British Biotechnology Journal
4(2): 173-184, 2014

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Exploration of Actinobacteria from Mangrove Ecosystems of Nizampatnam and Coringa for Antimicrobial Compounds and Industrial Enzymes

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Authors' contributions

This work was carried out in collaboration between all authors. Author UKM performed the experimental part, author MV designed the study and author SP performed literature search and statistical analysis. All authors read and approved the final manuscript.

Original Research Article

Received 16th August 2013
Accepted 28th September 2013
Published 11th January 2014

ABSTRACT

Aim: A study was made to examine the kinship between the seasonal distribution of actinobacteria and the physico-chemical properties of the mangrove sediments of Nizampatnam and Coringa located along the South East coast of Andhra Pradesh, India.
Place and Duration of Study: Department of Botany and Microbiology, between April 2010 to February 2011.
Methodology: Seasonal enumeration of actinobacteria from two different stations 1 (Nizampatnam) and 2 (Coringa) accorded by four different pre-treatments of soil sediments followed by plating onto three different media showed high incidence of actinobacteria in the month of February and least in December. Pretreatment with calcium carbonate and plating on starch casein agar yielded maximum number of actinobacteria. The strains were identified based on the morphological characteristics such as aerial mycelium, substrate mycelium, diffusible pigments and micro morphological features.
Results: The present investigation revealed that majority of the mangrove actinobacteria

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(69%) belongs to *Streptomyces* spp. Among the 55 isolates screened for antimicrobial compounds, 28 were found to be potential producers. The isolates could also produce commercially important enzymes such as L-asparaginase, cellulase and amylase. In addition the statistical study also revealed that positive correlation between the distribution of the actinomycetes and influence of physico-chemical parameters and the organic matter of the soil.
Conclusion: Our study revealed that the unexplored regions like Nizampatnam and Coringa mangrove ecosystems are proved as potential sites for antimicrobial and industrial enzyme producing actinobacteria.

Keywords: Mangrove ecosystem; actinobacteria; seasonal distribution; antimicrobial potential; industrial enzymes.

1. INTRODUCTION

Actinobacteria, the Gram positive filamentous bacteria are well known for the production of antimicrobial metabolites in drug discovery programmes [1]. Many species especially those belong to *Streptomyces* and *Micromonospora* have been recorded as potential producers of metabolites with diverse chemical structures and biological activities [2,3]. Thousands of such bioactive compounds were isolated and characterized, many of which have been developed into drugs for treatment of a wide range of diseases in human and agriculture sectors [4,5,6]. Thus searching for novel actinobacteria constitutes an essential component in natural product based drug discovery.

A significant effort has been focused on the successful isolation of novel actinobacteria from terrestrial habitats for drug discovery during the past five decades. In recent years the discovery of commercially significant novel secondary metabolites from terrestrial actinobacteria has decreased, as this practice leads to wasteful rediscovery of the known bioactive compounds [7]. Isolation and characterization of novel actinobacteria from unexplored habitats are proving to be a valuable source of new bioactive metabolites [1,3,8]. Hence there is a need to explore the unexplored marine ecosystems that are a wealthy source of new important bioactive compounds including antibiotics and commercially important industrial enzymes. Potent actinobacteria not only exist in oceans but are also widely distributed in different marine ecosystems [9].

Mangroves are unique intertidal ecosystems of the tropics, which support genetically diverse groups of aquatic and terrestrial microorganisms [10]. As mangrove environments differ greatly from terrestrial habitats, the distribution and biological characteristics of mangrove actinobacteria are expected to be different from those of terrestrial ones. Studies on the biodiversity of mangrove actinobacteria are important not only in terms of basic research, but also for the biotechnological exploitation of such organisms [11].

Research towards the exploration of actinobacteria in mangrove ecosystems has not progressed much in India. The mangrove ecosystems of Nizampatnam and Coringa regions of South East coast of Andhra Pradesh are not yet explored for microbial diversity and microbial metabolites. Hence there is a possibility to identify novel actinobacteria in these two mangrove ecosystems. Accordingly, the present study was designed to investigate the diversity, distribution and seasonal variation of actinobacterial population of Nizampatnam

and Coringa mangrove ecosystems with the ultimate objective of screening for antimicrobial metabolites and enzymes.

2. MATERIALS AND METHODS

2.1 Sampling Locations

Mangrove sediments were collected at bimonthly intervals from April 2010 to February 2011 from Nizampatnam mangrove ecosystem (Station 1 - Lat.15° 54 ’0N ; Long.80° 40’0E) and Coringa mangrove ecosystem (Station 2 - Lat.16°44 to 16°53’N; Long.82°14’ to 82°22’E) situated along the south east coast of Andhra Pradesh, India. Samples were collected from 6-10 cm depth and transported to laboratory in sterile bags and air-dried at room temperature. The samples taken from each station were analyzed for physico-chemical parameters such as pH, electrical conductivity, organic carbon, nitrogen, phosphorous, potassium, zinc, iron, manganese, copper and sulfates [12,13].

2.2 Pretreatment of Samples for the Isolation of Mangrove Actinobacteria

The air-dried samples were subjected to several pretreatments to enrich the actinobacterial population in the samples as well as to reduce the unwanted contaminants like fungi and bacteria. These methods include:

[P-1] Dry heat treatment at 120°C per one hour [14].
[P-2] Pretreatment with 1.5% phenol [15].
[P-3] Calcium-carbonate pretreatment [16] and
[P-4] Pretreatment with an osmoprotectant such as quarter strength Ringers solution [17].

The pretreated sample (1g) was suspended in 100 ml of sterile distilled water. Serial dilutions were prepared and 100 µl of 10^{-4} dilution obtained from the four different methods of pretreatment were spread onto the surface of three selective media, including Starch casein agar [M-1] [18]; Asparagine glucose agar [M-2] [19] and Glycerol-asparagine agar [M-3] [20]. These media were supplemented with nalidixic acid (25 µg/ml) and secnidazole (25 µg/ml) to retard the growth of bacteria and fungi respectively. The inoculated plates were incubated at 30°C for 10 days. After incubation, actinobacteria were isolated and enumerated [21]. In the present investigation the total number of actinobacteria were obtained by summing up all the colonies obtained in three different media by four different pretreatment techniques. Pure cultures of actinobacteria were maintained on YMD-agar slants and stored at 4°C [22].

2.3 Identification of the Isolates

Pure cultures of actinobacterial isolates were identified using morphological and cultural characteristics by the methods suggested in International *Streptomyces* Project (ISP) [23]. The color of aerial and substrate mycelia was examined. The morphology of spore chain, spore bearing hyphae and the structure and arrangement of spores were observed using inverted microscope at 40 X magnification (Olympus) by cover slip culture technique [24].

2.4 Screening of Mangrove Actinobacteria for the Production of Antimicrobial Metabolites and Extracellular Enzymes

The actinobacterial isolates were screened for antimicrobial activity by agar well diffusion method. The secondary metabolites produced by actinobacteria were extracted [25]. The pure culture of each actinobacterial isolate was transferred aseptically into YMD broth and incubated for 48 h followed by inoculation into the production medium of the same composition at a rate of 10%. The fermentation was carried out at 30°C for 96 h under agitation at 120 rpm. The culture filtrate obtained was extracted twice with ethyl acetate and the pooled solvent extracts were evaporated to dryness under vacuum in a Rotovap (Heidolph Germany), to yield a crude residue. The residue obtained was used to determine antimicrobial activity by agar well diffusion method [26] and effectiveness was measured in terms of zone of inhibition. *Staphylococcus aureus* (MTCC 3160), *Escherichia coli* (ATCC 35218) and *Candida albicans* (ATCC 10231) were employed as test microorganisms for antimicrobial assay.

The mangrove actinobacteria were screened for the production of three important enzymes such as amylase, cellulase and L-asparaginase. Each actinobacterial isolate was streaked on agar plates amended with starch, carboxy methyl cellulose and L-asparagine for amylase, cellulase and L-asparaginase and incubated for 48-96 h at 30°C. The plates were flooded with relevant indicator solutions and the development of clear zone around the growth of organism was considered positive for enzyme activity.

2.5 Statistical Analysis

The relation between variables like pH, electrical conductivity and mineral nutrients of sediment samples and the distribution of actinomycetes in station 1 and station 2 was analysed with Pearson's correlation. The statistical analysis was carried out using Microsoft Excel software 2007 version (Microsoft, USA) and SPSS 17 (Statistical Package for the Social Science, Chicago, USA).

3. RESULTS AND DISCUSSION

The mangrove ecosystems of South East coast of Andhra Pradesh (Nizampatnam and Coringa) were selected for studying the diversity of actinobacteria and their antimicrobial compounds and industrially important enzymes.

The present study was carried out to assess the actinobacterial diversity in two different mangrove ecosystems. The number of actinobacterial strains isolated from station 1 and station 2 was 31 and 24 respectively in which the Nizampatnam mangrove ecosystem supported maximum counts when compared to Coringa ecosystem. This might be due to the rich source of nutrients present in the Nizampatnam compared to Coringa mangrove ecosystem.

The influence of season on the distribution of actinobacteria was investigated (Fig.1). The highest number of actinobacteria in two stations was observed in February while the counts were minimum in December. This is in agreement with the findings of Li-Hua xu et al. [27] who reported the low incidence of actinobacteria in cooler climates. Ravi Kumar and Sugandhi [28] also reported high counts of actinobacteria in Thondi in July and January and

low in September and October. The actinobacterial population in two mangrove ecosystems exhibited common seasonal variation with high counts in dry, warm seasons.

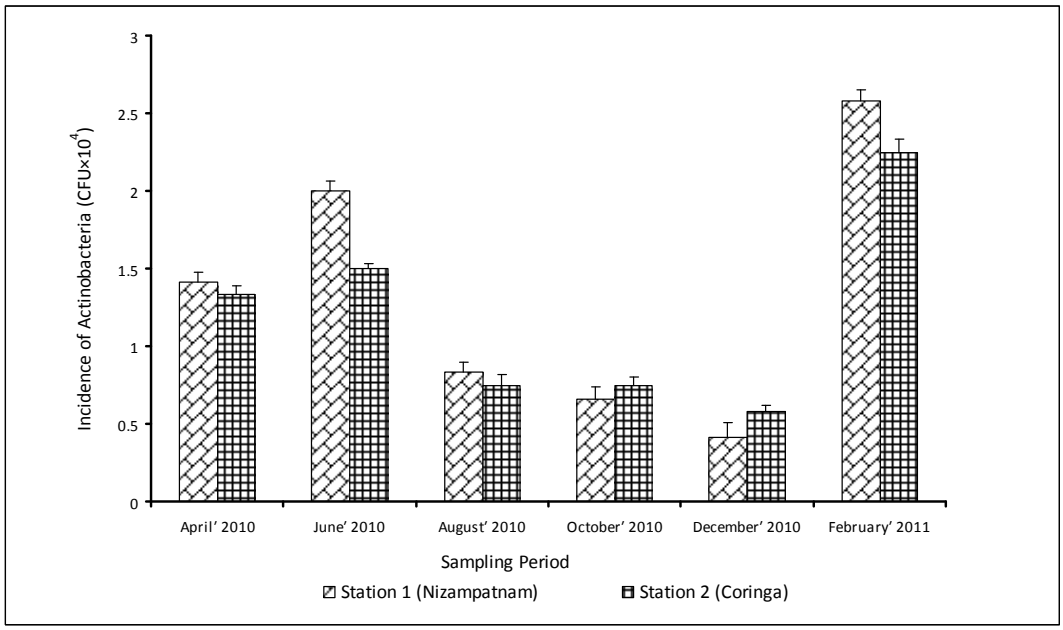

Fig. 1. Incidence of actinobacteria in Nizampatnam and Coringa mangrove ecosystems

The occurrence of actinobacteria in two stations was studied using four different pretreatment techniques and three different selective media (Table 1). High actinobacterial count was found in samples pretreated with calcium carbonate as compared to other pretreatments with dry heat, phenol and Ringers solution. Among the three different selective media used, starch-casein agar yielded high counts of actinobacteria followed by asparagine glucose agar and glycerol-asparagine agar. Anindita et al. [29] also reported maximum isolates of actinobacteria on starch casein agar.

The occurrence of different genera of actinobacteria in mangrove sediment samples of Nizampatnam and Coringa regions are represented in Fig. 2. All the 55 isolates were identified up to generic level based on colony morphology, micro morphology and microscopic features. Out of 55 isolates, 38 were assigned to *Streptomyces*, four to *Streptosporangium*, three to *Streptoverticillium,* three to *Actinopolyspora,* two to *Actinomadura,* two to *Micromonospora* and one each to *Pseudonocardia, Actinoplanes* and *Nocardiopsis*. The present investigation revealed that majority of the mangrove actinobacteria (69%) belongs to *Streptomyces* spp. It has been reported that *Streptomyces* spp. are common inhabitants of marine environments, though other actinobacteria are also present [21,30,31,32,33,34,35].

Table 1. Enumeration of actinobacteria by four different methods of pretreatment of mangrove sediment samples followed by plating on three different growth media

Station 1Nizampatnam							
Counts of actinobacteria							
Pretreatment	**Media**	**April**	**June**	**August**	**October**	**December**	**February**
P-1	M1	$3x10^4$	$4 x10^4$	$1 x10^4$	0	$1 x10^4$	$3 x10^4$
(Dry Heat)	M2	$1 x10^4$	$2 x10^4$	$1 x10^4$	$1x10^4$	0	$2 x10^4$
	M3	$1 x10^4$	0	$1 x10^4$	$1x10^4$	0	$2 x10^4$
P-2	M1	$1 x10^4$	$2 x10^4$	$2 x10^4$	$2x10^4$	$1 x10^4$	$3 x10^4$
(Phenol)	M2	$2 x10^4$	$1 x10^4$	0	0	$1 x10^4$	$2 x10^4$
	M3	$1 x10^4$	$2 x10^4$	0	0	0	$2 x10^4$
P-3	M1	$2 x10^4$	$3 x10^4$	$1 x10^4$	$2 x10^4$	$1 x10^4$	$5 x10^4$
($CaCo_3$)	M2	$1 x10^4$	$3 x10^4$	$2 x10^4$	$1 x10^4$	0	$3 x10^4$
	M3	$2 x10^4$	$2 x10^4$	0	0	$1 x10^4$	$2 x10^4$
P-4	M1	$2 x10^4$	$2 x10^4$	$1 x10^4$	$1 x10^4$	0	$4 x10^4$
(Ringers	M2	$1 x10^4$	$2 x10^4$	0	0	0	$1 x10^4$
Solution)	M3	0	$1 x10^4$	$1 x10^4$	0	0	$2 x10^4$
Station 2 Coringa							
Counts of actinobacteria							
Pretreatment	**Media**	**April**	**June**	**August**	**October**	**December**	**February**
P-1	M1	$2 x 10^4$	$3 x10^4$	$1 x10^4$	$1 x10^4$	$1x10^4$	$3 x10^4$
(Dry Heat)	M2	$2 x10^4$	$1 x10^4$	0	$1 x10^4$	$1 x10^4$	$2x10^4$
	M3	$1 x10^4$	$1 x10^4$	$1 x10^4$	0	0	$2 x10^4$
P-2	M1	$2 x10^4$	$1 x10^4$	0	$1 x10^4$	$1 x10^4$	$2 x10^4$
(Phenol)	M2	0	$1 X10^4$	$1 X10^4$	$1 X10^4$	0	$3 X10^4$
	M3	$1 x10^4$	$1 x10^4$	$1 x10^4$	0	$1 x10^4$	$1 x10^4$
P-3	M1	$2 x10^4$	$2 x10^4$	$1 x10^4$	$1 x10^4$	0	$4 x10^4$
($CaCo_3$)	M2	$2 x10^4$	$2 x10^4$	$2 x10^4$	0	$1 x10^4$	$3 x10^4$
	M3	$1 x10^4$	$1 x10^4$	0	$1 x10^4$	$1 x10^4$	$2x10^4$
P-4	M1	$1 x10^4$	$2 x10^4$	$1 x10^4$	$2 x10^4$	0	$2 x10^4$
(Ringers	M2	$2x10^4$	$1 x10^4$	0	$1 x10^4$	0	$2x10^4$
Solution)	M3	0	$2 x10^4$	$1 x10^4$	0	$1 x10^4$	$1x10^4$

M1- Starch casein agar; M2-Asparagine glucose agar; M3- Glycerol asparagine agar

The present study also determined the antimicrobial activity of the actinobacterial strains. Out of 55 strains, 28 possessed antimicrobial activity, of which 19 isolates were active against all the test microorganisms including *S. aureus*, *E. coli* and *C. albicans*; four against *E. coli* and *C. albicans* and five against *S. aureus* and *E. coli* (Table 2). On the other hand, 27 isolates did not show any antimicrobial activity. Several researchers have reported antimicrobial activity of actinobacteria against various human pathogens. Ramesh et al. [36] isolated 208 actinobacterial strains from different locations of the Bay of Bengal and found that 111 isolates produced antimicrobial compounds. Mitra et al. [37] obtained several actinobacteria from Sunderban mangrove ecosystem, of which 50.84% exhibited antimicrobial activity. Remya and Vijay kumar [38] isolated 64 different actinobacterial strains from marine and mangrove sediments of West coast of India and reported that 32.8% had antimicrobial activity.

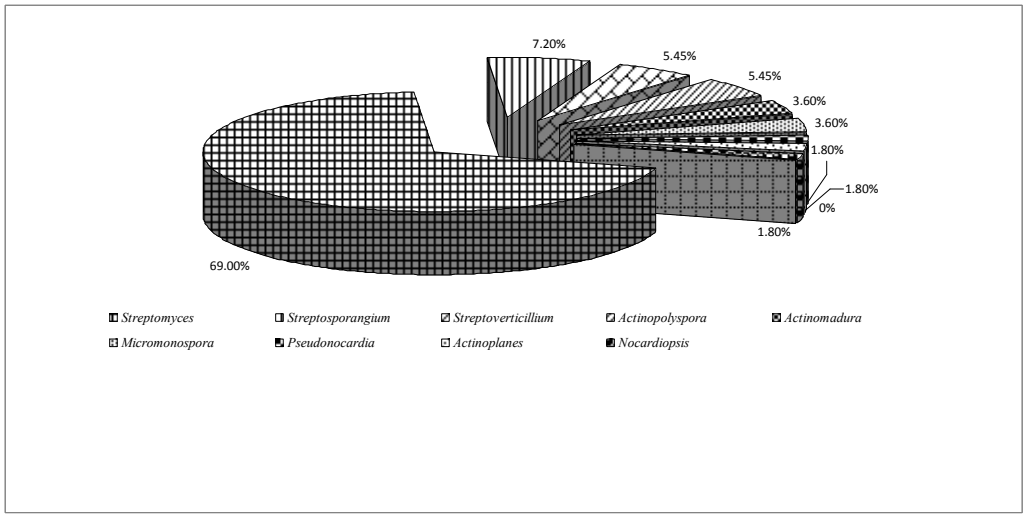

Fig. 2. Frequency and occurrence of actinobacteria in mangrove sediments of Nizampatnam and Coringa regions

Table 2. Antimicrobial activity of mangrove actinobacterial isolates against human pathogens (Inhibition zone represented in mm)

S.No	Isolate code	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
1	VUK-4	12	12	11
2	VUK-7	14	11	10
3	VUK-8	-	10	14
4	VUK-10	19	18	17
5	VUK-12	19	16	12
6	VUK-15	17	14	13
7	VUK-17	15	12	12
8	VUK-18	15	13	-
9	VUK-19	-	15	13
10	VUK-21	18	11	15
11	VUK-24	19	17	12
12	VUK-27	14	12	10
13	VUK-28	20	12	-
14	VUK-29	-	12	11
15	VUK-31	18	15	-
16	VUK-A	20	19	19
17	VUK-B	19	17	17
18	VUK-C	17	15	15
19	VUK-D	17	16	16
20	VUK-F	17	18	15
21	VUK-H	17	14	11
22	VUK-I	15	10	10
23	VUK-K	16	12	12
24	VUK-N	18	15	13
25	VUK-O	-	13	12
26	VUK-R	16	14	-
27	VUK-S	15	12	10
28	VUK-T	12	12	-

**Isolates from Nizampatnam represented in numbers while from Coringa in alphabets*

Out of 55 isolates screened for enzyme production, amylases were produced by 18, cellulases by 19 and L-asparaginases by 11 isolates, while five isolates produced all the three enzymes (Fig. 3). In recent years, there has been increasing interest in microbial enzymes as biocatalysts with novel features in many industrial processes. Microorganisms are the first choice as the source of enzymes because of their rapid growth, broad biochemical diversity and ease of genetic manipulation. Actinobacteria are physiologically diverse group as evident by their production of numerous extra cellular enzymes and metabolic products. Although they have provided many important bioactive compounds of high commercial value, exploration of their bio-catalytic potential is a relatively new phenomenon [38]. Many researchers reported the production of industrially important enzymes from terrestrial actinomycetes [39,40,41]. However, it is evident from the literature that the exploration of the enzymatic potential of marine actinobacteria is just the beginning and till date only few enzymes are investigated. Based on the screening results for antimicrobial compounds and enzymes, it has been shown that salt tolerant alkaliphilic actinobacteria isolated form mangrove sediments of Nizampatnam and Coringa region may be tapped as one of the potential source for bioactive compounds.

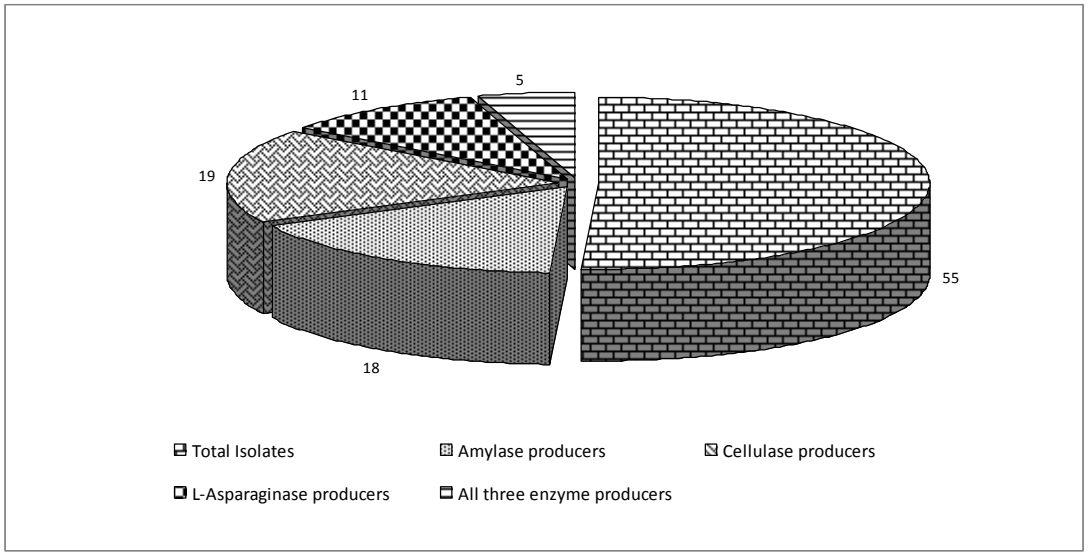

Fig. 3. Production of extracellular enzymes by mangrove actinomycetes

3.1 Statistical Analysis

The fact that in spite of the wide distribution of actinobacteria, there is a great variation in the dynamics of their population. The present investigation was found that there was correlation between the incidence of actinobacteria and the physico-chemical properties of the sediment. Table 3 showed the positive correlation between the number of actinobacteria, pH ($r = 0.452$, $P < .05$), conductivity ($r = 0.627$, $P < .05$), organic carbon ($r = 0.564$, $P < .01$) Potassium ($r = 0.752$, $P < .01$) and Iron ($r = 0.551$, $P < .05$) in station 1. There was a negative correlation between the number of actinobacteria, Zinc ($r = - 0.642$, $P < .05$) and Sulphate ($r = - 0.721$, $P < .01$). A positive correlation between the number of actinobacteria, pH ($r = 0.672$, $P < .05$), organic carbon ($r = 0.325$, $P < .01$), Zinc ($r = 0.421$, $P < .01$) and Copper ($r = 0.326$, $P < .05$) was found in Station 2 (Table 4). But negative correlation was reported

between number of actinomycetes, electrical conductivity (r = - 0.437, P< .05), Potassium (r = - 0.456, P< .05) and Manganese (r = - 0.324 P <0.5). Similar results were reported by Mansour [42], Saadoun and Al-Momoni [43] and Ndonde and Semu [44] from different habitats.

Table 3. Correlation between the Physico-chemical properties and actinobacteria isolated from station 1

	pH	EC	OC	P	K	Fe	Mn	Zn	S	Cu	Act
pH	1										
EC	0.823*	1									
OC	-0.156	-0.119	1								
P	0.477	0.877*	0.000	1							
K	0.012	0.257	-0.765	0.267	1						
Fe	-0.347	0.232	-0.115	0.604	0.574	1					
Mn	0.055	0.519	-0.454	0.667	0.857*	0.848*	1				
Zn	0.940**	0.788	-0.399	0.437	0.180	-0.284	0.169	1			
S	-0.260	0.135	-0.206	0.339	0.751	0.779	0.796	-0.281	1		
Cu	0.677	0.526	0.402	0.218	-0.189	-0.353	-0.098	0.484	-0.063	1	
Act	0.452*	-0.627*	0.564**	0.312	0.752**	0.551*	0.324	-0.642*	-0.721**	0.216	1

EC- Electrical Conductivity; OC- Organic Carbon; P- Phosphorus; K- Potassium; Fe- Iron; Mn-Manganese; Zn-Zinc; S- Sulphate and Cu- copper, Act-Actinomycetes

**. Correlation is significant at the 0.05 level (2-tailed)*

***. Correlation is significant at the 0.01 level (2-tailed)*

Table 4. Correlation between Physico-chemical properties and Actinobacteria isolated from Station 2

	pH	EC	OC	P	K	Fe	Mn	Zn	S	Cu	Act
pH	1										
EC	-0.163	1									
OC	-0.067	-0.836*	1								
P	-0.098	0.909*	-0.838*	1							
K	-0.547	-0.166	0.454	-0.189	1						
Fe	0.201	-0.581	0.142	-0.632	-0.206	1					
Mn	-0.091	0.000	-0.397	-0.118	-0.326	0.787	1				
Zn	0.188	-0.868*	0.847*	-0.986**	0.171	0.555	0.025	1			
S	-0.674	0.651	-0.413	0.676	0.000	-0.638	-0.138	-0.713	1		
Cu	-0.218	0.137	0.221	-0.230	-0.017	-0.163	-0.067	0.308	0.160	1	
Act	0.672*	-0.437*	0.325**	-0.681	-0.456*	-0.834	-0.324*	0.421**	0.431	0.326*	1

EC- Electrical Conductivity; OC- Organic Carbon; P- Phosphorus; K- Potassium; Fe- Iron; Mn-Manganese; Zn-Zinc; S- Sulphate and Cu- copper, Act- Actinomycetes

**. Correlation is significant at the 0.05 level (2-tailed)*

***. Correlation is significant at the 0.01 level (2-tailed)*

4. CONCLUSION

By combining several pretreatment techniques with suitable media supplemented with antibiotics, diverse actinobacterial genera were isolated from Nizampatnam and Coringa regions located at South East coast of Andhra Pradesh. They are capable of synthesizing several antimicrobial compounds and industrially important enzymes.The relationship between the actinobacterial community, soil pH, organic matter and nutrient content was also assessed in two mangrove regions at bimonthly intervals and are statistically analyzed.

ACKNOWLEDGEMENTS

This work is supported by the financial assistance from the Council of Scientific and Industrial Research, New Delhi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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