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Research Article
Characterization of Actinobacterial Population in the Seagrasses Rhizosphere Soils of the Gulf of Mannar Biosphere Reserve, India

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Abstract

Actinobacterial species diversity and population characterize of rhizosphere soil of six seagrasses species of the Gulf of Mannar Biosphere Reserve India was studied using Kuster's agar (KUA) and Actinomycetes Isolation Agar (AIA) for the first time. Higher population density was recorded in the, *Halophila* sp. rhizosphere soil (67×10^2 CFU g^{-1}) and the lower density was recorded in *Syringodium* sp. rhizosphere soil (24×10^2 CFU g^{-1}) in Kuster's agar. Whereas in AIA medium, higher population density was recorded in the *Halophila* sp. and *Thalassia* sp. rhizosphere soils (49×10^2 CFU g^{-1}) and the lower density was recorded in the *Syringodium* sp. rhizosphere soils (38×10^2 CFU g^{-1}). Out of the 59 strains, characteristically distinct 30 strains were selected for further taxonomic identification based on the morphological and good growth characterization. On the basis of spore mass colour, reverse side colour, aerial and substrate mycelia formation, production of diffusible pigment sporophore morphology and examination of spores by using SEM results all the 30 isolates were taxonomically identified as different *Streptomyces* species.

Key words: Marine actinobacteria, chemotaxonomy, identification, population density, microbial community

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Marine actinobacteria provide with many important bioactive compounds of high commercial value and hence they are routinely screened for new bioactive substances. These searches have been remarkably successful and approximately two-third of naturally occurring antibiotics including many of the medically important antibiotics has been isolated from actinobacteria (Okami and Hotta, 1988). Results of the intensive screening programmes carried out over the past several decades indicate that there is a growing trend in the rediscovery of already known bioactive compounds (Nolan and Cross, 1988). However, it has not yet been resolved whether actinobacteria form part of the autochthonous marine microbial community of the sediments or the actinobacteria isolated from the marine sediments have originated from terrestrial habitats and have been simply carried over to the sea in the form of resistant spores (Jensen *et al*., 1991).

It was felt in the early 1950s that better taxonomic criteria are required for the classification of actinobacteria. Advances in the biochemistry of microorganisms have revealed that the cell component analysis can be effectively applied to bacterial systematics, which provides the basis for chemotaxonomy. Analysis of cell wall amino acids and whole cell sugars provide the door-step for the actinobacteriologists to identify these organisms at generic level (Sivakumar, 2001). Similarly, Maldonado *et al*. (2005) selected carbon sources for the targeted isolation of marine actinomyces based on data generated by culture-independent studies on the *in situ* diversity as seen by ribosomal analysis of the actinomycetes present in the samples.

A total of 20 different actinomycetes were recovered from salt pan region of Kodiakarai, Nagapattinam District using starch casein agar medium (Gayathri *et al*., 2011). Sharma and David (2012) reported the actinomycetes diversity of the marine sediments from Pulicat estuary, Muttukadu and Ennore estuaries, Tamil Nadu. Totally, 227 isolate were morphologically distinct on the basis of spore mass colour, aerial and substrate mycelia formation and production of diffusible pigments. The majority were assigned genus Streptomyces (60%; 162 isolates) and Actinopolyspora (5%; 11 isolates). A Gram-stain-positive, non-spore-forming bacterium was isolated from the marine sponge *Aplysina aerophoba* (Kampfer *et al*., 2014). Actinomycetes have been looked upon as potential sources of bioactive compounds andthe work done earlier has shown that these microbes are the richest sources of secondary metabolites. They hold a prominent position as targets in screening programs due to their diversity and their proven ability to produce novel metabolites and other molecules of pharmaceutical importance. This great diversity of marine actinomycetes has offered greater chemical diversity. The diverse chemical compounds of marine actinomycetes have been found to have various biological activities such as antimicrobial, antitumor, anti-malarial, anti-algal, antioxidant and anti-inflammatory (Baskar *et al*., 2015).

A number of new and innovative techniques have been developed in recent years to increase the efficiency of the isolation of novel microorganisms from the marine biosphere (Toledo *et al*., 2006). Since, then a number of works on actiobacteria of India were carried out by different groups over a period of time (Umamaheswary *et al*., 2005; Sivakumar *et al*., 2006; Gunalakshmi *et al*., 2008; Sahu *et al*.,2009; Manivasagan *et al*., 2010; Rajkumar *et al*., 2012). However, actinobacteria of rhizosphere of seagrasses remains not attempted. Therefore, the main purpose for this study gain preliminary insight in the population diversity and density of marine actinobacteria of the seagrass rhizosphere soil samples of Gulf of Mannar Marine Biosphere reserve, India.

MATERIALS AND METHODS

Isolation of actinobacteria: Rhizosphere soil samples of *Cymodaceae* sp., *Enhalus* sp., *Halophila* sp., *Halodule* sp., *Syringodium* sp. and *Thalassia* sp. were collected from the shallow coastal waters of Pamban (latitude 8°35'-9°25' N; longitude 78°08'-79°30' E) in the Gulf of Mannar region of Tamil Nadu, India. Aseptically air-dried sediment samples were incubated at 55°C for 5 min (Balagurunathan, 1992) and pre-treated samples (1 g) were serially diluted (Jensen *et al*., 1991) and spread on Actinomycetes Isolation agar (AIA, Hi-Media, Mumbai) and Kuster's agar (Kuster and Williams, 1964) by spread plate method. To minimize the bacterial and fungal contaminations, all the agar plates were supplemented with 20 and 100 mg L^{-1} of nystatin and cycloheximide, respectively (Kathiresan *et al*., 2005). The actinobacterial colonies that appeared on the media were counted from 5th day on wards, upto 28th day. All the colonies that grew on the media were subcultured and maintained in slants culture. Based on the morphological distinctiveness and growth, actinobacterial colonies were selected for identification.

Identification (Genus affiliation)

Hydrolysis: Hydrolysis of the strains was done for releasing the amino acids. Harvested cells of each strain weighing 20 mg (fresh) were placed in a screw capped test tube and 1 mL of 6 N HCl was added and sealed with alcohol. The samples were kept at 121°C for 20 h in a sand bath. The bottles were cooled by keeping them at the room temperature of 28±2°C.

Hydrolysis was also done for releasing sugars separately. Harvested cells of each strain weighing 50 mg (fresh) were placed in an amber bottle and 1 mL of 5 N H_2SO_4 was added and sealed with alcohol. The samples were kept at 110°C for 12 h. The bottles were then cooled by keeping them at the room temperature of 28+2°C.

Thin Layer Chromatography (TLC): Spotting of the whole cell hydrolysis was made carefully on cellulase coated TLC plate (Merck, Pvt. Ltd. Kolkata) using a micropipette. Spots were of 0.5-1.0 cm. This was done by multiple applications on the same spot of very small portions of the sample, which were dried by a hand drier (Lechevalier and Lechevalier, 1970).

Amino acids: Each sample (10 µL) was applied on the base lines of silica TLC plate (20×20 cm). Adjacent to this, 3 µL of DL-diaminopimelic acid (an authentic material mixture of DAP isomers) and 3 µL of amino acetic acid (glycine) were spotted as standards. The TLC plate was developed with the solvent system containing; methanol, pyridine, glacial acetic acid, water (5: 0.5: 0.125: 2.5 v/v). It took approximately more than 2 h for development. The spots were visualized by spraying with 0.2% ninhydrin solution in water-saturated n-butanol, followed by heating at 100°C for 5 min. Spots of amino acid ran faster than DAP. The sample spots were immediately compared with the spots of the standards as the sample spots gradually disappeared in few hours (Lechevalier *et al*., 1966).

Species affiliation-cultural characteristics (Nonomura, 1974)

Aerial mass colour: The colour of the mature sporulating aerial mycelium was recorded in a simple way (white, grey, red, green, blue and violet) on the ISP2 medium. When the aerial mass colour fell between two colour series, both the colours were recorded. If the aerial mass colour of a strain to be studied showed intermediate tints, then also, both the colour series were noted.

Melanoid pigments: The grouping was made on the production of melanoid pigments (i.e., greenish brown, brownish black and distinct brown, the pigment modified by other colours) on the medium. The strains were grouped as melanoid pigment produced (+) and not produced (-). This test was carried out on tyrosine agar medium-ISP7 as recommended by International Streptomyces Project (Shirling and Gottlieb, 1966). Reverse side pigment (distinctive (+) and not distinctive or none (-), soluble pigments (produced (+) not produced (-) were determined by following Nonomura (1974) and using ISP2 and ISP7 media, respectively.

Spore chain morphology: The species with spore bearing hyphae are reported to be three types: Flexible-Rectiflexibiles (RF), open loops-Retinaculiaperti (RA) and spira-Spirales (S).

Spore morphological characters of the strains were studied by inserting 3-4 sterile cover slips at an angle of 45°C in the ISP2 medium. A characteristic of the spore bearing hyphae and spore chains is determined by the direct microscopic examination of the culture area. Adequate magnification used to establish the presence or absence of spore chains and to observe the nature of spore chains is 40X. By the standard protocol of cover slip culture technique the plates were prepared and after the incubation of 7-10 days it is observed under the binocular microscope (Leica ATC 2000) for the formation of aerial mycelium. Sporophore structure and spore morphology were studied under 400X magnification.

Spore surface: Spore morphology and the surface features were observed under the scanning electron microscope (Hitachi-s-450-SEM). The cross hatched cultures prepared for observation under the light microscope were used for this purpose. The electron grid was cleaned and adhesive tape was placed on the surface of the grid. The mature spores of the strains were carefully placed on the surface of the adhesive tape and gold coating was applied for half-an-hour and the specimens were examined under the electron microscope under different magnifications. The spore surfaces were characterized as smooth, spiny, hairy and warty.

Carbon source utilization: Ability of different actinobacterial strains in utilizing various carbon (arabinose, xylose, inositol, mannitol, fructose, rhamnose, sucrose, glucose and raffinose) compounds as source of energy was tested.

The utilization is expressed as, (I) Strongly positive (++), when growth on tested carbon in basal medium was equal to

or greater than growth on basal medium plus glucose, (II) Positive (+), when growth on tested carbon was significantly better than on basal medium without carbon, but somewhat less than on glucose and (III) Doubtful (±), when growth on tested carbon was only slightly better than on the basal medium without carbon and significantly less than with glucose andiv) negative (-), when growth was similar to or less than the growth on basal medium without carbon. Identified culture conditions and carbon source characteristics were compared with the conventional keys given by Nonomura (1974) and Bergy's Manual of Determinative Bacteriology (Bergey *et al*., 1974) and the strains were affiliated to specific species.

RESULTS AND DISCUSSION

Actinobacterial population density: Seagrasses rhizosphere soil samples showed varied actinobacterial population (Fig. 1); in the KUA medium, population density ranged from 24×0^2-67×10^2 CFU g^{-1} higher population density was recorded in the Halophila rhizosphere sediments (67×10^2 CFU g^{-1}) and the lower density was recorded in the Syringodium rhizosphere sediments (24×10^2 CFU g^{-1}). In the case of AIA medium, population density was ranged from 38×10^2-49×10^2 CFU g^{-1} registering the higher population density in the Halophila and Thalassia rhizospheres (49×10^2 CFU g^{-1}) and the lower density was in the Syringodium rhizosphere sediments (38×10^2 CFU g^{-1}). Totally, 59 strains were selected from the plates based on the colony morphology and are subculture. The strains are designated as RSG1-RSG 59. Among the 59 strains, 30 strains were selected based on colony morphological variability and aerial mycelium with white powder mass colour for chemotaxonomy.

Among the microbes, actinobacteria are widely dispersed throughout the marine environment as a small but significant fraction with higher levels of diversity (Ward and Bora, 2006). Present study has recorded impressive actinobacterial density in the seagrass rhizosphere environment. These ranges are similar to those reported by previous workers (Sahu *et al*., 2007; Sivakumar, 2001; Senthilkumar *et al*., 2005) from various other sources. Actinobacterial density in the marine sediment samples was 15×10^4 CFU g^{-1} in Cochin, India (Kala and Chandrika, 1995), 1-4×10^4 CFU g^{-1} in Pichavaram mangroves (Sivakumar *et al*., 2005) and 40×10^4 CFU g^{-1} (highest record available) in New Mexico, USA (Weyland and Helmke, 1988). However, Das *et al*. (2007) have recorded lower actinobacterial density (9.338-45.22$\times10^2$ CFU g^{-1}) in the deeper waters (1000 m) of the Bay of Bengal. It is quite natural and also proved that the population of actinobacteria would decrease in numbers as distance from the shore increases (Walker and Colwell, 1975; Weyland, 1969, 1981).

Though the microbes and actinobacteria have been widely studied in different marine environments, there are no reports on the microbial interaction in the rhizosphere of seagrasses of India and there are also not many studies on actinobacteria from the seagrass environs of the other parts of the world. Under these conditions, this study throws light on the actinobacterial population density of the rhizosphere region of the seagrasses. Higher population density of actinobacteria in both media used was recorded in the Halophila a low biomass producer rhizosphere than the other genera. This indicates that higher biomass producing seagrass environs are not much suitable for actinobacteria. This could be due to the leaching of lignin and tannins from the heavy seagrass biomass which would hinder the growth of actinobacteria, as reported for bacteria from mangrove environment (Ravikumar *et al*., 2007).

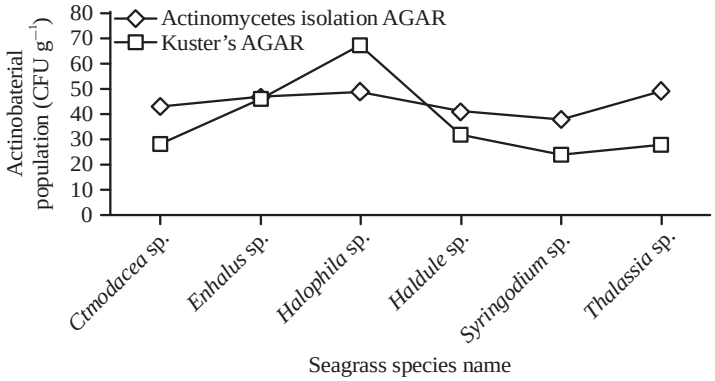

Fig. 1: Population of Actinobacteria from seagrasses rhizosphere soil

Table 1: Identification of chemotaxanomical characters of the selected actinobacterial isolates from seagrasses rhizosphere soil

	DAP						
Strains names	LL-DAP	Meso DAP	Glycine	Cell wall type	Spore chains	Spore surface	Presumed genus and species
RSG 1	+	-	+	I	Spirales	Smooth	*Streptomyces chraceiscleoticus*
RSG 2	+	-	+	I	Retinaculiaperti	Smooth	*Streptomyce spiroverticillatus*
RSG 3	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces aureofasciculus*
RSG 4	+	-	+	I	Rectiflexibiles	smooth	*Streptomyces spadicis*
RSG 5	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces alboniger*
RSG 6	+	-	+	I	Rectiflexibiles	smooth	*Streptomyces orientalis*
RSG 7	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces sindensis*
RSG 8	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces citrus*
RSG 9	+	-	+	I	Spirales	Smooth	*Streptomyces diastochromogenes*
RSG 10	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces puniceus*
RSG 11	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces alni*
RSG 12	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces nobilis*
RSG 13	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces kanamyceticus*
RSG 14	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces albolongus*
RSG 15	+	-	+	I	Spirales	Warty	*Streptomyces graminofaciens*
RSG 16	+	-	+	I	Spirales	Warty	*Streptomyces thermoflavus*
RSG 17	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces griseus*
RSG 18	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces godanensis*
RSG 19	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces galtieri*
RSG 20	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces sulphureus*
RSG 21	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces roscisclerotícus*
RSG 22	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces albidoflavus*
RSG 23	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces flavofungim*
RSG 24	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces moderatus*
RSG 25	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces gougeroti*
RSG 26	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces selanii*
RSG 27	+	-	+	I	Spirales	Spiny	*Streptomyces albus*
RSG 28	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces candidus*
RSG 29	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces craterifer*
RSG 30	+	-	+	I	Rectiflexibiles	Smooth	*Streptomyces tanashiensis*

+: Presence, -: Absence

Genus affiliation: Analysis of cell wall components was done for 30 strains viz. RSG-1 to RSG-30 (Table 1). TLC studies confirmed the presence of LL-DAP (Diaminopimeilic acid) and Glycine in all the strains and none of the strains has registered the presence of meso-DAP among all the 30 strains tested. Presence of LL-DAP along with glycine indicates the cell wall chemotype-I and the strains consisting of this wall type do not have characteristic pattern of sugars. The strains belonging to the wall type-I are Streptomyces, Streptoverticillium, Actinopycnidium, Actinosporangium, Elyptrosporangium, Microellobosporia, Sporichthya and Streptoverticillium, Actinopycnidium, Actinosporangium, Elyptrosporangium, Microellobosporia, Sporichthya and Intrasporangium. Micromorphological features of all the strains indicate that all of them belong to the genus Streptomyces.

Species affiliation: All the strains viz. RSG 1 to RSG 30 belonging to the genus Streptomyces were further analyzed to identify the species, using the conventional keys (Nonomura, 1974) and the results are given in Table 2. Further, all the 30 strains which were investigated for their taxonomy and species are tentatively ascribed to *Streptomyces* species (Table 2). Predominance of Streptomyces in the actinobacterial population has been reported in the several studies. Ramesh and Mathivanan (2009) reported a total of 288 marine samples from different locations of the Bay of Bengal starting from Pulicat lake to Kanyakumari and among all the marine actinomycetes, Streptomyces spp. were present in large proportion (88%). Vijayakumar *et al*. (2007) have also reported the dominance of Streptomyces from the marine samples of Palk Strait and Rajkumar *et al*. (2012) was studied a total of 116 actinobacterial colonies were recorded from 30 mangrove and marine sediment samples of Bhitherkanikka mangrove environment East coast of Orissa and Population, morphological and chemotaxonomical characterization of diverse rare actinomycetes in the mangrove and medicinal plant rhizosphere (Ara *et al*., 2013) reported the isolation and

Table 2: Comparison of culture characteristic by using the conventional keys

Character studied															
Strain No.	Colour of aerial mycelium	Melanoid pigment	Reverse side pigment	Soluble pigment	Spore chain (Fig. 2, 3)	Spore surface (Fig. 2, 3)	Carbon source assimilation								Species name (As per the Nanomura key)
							Arabinose	Xylose	Inositol	Mannitol	Fructose	Rhamnose	Sucrose	Raffinose	
RSG 1	Yellow	-	+	-	Spirales	Spiny	+	+	+	+	+	+	+	+	*S. ochraceiscleoticus*
RSG 2	Powdery white	-	+	-	Retinaculiaperti	Smooth	+	+	-	+	+	-	+	-	*S. spiroverticillatus*
RSG 3	White	+	+	-	Rectiflexibiles	Smooth and warty	+	+	+	+	+	+	+	+	*S. aureofasciculus*
RSG 4	Gray	+	+	-	Rectiflexibiles	Smooth	+	+	+	+	+	+	-	+	*S. spadicis*
RSG 5	White	-	-	+	Rectiflexibiles	Smooth	+	+	+	+	-	-	-	+	*S. alboniger*
RSG 6	Powdery white	-	-	-	Rectiflexibiles	Smooth	+	+	+	+	+	+	-	+	*S. orientalis*
RSG 7	White	-	-	-	Rectiflexibiles	Smooth and warty	+	+	-	+	+	-	+	-	*S. sindensis*
RSG 8	Yellow	-	+	-	Rectiflexibiles	Smooth	+	+	+	+	+	-	+	+	*S. citreus*
RSG 9	Gray	+	-	-	Spirales	Smooth	+	+	+	+	+	+	+	+	*S. diastochromogenes*
RSG 10	Yellow	-	+	-	Rectiflexibiles	Smooth	-	+	-	+	+	-	+	+	*S. puniceus*
RSG 11	Gray	-	-	+	Rectiflexibiles	Smooth and warty	+	+	-	+	+	+	+	+	*S. alni*
RSG 12	Powdery white	+	+	-	Rectiflexibiles	Smooth	+	+	+	+	+	+	+	+	*S. nobilis*
RSG 13	White	-	-	-	Rectiflexibiles	Smooth	+	+	-	+	+	-	+	+	*S. kanamyceticus*
RSG 14	White	+	-	-	Rectiflexibiles	smooth	+	+	-	-	-	+	-	+	*S. albologus*
RSG 15	Gray	+	-	-	Spirales	Warty	+	+	+	+	+	+	+	+	*S. graminofaciens*
RSG 16	Gray	-	+	-	Spirales	Smooth and warty	+	+	-	+	+	+	+	+	*S. thermoflavus*
RSG 17	Yellow	-	-	-	Rectiflexibiles	Smooth	-	+	-	+	+	+	-	+	*S. griseus*
RSG 18	White	-	+	-	Rectiflexibiles	Smooth	+	-	+	+	+	+	-	-	*S. godanensis*
RSG 19	White	-	-	-	Rectiflexibiles	Smooth	-	-	-	-	+	-	+	-	*S. galtieri*
RSG 20	Yellow	-	-	-	Rectiflexibiles	Smooth	+	+	+	+	+	+	-	+	*S. sulphureus*
RSG 21	White	-	+	+	Rectiflexibiles	Smooth	+	+	+	+	+	+	-	+	*S. rosciscleroitcus*
RSG 22	Powdery white	-	-	-	Rectiflexibiles	Smooth	+	+	-	+	+	-	-	+	*S. albidoflavus*
RSG 23	White	+	+	+	Rectiflexibiles	Smooth and warty	+	+	+	+	+	+	-	+	*S. fungim*
RSG 24	White	-	+	+	Rectiflexibiles	Smooth	+	+	+	+	+	+	+	+	*S. moderatus*
RSG 25	White	-	+	-	Rectiflexibiles	Smooth	+	+	-	+	+	-	-	-	*S. gougeroti*
RSG 26	Powdery white	-	-	-	Rectiflexibiles	Smooth	+	+	-	+	+	+	-	-	*S. selanii*
RSG 27	White	-	-	-	Spirales	Spiny	+	+	-	+	+	-	-	-	*S. albus*
RSG 28	White	-	-	-	Rectiflexibiles	Smooth	+	+	-	+	+	+	-	-	*S. candidus*
RSG 29	Gray	-	-	-	Rectiflexibiles	Smooth	+	+	-	+	+	+	+	+	*S. craterifer*
RSG 30	Gray	+	-	-	Rectiflexibiles	Smooth and warty	+	+	-	-	+	-	-	-	*S. tanashiensis*

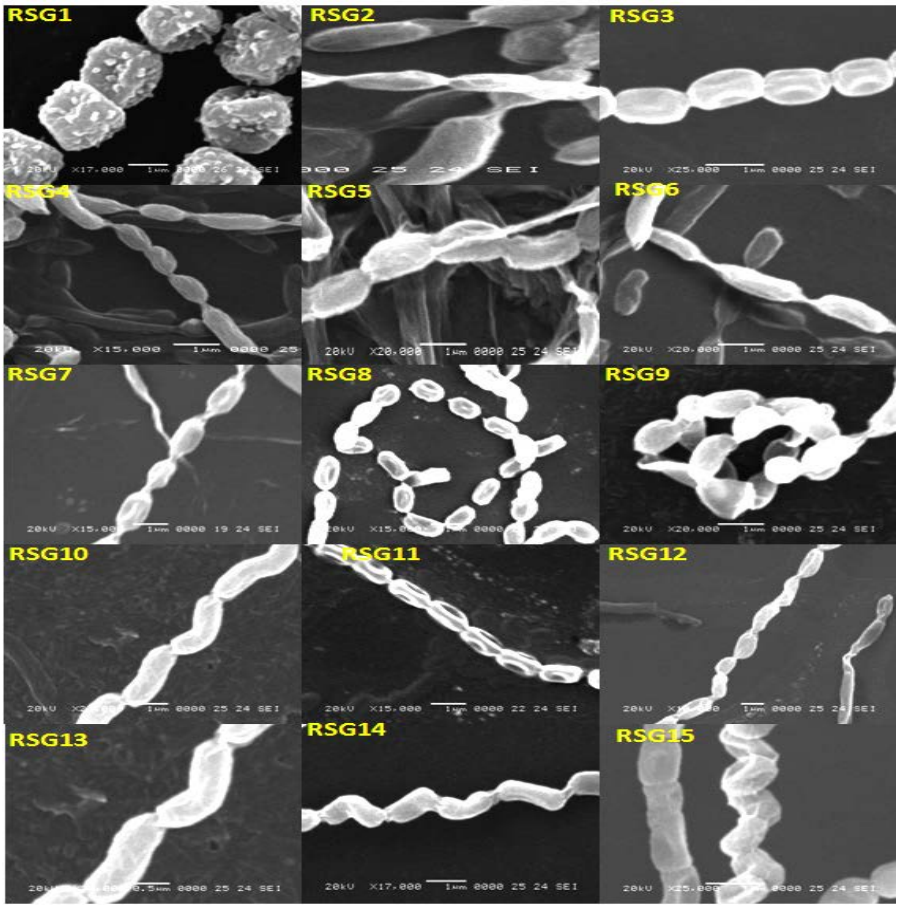

Fig. 2: Scanning electron micrographs (SEM) of actinobacteria from seagrass rhizosphere soil of the Gulf of Mannar Biosphere Reserve India. (RSG 1-15), RSG 1: Spirales spore chain and smooth spore surface of *Streptomyces chraceiscleoticus*, RSG 2: Retinaculiaperti spore chain and smooth spore surface of *S. spiroverticillatus*, RSG 3: Rectiflexibiles spore chain and smooth spore surface *S. aureofasciculus*, RSG 4: Rectiflexibiles spore chain and smooth spore surface of *S. spadicis*, RSG 5: Rectiflexibiles spore chain and smooth spore surface of *S. alboniger*, RSG 6: Rectiflexibiles spore chain and smooth spore surface of *S. orientalis*, RSG 7: Rectiflexibiles spore chain and smooth spore surface of *S. sindensis*, RSG 8: Rectiflexibiles spore chain and smooth spore surface of *S. citrus*, RSG 9: Spirales spore chain and smooth spore surface of *S. diastochromogenes*, RSG 10: Rectiflexibiles spore chain and smooth spore surface of *S. puniceus*, RSG 11: Rectiflexibiles spore chain and smooth spore surface of *S. alni*, RSG 12: Rectiflexibiles spore chain and smooth spore surface of *S. nobilis*, RSG 13: Rectiflexibiles spore chain and smooth spore surface of *S. kanamyceticus*, RSG 14: Rectiflexibiles spore chain and smooth spore surface of *S. albolongus* and RSG 15: Spirales spore chain and warty spore surface of *S. graminofaciens*

characterization of actinobacteria from different sites in the Western Gulf of California collected for 126 sediment samples and isolated on average 3.1-38.3 actinobacterial strains from each samples (Becerril-Espinosa *et al*., 2013). Much more attention has been paid to the actinobacterial community in soils or water columns of aquatic habitats in the surface sediments of Taihu Lake, China (Wang *et al*., 2013). Depending up on the effect of plant rhizosphere on microbial diversity and counting, the diversity of actinomycetes in mangrove ecosystem and their counting were analyzed by studying three soils rhizospheres of old mangrove, young mangrove and non mangrove rhizosphere (Reyad, 2013).

Scanning Electron Micrographs (SEM) of actinobacteria from seagrass rhizosphere soil of the Gulf of Mannar Biosphere Reserve India were presented in Fig. 2 and 3. Therefore it is imperative to record and quantify the abundance of marine actinobacteria in the seagrass rhizosphere soils and to culture them to ensure their conservation for future biological, genetic and molecular studies.

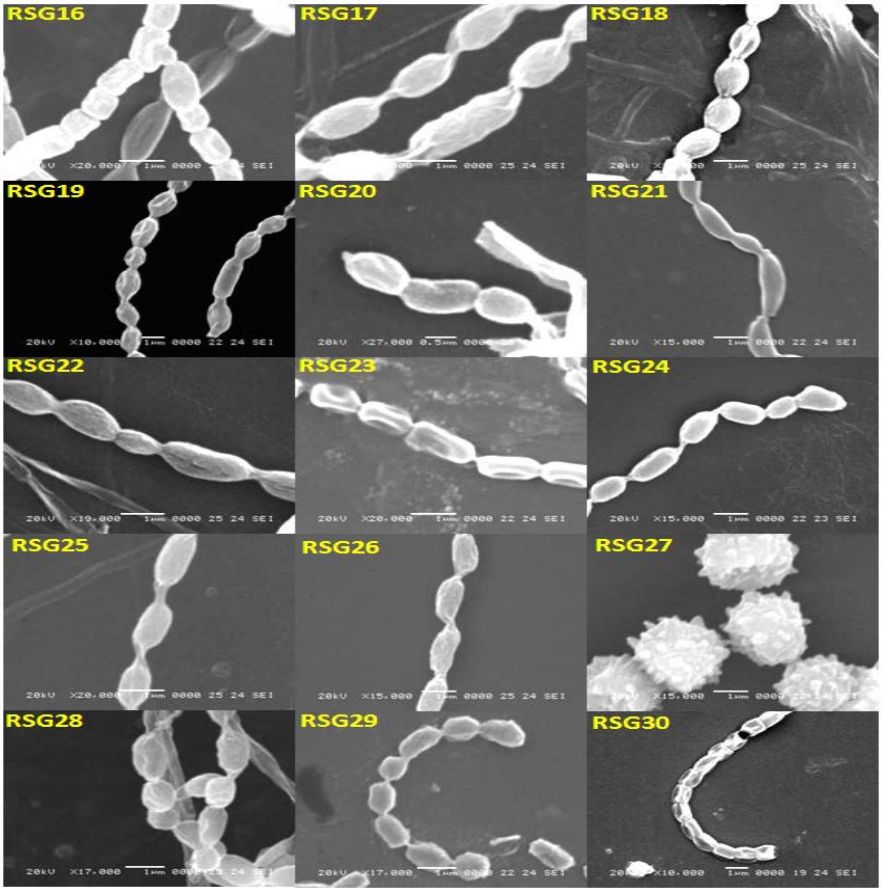

Fig. 3: Scanning Electron Micrograph (SEM) of actinobacteria from (RSG 16-30), RSG 16: Spirales spore chain and warty spore surface of *S. thermoflavus*, RSG 17: Rectiflexibiles spore chain and smooth spore surface of *S. grise*, RSG 18: Rectiflexibiles spore chain and smooth spore surface of *S. godanensis*, RSG 19: Rectiflexibiles spore chain and smooth spore surface of *S. galtieri*, RSG 20: Rectiflexibiles spore chain and smooth spore surface of *S. sulphureus*, RSG 21: Rectiflexibiles spore chain and smooth spore surface of *S. roscisclerotícus*, RSG 22: Rectiflexibiles spore chain and smooth spore surface of *S. albidoflavus*, RSG 23: Rectiflexibiles spore chain and smooth spore surface of *S. flavofungim*, RSG 24: Rectiflexibiles spore chain and smooth spore surface of *S. moderatus*, RSG 25: Rectiflexibiles spore chain and smooth spore surface of *S. gougeroti*, RSG 26: Rectiflexibiles spore chain and smooth spore surface of *S. selanii*, RSG 27: Spirales spore chain and spiny spore surface of *S. albus*, RSG 28: Rectiflexibiles spore chain and smooth spore surface of *S. candidus*, RSG 29: Rectiflexibiles spore chain and smooth spore surface of *S. craterifer* and RSG 30: Rectiflexibiles spore chain and smooth spore surface of *S. tanashiensis*

CONCLUSION

Though the microbes and actinobacteria have been widely studied in different marine environments, there are no reports on the microbial interaction in the rhizosphere of seagrasses of India and there are also not many studies on actinobacteria from the seagrass environs of the other parts of the world. Under these conditions, the present study throws light on the actinobacteria of the rhizosphere region of the seagrasses with an impressive actinobacterial density. These *Streptomyces* sp. isolated from the present study will be explored for wide range of applications for the production of enzymes, bioactive compounds, pigments etc.

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