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ISOLATION OF ENDOPHYTIC BACTERIA FROM MANGROVE, BANANAS AND SUGARCANE FOR THEIR BIOLOGICAL ACTIVITIES

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ABSTRACT

Endophytes - Microbes that colonize living internal tissues of plants without causing any immediate, overt negative effect. The phrase Bioprospecting is today most frequently used to describe the collection and screening of biological material for commercial purpose. Natural products have been the traditional path finder compounds offering an untold diversity of chemical structures. Endophytic bacteria could be better protected from biotic and abiotic stresses than rhizosphere bacteria. Further most the discovery of plethora of microbes for application that span a broad spectrum of utility in medicine, agriculture and industry is now practical. The growth stimulation of host plant by the endophytic microbes can be a consequence of nitrogen fixation or the production of phytohormones, biocontrol of phytopathogens in the root zone or by enhancing availability of nutrients and minerals. In this present study we collected roots, stems and leaf samples of mangrove, banana and sugarcane plants in sterile cover from pichavaram and Annamalai university, agriculture faculty trial field, Chidambaram. Samples transferred to lab and processed immediately after surface sterilization by standard procedure. Isolation and purification of endophytic bacteria was done by using starch casein agar with antibiotics to inhibit the growth of fungi. Totally 41 isolates were obtained from all the samples, 24 from mangrove, 7 from banana and 10 from sugarcane. All the isolates were taken to test the PGPR activity. We have done PGPR activity test *viz.*, phosphate solubilisation, silicate solubilisation, starch hydrolysis, salt tolerance on growth, growth on NFB medium (N2 fixation), and antimicrobial activity of endophytic bacteria. Most of the isolates show good biological activity. Based on the results 4 isolate from mangrove, 3 isolate from sugarcane and 2 isolate from banana was selected as potential for PGPR activity. Those potential isolates were taken for future work.

KEY WORDS

Endophytes, Bacteria, Mangrove, Banana, Sugarcane and Biological activity.

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INTRODUCTION

Natural products have been the traditional path finder compounds offering an untold diversity of chemical structures[1].

The phrase bioprospecting is today most frequently used to describe the collection and screening of biological material for commercial purposes[2].

Endophytic bacteria are bacteria that live in plant tissue without doing substantive harm or gaining befit other

than residency (Kado.l992, Kobayashi and Palumbo). Since, the discovery of endophytes by Darnal, Germany in 1904[3]. Bacon and white (2000)[4] give an inclusive and widely accepted definition of endophytes - "Microbes that colonize living, internal tissues of plants without carrying any immediate over negative effects"

Various investigations had done on endophytes in different ways, which is usually dependent on the prospective from which the endophytes were being isolated and subsequently examined[5]. Endophytic bacteria could be better protected from biotic and abiotic stresses than rhizosphere bacteria[6].

Bacterial endophytes can be isolated from surface disinfected plant tissue or extracted from internal plant tissue[6]. Both Gram positive and Gram negative bacterial endophytes have been isolated from several tissue types in numerous plant species. Furthermore, several different bacterial species have been isolated form a single plant (Kobayashi and Palumbo, 2000).

Endophytes enter plant tissue primarily through the root zone; however, aerial portions of plants such as flowers, stems and cotyledons, may also be used for entry. Specifically the bacteria enter tissues via germinating radicals (Gagnc, *et al*, 1987) secondary roots, stomates or as a result of foliar damage (Leben, *et al*, 1968) Endophytes inside a plant may either become localized at the point of entry or spread throughout the plant[6].

Secondary metabolite produced by endophytes provides a variety at fitness enhancements such as increased resistant to herbivora, parasitism trough as well as growth enhancements[7].

A specific rationale for the collection of each plant for endophyte isolation and natural product discovery is used[1].

Mangroves, unique woody plant communities of intertidal coasts in tropical and subtropical coastal regions, are highly productive ecosystems[8, 9].

Banana is a giant perennial herb, it is one of the main fruits cultivated in subtropical and tropical regions (L.Cao *et al.,* 2005).

Sugarcane is an annual plant, which is primarily used to produce sugar and alcohol (Mendes *et al*., 2007).

All the above three plants are have different ethnobiological history; in this present study we select a tree, annual plant and perennial herb with great biodiversity for this research.

MATERIALS AND METHOD

Sample collection and transport

Samples were collected from Parangipettai (protonova) and Annamalai nagar. All the Samples were collected in a sterile plastic covers, transferred to laboratory and processed immediately.

Sample pretreatment and endophytic bacterial isolation

For the pretreatment of samples and isolation of endophytic bacteria, the method described by[10] was adopted with some modifications. All the samples were excised and subjected to a three step surface sterilization procedure. All the samples were cut into bits (0.5-1.0 cm), Washed in running tap water and Rinsed in 70% ethanol for 30 sec then Rinsed in sodium hypochloride (3-5%) for 3 min finally Washed in sterile water 3 times thoroughly. Plated on starch casein agar and nutrient agar with nystatin and cycloheximide (50µg/ml) to suppress fungal growth and Incubated at 28ºC for 3 days after incubation plates were Observed for the growth of endophytic bacteria. Morphologically different colonies were selected, Pure culture were prepared and stored in refrigerator.

Phosphate Solubilization

Phosphate solubilising activity of endophytic bacteria was studied by the method described by[11] all the endophytic isolates were spot inoculated on Pikovaskaya's agar plates and incubator at 28°C for 3-5 days. After incubation all the plates were observed for clear halo zone formation around the bacterial growth.

Silicate Solubilization

Silicate solubilising activity of endophytic bacteria was studied by the method described by (Bunt and Rovira (1955) all the endophytic isolates were spot inoculated on Bunt and Rovira medium plates and incubator at 28°C for 5-7 days. After incubation all the plates were observed for clear halo zone formation around the bacterial growth.

Effect of sodium chloride on growth of endophytic bacteria

To study the effect of sodium chloride on the growth of endophytic bacteria, nutrient agar medium was prepared by supplementing with different concentration of Nacl and the endophytic bacterial isolates were inoculated into it. All the plates were incubated at 28°C for 5 days and observed for every 24 hours[12].

Starch hydrolysis

All the endophytic bacterial isolates were screened for starch hydrolysis starch agar. All the isolates were spot inoculated on starch agar media and incubated at 28° C for 5-7 days. After incubation Results were noted by using an reagent 1% iodine[13].

Antimicrobial activity

For the preparation of 18 hrs culture, nutrient broth was prepared and all the bacterial isolates were inoculated and incubated at 28°C for the production of antimicrobial compounds by using no 3 medium15 was prepared and about 10% of inoculums was transferred into it . All the test tubes were incubated in rotary shaker with 95 rpm for 120 hours at 28°C. After incubation, 2 ml of culture broth was taken and separated by centrifugation at 10,000 rpm for 10 minutes. After centrifugation, the culture supernatant was collected and used for antimicrobial activity testing. Antimicrobial activity of culture supernatant was tested by agar well diffusion method using nutrient agar medium. Test bacterial strains used in this study include human pathogens *Staphylococcus aureus*, *Escherichia col*i, *Proteus* s*pecies, Klebsiella species, Pseudomonas species, Salmonella typhi, Candida* and plant pathogens such as *Fusarium, Curvalaria, Penicillium, A.niger.* All the pathogenic cultures were obtained from Research laboratory, Department of Microbiology, Faculty of agriculture, Annamalai University, Chidambaram. 18 hours broth cultures of test organism were inoculated into Nutrient agar plates using sterile cotton swab. About 5 mm size well was made and 100 µl of culture supernatant was added into it. All the plates were observed for zone of inhibition after incubation at 37°C for 24 hours[14].

Growth on NFB medium of endophytic bacteria

To study the growth on NFB medium of endophytic bacteria, Nitrogen free Basal medium were prepared by supplementing with Malicacid and the endophytic bacterial isolates were inoculated into it. All the plates were incubated at 28°C for 3 days and observed for every 24 hours.

RESULTS AND DISCUSSION

Since the discovery of endophytes by Darnel, in 1904. Various investigators reported endophytic microbes from various plant exists in different ecosystems. It is not worthy that of the nearly 3,00,000 plant species that exists on earth each individual plant is host to one or more endophytes. Only a few these plants have ever been completely studied relative to their endophytic biology. Consequently the opportunity to find new and interesting microorganism among myriads of plants in different settings and ecosystems is great[1].

In this present study Totally 41 bacterial strains were recovered from all the collected samples from selected plants. All the bacterial strains are markable different from terrestrial bacterial isolates. So far these are numerous reports are available on endophytic fungi in mangroves[15,16]. In India also countable number of report showed on diversity of endophytic bacteria, fungi in medicinal plants[17] but from available literature there is no report on endophytic bacteria from sugarcane and Banana particularly in Tamil nadu. In general endophytic bacteria occur as lower population densities than rhizospheric bacteria or bacterial pathogens[6,18].

Endophytes are the chemical synthesizers with in plants. Many of them are capable of synthesizing bioactive compounds that can be used by plants for defence against pathogens and some of these compounds have been proved for useful drug discovery. Up to now most of the natural products from endophytes are antibiotics, anticancer agents, biological control agents antivirals, antidiabetic agents and other bioactive compounds by their different functional roles[19]. In the present study antimicrobial activity of endophytic bacteria were tested by agar well diffusion method. Most of the

endophytes from all the three plants show good antimicrobial activity.

[20]Studied the endophytic assemblage in young mature and senescent leaves of *Rhozospora apiculata* and their possible role in mangrove litter degradation the results of the present study indicates litter degradation, since it has wide range of enzymatic activities. Further these endophytic isolates will be a potential source for extracellular enzymes.

Endophytic bacteria reside within plant tissues have been reported to promote plant growth. They promote plant growth directly or indirectly via production of phyto hormones, biocontrol of host plant diseases or improvement of plant nutritional status[21]. Endophytic bacteria posses the capacity to solubilize phosphates and it was suggested by the authors that the endophytic bacteria from soyabean may also participate in phosphate assimilation[18]. In our present study most of the isolates from all the samples shows good PGPR activity.

Which proves that endophytic bacteria isolated from root, stem and leaf of Mangrove, Banana and Sugarcane may act as a good plant growth promoting bacteria than other normal PGPR organisms (Table No.1-8).

Table No.1: Phosphate solubilisation

S.No	Root Isolates		Stem Isolates		Leaf Isolates	
	Culture No	Hydrolysis	Culture No	Hydrolysis	Culture No	Hydrolysis
1	M1R1	+	M1S1	-		
2	M1R2	+	M1S2	+		
3	M2R1	-	M2S1	+	M1L1	-
4	M2R2	+	M2S2	+	M1L2	+
5	M3R1	+	M3S1	-	M2L1	+
6	M3R2	+	M3S2	+	M3L1	++
7	M3R3	-	M4S1	+	M3L2	-
8	M3R4	-	M4S2	-	M4L1	-
9	M4R1	+	S1S1	+	M4L2	+
10	S1R1	+	S1S2	+	S1L1	-
11	S1R2	+	S1S3	+	S1L2	-
12	S1R3	-	S1S4	-	B1L1	+
13	S1R4	-	B1S1	+	B1L2	-
14	B1R1	-	B1S2	+		
15	B1R2	+	B1S3	-		

Table No.2: Silicate solubilization

S.No	Root Isolates		Stem Isolates		Leaf Isolates	
	Culture No	Hydrolysis	Culture No	Hydrolysis	Culture No	Hydrolysis
1	M1R1	-	M1S1	+		
2	M1R2	-	M1S2	+		
3	M2R1	-	M2S1	+	M1L1	-
4	M2R2	++	M2S2	-	M1L2	-
5	M3R1	+	M3S1	+	M2L1	-
6	M3R2	+	M3S2	-	M3L1	-
7	M3R3	+	M4S1	+++	M3L2	+
8	M3R4	-	M4S2	+++	M4L1	+++
9	M4R1	-	S1S1	-	M4L2	+
10	S1R1	+	S1S2	-	S1L1	-
11	S1R2	+	S1S3	-	S1L2	-
12	S1R3	-	S1S4	++	B1L1	-
13	S1R4	-	B1S1	+++	B1L2	++
14	B1R1	-	B1S2	+		
15	B1R2	+++	B1S3	-		

Table No.3: Starch hydrolysis

S.No	Root Isolates		Stem Isolates		Leaf Isolates	
	Culture No	Hydrolysis	Culture No	Hydrolysis	Culture No	Hydrolysis
1	M1R1	++	M1S1	-		
2	M1R2	-	M1S2	-		
3	M2R1	-	M2S1	+	M1L1	-
4	M2R2	-	M2S2	+++	M1L2	+++
5	M3R1	+++	M3S1	+	M2L1	+++
6	M3R2	+	M3S2	++	M3L1	++
7	M3R3	++	M4S1	+++	M3L2	+++
8	M3R4	+	M4S2	-	M4L1	-
9	M4R1	+++	S1S1	+	M4L2	-
10	S1R1	+++	S1S2	++	S1L1	++
11	S1R2	+	S1S3	-	S1L2	-
12	S1R3	+++	S1S4	-	B1L1	-
13	S1R4	-	B1S1	-	B1L2	-
14	B1R1	-	B1S2	-		
15	B1R2	-	B1S3	-		

Table No.4: Salt Tolerance of root isolates

S.No	Root samples					
	Culture no	2.5%	5%	7.5%	10%	12.5%
1	M1R1	+++	+++	+++	++	+
2	M1R2	+++	++	+	-	-
3	M2R1	+++	++	+	-	-
4	M2R2	+++	++	+	-	-
5	M3R1	+++	+++	++	++	+
6	M3R2	+++	+++	+++	++	++
7	M3R3	+++	+++	++	+	-
8	M3R4	+++	+++	++	+	-
9	M4R1	+++	+++	++	+	-
10	S1R1	+++	+++	+	-	-
11	S1R2	+++	+++	+	-	-
12	S1R3	+++	+++	++	-	-
13	S1R4	+++	+++	+	-	-
14	B1R1	+++	+++	+	+	-
15	B1R2	+++	+++	++	++	+

Table No.5: Salt Tolerance of stem isolates

S.No	Stem samples					
	Culture no	2.5%	5%	7.5%	10%	12.5%
16	M1S1	+++	+++	+++	+++	++
17	M1S2	+++	+++	+++	++	+
18	M2S1	+++	+++	++	++	++
19	M2S2	+++	+++	++	-	-
20	M3S1	+++	+++	++	-	-
21	M3S2	+++	+++	++	-	-
22	M4S1	+++	+++	++	+	-
23	M4S2	+++	+++	+	-	-
24	S1S1	+++	+++	++	+	+
25	S1S2	+++	+++	++	-	-
26	S1S3	+++	+++	++	+	+
27	S1S4	+++	+++	+++	++	++
28	B1S1	+++	+++	++	+	-
29	B1S2	+++	++	++	+	+
30	B1S3	+++	++	+	-	-

Table No.6: Salt Tolerance of leaf isolates

S.No	Leaf sample					
	Culture no	2.5%	5%	7.5%	10%	12.5%
31	M1L1	+++	+++	++	+	-
32	M1L2	+++	+++	++	++	+
33	M2L1	+++	+++	++	-	-
34	M3L1	+++	++	-	-	-
35	M3L2	+++	+++	++	++	+
36	M4L1	++	++	++	++	+
37	M4L2	+++	+	+	+	-
38	S1L1	+++	++	++	+	+
39	S1L2	+++	++	++	-	-
40	B1L1	+++	++	+	-	-
41	B1L2	+++	++	+	-	-

Table No.7: Growth on NFB medium

S.No	Root Isolates		Stem Isolates		Leaf Isolates	
	Culture No	Hydrolysis	Culture No	Hydrolysis	Culture No	Hydrolysis
1	M1R1	-	M1S1	-		
2	M1R2	+	M1S2	+		
3	M2R1	-	M2S1	-	M1L1	-
4	M2R2	+	M2S2	-	M1L2	-
5	M3R1	-	M3S1	-	M2L1	+
6	M3R2	-	M3S2	+	M3L1	-
7	M3R3	-	M4S1	-	M3L2	-
8	M3R4	+	M4S2	-	M4L1	+
9	M4R1	-	S1S1	+	M4L2	-
10	S1R1	-	S1S2	-	S1L1	-
11	S1R2	+	S1S3	-	S1L2	+
12	S1R3	-	S1S4	+	B1L1	-
13	S1R4	+	B1S1	-	B1L2	-
14	B1R1	-	B1S2	-		
15	B1R2	+	B1S3	-		

Table No.8: Antimicrobial activity

S.No	Root Isolates		Stem Isolates		Leaf Isolates	
	Culture No	Hydrolysis	Culture No	Hydrolysis	Culture No	Hydrolysis
1	M1R1	+	M1S1	-		
2	M1R2	-	M1S2	+		
3	M2R1	+	M2S1	-	M1L1	-
4	M2R2	+	M2S2	-	M1L2	+
5	M3R1	-	M3S1	-	M2L1	-
6	M3R2	-	M3S2	+	M3L1	-
7	M3R3	+	M4S1	-	M3L2	+
8	M3R4	+	M4S2	-	M4L1	-
9	M4R1	-	S1S1	+	M4L2	-
10	S1R1	-	S1S2	-	S1L1	-
11	S1R2	+	S1S3	+	S1L2	-
12	S1R3	-	S1S4	-	B1L1	+
13	S1R4	+	B1S1	+	B1L2	-
14	B1R1	-	B1S2	-		
15	B1R2	+	B1S3	-		

CONCLUSION

This present study evidence that Mangrove, Sugarcane and Banana are the potential but under exploited resource for bioactive endophytic bacteria. This study showed promising phosphate solublization, silicate solublization, starch hydrolysis, NaCl tolerance on growth, growth on nitrogen free media and antimicrobial activity. Based on the biological activity of isolated endophytes, 9 was identified as potential. Those Endothytic bacteria may be a effective PGPR for the growth, yield and stress tolerance of the plants in future. Detailed investigations on bacterial endophytes of Mangrove, Sugarcane and Banana were needed to prove its potential further and it will leads to discovery of numerous high value products.

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