

## A Contribution to the Study of Streptomycete Diversity and Bioactive Potential in Unexplored Agricultural Landforms

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### Abstract

Actinomycetes are among the most significant prokaryotes due to their attribute in synthesizing bioactive secondary metabolites. Soil-derived actinomycetes, in particular, are notable candidates for the production of valuable compounds for pharmaceutical and industrial applications. In this investigation, actinomycetes were successfully isolated from the soil sample of agricultural landforms. Four different culture media were utilized to examine the influence of medium composition on the actinomycetes count. Among the tested media, starch casein agar yielded the highest actinomycete densities, ranging from  $1.4 \times 10^6$  to  $6.0 \times 10^6$  cfu g<sup>-1</sup> across all sampling sites. The lowest counts were recorded on Czapek-Dox agar, with values between  $1.0 \times 10^6$  to  $2.3 \times 10^6$  cfu g<sup>-1</sup>. Sample 2 exhibited the maximum average actinomycete density ( $6.0 \times 10^6$  cfu g<sup>-1</sup>), whereas sample 10 showed the lowest density of  $1.4 \times 10^6$  cfu g<sup>-1</sup> on starch casein medium. The investigation revealed that 91.2% of the total isolates were tentatively classified as *Streptomyces* species and 8.8% as non-*Streptomyces* species. Out of 68 isolates, 15 strains were prioritized based on their bioactive potential and underwent morphological, physiological, and biochemical characterization. IA10 and IA17 were found to be more potent against tested clinical pathogens in the right-angle streak method and in 16s rRNA sequencing; both the isolates showed close resemblance with *Streptomyces toxytricini* and *Streptomyces maritimus*, respectively. This study highlights exploring the prevalence of the actinomycetes population in agricultural landforms and isolation of strains with antagonistic properties.

**Keywords:** actinomycetes, starch casein medium, 16s rRNA sequencing, *Streptomyces toxytricini*, *Streptomyces maritimus*

### Резюме

Актиномицетите са сред най-значимите прокариоти поради способността им да синтезират биоактивни вторични метаболити. Получените от почвата изолати са особено забележителни кандидати за производството на ценни съединения за фармацевтични и промишлени приложения. В това изследване актиномицетите са успешно изолирани от почвена проба от земеделски релефни форми. Използвани са четири различни хранителни среди, за да се изследва влиянието на състава на средата върху броя на актиномицетите. Сред тестваните среди, нишестено-казеиновият агар даде най-висока плътност на актиномицетите, варираща от  $1.4 \times 10^6$  до  $6.0 \times 10^6$  cfu g<sup>-1</sup> във всички места за вземане на проби. Най-ниският брой е регистриран в агар Чапек-Докс, със стойности между  $1.0 \times 10^6$  и  $2.3 \times 10^6$  cfu g<sup>-1</sup>. Проба 2 показва максимална средна плътност на актиномицетите ( $6.0 \times 10^6$  cfu g<sup>-1</sup>), докато проба 10 показва най-ниска плътност от  $1.4 \times 10^6$  cfu g<sup>-1</sup> върху среда от нишестен казеин. Изследването разкрива, че 91.2% от общия брой изолати са предварително класифицирани като видове *Streptomyces*, а 8.8% - като видове, различни от *Streptomyces*. От 68 изолати, 15 щам са приоритизирани въз основа на техния биоактивен потенциал и са подложени на морфологична, физиологична и биохимична характеристика. IA10 и IA17 се оказват по-силни срещу тествани клинични патогени при метода на правоъгълната ивица и при 16s rRNA секвениране. Двата изолата

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Acta Microbiol. Bulg. 2026; 42(01). <https://doi.org/10.59393/amb26420116>

показват близко сходство съответно със *Streptomyces toxytricini* и *Streptomyces maritimus*. Това проучване акцентира върху изследването на разпространението на популацията на актиномицетите в земеделските релефни форми и изолирането на щамове с антагонистични свойства.

## Introduction

Actinobacteria have been widely acknowledged as a bountiful source of biopharmaceuticals, particularly antibiotics (Amin *et al.*, 2018; Amin *et al.*, 2019). Actinomycetes constitute the majority of free-living microorganisms that thrive in diverse ecosystems. Actinomycete's hostile behaviour provides proof that the ecosystem is a significant source of secondary metabolites with biological activity. The distinctive nature of actinomycetes in the transformation of organic and inorganic substrates has drawn the attention of researchers to explore open field (Murtaza *et al.*, 2024). Soil serves as the principal habitat for Actinomycetes and acts as a critical site for their interactions with other life forms (Singh and Dubey, 2018).

Actinomycetes are characterized as Gram-positive and heterotrophic saprophytes, which are recognized by significantly synthesizing extracellular enzymes to withstand competing environments (Sapkota *et al.*, 2020) and have a high proportion of G+C base pairing. They exhibit a stable hyphae network formed by filamentous branching. They represent a vital component of microbial communities and have been discovered across a variety of natural terrains, including extreme and harsh environments. Isolation and cultivation of *Actinomycetes* remain challenging by virtue of their ability to produce comprehensive and multifaceted natural compounds with curious bioactivity. Microbiomes from overlooked domains are being looked for in drug discovery (Nouioui *et al.*, 2024).

Recent breakthroughs in molecular biology, genomics, and metagenomics have enabled researchers to tap into the vast untapped potential of actinomycete diversity, revealing new insights into their evolution, ecology, and biochemistry (Baltz, 2017). This has spawned alluring chances for the identification of new bioactive substances, including enzymes and bioactive compounds that can be used in a variety of industrial, pharmaceutical, and agricultural applications. A study by Singh *et al.* (2022) accentuated the sense of Indian soils as a rich source of actinomycete diversity, with a focus on the isolation of novel species from uncharted regions.

Evident from these outcomes, our current investigation spotlights the study on the biodiversity of actinomycetes in and around Namakkal district, India, since it has been given inadequate considera-

tion in microbial diversity research. This territory's unacknowledged morality makes it a desirable topic for current research, which is primed for insights and improvements to the discipline of microbiology. In this research, ten different unresolved agroecological niches in Namakkal district, India, were investigated to isolate *Streptomyces* sp., which has guaranteed bioactivity.

## Materials and Methods

### Sample collection

On a track to isolate novel actinomycetes with high-grade bioactivity, a total of 10 soil samples were collected in and around the Namakkal district, India. The samples were obtained based on the parameters of soil such as pH, Electrical conductivity, moisture content, and organic matters including carbon and nitrogen. Under sterile circumstances, the samples were obtained from a depth of 10 to 15 cm, where the humus content was found to be good. The obtained samples were transferred to the laboratory in sterile zip-lock covers and kept at refrigerated conditions until processing (Kumari *et al.*, 2019).

### Physico-chemical parameter analysis of the soil sample

Physical parameters such as pH, temperature, moisture content, and electrical conductivity were examined for the soil samples using Handheld instruments. TOC was determined by the Walkley–Black wet oxidation method using 0.5 g of soil digested with 1 N  $K_2Cr_2O_7$  (Merck) and concentrated  $H_2SO_4$  (Sigma-Aldrich), followed by titration with 0.5 N  $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$  (HiMedia) using diphenylamine indicator. TN was estimated by the Kjeldahl method, in which 0.5 g of soil was digested with  $H_2SO_4$  (Merck) and a  $K_2SO_4$ : $CuSO_4$ :Se (10:1:0.1) catalyst (HiMedia), distilled using a Kjeltect™ 8200 unit (Foss Analytical), and titrated with 0.01 N  $H_2SO_4$  after ammonia absorption in 4% boric acid. Results were expressed as % TOC and % TN on a dry-weight basis (Dandwate *et al.*, 2020). The relationship between the physicochemical parameters of soil and the population density of actinomycetes was evaluated using Pearson's correlation coefficient.

### Pretreatment of soil samples

The soil samples obtained from the sampling sites were pre-treated before processing to isolate actinomycetes. The step was initiated by mixing

the soil sample in a solution containing 6% yeast extract (YE) and 0.05% sodium dodecyl sulphate (SDS) at 40°C for 20 minutes (Hayakawa and Nonomura, 1989). One gram of processed soil was admixed with 9.0 ml of sterile distilled water. 1.0 ml of this preparation was serially diluted up to 10<sup>-6</sup> logarithmic dilutions and used for plating purposes to isolate a pure culture.

#### *Growth promotion medium selection*

Starch Casein Agar (SCA) (g/l) Starch 10.0; Casein 1.0; K<sub>2</sub>HPO<sub>4</sub> 2.0; KNO<sub>3</sub> 2; MgSO<sub>4</sub>·7H<sub>2</sub>O 0.05; FeSO<sub>4</sub>·7H<sub>2</sub>O 0.01; CaCO<sub>3</sub> 0.02; NaCl 2; Agar 20.0; distilled water; pH 7.2.

Actinomycetes Isolation Agar medium (AIA) (g/l) (Sodium caseinate 2; L-Asparagine 0.1; C<sub>3</sub>H<sub>5</sub>NaO<sub>2</sub> 4; K<sub>2</sub>HPO<sub>4</sub> 0.5; MgSO<sub>4</sub> 0.1; FeSO<sub>4</sub> 0.001; Agar 20; distilled water 1L.

Glycerol Asparagine Agar (GAA) (g/l) (L-asparagine, 1.0; glycerol, 10.0; K<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O 1.0; agar 20.0).

Czapek-Dox Agar (g/l) (Sucrose 30; NaNO<sub>3</sub>, 3; K<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O 1.0; MgSO<sub>4</sub> 0.5; KCl, 0.5; FeSO<sub>4</sub> 7H<sub>2</sub>O 0.01; Agar 20.0.

These media were infused with nalidixic acid 30 µg/ml and cycloheximide 50 µg/ml (Van Hop *et al.*, 2011). Prepared media were autoclaved, cooled, and dispensed into petri dishes. The sterile media plates were maintained under refrigeration in a sterile condition until use. The spread plate technique was employed for plating of soil samples. 100 µl of the prepared dilutions were disseminated on the prepared selective agar media in Petri plates. After inoculation, the processed plates were incubated at 30°C for 1 to 2 weeks. Following the period of incubation for 7 days, prototypical actinomycete colonies were enumerated for the determination of total actinomycete populations.

#### *Screening of a potent strain*

Repeated sub-culturing on Actinomycetes Isolation Agar medium was used to purify the culturable actinomycetes colonies cultivated from each soil sample until pure cultures were obtained and verified by microscopic inspection and colony morphology. The right-angle streak method was initially carried out to evaluate the actinomycetes strain's antibacterial properties (Madigan *et al.*, 1997). After streaking the isolates' axenic cultures on assay media and leaving them undisturbed at room temperature for a four to seven-day period, test bacterial strains *Escherichia coli* MTCC 3261, *Pseudomonas aeruginosa* MTCC 741, *Klebsiella pneumoniae* MTCC 3040, *Enterobacter aerogenes*

MTCC 7724, *Staphylococcus aureus* MTCC 3103, and *Streptococcus pyogenes* MTCC 9724 were sown at a 90° angle. The inhibitory zones were measured in millimetres after the plates were kept undisturbed for a period of 1 to 2 days at 28°C. For additional characterization, isolates with moderate to good inhibitory activity were chosen.

#### *Phenotypic identification*

##### *Spore chain morphology*

A light microscope with a 40x objective lens and a scanning electron microscope (SEM) were used to view the morphology of the spore chains. Spore chain-bearing aerial mycelium was placed on a glass slide suspended in a drop of water, covered with a coverslip, and examined with the aid of a light microscope. Samples were dehydrated using graded ethanol, coated with 2.5% glutaraldehyde, and fixed for SEM (Konwar *et al.*, 2023).

Morphological and cultural analysis of actinomycetes on various growth media

The method framed by Shirling and Gottlieb (1966) and Kampfer (2006) was used to assess the producer strain's cultural traits. The strains were likely nurtured on various International Streptomyces Project (ISP) media, including Oat Meal Agar (ISP-1), Yeast extract-Malt extract Agar (ISP-2), Tryptone-Yeast Extract Agar (ISP-3) Inorganic salt Starch Agar (ISP-4), Glycerol Asparagine Agar (ISP-5), Peptone Yeast extract Iron agar (ISP-6), and Tyrosine Agar (ISP-7) (HiMedia Laboratories Pvt.Ltd., Mumbai, India). The synthesis of diffusible pigments, chromatic features of the colony, aerial and substrate mycelial color, growth pattern, and growth intensity were among the cultural traits noted from ISP2 medium, which provides optimum pigment differentiation. The colony colors were visually determined under natural light using the ISCC-NBS Color System (Kelly and Judd, 1976) as a standard color reference (Sun *et al.*, 2020)

##### *Physiological and biochemical analysis*

Biochemical and physiological testing were conducted using the procedures outlined by Williams *et al.* (1983). Tests were conducted to determine physiological characteristics, including ideal pH (6–10), temperature (25°–40°C), and salinity (1–8%). Furthermore, biochemical characterization, i.e., Voges-Proskauer, citrate, methyl red, and indole production tests, and sugar utilization tests were conducted.

##### *Screening for enzymatic activity*

The actinomycetes strains were investigated on harnessing the enzymatic potential in synthesiz-

ing a range of extracellular enzymes, viz., amylase using Starch Agar Medium (HiMedia M107), protease (Skim Milk Agar, HiMedia M557), cellulase (Carboxymethyl Cellulose Agar, HiMedia M1651), lipase (Tributyryn Agar, HiMedia M043), gelatinase (Nutrient Gelatin Medium, HiMedia, M009), urease (Urea Agar Base, HiMedia, M112), and catalase using 3% of H<sub>2</sub>O<sub>2</sub> (Prescott *et al.*, 2002).

### Hierarchical clustering

Strains ascertained to be potent from primary screening were subjected to hierarchical clustering using Python (V3.10) with Scipy and Matplotlib libraries. Dissimilarities were calculated using the Euclidean distance metric. Clustering was conducted using Ward's method to minimize within-cluster variance. A dendrogram was generated to visualize relationships among strains.

### Phylogenetic identification of a potent strain

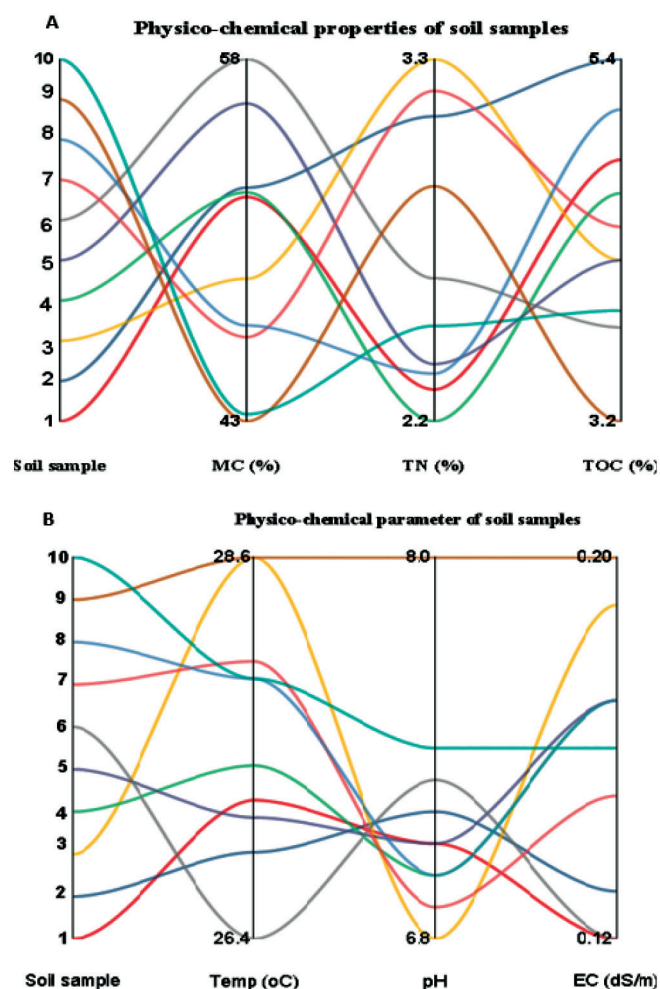
Based on the antimicrobial activity of the crude extract of the selected strain, the genotypic identification of the strains IA10 and IA17 was done by sequencing the 16S rRNA gene. Extraction of genetic material was carried out with the CTAB method according to the instructions of the manufacturer. Two universal primers (27F 5'-AGAGTTTGATCCTGGCTCAG-3'; 1492R 5'-GGTTACCTTGTTACGACTT-3') were selected for amplification of PCR reactions. By comparing and investigating the similarity and the homology of the obtained sequence of the isolate IA10 and IA17 with the equivalent gene sequences available in the NCBI database by using the BLAST online search tool and phylogenetic tree was constructed using MEGA 11 software (Zeng *et al.*, 2024).

## Results and Discussion

The physico-chemical properties of soils from sampling sites of Namakkal district, Tamil Nadu, were analyzed, revealing variations in soil texture, organic matter concentration, pH, and nutrient content (Fig. 1A and 1B).

The total actinomycete populations in the collected soil samples were determined. The results indicated that from the total isolation highest population of actinomycetes was recorded in sample 2 (15.2%), followed by sample 3 (13.8%) and sample 5 (11.6%). The lowest population density was found in sample 6 and 10 (5.8%) (Fig. 2). A correlation analysis between the physico-chemical properties of the bulk soil samples and the population density of actinomycetes revealed significant positive correlations between actinomycetes population density and pH ( $r = 0.82, p < 0.05$ ) which shows the

indication of alkaline conditions favor the proliferation of actinomycetes, organic matter content (carbon) exhibited positive correlation ( $r = 0.67, p < 0.05$ ) suggesting higher organic matter supports the microbial growth and metabolic activity and moisture content ( $r = 0.38, p < 0.05$ ) implying adequate soil moisture enhances survival of actinomycetes. The data obtained from the investigation showed that the physico-chemical parameter of soil has impact on microbial population dynamics.

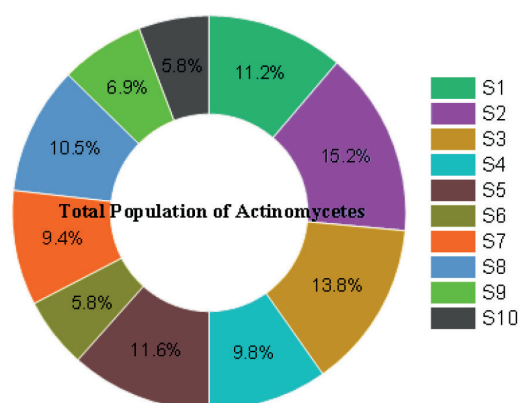


**Fig. 1.** Physicochemical properties of different soil samples: A. Dependence on moisture content (MC), total nitrogen (TN), and total organic carbon (TOC); temperature (°C), pH, and electrical conductivity (EC)

In particular, the pH of the soil has a positive correlation with the population of actinomycetes obtained from the sample. Actinobacteria predominantly bloom in slightly alkaline and neutral pH. Actinomycetes populations are remarkably higher in soil with pH levels of 7.0 to 8.0 (Zanane *et al.*, 2024). In extremely acidic soils with a pH less than 5.0, the actinomycetes population tends to be reduced more likely due to reduced nutrient availability and in harsh environments. The moisture content of the soil is also known to influence microbial activity, particularly actinobacteria, and it

prefers moderately damp soil. Reduced moisture level of the soil found to have a decreased number of actinomycetes (Chungopast *et al.*, 2023). The population density of actinomycetes may decline in extreme dry conditions due to water stress, and increased moisture levels may restrict the availability of oxygen; as a result, it lowers the activity of actinomycetes (Borowik and Wyszowska, 2016). Actinomycetes prevail in soils with balanced moisture content, which reinforces both the spore development and their metabolic activities.

Soil sample vs Population of Actinomycetes



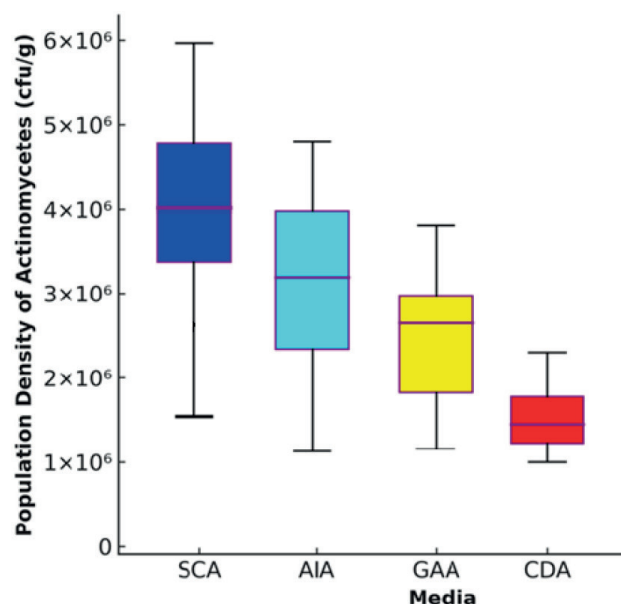
**Fig. 2.** Actinomycetes population in different soil samples (S1-S10 – Soil sample 1 to 10)

Another critical factor that reinforces the growth and dispersion of actinomycetes in soil is temperature. Actinomycetes are ordinarily boon in moderate temperatures between 25°C and 35°C, and temperatures outside of this range may lead to a decline in the actinomycetes population (Barka *et al.*, 2016). Cold and excessively hot soils inhibit the development of actinomycetes due to their sensitivity to extreme ranges of temperature. Research has indicated a notable increase in actinomycete diversity and population in temperate and tropical soils with moderate temperature ranges (Adegboye and Babalola, 2012).

Soil organic material is found to be a crucial factor for the growth of actinomycetes as it provides a source of carbon and energy. An affirmative correlation between soil organic matter and actinomycetes population has been reported in several studies (Binayke *et al.*, 2018). Actinomycetes, particularly *Streptomyces* species, are exceptionally productive in decomposing organic materials like plant residues, contributing to nutrient cycling in the soil. Soils productive of organic matter usually support a higher diversity of actinomycetes due to the abundance of substrates available for microbial breakdown (Barka *et al.*, 2016). Organic matter content is a supreme indicator of soil quality degra-

dation. This organic matter contributes to soil stability, high water retention ability, mineral element fixation, and substrate for the microorganisms of the soil (Mohammadi *et al.*, 2011).

In general, the actinomycete population in the study area varied depending on the media, which served as the exploration test to expound the impact of the medium components on the actinomycete count and to select the one that allows the maximum counts. The population density of actinomycetes varied significantly among the different soil samples and culture media (Fig. 3).



**Fig. 3.** Population density of Actinomycetes with respect to different growth media

SCA- Starch Casein Agar, AIA- Actinomycetes Isolation Agar, GAA – Glycerol Asparagine Agar and CDA – Czapek-Dox Agar

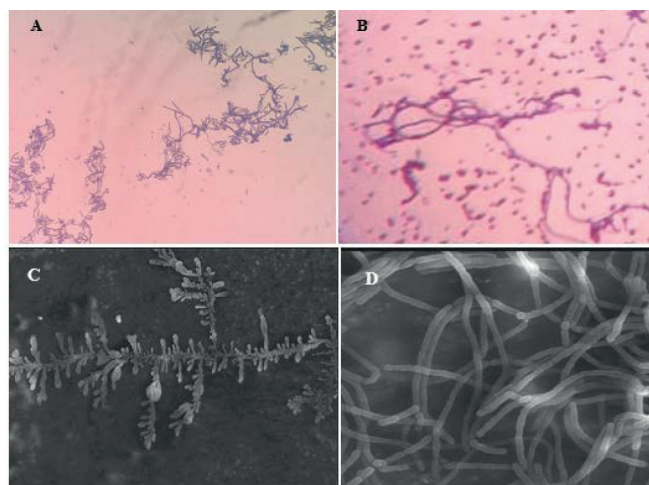
The starch casein agar medium supported the highest density of actinomycetes, ranging from  $1.4 \times 10^6$  to  $6.0 \times 10^6$  cfu g<sup>-1</sup> across all sampling sites. In contrast, the Czapek Dox agar medium exhibited comparatively lower densities, ranging between  $1.0 \times 10^6$  and  $2.3 \times 10^6$  cfu g<sup>-1</sup>. Among the samples, Sample 2 showed the maximum actinomycete density ( $6.0 \times 10^6$  cfu g<sup>-1</sup>), followed by Sample 3 ( $5.6 \times 10^6$  cfu g<sup>-1</sup>) on starch casein agar. The lowest population density was observed in Sample 10 ( $1.4 \times 10^6$  cfu g<sup>-1</sup>). These results indicate that starch casein agar was more effective in supporting the growth of actinomycetes than other tested media. Our outcomes illustrate that starch casein agar was the preeminent medium in yielding a far greater number of actinomycetes colonies compared to other media. The outcome of the research illustrated the starch casein agar medium as being preeminent in buttressing the development of actinomycetes, with a greater

number of colonies compared with other media. This output highlighted the efficacy of starch casein agar for the isolation of actinomycetes (Ayoyib *et al.*, 2024). Jessy Padma *et al.* (2018) concluded that actinomycetes strains isolated from terrestrial soils flourished successfully on starch casein media. An analogous study by Ahmad *et al.* (2017) determined that a biodiversity assessment of actinomycetes from different circumstances in the Mediterranean Sea reported the greatest number of isolates and the highest count on starch casein agar. The efficacy of starch casein agar with enhanced effectiveness can be attributed to its distinctive composition, which includes starch, an accessible carbon source, while casein in the medium supplies essential nitrogen.

#### Characterization of the strain

A deliberate survey of actinomycetes in Namakkal district has led to the isolation of 276 isolates, among which 68 showed distinguished colony features and were grouped for preliminary identification. The isolates were recognized based on their aerial and substrate mycelial color, microscopic inspection of spore chain sequences (spirals, rectiflexibles, flexibles, and monospore chains), and pigment production. Microscopic examination following Gram staining revealed branched filamentous hyphae with purple and deep violet color (Fig. 4A, 4B). The outcome of the research revealed that 62 (91.2%) of the 68 isolates were designated as *Streptomyces* species, characterized by spiral, hooked, and rectiflexible spore chain patterns. The active isolate IA10 under SEM exhibited well-developed mycelia with spores arranged along the filament surface, and appeared to be rectiflexible types. IA17 revealed a compact, extensively interwoven mycelial structure (Fig. 4C, 4D). The residual six isolates (8.8%) have been confirmed as non-*Streptomyces* species. *Streptomyces* spp. comprised the soil sample examined in the current research, which is concordant with previous findings (Singh *et al.*, 2016; Ahmad *et al.*, 2017; Dar *et al.*, 2024).

This finding reinforces the vital role of *Streptomyces* spp. in soil ecosystems, as they are the biggest contributors to decomposing organic matter and fixing nitrogen, as well as producing secondary metabolites with antimicrobial activity and antifungal abilities (Javed *et al.*, 2021). The diversity of *Streptomyces* spp. in soil is influenced by a range of factors, which include the type of soil, pH, temperature, moisture level, and its organic matter. In this work, the soil sample was obtained in a tropical climate, which may have facilitated the growth of certain *Streptomyces* species.



**Fig. 4.** Light microscope and SEM observation of *Streptomyces*: A – *S. toxytricini* IA10, B – *S. maritimus* IA17, C – *S. toxytricini* IA10, D – *S. maritimus* IA17

#### Screening of the active strain

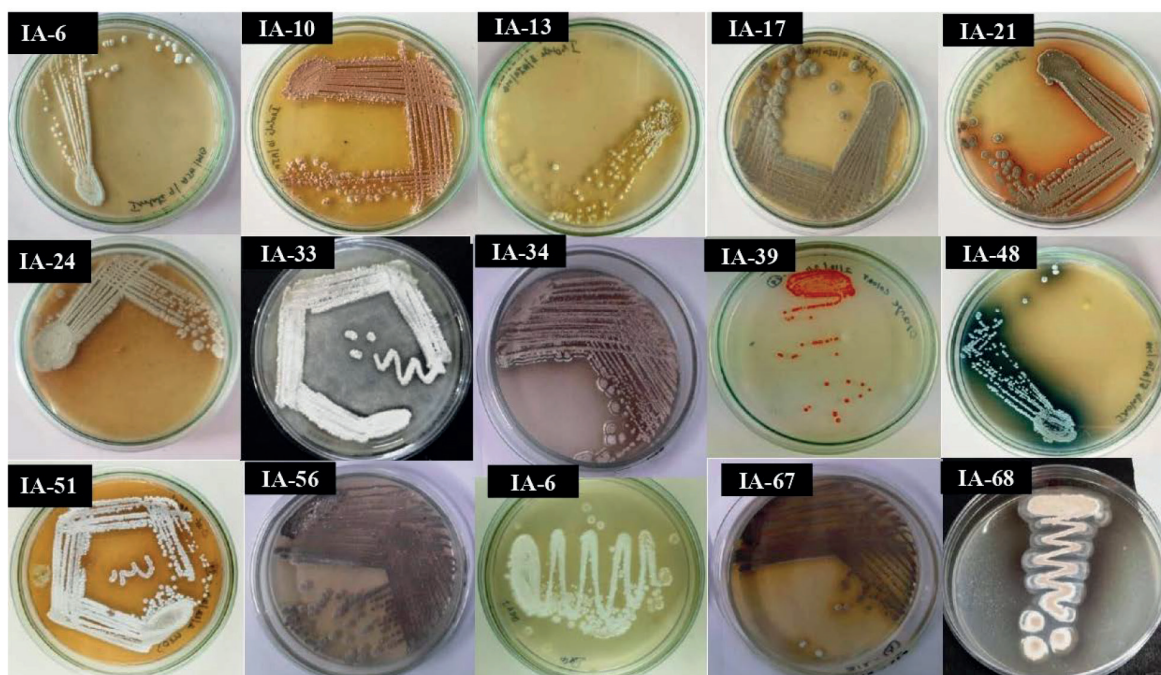
Actinomycetes colonies displaying unique morphological attributes had been selected for primary screening (Fig. 5).

Among the 68 total isolates, 15 showed prominent antimicrobial effect against both Gram-positive and Gram-negative bacteria, with IA10 and IA17 displaying the highest activity (Fig. 6A, 6B). The screening results revealed that the majority of the isolates had antibiotic activity, prompting further investigation. As a result, the active isolates were chosen to receive additional taxonomic, biochemical, and physiological characterization to determine their antibacterial potential.

#### Biochemical and physiological characterization

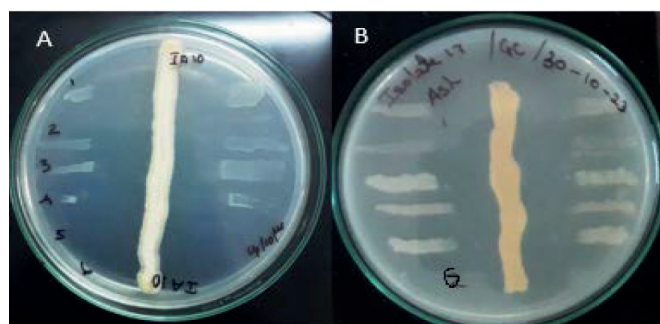
The cultural facets, which include microbial growth pattern and pigment formation, were assessed on different International *Streptomyces* Project (ISP) media, and their results are summarized in Table 1. All the chosen actinomycete isolates demonstrated moderate to abundant growth with different growth patterns. The pigment of the substrate mycelium showed variation and was found mostly to be white and grey. Two isolates express beige pigment, one produces blue pigment, three produce yellow pigment, one strain shows orange, and one produces brownish-black pigment. The presence of diffused brown-to-black coloration indicates the presence of melanin pigment formation. This illustrates the capability of the medium in supporting pigment production due to the source of nutrient composition of the media (Kazi *et al.*, 2022).

All the isolates grew well on oatmeal agar medium and optimally between 25°C -40°C and the majority of the isolates showed good growth at 30°C, with the optimum pH value between 7-8,



**Fig. 5.** Representatives of isolated Streptomyces from soil

which was related to the study by Bhanu Shrestha *et al.* (2021). All the tested isolates produced amylase, while the majority produced protease and cellulase. Except for the strains IA6, IA24 and IA33 the selected strains possessed the ability to tolerate 4% NaCl in the medium with the exception of strains.



**Fig. 6.** Antagonistic activity of potent strains against test organisms: A: *S. toxytricini* IA10, B: *S. maritimus* IA17  
Test organisms in A and B: 1. *E. coli* MTCC 3261, 2. *P. aeruginosa* MTCC 741, 3. *K. pneumoniae* MTCC 3040, 4. *E. aerogenes* MTCC 7724, 5. *S. aureus* MTCC 3103 and 6. *S. pyogenes* MTCC 9724

#### Hierarchical Cluster analysis

A hierarchical cluster analysis of 15 Actinomycetes strains showed that they can be grouped into four main categories when the dissimilarity level is about five (Fig. 7).

Cluster I included IA10, IA17, IA39, IA21, and IA56, with IA10 and IA17 being the most similar to each other in this group. Cluster I possessed IA10, IA17, IA39, IA21, and IA56, with IA10 and IA17 sharing the highest similarities within the group. Cluster II comprised IA6 and IA24, which were closely interrelated and remarkably similar

to Cluster I. Cluster III displayed IA63 and IA67, whereas Cluster IV comprised IA51 and IA68, the most strongly connected pair overall. Strains IA33, IA13, IA34, and IA48 did not fit into the main clusters and integrated at higher dissimilarity levels, exhibiting significant genetic diversity.

#### Molecular characterization

Genomic DNA of the isolates IA10 and IA17 was extracted according to the standard protocols, and 16S rRNA sequences were amplified with the appropriate primers in PCR. A single discrete PCR amplicon band of □1163bp and □1047bp was observed in the isolates IA10 and IA17, respectively, when resolved on agarose gel (Fig. 8).

According to 16S rRNA sequence analysis, isolate IA10 and IA17 were analyzed in Blast and revealed the highest homology. The results demonstrated that the isolate IA 10 was similar to *S. toxytricini* with 95.37% similarity, and IA17 was similar to *S. maritimus* with 97.32% similarity. The sequence of the isolates was deposited in the GeneBank depository as *S. toxytricini* IA10 and *S. maritimus* IA17 with the accession numbers PP758842 and PP758807, respectively, and the phylogenetic tree was constructed as shown in (Fig. 9A, 9B). This result is in line with previous research, where *Streptomyces* sp. recovered from different geographical niches of India was superior among distinct genera with antimicrobial potential (Singh *et al.*, 2016).

#### Conclusion

This study firmly supports the idea that the viable count of actinomycetes showed substantial

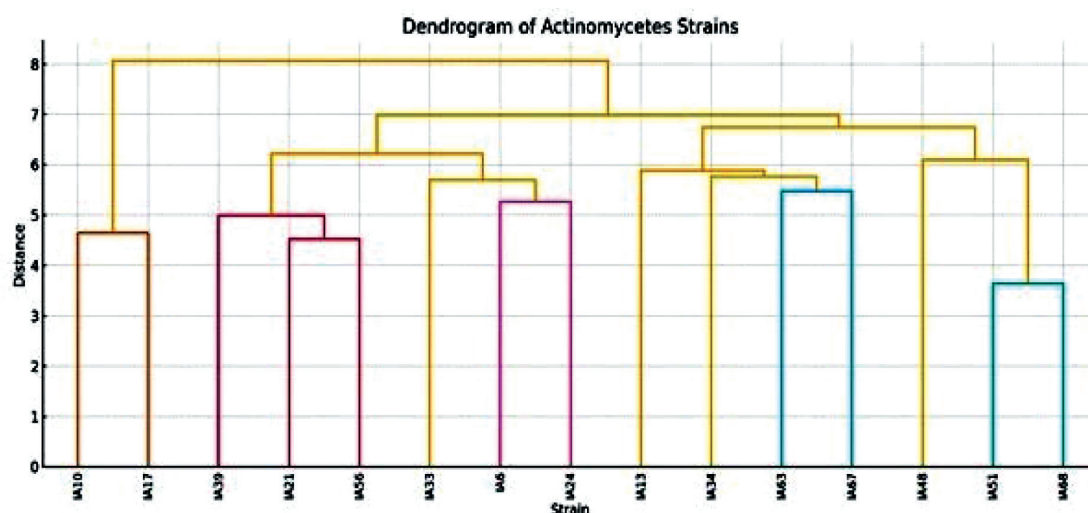
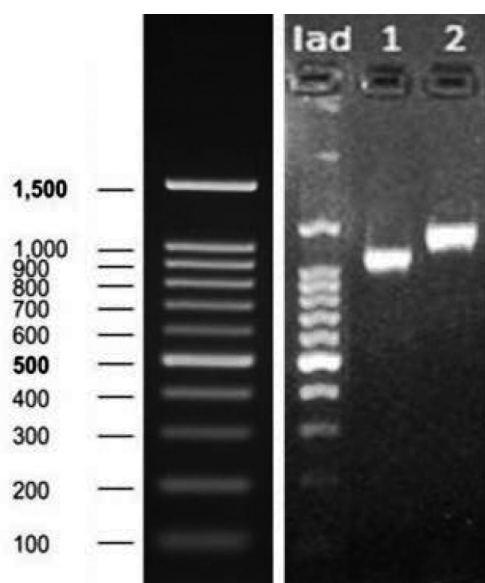
**Table 1.** Biochemical and Physiological characteristics of Streptomycete isolates

|                                                                   |                    | Characteristics |       |              |       |               |               |                |                |        |       |       |       |              |                |       |
|-------------------------------------------------------------------|--------------------|-----------------|-------|--------------|-------|---------------|---------------|----------------|----------------|--------|-------|-------|-------|--------------|----------------|-------|
|                                                                   |                    | IA6             | IA10  | IA13         | IA17  | IA21          | IA24          | IA33           | IA34           | IA39   | IA48  | IA51  | IA56  | IA63         | IA67           | IA68  |
| Growth Pattern                                                    | ISP1               | 1               | 3     | 3            | 3     | 2             | 3             | 3              | 1              | 2      | 1     | 2     | 3     | 2            | 1              | 2     |
|                                                                   | ISP2               | 2               | 3     | 2            | 3     | 3             | 3             | 2              | 1              | 3      | 1     | 2     | 2     | 2            | 2              | 2     |
|                                                                   | ISP3               | 2               | 2     | 2            | 3     | 2             | 2             | 2              | 2              | 2      | 2     | 3     | 2     | 2            | 3              | 2     |
|                                                                   | ISP4               | 2               | 3     | 1            | 2     | 2             | 1             | 1              | 2              | 1      | 2     | 2     | 2     | 1            | 2              | 3     |
|                                                                   | ISP5               | 1               | 2     | 1            | 2     | 2             | 2             | 1              | 2              | 1      | 1     | 1     | 1     | 1            | 1              | 2     |
|                                                                   | ISP6               | 2               | 3     | 2            | 3     | 1             | 3             | 2              | 2              | 1      | 2     | 2     | 2     | 2            | 2              | 2     |
|                                                                   | ISP7               | 2               | 3     | 2            | 3     | 1             | 3             | 2              | 1              | 2      | 2     | 1     | 3     | 1            | 2              | 3     |
| Colony color                                                      | Aerial mycelium    | White           | Beige | White        | Gray  | Gray          | Grayish white | Milky white    | Gray           | Orange | White | White | Gray  | White        | Gray           | Beige |
|                                                                   | Substrate mycelium | Pale yellow     | Gray  | Creamy white | Brown | Reddish brown | Brown         | Greenish black | Blackish brown | Yellow | Blue  | Brown | White | Creamy white | Yellowish gray | Black |
|                                                                   | Melanin production | No              | No    | No           | Yes   | Yes           | Yes           | Yes            | Yes            | No     | No    | Yes   | No    | No           | Yes            | Yes   |
| pH tolerance                                                      | 6                  | 1               | 2     | 1            | 2     | 2             | 2             | 1              | 1              | 2      | 2     | 2     | 2     | 2            | 1              | 2     |
|                                                                   | 7                  | 3               | 3     | 2            | 3     | 3             | 3             | 2              | 3              | 3      | 3     | 3     | 3     | 3            | 3              | 3     |
|                                                                   | 8                  | 2               | 3     | 3            | 3     | 2             | 3             | 3              | 2              | 2      | 3     | 2     | 3     | 2            | 2              | 2     |
|                                                                   | 9                  | 1               | 1     | 2            | 2     | 1             | 1             | 2              | 1              | 2      | 2     | 1     | 2     | 2            | 1              | 1     |
|                                                                   | 10                 | 1               | 1     | 1            | 1     | 1             | 1             | 2              | 1              | 1      | 1     | 0     | 1     | 1            | 1              | 0     |
| NaCl tolerance                                                    | 1%                 | 3               | 3     | 3            | 3     | 2             | 3             | 2              | 3              | 2      | 3     | 2     | 2     | 3            | 3              | 2     |
|                                                                   | 2%                 | 1               | 3     | 3            | 3     | 1             | 2             | 2              | 3              | 2      | 3     | 2     | 2     | 3            | 2              | 2     |
|                                                                   | 4%                 | 0               | 2     | 2            | 3     | 1             | 0             | 0              | 3              | 1      | 2     | 1     | 1     | 2            | 1              | 1     |
|                                                                   | 6%                 | 0               | 2     | 1            | 2     | 0             | 0             | 0              | 2              | 0      | 1     | 0     | 1     | 2            | 1              | 0     |
|                                                                   | 8%                 | 0               | 2     | 1            | 1     | 0             | 0             | 0              | 1              | 0      | 0     | 0     | 0     | 1            | 0              | 0     |
| Growth temperature                                                | 25°C               | 2               | 2     | 3            | 2     | 3             | 3             | 2              | 2              | 2      | 2     | 2     | 3     | 2            | 2              | 2     |
|                                                                   | 30°C               | 3               | 3     | 3            | 3     | 3             | 3             | 3              | 3              | 3      | 3     | 3     | 3     | 3            | 3              | 3     |
|                                                                   | 35°C               | 2               | 3     | 3            | 3     | 2             | 2             | 2              | 2              | 2      | 3     | 3     | 2     | 3            | 3              | 3     |
|                                                                   | 40°C               | 1               | 2     | 2            | 2     | 1             | 1             | 2              | 1              | 1      | 1     | 1     | 2     | 2            | 2              | 1     |
| Enzyme Production                                                 | Amylase            | 2               | 3     | 3            | 3     | 2             | 2             | 2              | 3              | 2      | 3     | 3     | 2     | 2            | 2              | 3     |
|                                                                   | Urease             | 0               | 3     | 2            | 3     | 0             | 0             | 0              | 0              | 1      | 0     | 0     | 1     | 1            | 0              | 0     |
|                                                                   | Protease           | 2               | 3     | 1            | 2     | 3             | 2             | 2              | 1              | 2      | 3     | 1     | 3     | 1            | 1              | 2     |
|                                                                   | Cellulase          | 1               | 2     | 2            | 3     | 2             | 2             | 2              | 1              | 2      | 1     | 2     | 3     | 1            | 2              | 2     |
|                                                                   | Lipase             | 0               | 3     | 1            | 2     | 1             | 0             | 1              | 0              | 1      | 2     | 0     | 0     | 1            | 0              | 1     |
|                                                                   | Gelatinase         | 0               | 1     | 0            | 1     | 0             | 0             | 0              | 0              | 1      | 1     | 1     | 0     | 1            | 1              | 1     |
|                                                                   | Catalase           | 0               | 3     | 1            | 3     | 1             | 1             | 1              | 0              | 1      | 0     | 1     | 1     | 0            | 1              | 0     |
| 0- No growth, 1- Poor growth, 2- Moderate growth, 3- Heavy growth |                    |                 |       |              |       |               |               |                |                |        |       |       |       |              |                |       |

**Table 1.** Biochemical and Physiological characteristics of Streptomycete isolates

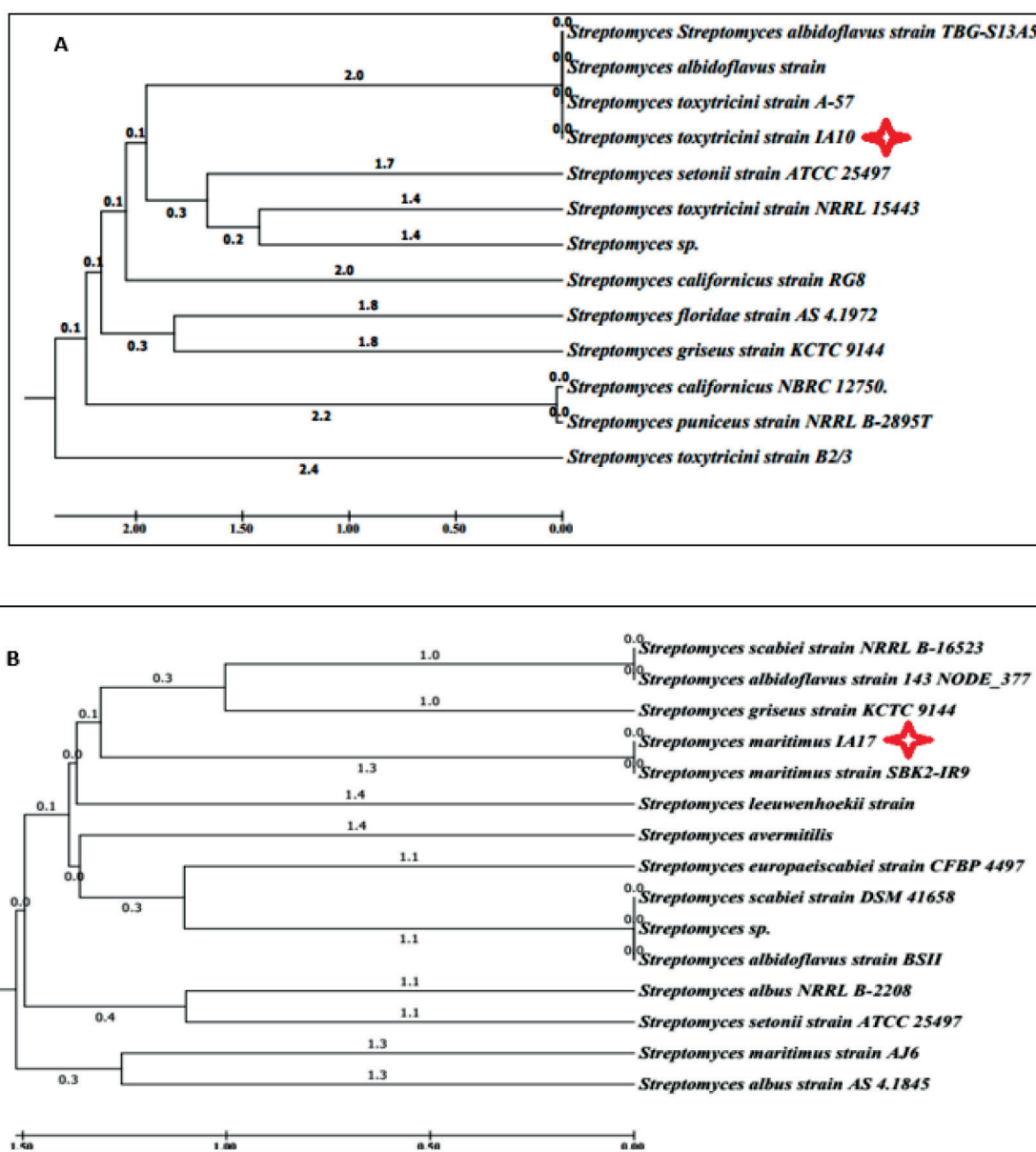
| Characteristics   |           | IA6 | IA10 | IA13 | IA17 | IA21 | IA24 | IA33 | IA34 | IA39 | IA48 | IA51 | IA56 | IA63 | IA67 | IA68 |
|-------------------|-----------|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Biochemical test  | Indole    | +   | +    | +    | +    | -    | -    | -    | -    | +    | +    | +    | -    | +    | -    | -    |
|                   | MR        | -   | -    | +    | +    | +    | -    | -    | +    | +    | -    | -    | +    | +    | +    | -    |
|                   | VP        | +   | -    | -    | -    | -    | +    | +    | -    | -    | -    | -    | -    | -    | -    | +    |
|                   | Citrate   | +   | -    | +    | +    | -    | -    | +    | +    | -    | +    | +    | -    | -    | +    | +    |
|                   | Glucose   | +   | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Sugar Utilization | Galactose | +   | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
|                   | Lactose   | +   | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
|                   | Maltose   | -   | +    | +    | +    | +    | +    | -    | +    | +    | +    | +    | -    | +    | +    | +    |
|                   | Sucrose   | +   | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | -    | +    | +    | +    |
|                   | Starch    | +   | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
|                   | Raffinose | -   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |

‘+’ indicates positive and ‘-’ indicates negative

**Fig. 7.** Hierarchical clustering based on physiological-biochemical characterization**Fig. 8.** Identification of PCR product in agarose gel electrophoresis.

Lane 1 – *S. toxytricini* IA10; Lane 2 – *S. maritimus* IA17

variation across regions. The actinomycete species isolated from various agro-ecological landforms exhibit an outstanding ability to produce bioactive compounds that are active against drug-resistant bacterial pathogens. Phylogenetic analysis of the strain found that the strain is in the genus *Streptomyces*. This research despite the great deal of available research data on exploration of actinomycetes and their secondary metabolites, there remains a tremendous source for discovering new bioactive agents from the genus with respect to diversity investigation, abundance of the unexplored soil in Namakkal district, India. Future interdisciplinary initiatives featuring the fields of microbiology, genomics, metabolomics, chemistry of bioactive compounds, and drug science are needed to thoroughly investigate this untapped resource in remote regions.



**Fig. 9.** Phylogenetic tree based on 16s rRNA sequencing: A – *S. toxytricini* IA10; B – *S. maritimus* IA17

## Acknowledgment

The author would like to acknowledge K. S. Rangasamy College of Arts and Science (Autonomous), Tiruchengode, Namakkal, Tamil Nadu, India, for supporting and facilitating the exploration of the research.

## References

- Adegboye, M. F., O. O. Babalola (2012). Taxonomy and ecology of antibiotic producing actinomycetes. *Afr. J. Agric. Res.* **7**: 2255-2261. <https://doi.org/10.5897/AJARX11.071>.
- Ahmad, M. S., A. O. El-Gendy, R. R. Ahmed, H. M. Hassan, H. M. El-Kabbany, A. G. Merdash (2017). Exploring the antimicrobial and antitumor potentials of *Streptomyces* sp. AGM12-1 isolated from Egyptian soil. *Front. microbiol.* **8**: 438. <https://doi.org/10.3389/fmicb.2017.00438>.
- Amin, D., A. Abolmaaty, S. Tolba, N. Abdallah, E. M. Wellington (2018). Phylogenetic characteristics of a unique antagonistic *Micromonospora* sp. Rc5 to *Staphylococcus aureus* isolated from Sinai Desert of Egypt. *Annu. Res. Rev. Biol.* **22**: 1–5. <https://doi.org/10.9734/ARRB/2018/38318>.
- Amin, D. H., A. Abolmaaty, C. Borsetto, S. Tolba, N. A. Abdallah, E. M. Wellington (2019). In silico genomic mining reveals unexplored bioactive potential of rare actinobacteria isolated from Egyptian soil. *Bull. Natl. Res. Cent.* **43**: 78. <https://doi.org/10.1186/s42269-019-0121-y>.
- Ayoib, A., S. C. Gopinath, N. S. Zambry, A. R. Yahya (2024). Screening biosurfactant-producing actinomycetes: Identification of *Streptomyces* sp. RP1 as a potent species for bioremediation. *J. Basic Microbiol.* **64**: 2300585. <https://doi.org/10.1002/jobm.202300585>.
- Baltz, R. H. (2017). Gifted microbes for genome mining and natural product discovery. *J. Ind. Microbiol. Biotechnol.* **44**: 573-588. <https://doi.org/10.1007/s10295-016-1815-x>.
- Barka, E. A., P. Vatsa, L. Sanchez, N. Gaveau-Vaillant, C. Jacquard, H. P. Klenk, C. Clement, Y. Ouhdouch, G. P. van Wezel (2016). Taxonomy, physiology, and natural products of *Actinobacteria*. *Microbiol. Mol. Biol. Rev.* **80**: 1-43. <https://doi.org/10.1128/mmbr.00019-15>.
- Binayke, A., S. Ghorbel, N. Hmidet, A. Raut, A. Gunjal, A.

- Uzgare, N. Patil, M. Waghmode, N. Nawani (2018). Analysis of diversity of actinomycetes from arid and saline soils at Rajasthan, India. *Environ. Sustain.* **1**: 61-70. <https://doi.org/10.1007/s42398-018-0003-5>.
- Borowik, A., and J. Wyszowska (2016). Soil moisture as a factor affecting the microbiological and biochemical activity of soil. *Plant Soil Environ.* **62**: 250-255. <https://doi.org/10.17221/158/2016-PSE>.
- Chungopast, S., N. Phankamolsil, W. Thaymuang, A. Phommuangkhu, S. Karuna, T. Inboonchuay (2023). Correlation of soil physiochemical properties, microorganism numbers, and bacterial communities following unburned and burned sugarcane harvest. *Appl. Environ. Soil Sci.* **2023**: 9618349. <https://doi.org/10.1155/2023/9618349>.
- Dandwate, S. C. (2020). Analysis of soil samples for its physicochemical parameters from Sangamner city. *GSC Biol. Pharm. Sci.* **12**: 123-128. <https://doi.org/10.30574/gscbps.2020.12.2.0243>.
- Dar, M. S., I. Ahmad (2024). Screening and evaluation of antibacterial active strains of actinomycetes isolated from Northern Indian soil for biofilm inhibition against selected ESKAPE pathogens. *J. Umm Al-Qura Univ. Appl. Sci.* **11**: 340-355. <https://doi.org/10.1007/s43994-024-00164-8>.
- Hayakawa, M., H. A. Nonomura (1989). New method for the intensive isolation of actinomycetes from soil. *Actinomycetologica*. **3**: 95-104. [https://doi.org/10.3209/saj.3\\_95](https://doi.org/10.3209/saj.3_95).
- Hegazy, G. E., Z. A. Olama, G. M. Abou-Elela, H. S. Ramadan, W. M. Ibrahim, D. E. El Badan (2023). Biodiversity and biological applications of marine actinomycetes-Abu-Qir Bay, Mediterranean Sea, Egypt. *J. Genet. Eng. Biotechnol.* **21**: 150. <https://doi.org/10.1186/s43141-023-00612-8>.
- Javed, Z., G. D. Tripathi, M. Mishra, K. Dashora (2021). Actinomycetes - the microbial machinery for the organic-cycling, plant growth, and sustainable soil health. *Biocatal. Agric. Biotechnol.* **31**: 101893. <https://doi.org/10.1016/j.bcab.2020.101893>.
- Jessy Padma, B., K. Saraswathi, P. Arumugam, R. Anna Shiny (2018). Isolation, characterization and evaluation of antioxidant activities of secondary metabolites producing actinomycetes of terrestrial origin. *Int. J. Res. Med. Sci.* **6**: 1017-1028. <http://dx.doi.org/10.18203/2320-6012.ijrms20180643>.
- Kampfer, P. (2006). The family Streptomycetaceae, part I: taxonomy. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (eds.), *The Prokaryotes*, Springer, New York, NY, pp. 538-604. [https://doi.org/10.1007/0-387-30743-5\\_22](https://doi.org/10.1007/0-387-30743-5_22).
- Kazi, Z., B. S. Hungund, J. S. Yaradoddi, N. R. Banapurmath, A. A. Yusuf, K. L. Kishore, K. A. Buyondo (2022). Production, characterization, and antimicrobial activity of pigment from *Streptomyces* species. *J. Nanomater.* **2022**: 3962301. <https://doi.org/10.1155/2022/3962301>.
- Kelly, K.L., D. B. Judd (1976). Color: *Universal Language and Dictionary of Names*. U.S. Department of Commerce, National Bureau of Standards, Washington DC.
- Konwar, A. N., S. Basak, S. Gurumayum, J. C. Borah, D. Thakur (2023). *Streptomyces* sp. MNP32, a forest-dwelling Actinomycetia endowed with potent antibacterial metabolites. *3 Biotech.* **13**: 257. <https://doi.org/10.1007/s13205-023-03670-4>.
- Kumari, N., E. Menghani, R. Mithal (2019). Bioactive compounds characterization and antibacterial potentials of actinomycetes isolated from rhizospheric soil. *J. Sci. Ind. Res.* **78**: 793-798.
- Madigan, M. T., J. M. Martinko, J. Parker (1997). *Brock Biology of Microorganisms*, 8th Edition. Prentice Hall International, Inc., New York.
- Mohammadi, K., G. Heidari, S. Khalesro, Y. Sohrabi (2011). Soil management, microorganisms and organic matter interactions: A review. *Afr. J. Biotechnol.* **10**: 19840-19849. <https://doi.org/10.5897/AJBX11.006>.
- Murtaza, M., V. Abrol, E. Nehra, P. Choudhary, S. K. Singh, S. Jaglan (2024). Biodiversity and bioactive potential of actinomycetes from unexplored high altitude regions of Kargil, India. *Indian J. Microbiol.* **64**: 110-124. <https://doi.org/10.1007/s12088-023-01133-1>.
- Nouioui, I., A. Zimmermann, O. Hennrich, S. Xia, O. Rossler, R. Makitrynsky, J. Pablo Gomez-Escribano, G. Potter, M. Jando, M. Doppner, J. Wolf, M. Neumann-Schaal, C. Hughes, Y. Mast (2024). Challenging old microbiological treasures for natural compound biosynthesis capacity. *Front. Bioeng. Biotechnol.* **12**: 1255151. <https://doi.org/10.3389/fbioe.2024.1255151>.
- Prescott, L. M., J. P. Harley, D. A. Klein (2002). *Microbiology*, 5th Edition. McGraw-Hill, New York.
- Sapkota, A., A. Thapa, A. Budhathoki, M. Sainju, P. Shrestha, S. Aryal (2020). Isolation, characterization, and screening of antimicrobial-producing actinomycetes from soil samples. *Int. J. Microbiol.* **2020**: 2716584. <https://doi.org/10.1155/2020/2716584>.
- Shirling, E. B., D. Gottlieb (1966). Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* **16**: 313-340. <https://doi.org/10.1099/00207713-16-3-313>.
- Shrestha, B., D. K. Nath, A. Maharjan, A. Poudel, R. N. Pradhan, S. Aryal (2021). Isolation and characterization of potential antibiotic-producing actinomycetes from water and soil sediments of different regions of Nepal. *Int. J. Microbiol.* **2021**: 5586165. <https://doi.org/10.1155/2021/5586165>.
- Singh, R., A. K. Dubey (2018). Diversity and applications of endophytic actinomycetes of plants in special and other ecological niches. *Front. Microbiol.* **9**: 1767. <https://doi.org/10.3389/fmicb.2018.01767>.
- Singh, V., S. Haque, H. Singh, J. Verma, K. Vibha, R. Singh, A. Jawed, C. K. Tripathi (2016). Isolation, screening, and identification of novel isolates of actinomycetes from India for antimicrobial applications. *Front. Microbiol.* **7**: 1921. <https://doi.org/10.3389/fmicb.2016.01921>.
- Singh, T., V. Wahla, R. C. Dubey, D. K. Maheshwari (2022). Destructive mycolytic suppression of *Fusarium oxysporum* causing wilt in chickpea by fungicide tolerant actinobacteria. *Environ. Sustain.* **5**: 243-253. <https://doi.org/10.1007/s42398-022-00229-6>.
- Sun, B., L. Yuan, Z. Xia, C. Wan, L. Zhang (2020). *Streptomyces albicerus* sp. nov., a novel actinomycete isolated from the sediments of the Tailan River in Xinjiang, China. *Arch. Microbiol.* **202**: 1639-1646. <https://doi.org/10.1007/s00203-020-01871-6>.
- Van Hop, D., Y. Sakiyama, C. T. Binh, M. Otoguro, D. T. Hang, S. Miyadoh, D. T. Luong, and K. Ando (2011). Taxonomic and ecological studies of actinomycetes from Vietnam: isolation and genus-level diversity. *J. Antibiot.* **64**: 599-606. <https://doi.org/10.1038/ja.2011.40>.

- Williams, S. T., M. Goodfellow M, G. Alderson, E. M. H. Wellington, P. H.A.Sneath, M. J. Sackin (1983). Numerical classification of *Streptomyces* and related genera. *J. Gen. Microbiol.* 129: 1743-1813.
- Zanane, C., D. Mazigh, S. Mitro, T. Hakim, S. Lekchiri, R. El Othmany, M. El Louali, H. Latrache, H. Zahir (2024). Study of the physicochemical properties of *Streptomyces* cell surface and their relationship with soil salinity of origin. *Ecol. Eng. Environ. Technol.* **10**: 31-43. <https://doi.org/10.12912/27197050/191117>.
- Zeng, J., Y. Iizaka, M. Hamada, A. Iwai, R. Takeuchi, A. Fukumoto, T. Tamura, Y. Anzai (2024). *Actinoplanes kirromycinicus* sp. nov., isolated from soil. *J. Antibiot.* **77**: 657-664. <https://doi.org/10.1038/s41429-024-00756-w>.