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Original Article

INDUSTRIALLY IMPORTANT ENZYMES PRODUCING *STREPTOMYCES* SPECIES FROM MANGROVE SEDIMENTS

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ABSTRACT

Objective: To explore the systematic diversity of mangrove actinomycetes as well as screening of top valued enzymes

Methods: The target sampling region was selected on Muthupet mangrove forest, Tamilnadu, India. Soil samples were collected at a depth of 15-25 cm from the land to the marine. All isolates were identified by classical approach by means of morphological, physiological and biochemical characterization. In addition, the ability of selective *Streptomyces* isolates producing important enzymes such as amylase, protease, lipases, esterase and gelatinase were screened for industrial application.

Results: In total, 205 actinomycetes were isolated from mangrove sediments and most of the genus belongs to *Streptomycetes*. Among, 35 morphologically distinct *Streptomyces* species were exhibited positive results for amylase (25%), Protease (21%), Lipase (16%), Esterase (17%) and Gelatinase (21%)

Conclusion: The research focused towards exploring marine actinomycetes as a source for enzyme reservoir to compensate current industrial demands.

Keywords: Mangroves, Actinomycetes, Diversity, *Streptomyces*, Enzyme sources.

INTRODUCTION

Muthupet mangroves look an inimitable ecosystem, situated at the southern end of the Cauvery delta and covering an area approximately 13,500 ha [1]. Mangrove is an intertidal zone located in the changeover between land and sea. This ecosystem is categorized by periodic tidal flooding which makes environmental factors such as salinity and nutrient availability extremely variable, resulting in unique and precise characteristic [2]. Microbial diversity comprises an infinite group of novel chemistry, creating upon precious source for innovative biotechnology [3, 4]. Marine environments are enormously unusual from terrestrial ones; microorganisms living in this environment produce some unique properties. Actinomycetes are virtually unrestricted sources of novel compounds with many industrial applications. The distribution of actinomycetes are highly depends upon the uniqueness of environmental condition were studied [5, 6]. However, the distribution of actinomycetes in the marine is chiefly unexplored or underexploited habitats be pursued as sources of novel compounds for industrial usage [7, 8].

Actinomycetes are aerobic, gram-positive bacteria that have high GC content in their DNA (69-73%). They form extensive branching substrate, aerial mycelia with numerous pigmentations and widely distributed in soil [9]. Currently, increasing number of investigation has been done to identify ecological factors deciding for active compounds of soil actinomycetes in the estuarine mangrove ecosystem [10]. Addition of slightly acidic environment need to harbor a greater diversity of actinomycetes than neutral condition, the use of appropriate selective media for promoting the growth of rare actinomycetes and enhances the selection of members of the actinomycetales family [11, 12, 13].

Among actinomycetes, *Streptomyces* are dominant and are considered economically important because of more than thousands of antibiotics and manufacture of many commercially significant enzymes are produced by the genus. Industrial enzymes are naturally used as mass in detergents, textile, pulp and paper industries. Global market for industrial enzymes was $3.3 billion in 2010 and likely to reach $4.4 billion by 2015. Enzymes were treasured over $1 billion in 2010 by several research associations. It is predictable that the technical enzymes market will increase at a 6.6% compound annual growth rate (CAGR) to reach $1.5 billion in 2015 with the highest sales in leather market [14]. In recent years, microbial enzymes are replacing chemical catalysts in manufacturing chemicals, food, leather and pharmaceutical industries. Food industry is continuously looking for higher technologies to meet the demand. Microbial enzymes formed by fermentation technique can simply be scale up. Moreover, due to quick doubling time of microbes with special features than plants and animals, microbial fermentation process may possibly meet the current market demand for industrial enzyme [15].

Amylase and protease occupies the top most position in the world market and play a vital role in various industrial applications, the demand of these enzymes increasing day by day, thus it is vital to identify hyper producing microbial strains in order to meet the ever-growing industrial demand[16]. Lipases and esterases are a diverse group of enzymes that catalyze the hydrolysis of ester bonds in triacylglycerides to glycerol and fatty acids [17]. Lipases have extensive range of enzymatic properties and substrate specificities produce them very useful for industrial function such as processing of fat and oils, additives, detergents, cosmetics, paper manufacturing and pharmaceuticals [18]. Lipolytic enzymes have many industrial applications but still demand for the biocatalyst with novel and specific properties such as pH, temperature, specificity and stability is increasing some new molecular approach [19]. Another one important enzyme gelatinase have established significant attention as targets for drug development; because of their prospective function in connective tissue degradation linked with tumor metastasis[20]. Based on the valuable application of above all enzymes, the present research planned to elucidate the efficient diversity of actinomycetes distribution, potential usefulness of *Streptomyces* groups in enzyme production was achieved to address the actinomycetes diversity as an essential resource for industrial market.

MATERIALS AND METHODS

Sampling locations

The underneath soil samples (15-25 cm) were collected from mangrove locations as presented in the GPS point (Fig.1). The soil

samples were collected from different region of dense muthupet mangrove forest. The samples were sustained at suitable temperature with mangrove water and were transported to the laboratory.

Fig. 1: GPS map of mangroves

Isolation of Actinomycetes

Soil samples were subjected to 10-fold serial dilution and aliquots (0.1) were plated on starch casein agar medium supplemented with 50% seawater and antibiotics (10µg/ml Streptomycin and Gresiofulvin) [21]. Plates were incubated for 7-14 days at suitable temperature. Individual isolated colonies were selected for purification of actinomycetes. Purified isolates were kept in slant culture at 4°C.

Characterization of Actinomycetes

The selected foremost actinomycetes strains were studied for cultural, morphological, physiological and biochemical characteristics. The classical method for the identification of aerial mass color, reverse side pigmentation described by Nonomura [22] and Bergey's Manual of determinative Bacteriology[23]. The morphology of spore chain ornamentation, spore surface and spore bearing hyphae were identified using light microscope (oil immersion objective at 100x magnification).

Enzymatic Screening of *Streptomyces*

Based on the growth phase, rapid growing *Streptomyces* selected to evaluating the production of amylase, protease, gelatinase, lipase and esterase activity. In each of this analysis, the enzyme activities were calculated by the value obtained subtracting the halo size by the diameter of the *Streptomyces* colony.

Amylolytic activity

Screening of *Streptomyces* for amylase production was performed by starch hydrolysis reaction.

The *Streptomyces* isolates were inoculated on starch agar medium and the plates were incubated at 28°C for 4-7 days. After *Streptomyces* growth, 4 ml of iodine solution were loaded onto each plate and observed zone of clearance around the colonies. The results were indicated the presence of amylase enzyme [24].

Proteolytic activity

Streptomyces isolates were inoculated on to protease agar medium containing (1%) peptone and incubated 28°C for 4-7 days. Enzyme activity was detected by visualization of transparent zone around the colonies [25].

Lipolytic/ Esterase activity

Lipolytic activity was detected using a culture medium described by Sierra[26]. After sterilization of SCA medium, Tween 20 previously sterilized then added to the final concentration of 2% (v/v). After 7 days of incubation, growth was observed and the presence of digestive zone was indicates lipolytic activity. Sequentially to detect esterase activity, after sterilization of culture medium, Tween 80 was aseptically transferred to the final concentration 2% (v/v). The presence of degradation zone was indicated esterase activity

Gelatinase activity

Streptomyces isolates inoculated on gelatin agar medium containing 8% (w/v) gelatin, plates were incubated at 28°C for 4-7 days. After incubation, halos were observed the culture indicated gelatinase production[27].

RESULTS AND DISCUSSION

The systematic microbial diversity screening process was identified extremely large number of actinobacteria. Totally 205 actinomycetes was obtained from different location of muthupet mangrove regions.

The distribution of actinobacteria including *Streptomyces* sp. (73%), *Micromonospora* sp. (12%), *Nocordia* sp. (10%) and *Mycobacterium* sp. (5%) were identified (Fig. 2).

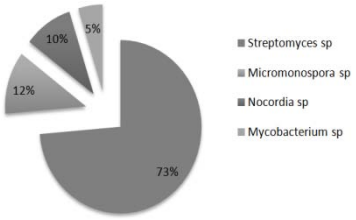

Fig. 2: Diversity of Actinobacteria

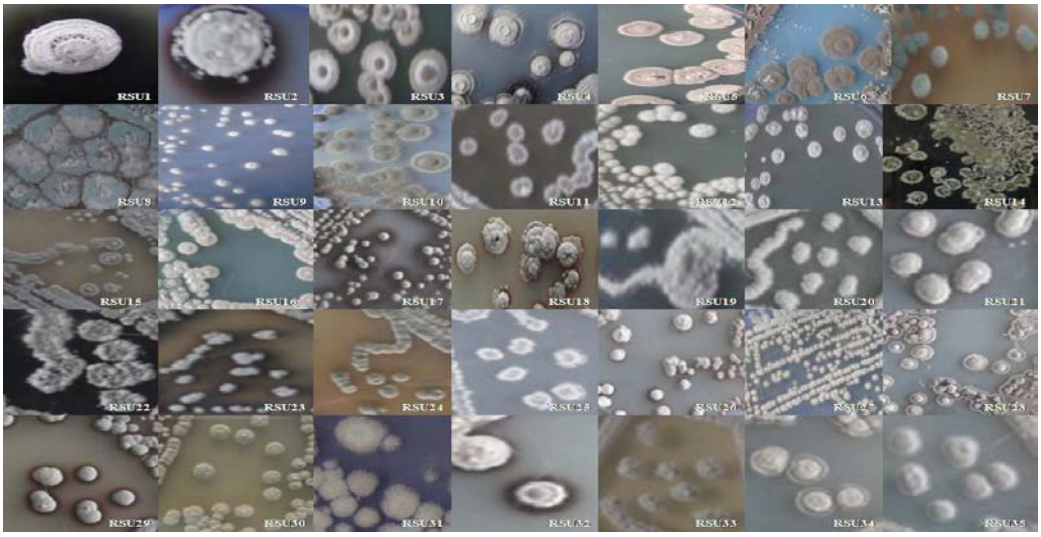

Fig. 3: Macroscopic observation of numerous *Streptomyces* species

The group of genus present in soil sample varied from one region to others. The majority of the actinomycetes isolates from sediment sample are belongs to the genera *Streptomyces*, the selective isolates is given in (Fig.3). Previous findings clearly reported the diversity and distribution of actinomycetes population were high in Muthupet mangroves [28]. Diverse group of Actinomycetes were isolated from mangrove environment of Palaverkadu [29]. Members of the actinomycetes, which live in marine environment, insufficiently understood and few reports are available pertaining actinomycetes from mangroves [30]. The diversity of actinomycetes was studied from the Palk Strait region of the Bay of Bengal[31, 32]. The present study was similarly corroborates with previous reports that *Streptomyces* are leading species in the marine atmosphere[33]. Among them 35 *Streptomyces* species, were selected based on cultural characterization. Every one of the isolates was identified as gram-positive aerobic and non-endospore forming organism. The color of substrate and aerial mycelium was recorded in a simple way (White, ash, gray, yellow, green, black, red, violet, brown and etc). Spore ornamentation, spore size, surface of the colonies (both smooth and rigid) appearance was observed (Table.1).

Table 1: Morphological characterization of Mangrove *Streptomyces* species

Streptomyces	**Appearance**	**Spore surface**	**Size**	**Pigmentation**
RSU1	Powdery with bulged white colonies	Smooth	9 mm	Dark Green
RSU2	Ash with gray color colonies	Smooth	6 mm	Violet
RSU3	White with cream circular colonies	Smooth	2 mm	Pale yellow
RSU4	Gray white with multiple circles	Watery	5 mm	Light Pink
RSU5	Cream with white fine powder	Dew	7 mm	Brown
RSU6	Shirking with ash color colonies	Smooth	3 mm	Violet
RSU7	Green with white colonies	Smooth	8 mm	Yellow
RSU8	Green with gray color	Smooth	2 mm	Brown
RSU9	Pure white spongy appearance	Rough	9 mm	-
RSU10	Ash with brown, multiple circular	Smooth	3-6 mm	-
RSU11	White with orange color	Smooth	1-4 mm	Orange
RSU12	Pure white color colonies	Rigid	5 mm	Yellow
RSU13	Pale white cotton like colonies	Rigid	1-4 mm	Brown
RSU14	Ash with green color powder colonies	Flexuous	3-5 mm	Green
RSU15	Ash color double circular colonies	Smooth	8 mm	Brown
RSU16	Cream color multiple colonies	Rough	6 mm	-
RSU17	Cotton like white colonies	Smooth	7 mm	Violet
RSU18	Gray with bulged pink color colonies	Smooth	5 mm	Pink
RSU19	Pure white with multiple layer	Tiny	2 mm	-
RSU20	White color colonies	Rough	4 mm	Orange
RSU21	Cream with ash double circular margin	Smooth	3 mm	Violet
RSU22	Flower like ash color cummy colonies	Smooth	1-3 mm	Green
RSU23	White with violet color bulged colonies	Smooth	4-7 mm	Violet
RSU24	Ash color branched colonies	Rough	5-6 mm	Orange
RSU25	Flower like white color colonies	Smooth	1-5 mm	Yellow
RSU26	Gray with white color colonies	Smooth	3-5 mm	Violet
RSU27	Gray color tiny powdery colonies	Smooth	4-6 mm	-
RSU28	Gray color double circular rigid margin	Smooth	1-6 mm	Violet
RSU29	Violet with white color colonies	Smooth	2 mm	Violet
RSU30	Creamy with tiny pores like appearance	Smooth	4 mm	-
RSU31	Cotton appearance	Smooth	5 mm	
RSU32	Button shape gray color colonies	Smooth	4-6 mm	Violet
RSU33	Powdery gray color colonies	Smooth	1-4 mm	Orange
RSU34	Two layer ash color circular margin	Smooth	4-6 mm	Pink
RSU35	White with bulged colonies	Smooth	7 mm	Light yellow

All the isolates were grown in the particular pH and temperature 7.2 and 28^{o}C respectively. The production of Citrate, Catalase, Urease, Nitrate and Carbohydrate fermentation has been considered for the identification of isolated *Streptomyces* (Table.2). Overall results established that there was significant diversity among the *Streptomyces* species, morphology as well as biochemical and physiological properties. Totally 35 morphologically distinct *Streptomyces* species were selected for screening of economically valuable enzymes.

A positive result for amylase (25%), protease (21%), lipase (16%) esterase (17%) and gelatinase (21%) was showed that isolates exhibited enzyme activity (Fig.4; Table.2).

These finding proved that *Streptomyces* to be the superior enzyme producing group within the actinomycetes family, mainly for the production of amylase, protease and gelatinase activity. The other two enzymes (lipase/esterase) also provide good result (Fig.4).

Table 2: Enzyme screening/Biochemical characterization of isolated Mangrove *Streptomyces* species

Streptomyces	**Average Zone of Clearance (mm)**					**Biochemical Characterization**			
	Amylase	**Protease**	**Lipase**	**Esterase**	**Gelatinase**	**Citrate**	**Catalase**	**Urease**	**Nitrate**
RSU1	14.1	18.0	4.5	6.5	12.0	+	+	+	+
RSU2	35.0	7.0	-	-	-	+	+	+	+
RSU3	9.4	13.4	14.5	-	9.0	+	+	-	+
RSU4	12.7	21.2	-	11.8	4.1	+	-	+	+
RSU5	7.0	27.4	-	9.2	13.0	+	-	-	+
RSU6	15.0	28.0	-	5.3	11.2	-	-	-	-
RSU7	24.9	4.1	9.3	7.4	4.2	-	-	-	-
RSU8	13.2	17.0	-	4.1	7.1	+	-	-	-
RSU9	5.0	15.9	10.3	3.9	-	+	-	+	-

RSU10	-	12.0	15.9	9.4	-	+	+	+	-
RSU11	27.1	-		-	9.4	-	+	-	-
RSU12	30.9	-	-	-	-	-	+	+	-
RSU13	21.5	15.7	-	-	9.5	+	+	+	-
RSU14	23.7	-	18.9	5.8	13.9	+	+	+	-
RSU15	31.0	-	7.3	9.1	-	+	-	+	+
RSU16	-	-	-	11.4	-	-	-	-	+
RSU17	-	7.9	-	-	-	-	-	-	+
RSU18	16.0	13.9	8.4	-	-	+	-	-	-
RSU19	8.9	16.2	21.0	-	14.9	+	+	-	-
RSU20	7.9	15.8	-	12.0	12.0	-	+	+	-
RSU21	20.7	3.9	-	14.5	19.0	+	+	+	-
RSU22	14.0	27.5	5.8	-	20.7	+	-	+	-
RSU23	27.3	32.0	4.7	13.9	16.0	+	+	+	+
RSU24	34.0	-	3.9	7.9	8.0	+	-	-	+
RSU25	29.7	-	-	3.5	15.7	+	+	-	+
RSU26	35.1	34.0	21.0	2.9	18.0	-	-	-	+
RSU27	11.0	-	14.9	-	3.9	-	+	-	+
RSU28	17.8	-	12.2	-	5.2	-	-	+	-
RSU29	5.7	-	11.0	-	4.8	+	+	+	+
RSU30	10.0	25.6	18.1	-	7.8	+	-	+	-
RSU31	21.9	20.5	-	7.3	16.5	+	-	+	-
RSU32	14.9	19.5	-	5.9	11.0	-	+	+	-
RSU33	23.7	17.2	-	4.2	13.9	-	+	+	-
RSU34	34.5	-	21.9	6.0	-	-	-	-	-
RSU35	-	15.9	-	-	17.4	-	+	-	+

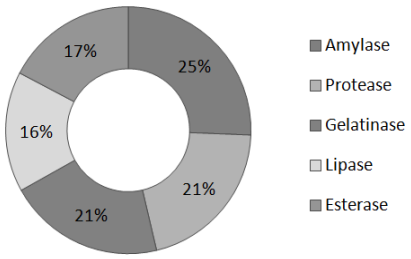

Fig. 4: Enzyme screening from *Streptomyces species*

The extensive variation among the different *Streptomyces* species on enzyme production was recorded. Microbial diversity is essential one for selecting hyper producing strains in order to meet the ever-growing demand. Likewise, previous findings also proved the production of enzymes from marine actinomycetes[34, 35].

Hence, the present data state that extremely various amounts of top valued enzymes were screened from numerous *Streptomyces* species isolated from mangrove environment.

CONCLUSION

Biological diversity of mangrove habitats is significantly identified that large number of enzymes has existed in the *Streptomyces* species. The outcome of the present study was actinomycetes diversity established a wide range of isolates in mangrove environment; especially *Streptomyces* species has been produced a great number of enzymes for industrial application and utilize them in all biotechnological industries. This finding has created a new avenue for mangrove *Streptomyces* species is essential one for enzyme market. Further development research can improving isolation strategies in the recovery of marine actinomycetes is of greatest significance for ensuring wide achievement in mangrove regions.

CONFLICT OF INTEREST

We declared that there is no conflict of interest.

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