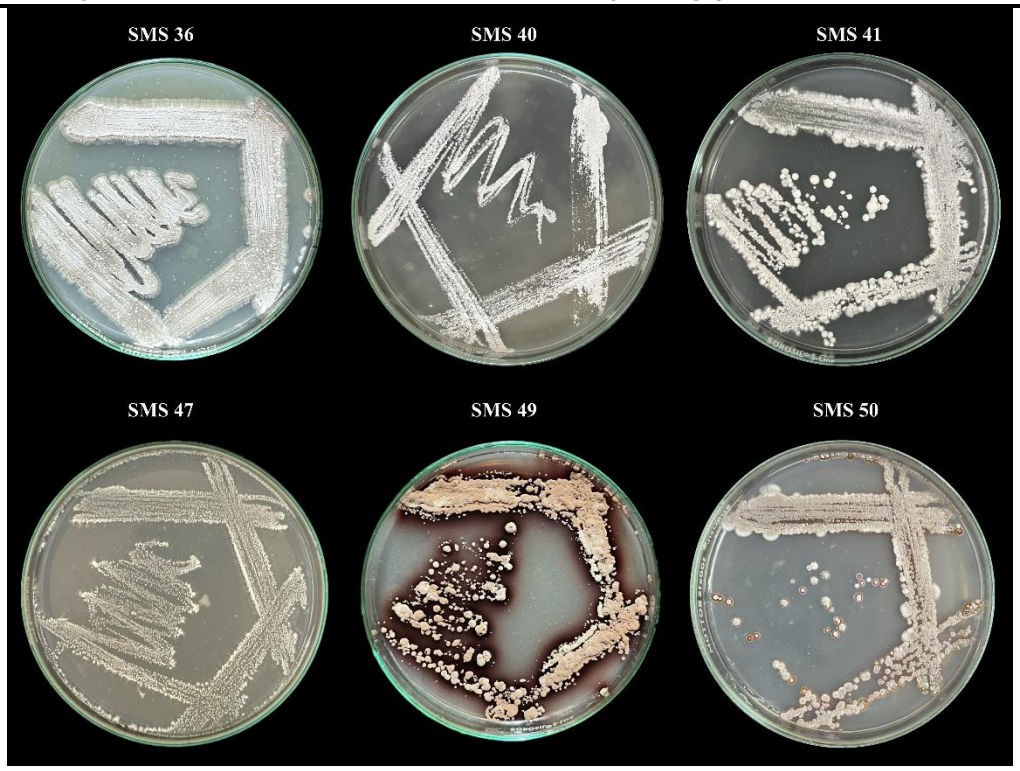

Fig 1: Quadrant streak of the actinobacterial isolates.

2.5 Biochemical characterization

Based on biochemical features, these actinomycetes have unique physiologies, and none of them produced gas in carbohydrate fermentation experiments. Most isolates had negative biochemical test results, except for a few minimal positives for citrate utilization, nitrate reduction, and catalase. With the data and Bergey's handbook, the genus may be identified.

2.6 Molecular characterization

The selected actinobacterial isolate was characterized by partial 16S rRNA gene sequencing using universal actinobacterial primers, yielding an approximate ~300-1300bp length 16S rRNA sequence suitable for phylogenetic analysis. The edited sequence was compared with NCBI GenBank entries using BLAST and aligned with closely related taxa in MEGA 12, and a neighbour-joining tree with 1000 bootstrap replications placed the isolate within the actinomycete clade, supporting its identification at the genus level in agreement with morphological and biochemical data.

Genus	Strain	Close type strain	Similarity %	Accession Number
Streptomyces	SMS 36	*Streptomyces flavogriseus*	97.88%	PV097181
Micromonospora	SMS 37	*Micromonospora thawaii*	98.79%	PV097182
Streptomyces	SMS 39	*Streptomyces noursei*	99.99%	PV097183
Streptomyces	SMS 40	*Streptomyces flavovirens*	98.27%	PV097184
Streptomyces	SMS 41	*Streptomyces griseus*	98.35%	PV097185
Rhodococcus	SMS 43	*Rhodococcus opacus*	99.63%	PV097186
Streptomyces	SMS 44	*Streptomyces olivaceus*	97.78%	PV097187
Streptomyces	SMS 46	*Streptomyces clavuligerus*	99.65%	PV097188
Streptomyces	SMS 47	*Streptomyces sampsonii*	99.77%	PV097189
Streptomyces	SMS 49	*Streptomyces flavogriseus*	99.71%	PV097190
Streptomyces	SMS 50	*Streptomyces olivaceus*	98.71%	PV097191

Table 2: Close relatedness of the isolate, percentage similarity and accession numbers.

DISCUSSION

The recovery of actinomycetes from various soil types using air-drying pretreatment effectively suppresses competing microflora, enriching for slow-growing filamentous actinobacteria. This method is common in actinomycete ecology, favoring desiccation-adapted spore-forming taxa, which explains the prevalence of dry, powdery colonies on starch casein agar. Colony-forming units were highest in Davangere brown soil, followed by Chitradurga black soil, indicating that local soil conditions significantly affect actinomycete populations. Notably, a single isolate from termite mound soil is ecologically significant, as these microhabitats are known to harbor actinomycetes that produce defensive metabolites.

The isolates exhibited morphological diversity in mycelial colors and diffusible pigments, suggesting a wide taxonomic range and potential for secondary metabolite diversity. The biochemical profiles were largely negative, aligning with the slow-growing, oxidative nature of many soil actinomycetes, which supports their classification into a limited number of genera based on Bergey's Manual.

Molecular analysis via 16S rRNA sequencing revealed a dominance of *Streptomyces* spp., along with *Micromonospora* and *Rhodococcus*. High sequence similarity (up to 99.99% with *Streptomyces noursei*) indicates close ties to known species, while similarities around 97–98% may point to novel or divergent taxa. The coexistence of these three genera is significant, as they are known for producing antibiotics, pigments, and extracellular enzymes, including those with clinical and industrial relevance. Although functional screening data is pending, the tax

CONCLUSION

The study demonstrates that mid Karnataka soils, particularly brown and black agricultural soils and termite mound microhabitats, harbour cultivable populations of morphologically and phylogenetically diverse actinomycetes. A combination of simple pretreatment, selective isolation on starch casein agar, phenotypic characterization and 16S rRNA gene sequencing successfully recovered and identified isolates predominantly belonging to *Streptomyces*, with additional *Micromonospora* and *Rhodococcus* strains showing up to 99.99% similarity to established type species.

These results confirm that the sampled environments are valuable reservoirs of actinomycete diversity with likely capacity for bioactive metabolite and enzyme production. Further work should focus on systematic screening of these isolates for antimicrobial, antioxidant and enzyme activities, along with advanced molecular and chemical analyses, to uncover novel compounds of pharmaceutical and industrial significance.

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