ASSESSMENT OF ANTIMICROBIAL PROFICIENCY AND ENZYMATIC SCREENING OF ACTINOMYCETES ISOLATED FROM RAJAKKAMANGALAM MANGROVES

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Abstract

Mangroves are a distinctive woody plant community that can be found on tropical and subtropical intertidal shores. The mangrove ecosystem, which is still completely unexplored, has tremendous opportunities for discovering unusual actinomycetes with distinctive characteristics that are capable of creating a variety of novel bioactive chemicals, including enzymes, antibiotics, and anticancer agents. Mangrove ecosystems are among the most productive and

have special environmental characteristics, although they have received the least attention. Mangrove soil-isolated actinomycetes have been shown to generate antimicrobials, antivirals, antioxidants, and anticancer chemicals in addition to industrial enzymes like lipase, cellulase, protease, and pectinase. The majority of the aerobic actinomycetes are gram-positive, filamentous, branching bacteria that develop slowly and are only partially acid-fast. Actinomycetes are capable of producing a wide range of biologically active secondary metabolites, including cosmetics, vitamins, food additives, antibiotics, insecticides, anti-parasitic agents, and enzymes.

In the current study, actinomycetes that were isolated from the soil sediments of the Rajakkamangalam mangrove in the Kanyakumai District of Tamilnadu, India, were tested for their enzymatic and antibacterial properties. On Starch Casein Agar, four actinomycete isolates were discovered using the spread plate method. When extracellular enzymes including amylase, protease, lipase, and gelatinase were screened for in the isolated actinomycetes, it was discovered that the majority of the isolates produced amylase and protease activity. By using the spot inoculation method, the isolate RE2 was discovered to have the maximum antibacterial activity against the pathogens of aquaculture, including *Stapylococcus aureus, Escherichia coli, Bacillus cereus, Vibrio harveyi,* and *Aeromonas hydrophila*. Using their biochemical and morphological characteristics, *Streptomyces sp.* was determined to be a very effective isolate.

Key words: Actinomycetes, Mangroves, enzymatic activity, antimicrobial activity

Introduction

At the meeting point of land and water, mangrove ecosystems make up a significant amount of tropical and subtropical coastlines. These ecosystems rely on tightly coupled exchanges between plants, microbes, and microbes to recycle the elements, with bacteria and archaea being key players in the biogeochemical cycling of carbon, nitrogen, and phosphorus Reis *et al*., (2017). The mangrove ecosystem, which is still completely unexplored, has tremendous opportunities for discovering unusual actinomycetes with distinctive characteristics that are capable of creating a variety of novel bioactive chemicals, including enzymes, antibiotics, and anticancer agents. Recently, there has been a lot of interest in using the mangrove microorganism resource, which has resulted in the identification of novel Streptomyces species (Hong *et al*., 2009; Lee *et al*., 2014). Actinomycetes are aerobic, Gram-positive bacteria having high DNA GC content (69–73%) and Streptomyces are the leading actinomycetes, and are regarded as economically significant due to the production of thousands of antibiotics and other commercially relevant enzymes. Detergents, the textile, pulp, and paper industries all naturally employ industrial enzymes in large quantities. Microbial enzymes have recently taken the place of artificial catalysts in the production of chemicals, food, leather, and pharmaceuticals. Lipases are particularly valuable for industrial functions such as processing fat and oils, additives, detergents, cosmetics, paper manufacture, and pharmaceuticals due to their wide range of enzymatic characteristics and substrate specificities (Hasan, 2006). Streptomyces are well known for producing secondary metabolites in addition to enzymes, making them of significant

medicinal and economic importance (Mitsuiki *et al*., 2002; Tsujibo *et al*., 2003). The source of about 80% of all antibiotic products is Streptomyces sp. According to Ogunmwonyi *et al*., (2010), actinomycetes have the ability to synthesize a wide variety of biologically active secondary metabolites, including cellulase and xylanase, which are employed in waste treatment, as well as vitamins, nutritional supplements, herbicides, antibiotics, insecticides, and anti-parasitic agents. Actinomycetes are widely acknowledged to be the primary manufacturers of antibiotics. Actinomycetes manufacture more than 80% of the antibiotics that are currently in use, including tetracycline, macrolide, chloramphenicol, nucleosides, and polyenes (Ibnouf, 2021; Raja and Prabakarana, 2011; Shrestha *et al*., 2021). They were said to create the majority of the antibiotics among them, with members of the genus Streptomyces in particular (Berdy *et al*., 2012). In order to treat and prevent illnesses brought on by bacterial infections, antibiotics are molecular molecules (De Simeis *et al*., 2021). In light of this, the current research was carried out to examine the enzymatic and antibacterial properties of actinomycetes isolated from the Rajakkamangalam mangrove in the Kanyakumari District.

Materials and Methods

Collection of samples

Mangrove soil sediment was collected from Rajakkamangalam mangrove (Latitude: 8.1290447; Longitude: 77.3640107), Kanyakumari District, Tamil Nadu. The collected sample was covered with sterile zip lock to avoid contamination. The sample was taken to the laboratory in an ice box, and the isolates were screened using SCA (starch casein agar) medium.

Screening of the isolated actinomycetes using enzymes

Amylase activity

By performing a starch hydrolysis test on a starch agar plate, actinomycetes isolates were evaluated for their amylolytic potential. The actinomycetes isolate was streaked on culture plate and incubated for 4–7 days at 28–2°C. Following growth, starch agar plates were flooded with 4 ml of newly made 1% iodine solution, and any apparent zones of hydrolysis were regarded as amylase producers (Hamilton *et al*., 1999).

Protease activity

Actinomycetes isolates were streaked on skim milk agar (SMA) plates, which were then cultured at 28 °C for 4 to 7 days. According to Dinarayana *et al*., (2003), a clear zone of skim milk hydrolysis indicated the presence of protease-producing microbes.

Lipase Activity

The medium was heated roughly at 50 ºC mixed with tributyrin (3 mL/L) was added as a substrate and forcefully stirred before being poured into petri dishes to measure lipase activity (Difco, NJ, USA). The isolates were inoculated to the plates, which were then cultured for three days at 28 ± 2 °C. The presence of halos around each streaked colony, which showed that positively digested lipids, demonstrated lipolysis (Gopinath *et al*., 2005).

Gelatinase Activity

Agar plates with 12 g of gelatin, 1 g of yeast extract, 4 g of peptone, and 18 g of agar per liter were used to assess the activity of gelatinase. Gelatinase activity was assumed to be present if there were clear zones surrounding the bacterial growth (Vigneshwari *et al*., 2021).

Antibacterial Screening of the Isolated Actinomycetes

Using the agar overlay method, the isolates were screened on nutrient agar plates, around 5-7 days old actinomycetes were spot-inoculated. The colony was then incubated for 3 days at 30°C. A 24-hour bacterial inoculum was prepared on the nutrient broth such as *Stapylococcus aureus, Escherichia coli, Bacillus cereus, Vibrio harveyi,* and *Aeromonas hydrophila*, were then added to 5 ml of semisolid nutritional agar, and incubated at 37°C for 24 hours. Gebreyohannes *et al*., (2013) examined the zone of inhibition (measured in cm) surrounding the colonies.

Spore chain and surface morphology of the isolated actinomycetes

By using the coverslip method and direct examination, the spore carrying hyphae and spore chain were identified. The actinomycete status of the isolated strains was established by

microscopically examining their morphology. On sterile slides, SCA (Starch Casein Agar) was poured and given time to set. After that, the organisms were streaked on it and left to incubate for 48 hours at 37 °C. The coverslips were carefully removed after incubation while keeping in mind their orientation and placed upwards on a slide. After adding around 2 drops of methylene blue dye, the isolated actinomycete strain's colony color was classed into light whitish, grayish, and light dark gray brownish colors. The morphology of these isolates was examined under a microscope after they had been identified, covered with a cover slip, and so on (Mohan *et al*., 2014).

Morphological and biochemical characteristics of the isolates

The morphological and biochemical properties of isolates of Actinomycetes from mangroves were characterized with color and morphological features. Biochemical properties such as Methyl-Red (MR) test, Voges-Proskaeur test, Indole production test, Nitrate reduction test, Citrate utilization test, Urease test, Triple Sugar Iron (TSI) test, and Catalase test are used to categorize the isolates.

RESULT

Isolation of the actinomycetes

From Rajakkamangalam mangrove sediments, 4 distinct morphologically active actinomycetes (RE1, RE2, RE3 and RE4) were scrutinized on starch casein agar (SCA) media that had been added with actidione and nalidixic acid to suppress common contaminants like fungus and bacteria, respectively, all of the strains were isolated under the ideal culture conditions.

Enzymatic screening of the selected isolates

Protease, amylase, lipase, and gelatinase were among the extracellular hydrolytic enzymes that were examined. *Streptomyces sp* RE2 and RE3 were found to produce proteases, while *Streptomyces sp* RE1 and RE4 were found to produce amylases, as indicated in (**Table: 1 and Figure: 1**).

Table. 1 Screening of extracellular hydrolytic enzymes from actinomycetes strains isolated from Rajakkamangalam mangrove

Actinomycetes strains	Extracellular hydrolytic enzymes			
	Protease	Amylase	Lipase	Gelatinase
RE1	_	+	_	_
RE 2	+	+	_	_
RE 3	+	+	_	_
RE 4	_	+	_	_

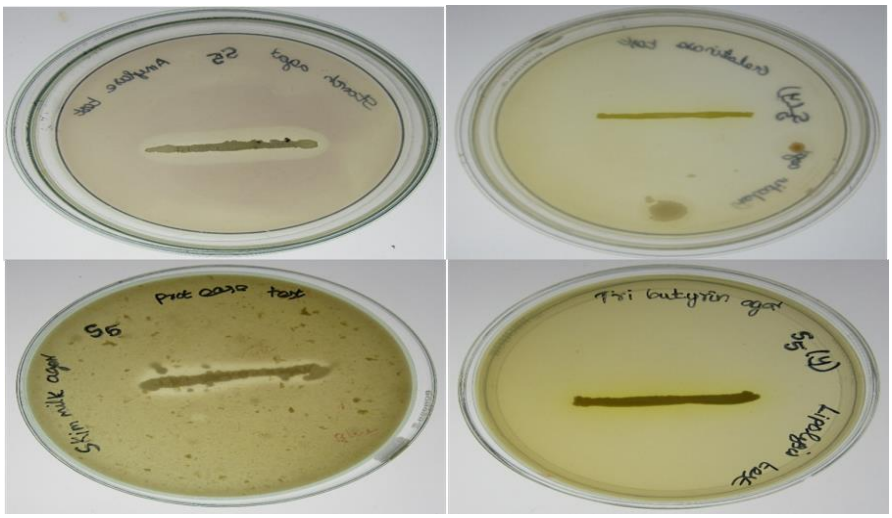

Figure: 1. Enzyme screening of selected *Actinomycetes sp* from Rajakkamangalam mangrove

Antibacterial Screening of Selected Isolates

Various aquaculture diseases were investigated for antibacterial effectiveness against different actinomycetes strains. In initial screening, *Stapylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Vibrio harveyi*, and *Aeromonas hydrophila* were all susceptible to all isolates of actinomycetes RE1, RE2, RE3, and RE4. One strain out of the four has more action against diseases in aquaculture. According to **Table: 2 and Figure: 2,** the strain RE2 was most effective against *Bacillus cereus* (1.6 0.12 cm) and *Vibrio harveyi* (2 ± 04 cm).

Table. 2 *In vitro* antagonistic activity actinomycetes strains (RE1 – RE4) against aquaculture bacterial pathogens by spot inoculation method.

Actinomycetes strains	Zone of inhibition (cm) against aquaculture bacterial pathogens				
	E. coli	***V. harveyi***	***B.cereus***	***S. aureus***	***A. hydrophila***
RE1	0.5 ± 0.11	0.5 ± 0.14	0.5 ± 0.12	0.6 ± 0.12	0.3 ± 0.15
RE 2	0.4 ± 0.05	2 ± 0.04	1.6 ± 0.12	0.5 ± 0.04	1.3 ± 0.16
RE 3	0.8 ± 0.04	0.7 ± 0.03	0.5 ± 0.04	0.1 ± 0.12	0.0 ± 0.0
RE 4	0.7 ± 0.04	0.9 ± 0.12	0.4 ± 0.04	0.8 ± 0.16	1.1 ± 0.14

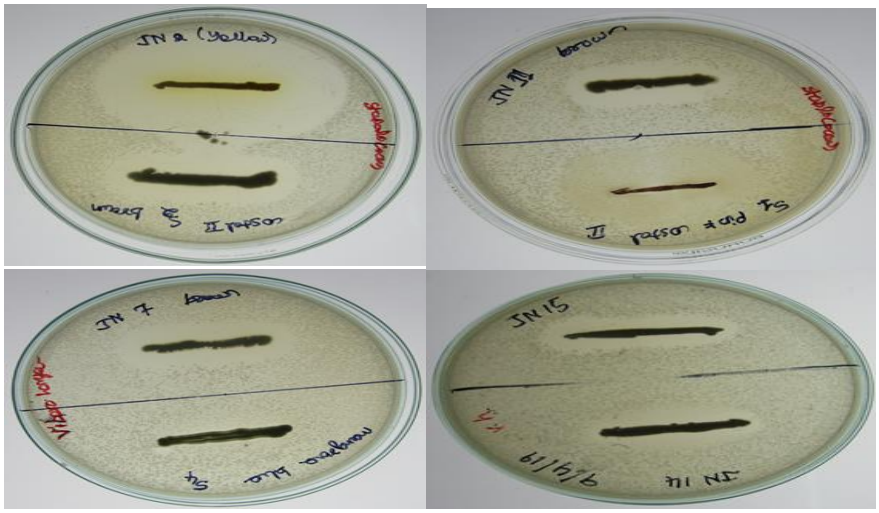

Figure: 2. Antibacterial activity of actinomycetes sp (RE1 to RE 4) against aquaculture pathogens by spot inoculation method

Morphological identification of isolated actinomycetes

The cover sip method was used to examine the morphology and cultural features of the four strains RE1, RE2, RE3, and RE4. The four strain isolates were all identified as Streptomyces sp. because they all belonged to the cream, ash, yellow, and pinkish ash color series (**Figure: 3**).

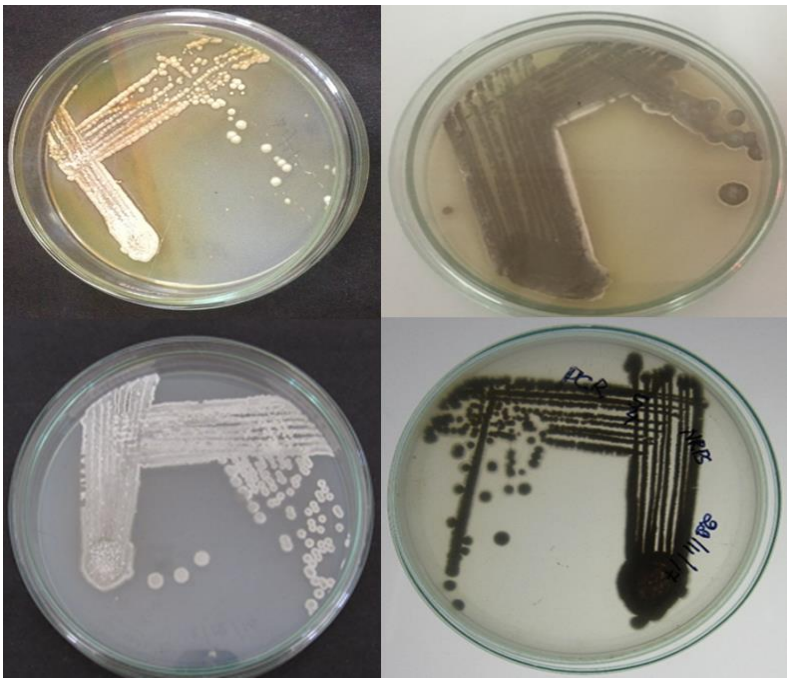

Figure: 3. Cultural observation of actinomycetes (RE1 – RE4) isolated from Rajakkamangalam mangrove

Biochemical characteristics of isolated actinomycetes

The Active four isolates (RE1, RE2, RE3, and RE4) were non-motile and gram positive in morphology. The strains RE1 and RE2 were a light cream color, RE3 was a yellow color, and RE4 was an ash color. The isolates displayed negative results for all of the actinomycetes tested for indole synthesis, methyl red, Voges Proskaur, and urease tests among the many biochemical features examined. The RE1, RE2, RE3 and RE4 strains all demonstrated positive results in the triple sugar ion, nitrate reduction, and oxidase test described in **Table: 3**.

Table: 3. Biochemical characterization of actinomycetes strains (RE1-RE4) isolated from Rajakkamangalam mangrove

S.No	Test used	RE 1	RE 2	RE 3	RE 4
1	Gram staining	+	+	+	+
2	Colony colour	White yellow	ash	grey	dark ash
3	Motility	Non motile	Non motile	Non motile	Non motile
4	Methyl red (MR)	-	-	-	-
5	Voges prosker(VP)	-	-	-	-
6	Indole production	+	-	+	-
7	Nitrate reduction	+	+	+	-
8	Urease activity	-	-	-	-
9	TSI test	+	+	+	+
10	Oxidase activity	+	+	+	+

Discussion

Actinomycetes continue to be frequently searched for new bioactive compounds and are unequalled sources of numerous significant bioactive chemicals with high commercial value (Okami and Hotta, 1988). Actinomycetes have been the subject of a remarkable amount of research that has resulted in the isolation of numerous naturally occurring antibiotics and therapeutically important enzymes (Hopwood and Wright, 1979). Overall findings showed that the *Streptomyces* species varied significantly in terms of appearance, biochemistry, and physiological traits. 2014 (Sathya and Ushadevi). The findings showed that actinomycetes had the ability to release a wide variety of enzymes, which may have evolved naturally as a result of the microbes' need to live in a hostile environment. Jeffery (2008).

Enzymes have received a lot of attention recently in industrial processes and have taken the position of chemical catalysts in a variety of pharmaceutical, textile, paper, food, and other industries. However, different enzymes from samples of terrestrial soil have been identified by numerous researchers (Sharma *et al*., 2005). The amylase enzyme that the marine isolate

produced was most active at pH 9.5 and 50 ºC. *Streptomyces rochei* is a potential microorganism to create the alkaline amylase needed for high-temperature and pH applications since its activity is comparable to that of thermostable, alkaline bacterial amylases. According to Thippeswamy *et al*., (2006), the functionality of the enzyme at higher temperatures increases the solubility of starch, lowers viscosity, restricts microbiological contamination, and speeds up reaction time.

Around 90% of the isolates produced one or more enzyme activities, according to studies on the enzymatic activity (Tsujibo *et al*., 2003; Muiru *et al*., 2007; Gulve and Deshmukh, 2011). Gelatin was discovered to be the optimum nitrogen source for the synthesis of protease in Streptomyces clavuligerus (Thumar and Singh, 2007). One of the most significant industrial enzymes, amylase is used in numerous industrial domains throughout Iran each year (Moghbeli and Noshiri, 2009). One of the most significant industrial enzymes, amylase is used globally in a variety of industrial fields. In light of this study, the ability of marine actinomycetes isolated from sediment to produce amylase was examined by Hwang *et al*., (2013) and Acharyabhatta *et al*., (2013).

Actinomycetes are the most capable group of organisms when it comes to producing industrial enzymes such cellulase, amylase, chitinase, gelatinase, caseinase, and lipase as well as antibacterial substances (antibiotics) (Bredholt *et al*., 2008). Aquatic actinomycetes were isolated in the Bengal gulf, and the results indicated that they may be a source of bioactive chemicals and industrial enzymes. (2006) Ward and Bora.

Conclusion

A speculative class of microorganisms known as "actinomycetes" has been identified as having numerous purposes in the synthesis of novel biologically active substances, including enzymes, antibiotics, and anticancer drugs. These microorganisms contain traits shared by both bacteria and fungi. The current investigation was focused on the isolation, primary screening of active enzyme production, and antibacterial potential against aquatic pathogens. In such favorable ways, the RE1, RE2, RE3, and RE4 isolates revealed promising enzymes and antibacterial compounds. This will be further enlightened the prospectives on developing antibiotics against vulnerable pathogens.

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