



Bioprospecting *Actinomycetes* from the Untapped Mangrove Sediments of North Kerala for Antimicrobial and Hydrolytic Enzyme Activities

Kiran Kishore, Vidya Pothayi, Sebastian Chempakassery Devasia*

Division of Molecular Biology, Department of Zoology, University of Calicut, Kerala 673635, India

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ABSTRACT

Actinomycetes from unexplored environments are regarded as a potential source of novel metabolites with a diverse array of biological activities. Sediments from mangrove habitats, characterized by their distinct environment, are regarded as essential for the identification of Actinomycetes strains with potent industrial, biotechnological, and therapeutic applications. The primary goal of the present study was to investigate the distribution, hydrolytic enzyme potential, and antimicrobial properties of actinomycetes strains from the mangrove sediments of north Kerala with respect to sediment characteristics. Sediment samples were collected from 8 stations in 5 districts along the northern coast of Kerala state. Sediments were subjected to different pretreatment methods to eliminate the growth of unwanted contaminants and plated on three different media for the selective isolation of actinomycetes. The pH of sediments ranged from 6.0-7.0, temperature from 28°C- 35°C, salinity from 33%- 55%, and percentage of organic matter from 1.0% - 9.2%. A total of 117 actinomycetes were isolated based on their distinct morphology, colony appearance, sporulation, and pigment production, out of which 86% of isolates exhibited hydrolytic enzyme potential and 64% isolates exhibited antagonistic activity against pathogenic strains. Based on 16S rRNA sequencing, the isolates exhibited the greatest resemblance to four distinct genera: *Streptomyces*, *Brevibacterium*, *Microbacterium*, and *Nonomuraea*. Further research into the isolation and identification of secondary bioactive compounds from these groups will be advantageous for uncovering new biomolecules that are beneficial to humans.

Keywords: 16SrRNA; Actinomycetes; Antimicrobial activity; Hydrolytic enzyme; Mangrove

1. Introduction

Mangrove ecosystems rank among the most productive habitats, supporting a vast array of life from bacteria and fungi to invertebrates, birds, and mammals. Mangrove ecosystems are located in tropical and subtropical places that are shielded from wave action and are typically highly sedimented. As a result, these sediments serve as the foundation for the mangrove forest and everything that lives within. Life in mangroves requires special adaptations to survive in areas that are periodically inundated with sea water [1].

Apart from flora and fauna, mangroves provide a unique habitat for a wide range of microorganisms [2]. These microbial communities govern the chemical environment of the mangrove ecosystem by cycling nutrients such as carbon, nitrogen, sulphur, and phosphorus. Due to the abundance of organic debris from plants and animals, mangrove sediments provide a suitable niche for diverse actinomycetes species. High index species diversity, limited consumable nutrient resources, and fluctuations in salinity and tidal gradient are typical of mangrove habitats. Such factors could be the driving force behind changes in metabolic pathways and the emergence of novel microbial characteristics [3].

On that account, research into mangrove actinomycetes can provide insight into the discovery of novel and distinct isolates, as well as their antimicrobial products. In the microbial world, actinomycetes represents one of the largest taxonomic units, accounting for one of the 18 major lineages identified within the domain Bacteria. These filamentous, spore forming, Gram positive bacteria with a high G+C content play a vital role in a wide range of habitats. Actinomycetes are prevalent in nature, found in terrestrial and aquatic habitats including mangrove and sea sediments [4]. They are the leading manufacturer of bioactive compounds, therapeutically active natural products, and several new antimicrobial compounds.

The hydrolytic enzyme potential of actinomycetes is involved in transformation activities and can be employed in a variety of industrial applications. Due to its biotechnological importance, scientist from all over the world study the community diversity in the phylum Actinomycetes from various countries and habitats [5].

With its vast geographical diversity, India provides completely distinct environmental dynamics that enable the flourishing of a wide group of microorganisms, including several strains of actinomycetes [6]. Kerala, on India's west coast, features vast swaths of luxuriant and unbroken mangrove habitats. Actinomycetes isolated from mangroves are a rich and underutilized source of pharmacological thrust. Researchers continue to investigate for novel molecules with antimicrobial, antitumor, and biochemical properties in mangrove microbes. Despite this, there is a paucity of knowledge on actinomycetes from Kerala mangroves, particularly those in the northern part. Therefore, the present study focused on investigating the diversity, hydrolytic enzyme potential, and antimicrobial activity of actinomycetes from the mangrove sediments of north Kerala.

2. Materials and Methods

2.1 Sediment sampling

Sediment samples were collected from the mangrove belt in the coastal areas along the five districts of north Kerala at eight sites:

Kasargod(12°05'32"N75°13'39"),
Edat(12°05'32"N75°13'39"E),
Pazhayangadi(12°02'72"N75°29'31"E),
Valapattanam(11°93'45"N75°35'35"E),
Elathur(11°19'43"N75°45'2"E),
Kadalundi(11°07'43"N75°49'48"E),
Ponnani(10°47'1"N75°55'3"E), and
Chetuva(11°1'41"N75°52'6"E) (Fig.1).

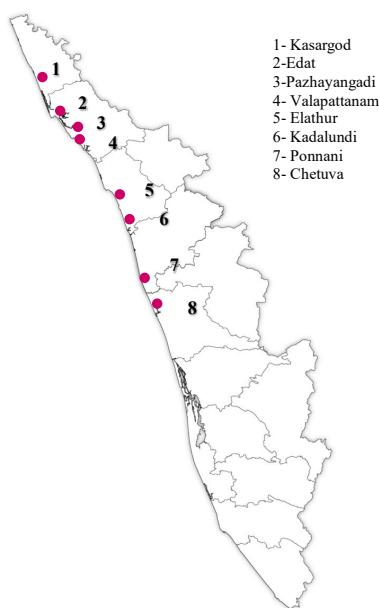


Fig. 1. Map of Kerala showing the mangrove sediment collection sites. Scale bar 1 mm = 1 Km.

Samples were collected during pre-monsoon season (April-May 2023). Mangrove sediment samples from the subsurface were collected using the hand-core method, transferred aseptically in to sterile polythene bags, and transported to the laboratory. The samples were air dried at room temperature. Physico-chemical characteristics like temperature, pH, and salinity of the samples were recorded during the sample collection using a mercury thermometer, digital pH meter, and salinometer, respectively. A total of 5 sub-samples were collected from each location and these sediments were homogenized into one composite sample for the analysis. The percentage organic matter and sediment texture were analyzed using modified Walkley and Black method in triplicates [7]. Sediment particle size was measured using HORIBA laser scattering particle size distribution analyzer LA-960.

2.2 Isolation of actinobacteria

For the selective isolation of actinomycetes, air dried samples were pretreated with wet heat at 75°C for 15 min, dry heat at 101°C for 1 hour and then underwent phenol

treatment in order to reduce the number of unwanted bacteria. Pretreated sediment samples were serially diluted to a factor of 10^{-3} and then spread over the surface of International *Streptomyces* medium (ISP2), Actinomycetes isolation medium, and starch casein agar medium using the spread plate technique in duplicates. Each medium was supplemented with Nystatin (25 µg/ml) and Nalidixic acid (25 µg/ml) to minimize microbial contamination. Plates were incubated at $28 \pm 2^\circ\text{C}$ for 2 weeks and actinomycetes colonies were selected on a morphological basis as per International Streptomyces Project [8] and Bergey's Manual of Systematic Bacteriology [9]. The total number of colonies in each plate were counted to calculate CFU/G dry sediment. Developed colonies were purified by quadrant streaking and transferred to starch casein agar slants for further analysis. In addition, Gram staining and acid-fast staining were used to observe the thin thread like mycelial and hyphal forms under the microscope and spores on the surface and spore chain morphology were observed by scanning electron microscope (SEM).

2.3 Hydrolytic enzyme production

Morphologically distinct isolates obtained during the study period were screened for the production of various hydrolytic enzymes such as protease, amylase, lipase, chitinase, DNase, and pectinase as per standard protocols in duplicates. Isolates were also screened for ligninolytic enzyme production potential. Nutrient agar supplemented with casein (2%), starch (1%), and tributyrin (1%) were used for the detection of protease, amylase, and lipase, respectively. Cellulose agar, pectin agar, chitin agar, and DNA agar were used for the detection of cellulase, pectinase, chitinase, and DNase activity, respectively. Crawford's agar supplemented with 0.5% tannic acid was used for the detection of ligninase activity. Plates were spot inoculated and incubated at 28°C for 7 days. Formation of a clearance /halo zone or brown colour around a colony indicated a positive

result; the diameter of halo zones were recorded to select for strains with the highest enzyme activity.

2.4 Screening for antimicrobial activity

The isolated actinomycetes strains were screened for both antibacterial and antifungal properties in duplicates. The following test microorganisms were used in this study: *Escherichia coli* (MTCC443), *Staphylococcus aureus* (MTCC96), *Vibrio cholerae* (MTCC3906), *Vibrio parahaemolyticus* (MTCC451), *Klebsiella pneumoniae* (MTCC109), *Candida albicans* (MTCC183), and *Aspergillus brasiliensis* (MTCC1344). Bacterial strains were cultured on nutrient agar and incubated at 37°C for 24 hours and fungal strains were cultured on malt extract agar medium and incubated at 37°C for 48 hrs. Primary screening was employed by the cross-streak method. Single streaks of actinomycetes isolates were made on the center of Muller Hinton agar medium plates and incubated at 28°C for 7 days. Each test strain was streaked perpendicularly and incubated at 37°C for 24 h in order to measure growth inhibition of the test strains by actinomycetes isolates. Secondary screening was done by the agar well diffusion method. Spores of actinomycetes were inoculated in starch casein medium prepared with sea water and incubated at 28°C for 5-7 days at 200 rpm. Muller Hinton agar plates were inoculated with test strains by the spread plate technique. Wells were made using sterile borer and 100 µl of cell free supernatant were poured to each well. Plates were incubated at 37°C for 24 hours and inhibition zones were measured.

2.5 Production and extraction of bioactive compounds

Actinomycetes isolates with the highest antagonistic activity were selected for the detection of antimicrobial metabolites using liquid-liquid extraction with ethyl acetate as the active solvent. Active strains were fermented in starch casein broth and incubated at 28°C for 7-14 days at 200 rpm. The

cultures were harvested and filtered using Whatman No.1 filter paper and an equal amount of ethyl acetate was added and then agitated overnight to complete the liquid-liquid extraction. The separation process was carried out in a separating funnel. The upper organic layer containing bioactive compounds was collected [9]. Crude extracts of selected actinomycetes strains were used for the quantitative assay by the agar well diffusion technique against *E. coli*, *S. aureus*, *V. cholerae*, *V. parahaemolyticus*, *K. pneumoniae*, *C. albicans*, and *A. brasiliensis*. Suspensions of the test strains were swabbed over the Muller Hinton agar plates and wells were made using sterile borer and 100 µl of solvent extracts were added to each well. Ethyl acetate was used as the control. Plates were incubated at 37°C for 24 hours and inhibition zones were measured.

2.6 Molecular identification

The most potent isolates were identified by molecular methods using the amplification of the 16S rRNA gene. Total genomic DNA from the selected bacterial strains was extracted as per standard protocol [10]. Molecular identification was carried out with 16SrRNA gene amplification using the actinomycetes specific primers S-C-Act-235-a-S-20 F and S-C-Act-878-a-A-19R [11]. The PCR products were electrophoresed to ensure that the fragment of the appropriate size had been amplified. The purified PCR product from representative isolates were sequenced by Sanger's dideoxy chain termination sequencing method [12], and the obtained nucleotide sequence were used for the sequence similarity search using NCBI BLAST. The identified sequences were deposited in GenBank and accession numbers of actinomycete isolates were obtained. Nucleotide sequences obtained were analyzed using Molecular Evolutionary Genetic Analysis Version XI (MEGA XI) software and a phylogenetic tree was constructed using the maximum likelihood method. *Penicillium*

chrysogenum was used as the outgroup for tree construction.

3. Results and Discussion

3.1 Isolation of actinobacteria

Three different pre-treatments were employed for the selective isolation of actinomycetes. Bhaskaran et al. [14] reported that, air dried and pretreated mangrove sediments inhibited the dominance of unwanted bacterial and fungal contaminants while compared to the untreated ones. Out of the three pretreatments in our study, treatment at 75°C for 15 min resulted in the isolation of

the most isolates. Regarding the media used, starch casein agar produced the highest number of isolates (98), followed by ISP2(53), and AIA (25); this result aligns with existing findings of other studies. A study by Meena et al. [15] reported the isolation of actinomycetes from marine ecosystem using starch casein medium prepared with sea water. The CFU/G from each sampling station was determined in duplicate. The CFU/G of actinomycetes in mangrove sediments differed significantly at each sampling site (Fig. 2). Mangrove sediments from Kasargod exhibited the maximum CFU/G and the Ponnani site showed the minimum.

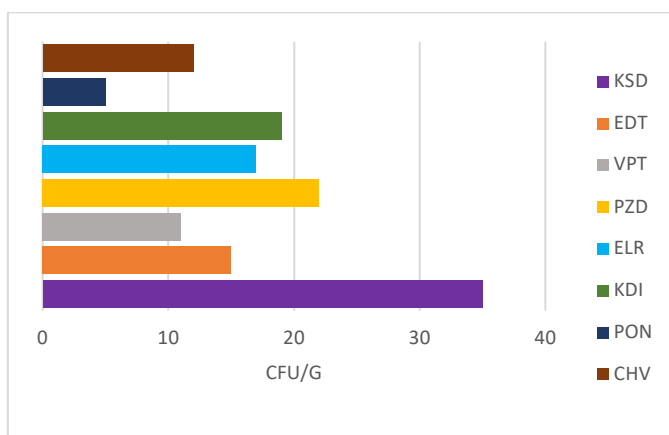


Fig. 2. CFU/G of actinobacteria from the collection sites KSD- Kasargod, EDT- Edat, VPT- Valapat-tanam, PZD- Pazhayangadi, ELR- Elatur, KDI- Kadalundi, PON- Ponnani, CHV- Chetuva.

3.2 Morphological characterization

Pigment production was observed in some isolates of our study and it has been reported by Shirling and Gottlieb [8] that such traits can serve as identification markers. Aerial mycelia of most isolates ranged from white to off-white, grey, orange, purple, brown, and black (Fig. 3). Most of the isolates exhibited reverse side pigments including from brown, black, pink, red, orange, and grey. All isolates were Gram positive and tested

negative for acid fast staining under microscopic examination. Isolated colonies were identified based on colony morphology and microscopic characteristics. Spore chain morphology and spore shape of the isolates were observed using the coverslip technique for microscopic and SEM analysis. The most prevalent spore chain morphology was the spiral type, rectiflexibles, and straight. Spore shape observed included smooth, warty, and irregular (Figs. 4a-4b).



Fig.3. Aerial mycelium of actinomycetes.

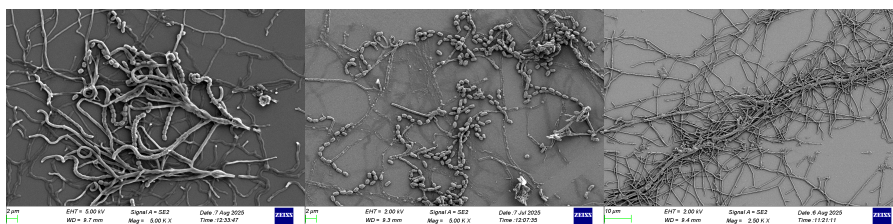


Fig. 4a. SEM images of actinomycetes strains.

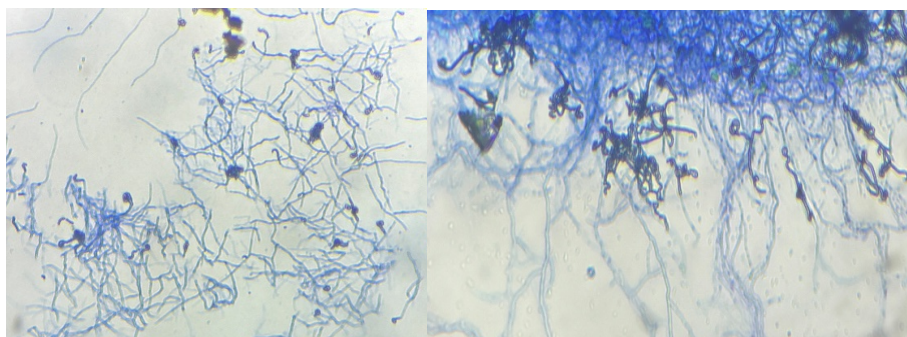


Fig. 4b. Microscopic observations of actinomycetes strains (40X).

3.3 Physico-chemical parameters

Physicochemical parameters of the sediment such pH, temperature, and salinity were determined in situ from each site. The pH of the sediment ranged from 6.0-7.0, temperature observed from 28-35°C, and salinity from 1.8-3.2‰. The sediment texture and organic matter content were analyzed later in the laboratory. The percentage of organic matter varied from 1.0-9.2% between the sampling sites. The Edat station showed the highest percentage of organic matter and the Kasargod station exhibited the lowest percentage of organic matter. Previous studies have established that the physicochemical characteristics of the sediment plays a vital role in the microbial population. A study by Poongodi et al. [16] showed a significant correlation ($r = 0.927$, $p < 0.01$) between the percentage of organic matter and the count of

actinomycetes in the soil. However, there are conflicting reports. According to Jensen et al. [17], actinomycetes population exhibited no correlation with the percentage of organic matter from marine sediments. Each station exhibited significant disparities among the sediment texture. Ponnani, Kasaragod, and Elatur exhibited higher sand percentages. The percentage of silt was higher at Edat followed by Kadalundi. Edat was abundant in clay deposits and Chetuvu displayed a comparable distribution of sand and silt in its sediment composition. Shepard's ternary diagram supported these classifications, with stations grouped into distinct sediment types—PON, KSD, and ELR plotted near the sand apex, KDI and VPT near silty sand area, EDT near the clay-silt edge, and CHV and PZD occupying intermediate zones (Fig. 5). Thus, findings from our study also

corroborate the impact of organic matter, sediment moisture, and sediment texture on

the prevalence of culturable actinomycete colonies.

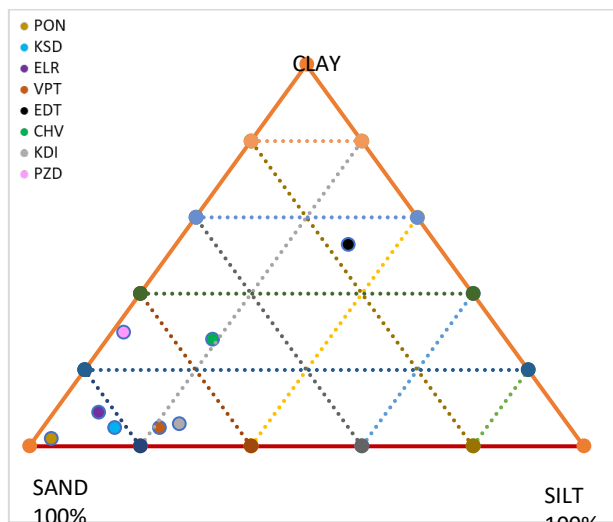


Fig. 5. Percentage of sediment texture from the collection sites.

3.4 Hydrolytic enzyme production

Enzymes serve as the optimal catalyst in various applications across several industries due to their specificity and nontoxic nature. As marine microbes possess unique properties like salt tolerance and thermostability, researchers have been drawn to the enzymes derived from these microbes for industrial use. Previous studies by Wakil and Adelegan [18] have shown that marine microbial enzymes are biodegradable, less toxic, and can be administrated on site at ease. Among the marine microbiota, actinomycetes are well known for their role in industrial importance as they produce a vast array of enzymes and valuable metabolites with diverse biological activities. Due to their complex metabolic composition and genomic organization, actinomycetes have enormous potential for biological breakdown of different and

complex substrates present in their natural habitats.

In the present study, all morphologically distinct isolates were screened for the production of different enzymes viz. protease, amylase, lipase, DNase, cellulase, pectinase, chitinase and ligninase (Fig. 6). Isolates from the mangrove sediments of Kadalundi and Ponnani showed the greatest enzyme activity, whereas the Elatur region showed the least. The percent enzyme activity of the isolates was observed to be in the following order. Pectinase > cellulase > amylase > protease > lipase > DNase > chitinase. None of the isolates exhibited ligninase activity (Fig. 7). This suggests that ligninase is not essential for mangrove actinomycetes, while the presence of carbohydrates, proteins, and lipids, and metabolic processes, are associated with actinomycetes in mangrove sediments.

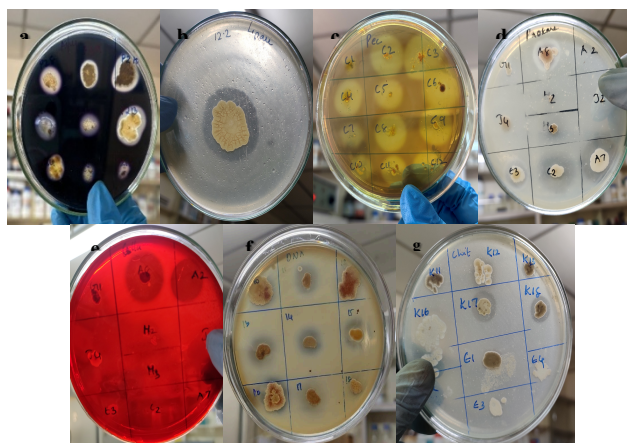


Fig. 6. Plates showing hydrolytic enzyme potential of isolates. a- Amylase, b- Lipase, c- Pectinase, d- Protease, e - Cellulase, f - DNase, g – Chitinase.

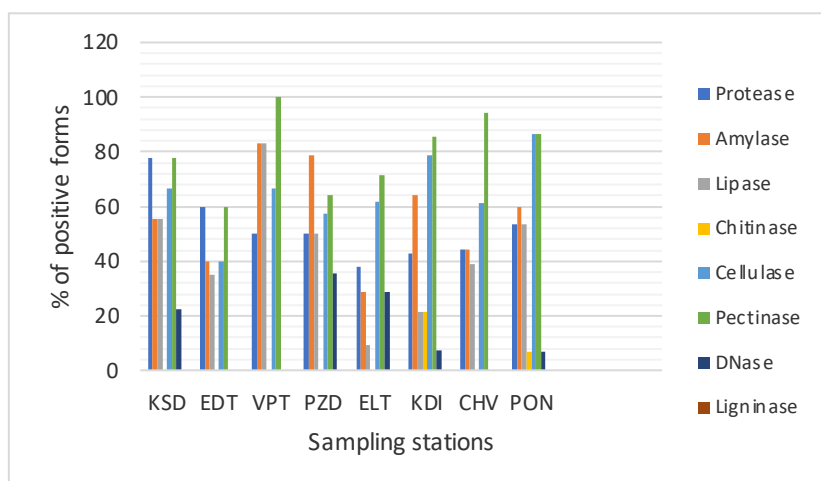


Fig. 7. Percentage of isolates (positive forms) showing various enzyme activities.

Pectinase serves as a sustainable tool utilized across multiple sectors including the wine paper industry for pulp bleaching and waste paper recycling, the food industry in the processing of fruits and vegetables, tea and coffee, the extraction of vegetable oil, etc. [19]. Arjith and co-researchers [20] isolated pectinolytic actinomycetes from the mangrove sediments of Valapattanam, Kerala and purified the pectinase enzyme of 32 kDa weight. However, reports on pectinase producing actinomycetes from mangrove sediments are limited [21]. Actinobacteria, recognized for their ability to produce

cellulase, have garnered increased attention in the field due to their promising applications [22]. Cellulase has a significant role in the biofuel, pharmaceuticals production, and pulp and paper sectors. The mangrove actinomycetes serve as a putative bioactive hub for amylase enzymes [23] and the present findings align with the existing literature. Amylases are mainly used in the food, textiles, paper, and detergent industries. Proteases comprise 60% of the global enzyme market and nearly two-thirds of commercially produced proteases come from microbial sources. Ramesh et al. [24] isolated

Streptomyces fungicides (MML1614) from the Pichavaram mangrove, which is a highly stable alkaline protease with potential use in detergent manufacturing. Lipases are the most versatile enzymes, which hydrolyze the ester bonds in triglycerides to glycerol and fatty acids. Microbial lipases find immense application in various industries like cosmetics, agrochemical, and diagnostics [25]. Ramesh and Mathivan [26] isolated 183 lipase-producing actinomycetes from mangrove sediments from the Bay of Bengal and reported that due to the presence of huge amounts of polymers such as polyethylene and polypropylene in the oceans, the population of lipase producing actinomycetes is comparatively vast in the marine environment. From this study it has been determined that the reports on the extracellular enzymes from marine actinomycetes is currently limited. However, findings from this research revealed that out of 117 isolates, 91 produced pectinase, 74 produced cellulase, 61 produced amylase, 59 produced protease, 44 produced lipase, 15 produced DNase, and 4 produced chitinase. Interestingly, 3 isolates exhibited hydrolytic enzyme potential for at least 5 enzymes and unfortunately none of the isolates exhibited ligninase activity. Insufficient substrates and their recycling in the sampling sediments could be the reason for the lower production of other enzymes such as DNase, chitinase and ligninase.

3.5 Antimicrobial potential of isolates

Microbial secondary metabolites exhibit a wide spectrum of biological activities including antibiotic, antiviral, anticancer, and immunomodulatory properties. Despite not being vital for growth or reproduction, certain filamentous microbes like actinomycetes produce substantial quantities of secondary metabolites. Actinobacteria have been thoroughly explored for a number of unique secondary metabolites, most notably antibiotics [2]. In the current research, the inhibitory zones against the test strains ranged from 12-34 mm and the strains exhibiting

zones exceeding 15 mm were considered potent. Initial screening of the actinomycetes isolates from our study found significant antibacterial and antifungal activity against at least one of the test strains. Out of 117 isolates, 72 exhibited antagonistic activity against both Gram-positive and Gram-negative bacteria, *C. albicans*, and *A. brasiliensis*, respectively, indicating broad spectrum activity. Isolates were subjected to primary screening using the cross-streak method and secondary screening using the agar well diffusion method (Fig. 8). Isolates were highly active against *V. parahaemolyticus* (51 isolates), followed by *A. brasiliensis* (40 isolates), *C. albicans* (39), *V. cholerae* (35 isolates), *E. coli* (35 isolates), *S. aureus* (32 isolates), and *K. pneumoniae* (28 isolates) (Fig. 9). The mangrove sediments of the Chetuva region yielded isolates with the most potent antagonistic activity against the test strains, whereas the least activity was observed in the Edat region. According to Meena et al. [15], such increased antagonistic activity is due to their adaptation to nutrient availability and osmotic flux influencing their intricate biochemical pathways. The antimicrobial activity of actinomycetes against various human pathogens has already been documented by a number of studies. Kripa et al. [27], isolated 126 actinobacteria from the mangrove sediments of Ponnani estuary, among which 4 streptomyces species showed best antagonistic activity against Gram negative bacteria.

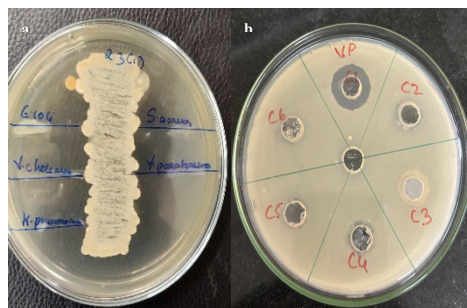


Fig. 8. Plates showing the inhibition zone by a-Cross streak method and b-Agar well diffusion method.

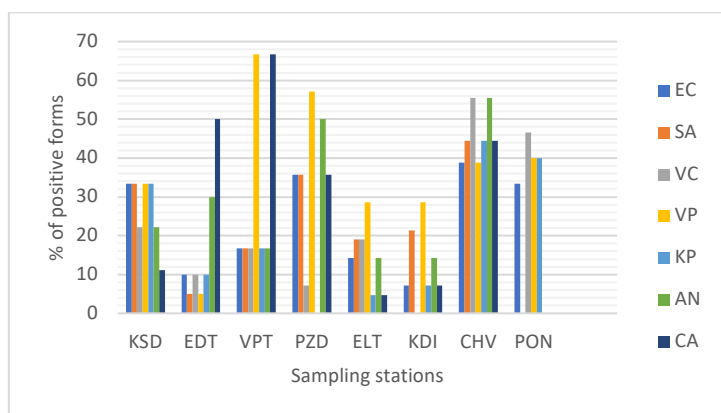


Fig. 9. Percentage of isolates (positive forms) showing antimicrobial activities.

Among the 72 isolates, 3 exhibited antagonistic activity against all the selected test strains. Therefore, these strains were subjected to liquid-liquid extraction to isolate the antimicrobial compounds. Ethyl acetate extracts from fermented broth of these strains were prepared and assessed for antimicrobial activity. Crude extracts of all 3 of the potent isolates exhibited the highest antimicrobial activity against all the test strains (Table 1, Fig. 10). Previous research has indicated that antimicrobial compounds extracted by ethyl acetate from actinobacteria exhibited significant antimicrobial properties [28]. Our report is the first to provide a comprehensive

analysis of antagonistic actinomycetes isolated from the mangrove sediments of North Kerala against human pathogens. Despite the presence of household and livestock farm waste and a low organic matter in the sediment, the Chetuva region of Thrissur district exhibited the strongest antagonistic activity against the pathogenic strains. The lowest antimicrobial activity was found at the Kasargod station. According to Ouchene et al. [29], inappropriate activity screening, cryptic biosynthetic gene clusters, or improper medium or method for metabolite formation could be contributing factors to the low rate of active strains.

Table 1. Antimicrobial activity of ethyl acetate extracts against the tested pathogenic microbes. Values in each column are the mean of triplicates $\pm$ SD.

Isolate	Zone of inhibition (mm)						
	<i>E. coli</i>	<i>S. aureus</i>	<i>V. cholerae</i>	<i>V. parahaemolyticus</i>	<i>K. pneumoniae</i>	<i>C. albicans</i>	<i>A. brasiliensis</i>
1	12.3 $\pm$ 0.47	15 $\pm$ 2.05	14 $\pm$ 1.41	13 $\pm$ 0.47	11.3 $\pm$ 1.24	14 $\pm$ 0.81	20 $\pm$ 0.47
2	15.3 $\pm$ 1.24	12 $\pm$ 0.8	12.3 $\pm$ 1.6	17.3 $\pm$ 1.24	16.2 $\pm$ 1.2	13 $\pm$ 0.9	24.3 $\pm$ 1.24
3	13.2 $\pm$ 0.94	19 $\pm$ 1.24	17 $\pm$ 0.8	12.3 $\pm$ 0.47	14.3 $\pm$ 0.47	18 $\pm$ 0.81	18.3 $\pm$ 0.94



Fig. 10. Plates showing the inhibition zone of ethyl acetate extracts against the pathogenic microbes. a- *S. aureus*, b- *V. parahaemolyticus*, c- *K. pneumoniae*, d- *E. coli*, e- *C. albicans*, f- *A. brasiliensis*, and g- *V. cholerae*. Wells marked with "C" represent the control, while the remaining wells contain the crude extracts.

3.6 Molecular identification

Identification of isolates up to genus level were performed based on their colony morphology and microscopic examinations. Genomic DNA of the selected isolates were extracted according to standard protocol, followed by the amplification of the 16S rRNA gene by PCR. Sequences obtained were compared with the GenBank database using BLAST analysis. 16SrRNA sequences of potent isolates exhibited the greatest resemblance to four distinct genera. Most of the isolates belonged to the genus *Streptomyces*, which is the most dominant actinobacteria found in the marine sediments. *Streptomyces* species are renowned as broad-spectrum antibacterial compound producers. Screening of antimicrobial activities in this study relied on the identification of a significant number of *Streptomyces*, since this genus has proven to be a prolific producer of antibiotics and various bioactive compounds [30]. BLAST

search of remaining 16SrRNA sequences of actinomycetes indicated 99-100% similarity with the rare actinomycetes genera *Nonomuraea*, *Brevibacterium*, and *Microbacterium* (Table 2). Phylogenetic analysis revealed clear evolutionary relationships among the examined actinobacterial species, with *Penicillium chrysogenum* serving as the outgroup to root the tree. The majority of the isolates clustered within the genus *Streptomyces*, forming several well-supported clades. Outside the *Streptomyces* clade, representatives of rare actinomycetes such as *Brevibacterium sanguini*, *Microbacterium maritipicum*, and *Nonomuraea coxensis* were positioned on separate branches, consistent with their taxonomic distinction from *Streptomyces*. These non-*Streptomyces* taxa were clearly separated from the main clade, reinforcing the robustness of the genus-level classification (Fig. 11).

Table 2. GenBank details of identified actinomycetes strains from mangrove sediments.

Sl. No.	Isolates	Organism	GenBank Accession
1	CUMB KK - 01	<i>Streptomyces anandii</i>	PV449464
2	CUMB KK - 02	<i>Streptomyces griseoviridis</i>	PV449466
3	CUMB KK - 03	<i>Streptomyces niveoruber</i>	PV449467
4	CUMB KK - 04	<i>Streptomyces fumigatiscleroticus</i>	PV449468
5	CUMB KK - 05	<i>Streptomyces niveoruber</i>	PV449469
6	CUMB KK - 06	<i>Streptomyces diastaticus</i>	PV449745
7	CUMB KK - 07	<i>Streptomyces ardesiacus</i>	PV449751
8	CUMB KK - 09	<i>Streptomyces mexicanus</i>	PV449775
9	CUMB KK - 10	<i>Streptomyces wuyuanensis</i>	PV449774
10	CUMB KK - 11	<i>Streptomyces rochei</i>	PV449776
11	CUMB KK - 12	<i>Streptomyces fumigatiscleroticus</i>	PV449778
12	CUMB KK - 14	<i>Streptomyces parvulus</i>	PV449787
13	CUMB KK - 15	<i>Nonomuraea coxensis</i>	PV449791
14	CUMB KK - 26	<i>Streptomyces althioticus</i>	PV449810
15	CUMB KK - 36	<i>Microbacterium maritipicum</i>	PV450418
16	CUMB KK - 27	<i>Brevibacterium sanguinis</i>	PV450194
17	CUMB KK - 33	<i>Streptomyces matensis</i>	PV450395
18	CUMB KK - 37	<i>Streptomyces shandongensis</i>	PV450420
19	CUMB KK - 35	<i>Streptomyces longispororuber</i>	PV450413

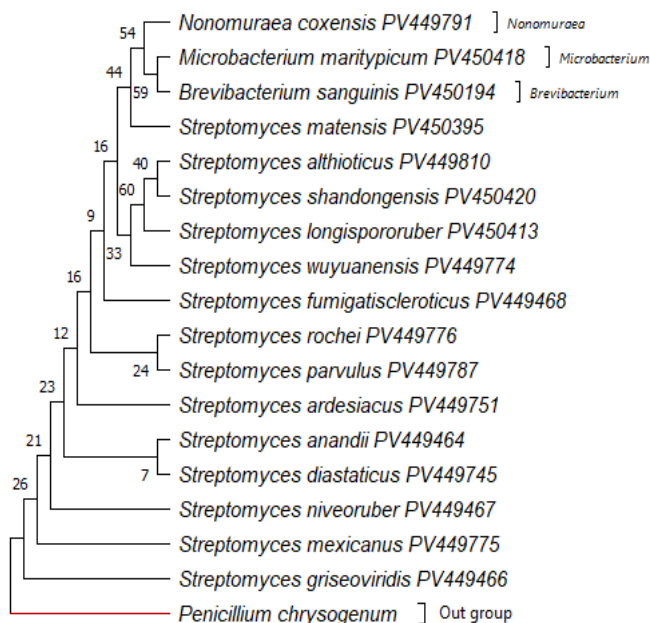


Fig. 11. Phylogenetic tree based on 16S rRNA gene sequences showing the evolutionary relationships among actinomycete isolates. The tree was rooted using *Penicillium chrysogenum* as an outgroup. Bootstrap values (expressed as percentages of 100 replicates) are indicated at the nodes, reflecting the confidence level of each branching point.

4. Conclusion

The present study highlights the diversity and functional potential of actinomycetes isolated from mangrove sediments along the northern coast of Kerala. A total of 117 isolates were obtained, the majority of which demonstrated hydrolytic enzyme activity and antagonistic properties against pathogenic strains. Molecular identification revealed representatives from four distinct genera, *Streptomyces*, *Brevibacterium*, *Microbacterium*, and *Nonomurae*, underscoring the richness of this unexplored environment. These preliminary findings establish a baseline for understanding the distribution and bioactivity of mangrove-associated actinomycetes. Further characterization of secondary metabolites and detailed functional analyses will be essential to uncover novel biomolecules with potential industrial, biotechnological, and therapeutic applications.

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