WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 7, 1285-1294. Research Article ISSN 2277– 7105

EXPLORATION OF ACTINOBACTERIA FROM MANGROVE ECOSYSTEM OF KRISHNA DISTRICT, ANDHRA PRADESH

Krishna Naragani*

Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

Article Received on
08 Feb. 2018,

Revised on 28 Feb. 2018,
Accepted on 21 March 2018,

DOI: 10.20959/wjpr20187-11700

***Corresponding Author**
Krishna Naragani
Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

ABTRACT

Total 120 actinobacterial strains isolated from the coastal region of Krishna district by using six different pretreatment techniques and two different selective media. High actinobacterial count was found in samples pretreated with calcium carbonate as compared to other pretreatments. The influence of season on the incidence of actinobacteria was investigated. The actinobacterial population in the mangrove ecosystems exhibited common seasonal variation. The highest number of actinobacteria was observed in Rainy season while the counts were minimum in Summer. High actinobacterial count was found on the Starch-casein agar medium (ISP-6), when compared to Yeast extract malt extract and dextrose agar medium (ISP-2). The santimicrobial efficacy of the strains was evaluated by using four solvents such as chloroform, ethyl acetate, methanol and acetone. Among the solvents used, ethyl acetate extract exhibited maximum antimicrobial activity where as the other solvent extracts showed moderate to minimum activity against the test microorganisms.

KEYWORDS: Mangrove Actinobacteria, Seasonal enumeration, antimicrobial activity.

1. INTRODUCTION

Mangroves are unique inter-tidal ecosystems of the tropics, which hold genetically diverse groups of micro organisms. This ecosystem is situated between the terrestrial and marine environment and supports a rich and diverse group of microorganisms. Among the microorganisms, Actinobacteria represent a significant constituent of the microbial population due to their capacity to produce bioactive secondary metabolites and enzymes. The increasing number of publications on the diversity of marine actinobacteria strongly

supports the view that the mangrove environment is a good source for actinobacterial diversity and secondary metabolites.

Mangrove sediments are rich source for new species of *Streptomyces*, *Nocardiopsis* and various strains of actinobacteria. It has rich biological diversity due to extreme conditions like high moisture, salinity, pH, temperature. (Amrita *et al*., 2012). All these circumstances have generated an evolutionary pressure on marine microorganisms, differentiating them from their terrestrial counterparts (Manivasagan *et al*., 2013). This environment is a virtually untapped source for novel and diverse microorganisms. Actinobacteria are outstanding group of microorganisms with different kinds of bioactive features and extensive commercial importance (Raja and Prabakarana 2011). It is essential that new groups of microbes from unexplored habitats are pursued as sources of novel antibiotics and other bioactive compounds (Goodfellow and Fiedler, 2010). The significance of cultivating these microorganisms is necessary for a viable opportunity to bio-discovery (Joint *et al.*, 2010). Hence, I have switched over to extreme environments to explore the actinobacteria in mangrove ecosystems. There is a possibility to identify novel actinobacteria in mangrove ecosystems. Accordingly, the present study was designed to study the diversity, distribution and seasonal variation of actinobacterial population of Krishna district Andhra Pradesh, India.

2. METERIAL METHODS

2.1 Collection of mangrove soil sample

Mangrove sediment samples were collected bimonthly intervals from mangrove ecosystem of coastal region, Krishna district of Andhra Pradesh. The samples were collected from 6-10 cm depth and transported to the laboratory in sterile bags and air dried at room temperature.

2. 2 Enumeration of actinobacteria from mangrove sediments

The air-dried mangrove soil samples were pretreated with various enrichment techniques to selectively isolate rare actinobacteria. Dry heat at 120^{o}C for 1 h (Hayakawa *et al*. (1991), SDS 0.05% and Yeast extract 5% (Hayakawa & Nonomura 1989), Phenol 1.5% (Hayakawa *et al*., 2004), Dry heat at 110^{o}C for 1 h and phenol 1.0% (Hayakawa *et al.*, 1995) Moist incubation and Drying (Matsukawa *et al*., 2007) and calcium carbonate (El-Nakeeb and Lechevalier, 1963) were incubate at 37°C for four days. The treated soil samples were suspend in sterile distilled water (1g in 100 ml), homogenized by vortexing and 0.1ml of serially diluted sample (10^{-3} dilution) were spread over the surface of various selective

culture media like Yeast extract-malt extract- dextrose (Naragani *et al*., 2014) Starch-Casein agar medium (ISP-6) (Wellington and Cross, 1983) containing 3% NaCl supplemented with nalidixic acid and secnidazole (Usha *et al*., 2011). After incubation for a week at 30°C, distinct strains were selected for sub culturing to maintain pure culture on agar slants.

2.4 Screening of potent actinobacterial strains for bioactive metabolites

Pure culture of the actinobacterial strains was tested for secondary metabolites by the method of agar well- diffusion (Cappuccino and Sherman, 2004). The pure culture of the strain was transferred aseptically into the seed medium. Fermentation was carried out at 30°C for one week under agitation at 120 rpm. At every 24 h interval, the flasks were harvested. The culture filtrate obtained from broth was extracted with equal volumes of different organic solvents (viz. chloroform, ethyl acetate, methanol and acetone) separately to extract the antimicrobial compounds. Solvent extracts were evaporated to dry in water bath and residues obtained were used to determine antimicrobial assay by employing seeded plate techniques. The inoculated plates were examined for zones of inhibition after incubation period. Diameter of the inhibition zone against the test microorganisms was taken as criteria for determining the antimicrobial potential of the actinobacterial strains.

Test microorganisms

The test bacteria, such as *Bacillus megaterium* (NCIM 2187), *B. cereus* (MTCC 430), *B. subtilis* (ATCC 6633), *Escherichia coli* (ATCC-15597)), *Staphylococcus aureus* (ATCC-6538), *Klebsiella pneumoniae* (ATCC-10031) *Vibrio paraheamoliticus* (ATCC-43996*)* and fungi, *C. albicans* (ATCC-10231).The inoculated plates were examined after 24-48h of incubation at 37^{o}C for bacteria and 48-72 h at 28^{o}C for fungi.

3. RESULTS AND DISCUSSION

Sample collection

The mangrove sediment samples were collected at bimonthly intervals from the different places of the coastal region of Krishna district mangrove ecosystems located along the east coast of Andhra Pradesh, India. Samples were collected from 6-10 cm depth and transported to the laboratory in sterile bags and air-dried at room temperature.

Enumeration of actinobacteria from the coastal region Krishna district

The mangrove ecosystem of Krishna district of Andhra Pradesh was selected for studying the diversity of actinobacteria. The number of actinobacterial strains isolated from the coastal

region of Krishna district was 120 by using six different pretreatment techniques and two different selective media (Table 1). The influence of season on the incidence of actinobacteria was investigated. The actinobacterial population in the mangrove ecosystems exhibited common seasonal variation. The highest number of actinobacteria was observed in August while the counts were minimum in April. High actinobacterial count was found on the Starch-casein agar medium (ISP-6) (Fig-1) when compared to Yeast extract malt extract and dextrose agar medium (ISP-2).

Table 1: Incidence of actinobacteria from samples collected from mangrove ecosystem of Krishna district

S.No	Pretreatment	Media used for isolation	Actinobacterial count at different months in the year 2016					
			April	**June**	**August**	**October**	**December**	**February**
1	Dry heat at 120°C	YMDA	$0x10^3$	$1x10^3$	$1x10^3$	$1x10^3$	$0x10^3$	$0x10^3$
		SCA	$0x10^3$	$2x10^3$	$2x10^3$	$2x10^3$	$2x10^3$	$2x10^3$
2	SDS 0.05% and Yeast extract 5%	YMDA	$2x10^3$	$2x10^3$	$2x10^3$	$2x10^3$	$2x10^3$	$1x10^3$
		SCA	$4x10^3$	$4x10^3$	$6x10^3$	$5x10^3$	$5x10^3$	$3x10^3$
3	Phenol 1.5%	YMDA	$1x10^3$	$1x10^3$	$1x10^3$	$1x10^3$	$0x10^3$	$0x10^3$
		SCA	$2\ x10^3$	$3x10^3$	$3\ x10^3$	$2x10^3$	$2x10^3$	$2x10^3$
4	Dry heat at 110°C and phenol 1.0%	YMDA	$0x10^3$	$1x10^3$	$1x10^3$	$1x10^3$	$0x10^3$	$0x10^3$
		SCA	$0x10^3$	$1x10^3$	$1x10^3$	$1x10^3$	$1x10^3$	$1x10^3$
5	Moist incubation and Drying	YMDA	$1x10^3$	$2x10^3$	$1x10^3$	$1x10^3$	$2x10^3$	$2x10^3$
		SCA	$1x10^3$	$1x10^3$	$2x10^3$	$1x10^3$	$3x10^3$	$2x10^3$
6	**Calcium carbonate**	YMDA	$3x10^3$	$7x10^3$	$10x10^3$	$5x10^3$	$7x10^3$	$5x10^3$
		SCA	$5x10^3$	$10x10^3$	$14x10^3$	$10x10^3$	$12x10^3$	$6x10^3$

YMDA: Yeast extract malt extract and dextrose agar medium.

SCA: Starch-Casein agar medium.

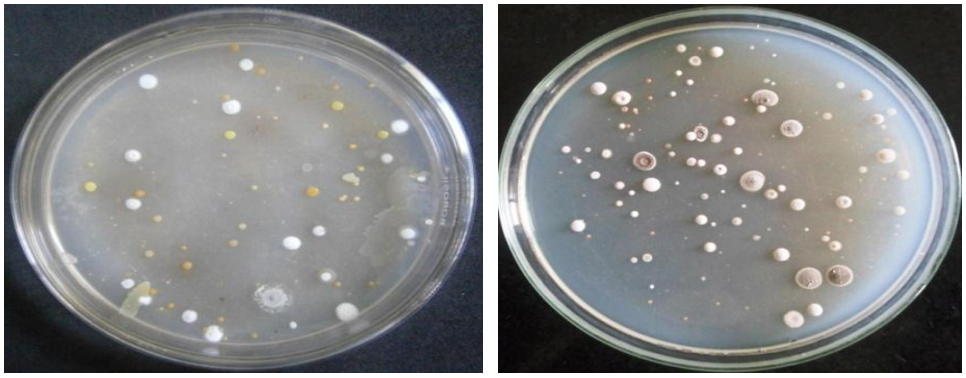


Figure 1: Isolation of actinobacteria colonies on ISP-6 agar plate.

Muvva *et al.* (2016**)** reported that the highest number of actinobacteria was observed on starch-casein agar medium from the mangrove ecosystem of south coastal Andhra Pradesh (Nizampatnam and Gilakaldindi). High actinobacterial strains isolated from mangrove ecosystems of Nizampatnam and Coringa, pretreated with calcium carbonate on starch-casein agar medium (Mangamuri *et al*., 2014). Anindita *et al*. (2008) also reported maximum isolates of actinobacteria on starch casein agar medium.

The present study also determined the antimicrobial activity of the actinobacterial strains. Out of 120 strains, 50 possessed antimicrobial activity, of which 25 isolates were active against all the test microorganisms including *Bacillus megaterium, B. cereus B. subtilis*, Escherichia *coli, Staphylococcus aureus, Klebsiella pneumoniae, Vibrio paraheamoliticus* and *Candida albicans* (Table 2). Several researchers have reported antimicrobial activity of actinobacteria against different pathogens. Out of 208 actinobacterial strains isolated from different locations of the Bay of Bengal and found that 111 isolates produced antimicrobial compounds (Ramesh *et al*., 2009). Mitra *et al.* (2008) reported several actinobacteria from mangrove ecosystem of Sunderban region, of which 50.84% exhibited antimicrobial activity. Remya and Vijay kumar (2008) isolated 64 different actinobacterial strains from marine and mangrove sediments of West coast of India and reported that 32.8% had antimicrobial activity.

Table 2: Screening of actinobacteria isolated from mangrove ecosystem of Krishna District.

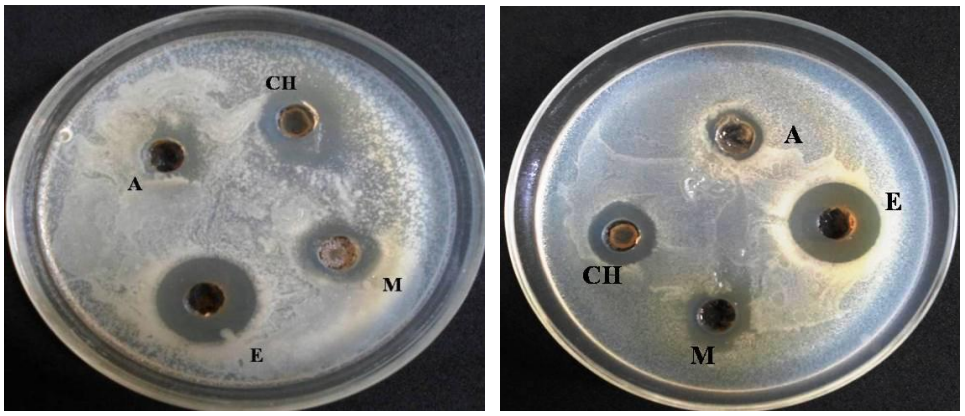
S.No	Isolate code	Zone of Inhibition (mm)							
		Bm	**Bc**	**Bs**	***Ec***	***Sa***	***Kp***	***Vp***	***Ca***
1	VLK-4	12	10	12	11	14	10	11	15
2	VLK-7	14	12	11	11	15	13	10	15
3	VLK-8	12	10	10	13	12	11	14	13
4	VLK-10	19	16	18	14	16	14	17	15
5	VLK-12	19	15	16	17	15	13	12	16
6	**VLK-15**	**21**	**20**	**21**	**20**	**24**	**20**	**22**	**20**
7	VLK-17	15	14	12	10	14	12	12	14
8	VLK-18	15	15	13	12	15	12	12	16
9	VLK-19	13	12	15	11	12	10	13	13
10	VLK-21	18	14	11	16	14	14	15	15
11	**VLK-24**	**22**	**24**	**20**	**19**	**19**	**18**	**20**	**19**
12	VLK-27	14	12	11	11	15	13	10	15
13	VLK-28	20	15	16	17	15	13	12	16
14	VLK-29	12	10	12	11	14	10	11	15
15	VLK-51	18	14	11	16	14	14	15	15
16	VLK-53	14	12	11	11	15	13	10	15
17	VLK-55	15	15	13	12	15	12	12	16
18	**VLK-56**	**22**	**24**	**20**	**19**	**19**	**18**	**22**	**18**
19	VLK-57	18	16	15	14	18	12	16	16
20	VLK-63	18	14	11	16	14	14	15	15
21	VLK-74	15	15	13	12	15	12	12	16
22	VLK-91	15	14	10	11	17	13	11	14
23	**VLK-102**	**24**	**22**	**23**	**22**	**24**	**23**	**22**	**30**
24	**VLK-104**	**28**	**26**	**29**	**24**	**26**	**22**	**24**	**25**
25	**VLK-119**	**20**	**22**	**20**	**19**	**20**	**19**	**21**	**20**

Bm: *Bacillus megaterium*, ***Bc:*** *B. cereus* ***Bs:*** *B. subtilis*, **Ec:** *Escherichia coli*, ***Sa:*** *Staphylococcus aureus*, ***Kp:*** *Klebsiella pneumoniae*, ***Vp:*** *Vibrio paraheamoliticus* and **Ca:** *Candida albicans*

The antimicrobial efficacy of the strains was evaluated by using four solvents such as chloroform, ethyl acetate, methanol and acetone (table 3 and fig. 2). Among the solvents used, ethyl acetate extract exhibited maximum antimicrobial activity where as the other solvent extracts showed moderate to minimum activity against the test microorganisms. Ethyl acetate extracts were more competent than other solvent extracts (Muvva *et al*., 2016 and Usha Kiranmayi *et al*., 2011). The ethyl acetate extract are highly effective against *Candida albicans*, *Staphylococcus aureus*, *Bacillus megaterium* and followed by, *Bacillus subtilis Escherichia coli* and *Klebsiella pneumoniae*.

Table 3: Antimicrobial Activity of the Strain 102 by using different solvents.

S.No	Test Organisms	Inhibition Zone (mm)			
		Chloroform extract	Ethyl acetate extract	Methanol extract	Acetone extract
1	*Bacillus megaterium*	18	**24**	14	16
2	*B. cereus*	15	**22**	11	14
3	*B. subtilis*	14	**23**	13	12
4	*Escherichia coli*	16	**22**	14	10
5	*Staphylococcus aureus*	18	**24**	12	16
6	*Klebsiella pneumoniae*	14	**23**	11	12
7	*Vibrioparaheamoliticus*	16	**22**	14	15
8	*Candida albicans*	19	**30**	17	13



A **B**

CH: Chloroform, **E**: Ethyl acetate, **M**: Methanol and **A**: Acetone

A: Antibacterial activity against Escherichia *coli*

B: Antifungal activity against C*andida albicans*

Fig. 2: Antimicrobial activity of the crude extract of the strain 102 with different Solvent extracts.

4. CONCLUSIONS

Actinobacterial population in the mangrove ecosystems were exhibited seasonal variation and the highest number of colonies was observed in Rainy season while the counts were minimum in Summer on the Starch-casein agar medium (ISP-6), when compared to Yeast extract malt extract and dextrose agar medium (ISP-2), pretreated with calcium carbonate as compared to other pretreatments from coastal region of Krishna district. It is evident from the study that mangrove habitats of South coast of Andhra Pradesh, India serve as a good source of potent actinobacteria with broad spectrum antimicrobial activity.

5. AKNOWLEDGEMENTS

This work was accomplished with the help of DST-SERB (**SCIENCE & ENGINEERING RESEARCH BOARD**), **YOUNG SCIENTIST START-UP GRANT**, in Sciences and Engineering, Govt. of India, New Delhi.

6. REFERENCES

1. Goodfellow, M., and Fiedler, H.P. A guide to successful bioprospecting: informed by actinobacterial systematics. Antonie Van Leeuwenhoek, 2010; 98: 119–142.
2. Manivasagan, P., Venkatesan, J., Sivakumar, K., and Kim, S.K. Marine actinobacterial metabolites: current status and future perspectives. Microbiol. Res., 2013; 168: 311–332.
3. Joint, I., Mühling, M., and Querellou, J. Culturing marine bacteria – an essential prerequisite for biodiscovery. Microb. Biotechnol, 2010; 3: 564–575.
4. Amrita, Kumari., Jain Nitin, C.Subathra Devi. Novel bioactive compounds from mangrove derived actinomycetes. International research Journal of Pharmacy, 2012; 3: 25-29.
5. Raja A, Prabakarana P. Actinomycetes and drug-an overview. Am J Drug Discovery Dev., 2011; 1: 75–84.
6. Hayakawa, M., Sadakata, T., Kajiura, T. & Nonomura, H. New methods for the highly selective isolation of Micromonospora and Microbispora from soil. J. Ferment. Bioeng, 1991; 72: 320–326.
7. Hayakawa, M. & Nonomura, H. A new method for the intensive isolation of actinomycetes from soil. Actinomycetol, 1989; 3: 95–104.
8. Hayakawa, M., Yoshida, Y. & Iimura, Y. Selective isolation of bioactive soil actinomycetes belonging to the Streptomyces violaceusniger phenotypic cluster. J. Appl. Microbiol, 2004; 96: 973–981.
9. Hayakawa, M., Momose, Y., Kajiura, T., Yamazaki, T., Tamura, T., Hatano, K. & Nonomura, H. A selective isolation method for Actinomadura viridis in soil. J. Ferment. Bioeng, 1995; 79: 287–289.
10. Matsukawa, E., Nakagawa, Y., Iimura, Y. & Hayakawa, M. A new enrichment method for the selective isolation of streptomycetes from the root surfaces of herbaceous plants. Actinomycetol, 2007; 21: 66–69.
11. El-Nakeeb, M. A. and Lechevalier, H. A. Selective isolation of aerobic actinomycetes. Appl. Microbiol., 1963; 11: 75-77.

12. Hayakawa, M., Yoshida, Y. and Iimura, Y. Selective isolation of actinomycetes belonging to the *Streptomyces violaceusniger* phenotypic cluster. *J.Appl. Microbiol.*, 2004; 96: 973-981.
13. Naragani, Krishna, Rajesh kumar Munaganti, Chandra Kala Sirigiri, Vijayalakshmi Muvva. Antimicrobial Potential of *Streptomyces violaceoruber* VLK-4 Isolated from South Coast of Andhra Pradesh, India. *Int. J. Pharm. Sci. Rev. Res.*, 2014; 25(1): 125-129.
14. Wellington, E.M.H. and Cross, T. Taxonomy of antibiotic producing actinomyces andnew approaches to their selective isolation.in: "progress in industrial microbiology?" Bushell, M. E(edc). Elsevier, Ameterdam, 1983; 36.
15. Usha Kiranmayi, M., Sudhakar, P., Krishna, N., Yellamanda, B. and Vijayalakshmi, M. Taxonomic Characterization of Potential Bioactive metabolite producing Actinomycetes from Mangrove Sediments of Coringa. *J. Phar. Res.*, 2011; 4: 4650-4653.
16. Shirling, E.B. and Gottlieb, D. Methods for characterization of *Streptomyces* sp. *Int. J.Syst. Bacteriol*, 1966; 16: 313-340.
17. Bozzola, J.J. and Russell, L.D. (1999). Electron microscopy principles and techniques for biologists. 2nd edn. Jones and Bartlettpublishers. Sudbury, MA.
18. Holding, A.J. and Collee, J.G. Routine biochemical tests *In*: Methods in Microbiology, 6A. Academic Press, London, 1971; 1-131.
19. Cappuccino, J. G. and Sherman, N. Microbiology, a laboratory manual. Pearson Education, Inc., New Delhi, 2004; 282-283.
20. Ramesh S, Rajesh M, Mathivanan N. Characterization of a thermo stable alkaline protease produced by marine *Streptomyces fungicidicus* MML 1614. Bioprocess Biosyst Engg, 2009; 32: 791-800.
21. Mitra A, Santra SC, Mukherjee J. Distribution of actinomycetes, their antagonistic behavior and the physico chemical characteristics of the World's largest tidal mangrove forest. Appl Microbiol Biotechnol, 2008; 80: 685-695.
22. Remya M, Vijayakumar R. Isolation and characterization of marine antagonistic actinomycetes from West coast of India. *Facta universitatis Series.* Medicine and Biology, 2008; 15: 13-19.
23. Krishna Naragani and Vijayalakshmi Muvva. Actinobacteria from mangrove habitats: Diversity and antimicrobial activity, European Journal of Biomedical and Pharmaceutical sciences, 2016; 3: 460-467.

24. Usha Kiranmayi Mangamuri, Muvva Vijayalakshmi and Sudhakar Poda. Exploration of Actinobacteria from Mangrove Ecosystems of Nizampatnam and Coringa for Antimicrobial Compounds and Industrial Enzymes. British Biotechnology Journal, 2014; 4: 173-184.
25. Anindita M, Subhas Chandra S, Mukherjee J. Distribution of actinomycetes, their antagonistic behavior and the physico-chemical characteristics of the world's largest tidal mangrove forest. Appl Microbiol Biotechnol, 2008; 80: 685-695.
26. Usha Kiranmayi M, Sudhakar P, Krishna N, Yellamanda B, Vijayalakshmi M. Taxonomic Characterization of Potential Bioactive metabolite producing Actinomycetes from Mangrove Sediments of Coringa. *J. Phar. Res.*, 2011; 4: 4650-4653.