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Antibacterial Potential of Secondary Metabolites Producing Actinomycetes from Mangrove Ecosystem of Kanyakumari District

S. Jeraldin Nisha*[1], G. Uma[2,3], V. Samuel Gnana Prakash[4], S. Jameer Ahamed[5],T. Citarasu[6]

[1]Research Scholar (Reg. No: 17217042082005), Centre for Marine Science and Technology, ManonmaniamSundaranar University Rajakkamangalam, Kanyakumari District, Tamilnadu, India 629 502

[2]Assistant Professor, Centre for Marine Science and Technology, ManonmaniamSundaranar University Rajakkamangalam, Kanyakumari District, Tamilnadu, India 629 502.

[3]Assistant Professor, Biotechnology, Udhaya College of Arts and Science, ManonmaniamSundaranar University Rajakkamangalam, Kanyakumari District, Tamilnadu, India 629 502.

[4]Professor, Centre for Marine Science and Technology, ManonmaniamSundaranar University Rajakkamangalam, Kanyakumari District, Tamilnadu, India 629 502.

[5]Research Scholar (Reg. No: 21114012271031), Centre for Marine Science and Technology, ManonmaniamSundaranar University Rajakkamangalam, Kanyakumari District, Tamilnadu, India 629 502.

[6]Associate Professor, Centre for Marine Scienceand Technology, ManonmaniamSundaranar University Rajakkamangalam, Kanyakumari District, Tamilnadu, India 629 502.

[*1]**Corresponding Author (**SJN) E.mail: jeraldinnisha@gmail.com

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Abstract

Mangrove ecosystem is ideally situated at the interphase between the terrestrial and marine environment and harbors a rich and diverse group of microorganisms. Actinomycetes are the most economically and biotechnologically important prokaryotes; hold a prominent position due to their diversity and proven ability to produce novel bioactive compounds. The aim of this study was to isolate the bioactive actinomycetes strains capable of producing antimicrobial compound from the Manakudy and Rajakamangalam mangroves of Kanyakumari District, India. Here, 25 isolates of actinomycetes were isolated from the two different mangrove environment and 11 morphologically distinct isolates selected for further analysis based on antibacterial activity and extracellular enzymes production and the potent strains were identified by morphological, biochemical and genomic level identification. The isolates were preceded to preliminary screening for antibacterial activity against the bacterial pathogens by the agar overly method and cross streak method and the S4 showed highest activity against *V. harveyi*, *S. aureus*, and *B. cereus* in both methods. The selected 4 isolates were found to be positive for the production of industrially important enzymes such as amylase, lipase, and proteases.

Keywords: Actinomycetes; Antagonistic; Mangrove Ecosystem; *Streptomyces*sp

1. INTRODUCTION

Mangrove forests are also called as “tidal forests”, “coastal woodlands”, or “oceanic rain forests” that grows at the interface between land and sea in tropical and subtropical latitudes. Mangrove forests are highly productive ecosystems which comprises of unique woody plant communities and located in tropical and subtropical coastal area [1,2 and 3]. Globally mangroves are mainly distributed in Asia (42%), Africa (20%), North and Central America (15%), Oceania (12%) and South America (11%) [4], and covers about 60%–75% of the global tropical and subtropical coastlines [5]. Microorganisms are adapted to survive in harsh conditions such as high moisture, high salinity, high temperature, low oxygen, and high organic matter content extreme tides [6].

Actinomycetes represent a high proportion of the soil microbial biomass and have the capacity to produce a wide variety of antibiotics and extracellular enzymes. Among these medications, actinobacteria, a Gram-positive bacterium with a high genomic G+C content, is the

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source of 75% natural compounds. Actinobacteria create secondary metabolites with a wide range of structural variations, including alkaloids, polyketides, terpenes, and macrolides, among others. The number of biosynthetic gene clusters in actinobacteria is significantly more than the number of chemicals that have been extracted from them, according to current genomics research. In order to increase the number of lead compounds for therapeutic development, it is important to identify all of the gene clusters and investigate the potentials of secondary metabolic biosynthesis in actinobacteria [7].These metabolites are known to possess antibacterial, antifungal, neuritogenic, anticancer, antialgal, antimalarial and anti-inflammatory activities [8].Actinomycetes secondary metabolites may one day serve as novel antibiotics, anticancer medicines, immunosuppressive agents, and enzyme inhibitors, according to numerous studies. More than 10,000 of the approximately 23,000 bioactive chemicals produced by microbes up to this point have been identified from actinomycetes. Hence the continued interest in screening such organisms for new bioactive metabolites. The current work is intended to isolate secondary metabolite producing microorganisms from mangrove environments of Kanyakumari District.

2. MATERIALS AND METHODS

2.1 Enumeration of actinomycetes

The samples such as mud, leaf litter and mangrove root associated soil were collected from Rajakkamangalam and Manakudy mangroves Kanyakumari District, Tamil Nadu. Isolation and enumeration of atinomycetes were performed on selective media such as Actinimycetes Isolation Agar (AIA) (Himedia Mumbai, India), Streptomyctes agar (Himedia, Mumbai, India), Knights media and Starch Caesin Agar (SCA) After incubation, actinomycetes colonies were selected and maintained by sub culturing and stored at 4 ^{o} C as well as at 20% (v/v) glycerol stock at -80^{o}C [9].

2.2 Identification of isolated actinomycetes

The isolates were morphologically and biochemically characterized by Gram's staining, motility, MR-VP, indole production, nitrate production, citrate utilization, urease, TSI, oxidase, catalase and haemolytic tests. The spore bearing hyphae and spore chain was determined by direct examination by coverslip method. Bergey's Manual of Determinative Bacteriology.

2.3*In vitro* screening for antimicrobial activity

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All the actinomycetes isolates were primarily screened for antibacterial activity against the test microorganisms (*Stapylococcus aureus, Escherichia coli, Bacillus cereus, Vibrio harveyi*and *Aeromonas hydrophila*) by Agar overlay method[10]and Cross streak method[11]

2.4 Enzyme screening in the selected isolates

Enzyme screening such as protease activity[12], amylase activity[13], lipase activity [14], gelatinase activity and xylanase activity were performed in the selected isolates.

3. RESULT

3.1 Isolation of actinomycetes sp

In the present study, 25 actinomycetes strains were isolated from mud, degraded plant, soil and samples were collected from the Rajakkamangalam, and Manakudy mangroves, Kanyakumari District, Tamil Nadu. Were from the 25 isolates high inhibitory active strains, ten actinomycetes strains (S1-S10) selected for further analysis. All the isolates were Gram positive and non-motile.

3.2 Morphological identification of actinomycetes sp

Identification of strains by both morphological and cultural characteristics revealed that most of the isolates belonged to the cream, ash, yellow, and greenish ash colour series show this (Figure 1) and that was why the isolates were assigned to the *Streptomyces* sp.

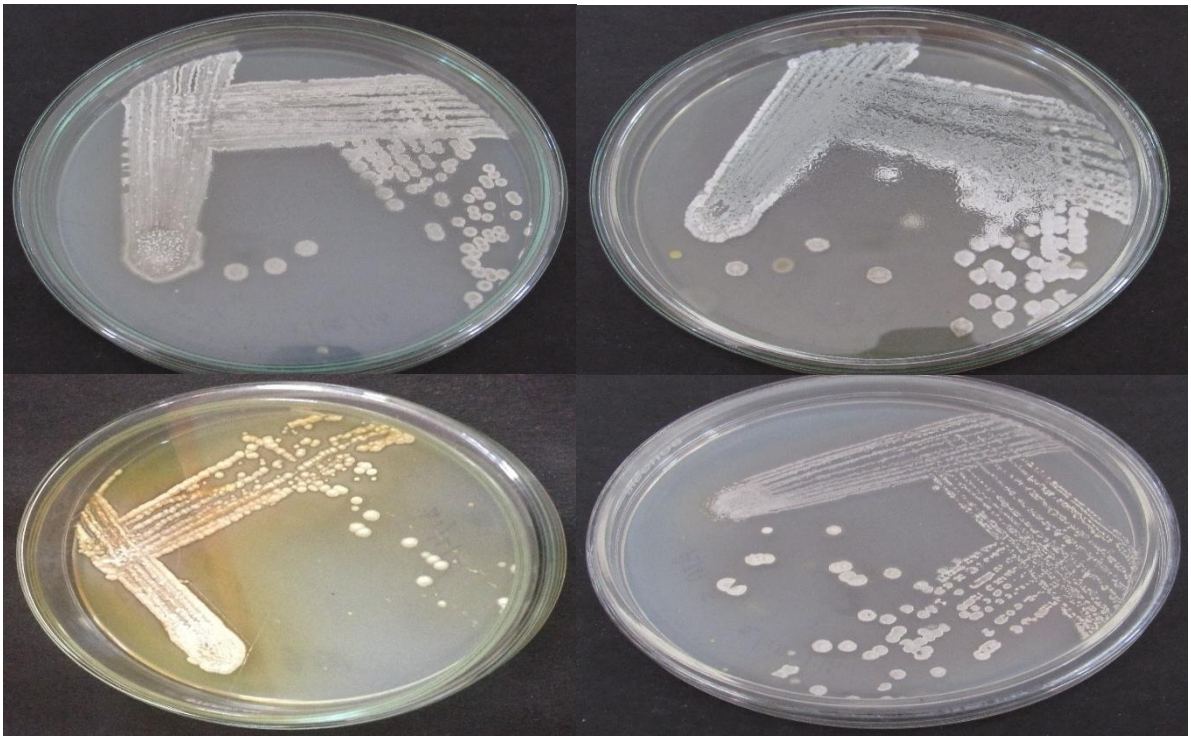

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Figure 1. The isolates selected for further processing actinomycetes strains are (S1, S2, S3 and S4)

3.3 Cultural characteristic of actinomycetes sp on different media

To study the cultural characteristics of actinomycetes isolates different media were selected. The cultural and morphological characteristics of the isolates in different media. The actinomycetes isolates (S1-S10) showed excellent growth and abundant aerial mycelium formation on starch casein agar.

3.4*In vitro* screening for antagonistic activity

All the actinomycetes isolates were primarily screened for antagonistic activity the test microorganisms such as bacterial pathogens which was done by cross streak method and spot inoculation method.

In the cross streak method among the different isolates tested against the bacterial species, S1, S2, S3 and S4 were effectively controlled and showed zone of around 2 ± 0.04 cm. The strain *Streptomyces* sp. S4 (Figure 2) showedhighest activity against *Vibrio harveyi, V. parahaemolyticus and A. hydrophila*. *Streptomyces* sp. S3 showed activity against the*Vibrio harveyi*and *A. hydrophila*.

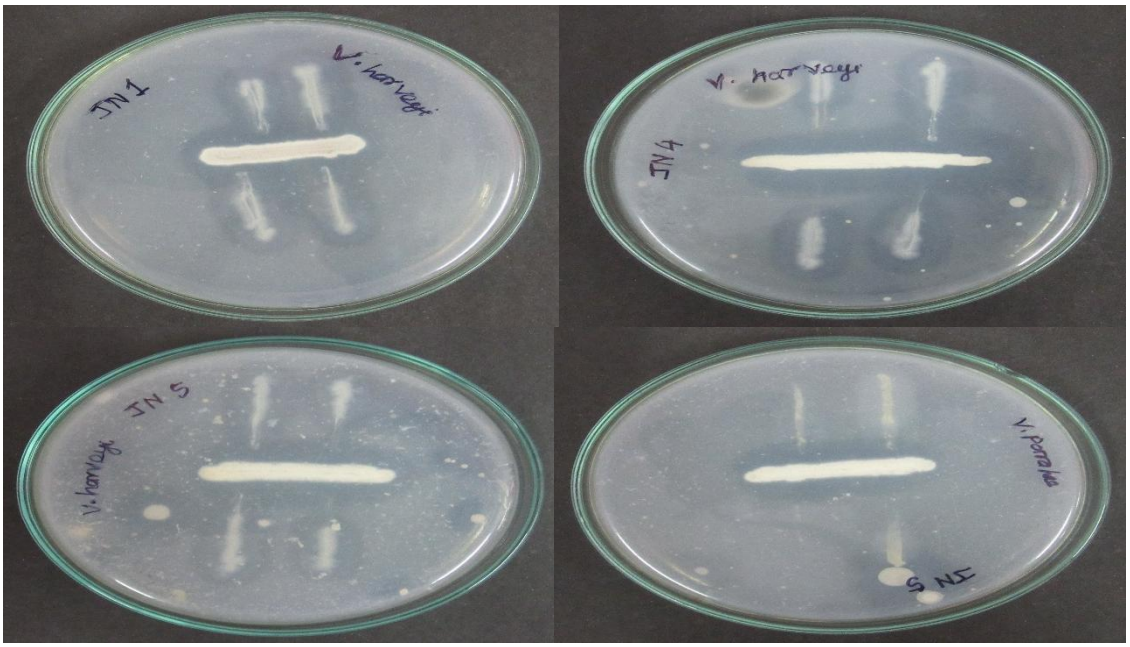

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Figure 2. *In vitro* antagonistic activity against bacterial pathogens by cross streak method

In the spot inoculation method, among the different isolates tested against the bacterial species, S1, S2, S3 and S4 were effectively controlled (Figure 3) andshowed zone of around 2.5 cm. The strain *Streptomyces* S4 showed highest activity against *Vibrio harveyi*, *Staphylococcus aureus* and *Bacillus*. *Streptomyces* sp. S3 showed activity against the*Vibrio harveyi and Bacillus cerus*.

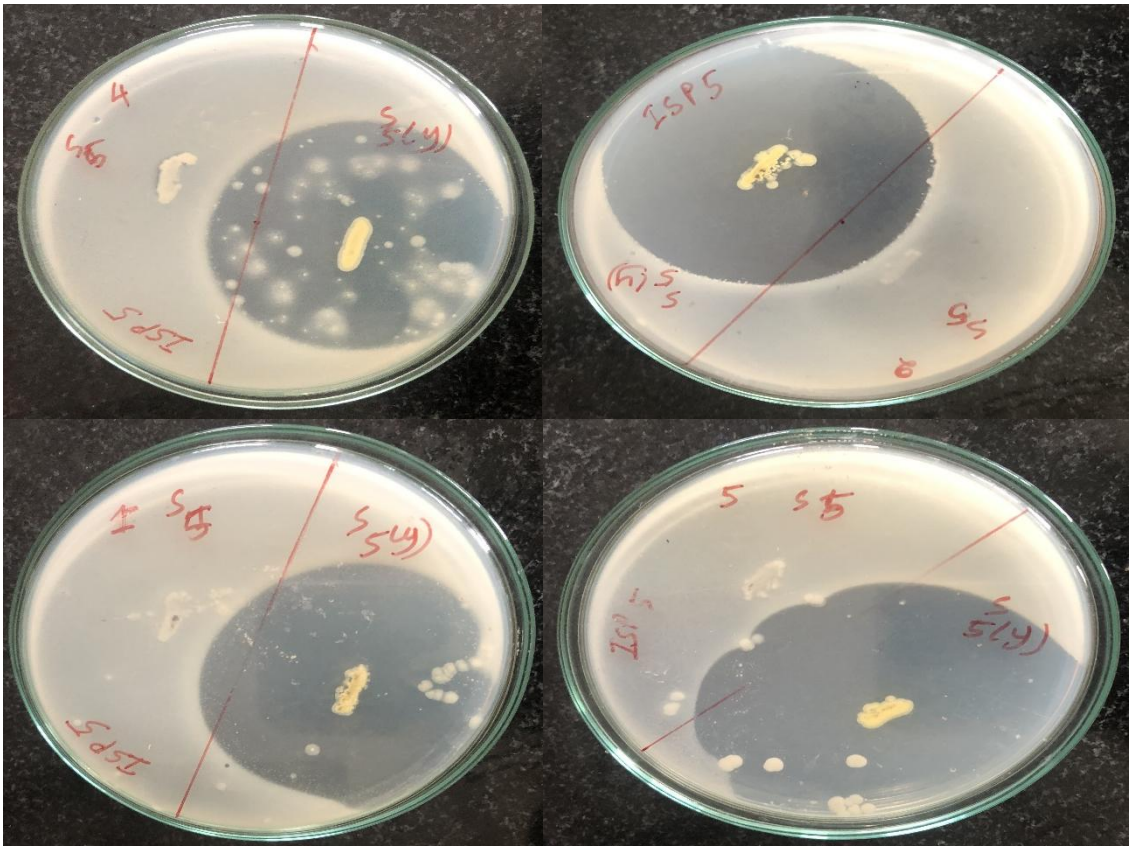

Figure 3. *In vitro* antagonistic activity against bacterial pathogens by spot inoculation method

3.5 Enzyme screening

The protease production were observed in *Streptomyces*sp S2 and *Streptomyces*sp S3; amylase production in *Streptomyces* sp S1, *Streptomyces* sp S2, *Streptomyces*sp S3 and *Streptomyces* sp S4 result were given in the (Table 1).

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Actinomycetes strains	Extracellular hydrolytic enzymes				
	Protease	Amylase	Lipase	Gelatinase	Xylanase
S1	–	+	–	–	–
S2	+	+	–	–	–
S3	+	+	–	–	–

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S4	–	+	–	–	–
S5	–	–	–	+	–
S6	–	–	–	–	–
S7	–	–	+	+	–
S8	–	–	+	–	–
S9	–	–	–	–	–
S10	–	+	–	–	–

Table. 1 Screening of enzymes

4. DISCUSSION

Actinomycetes strains from south Pacific coast of Philippines revealed that most (54%) of the isolates belonged to white and grey colour series [15]. In the present study all the ten actinomycetes strains (S1-S10) were Gram positive and non-motile organisms. The strain (S1 and S9) were pale cream colour, (S5 and S4) were yellow colour, (S6 and S10) were ash colour, (S3 and S7) pinikish ash colour, S2 were yellowish brown, and S8 light blue in colour. Initially, actinomycetes were characterized on the basis of morphological characters so as to have a preliminary determination of the genus. However, in the present study the maximum numbers of actinomycetes colonies were isolated on starch casein agar and actinomycetes isolation agar. Among the four-culture media, starch casein agar is the best medium for the isolation of actinomycetes strains. This performance can be explained by the presence of starch and casein in the media which stimulate the growth of actinomycetes in performance to other bacteria [16]. Actinomycetes screened from mangrove have proved *Streptomyces*sp as the potent in inhibiting Gram positive as was evidenced in the present study [17]. Also*Streptomyces* sp. exhibited significant antibacterial activity against pathogens i.e., *Bacillus* sp., *Staphylococcus* sp. and *Vibrio* sp. as reported earlier [18].

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The protease production were observed in *Streptomyces*sp S2 and *Streptomyces* sp S3; amylase production in *Streptomyces* sp. S1; *Streptomyces* sp. S2; *Streptomyces* sp. S3; *Streptomyces* sp. S4. Lipase production only in *Streptomyces* sp. S1. Industrial production application of alkaliphilic enzymes such as protease, cellulases, lipases were well documented [19]. The present studies, *Streptomyces* sp. S2 and *Streptomyces* sp. S3 were able to produce protease and amylase. *Streptomyces* sp. secretes a wide range of extracellular hydrolytid enzymes. To degrade lignocellulosic material in soil [20] actinomycetes are reservoir of many enzymes including cellulases, xylanases, amylases, lipases, collagenases, proteases, chitinases, ligninases etc.

5. CONCLUSION

The present research highlights the importance of mangrove actinomycetes which are quite active in producing antagonistic metabolites. In this study we found 10 out of 25 isolates are efficient in producing antimicrobial substance against *Stapylococcus aureus, Escherichia coli, Bacillus cereus, Vibrio harvey*iand *Aeromonas hydrophila.* The strain (S1-4) have an enzyme activity.

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