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Research Article

Antibacterial activity of Endophytic fungi extracts from the Mangrove plant *Avicennia marina* (Forsk) Vierh

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Abstract

The aim of this study was to identify the endophytic fungi of the mangrove plant *Avicennia marina* (Forsk) vierh. The investigate their potential antimicrobial activity. Ten species of entophytic fungi were successfully isolated from *Aspergillus awamori, Aspergillus favipes, Aspergillus chevalieri, Aspergillus flavus, Aspergillus clavatus, Aspergillus fumigatus, Penicillium candidum, Penicillium japonicum, Penicillium pupurogenum, Phoma* sp. *Aspergillus flavus* as well as highly dominant species. The fungal extracts were assessed for antibacterial activity against five standard pathogenic bacterial strains. *Baillus substilis, Enterobacter aerogenes. Enterococcus faecalis Escherichia coli* and *Klebsiella oxytoca*. Most of the extracts showed in vitro inhibition of bacterial growth. The Bio active compound screening revealed the existence of a diverse group of secondary metabolites in the methanolic extracts of endophytic fungi of highly activity.

Keywords: Endophytic fungi, Antibacterial activity, TLC screening.

Introduction

Endophytes are microbes that colonize living tissues of plants without causing any immediate, overt negative effects (Bacon and White, 2000). As almost all vascular plant species appear to be inhabited by endophytic bacteria or fungi, these represent important components of microbial diversity. The relationship between the host plant and its endophyte shows symbiotic characteristics as the endophytic occupant usually obtains nutritients and protection from the host plant and in return profoundly enhances the fitness of the host by producing certain functional metabolites (Tan and Zai, 2001).

During this period several aspects of endophyte biology were thoroughly studied, including the diversity, taxonomy, reproduction, host ecology and effects on the host (Eg. Saikkonen *et al.*,1998). Because natural selection favors the evolution of beneficial endophytic strains, several endophytes were found to secret secondary metabolites that protect plants against insect pests, pathogenetic organisms as well as herbivores (Saikkonen *et al.*, 2004) thus, endophytes represent a promising source of Noval Biologically active metabolites for pharmacological and agricultural applications (Dreyfuss and Chapela 1994; Schu12 *et al.*, 2002).

The discovery of novel antimicrobial metabolites from endophytes is an important alternative to surmount the increasing level of drug resistance by human pathogen, the inadequate number of effective antibiotics against diverse bacterial species, and few new antimicrobial agent in development is probably due to relatively unfavorable returns on asset (Song, 2008, Yu *et al.*, 2010).

Antimicrobial metabolites can be defined as low molecular weight organic natural substances made by microorganisms that are active at low concentrations against other microorganisms. (Wain *et al.*, 1971) Endophytes are believed to carry out a resistance mechanism to over come pathogenic invasion by producing secondary metabolites (Tan *et al.*, 2001).

Materials and Methods

Sample collection

The mangroves plants namely *Avicennia marina* were collected from mangrove environment of Karankadu. The collected leaves were carefully stored in polythene bags and transported to the laboratory for the mycological examination.

Sterilization of plant materials

For isolation of the endophytic fungi, selected mangrove plant leaves were subjected to surface sterilization. They were first washed with running water and then immersed in aqueous ethanol (3:7) for 1 minute, then in 3% aqueous Sodium hypochlorite solution for 4 minutes and gain in aqueous ethanol (0:2:9:8) for 0.5 minute. Finally ringing in distilled water three times, with further drying in sterilized paper in a biosafety chamber (Petrini*et.al.*, 1982).

Isolation of endophytic fungi

The potato dextrose agar medium (Potato – 200g, Dextrose -20g, Agar-18g, Distilled water – 1000ml and pH – 6.5) was used for isolation of endophytic fungal species. The slide was observed under a compound microscope. Microphotgraphy of the individual fungal species was also taken using Nikon phase contrast microscope (Nikon, Japan) Colony colour any morphology were observed besides hyphal structure, spore size, shapes and spore bearing structures. They were compared with the standard works of manual of soil fungi (Gillman, 1957).

Screening of fungi for antibacterial efficacy (Cuomo *et al.*, 1995)

The selected fungal endophytes were screened for antibacterial efficacy by agar well diffusion method. The five bacterial strain (*Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli* and *Klebsiella oxytoca*) were obtained from microbial type culture collection (MTCC). Chandigarh, India.

Solvent extraction of fungal isolates

The conical flasks were taken any 150ml of PD broth was prepared in each of the conical flask. The selected fungal cultures were inoculated in each of the conical flask separately any incubated at 28^{o}C for 5 days. After incubation, the fungal mats were taken from each of the flask and put in to the each of the beakers. To this each of the solvents. (Methanol, Ethanol, Distilled water) was added separately, crushed and centrifuged at 10,000 rpm for 15 mins. The fungal mat extracts were tested against human pathogenic bacteria.

Assay

Ethanol, Methanol and Distilled water extracts were tested for their antibacterial efficacy against the bacterial pathogens.

Separation of bioactive compounds from endophytic fungi

The separation of the bioactive compounds from the crude extract of selected endophytic fungi was performed by TLC (Wagner, 1995)

Results

The present investigation includes isolation and identification of endophytic fungi from mangroves leaves antibacterial efficacy of endophytes, separation of bioactive compounds by TLC.

In general, totally ten endophytic fungal species isolates from Karankadu mangrove forest, Ramanathapuram District. The fungal endophytes were presented in table.1

Table 1 Isolated fungal endophytes from mangrove plants

S.No	Name of the Fungi
1	*Aspergillus awamori*
2	*Aspergillus favipes*
3	*Aspergillus chevalieri*
4	*Aspergillus flavus*
5	*Aspergillus clavatus*
6	*Aspergillus fumigatus*
7	*Penicillium candidum*
8	*Penicillium japonicum*
9	*Penicillium purpurogenum*
10	*Phomo* sp.

Isolated endophytic fungi

The antibacterial efficacy of the selected fungal endophytes such as *Aspergillus flavus* and *Phomo* sp. were evaluated by agar well diffusion method. Among the three extract Ethanol, Methanol, Distilled water showed broad spectrum of antibacterial activity by exhibiting prominent zone of inhibition against the test pathogens such as *Enterococcus faecalis* (14.7±1.7mm) *Klebsiella oxytoca* (14.3±1.3mm) *Escherichia Coli* (13.3±0.71mm) *Bacillus subtillus* (13.1±1.1mm) *Enterobauter aerogenes* (10±1.3mm) Ethanol extract exhibited moderate activity any distilled water extract showed slight inhibitory effects on the test pathogens table 2.

Table.2 Antibacterial efficacy of *Aspergillus flavus*

S. No.	Bacterial Pathogen	Zone of inhibition (diameter mm) Concentration – 100 mg		
		Distilled water	Methanol	Ethanol
1.	*Bacillus subtilis*	-	13.1±1.1	5 ± 1.1
2.	*Enterobacter aerogenes*	-	10±1.3	3± 1.2
3.	*Enterococcus faecalis*	-	14.7±1.7	4± 1.6
4.	*Escherichia coli*	-	13.3±0.7	1.1± 0.1
5.	*Kebsiella oxytoca*	-	14.3±1.3	6±1.4

Antibacterial efficacy of *Phoma* sp.

The methanol extracts of *Phoma* sp. exhibited moderate zone of inhibition against *Escherichia coli* (12.5±1.7 mm) *Enterococcus faecalis* (10.5±1.1mm) *Bacillus subtilis* (10.3±0.5mm) *Enterobacter aerogenes* (9.7±1.8mm) and *Klebsiella oxytoca* (7.5±0.8). The distilled water extract did not show inhibition against rest of other tested pathogen.

Table: 3 Antibacterial efficacy of *Phoma* sp.

S.No.	Bacterial Pathogen	Zone of inhibition (diameter mm) Concentration – 100 mg		
		Distilled water	Methanol	Ethanol
1.	*Bacillus subtillus*	-	7.1±0.3	10.3±0.5
2.	*Enterobacter aerogenes*	-	6.5±0.8	9.7±1.8
3.	*Enterococcus faecalis*	-	8.2±1.7	10.5±1.1
4.	*Escherichia coli*	-	6.8±1.1	12.5±1.7
5.	*Kiebsiella oxytoca*	-	5.3±1.3	7.5±0.8

Discussion

During this study, a survey has been conducted for the diversity of entophytic fungi associated with the mangrove plant *Avicennia marina.* A total of ten fungal endophytes were isolated and cultured in the laboratory. The results are in accordance with Rajendran and Kathiresan (2007) who studied microbial flora associated with *Avicennia marina* and *Rhizophora apiculata*. Most of the species encountered in the present study was also observed by other works (Maria *et al.*, 2005; Liu *et al.*, 2007).

In this study an initial assessment was performed for the antibacterial activity of the isolated endophytes species. The methanolic extracts from the culture of endophytic fungi grown aerobically In nutrient agar medium displayed antibacterial activity. Some extracts were effective against all the bacterial strains included in the study. The effectiveness of the extracts method. Three different solvents were used for the extraction of antimicrobial metabolites from the culture filtrate of the selected fungi Distilled water extract showed least antibacterial activity while Methanol extraction of fungi. Shows higher antibacterial activity than Ethanol extracts. It is accepted widely that the use of organic solvents always provides a higher efficiency in extracting an antimicrobial compounds when compared with water extraction (Rosell and Srivastava, 1987).

The enormous chemical diversity and biological potential of endophytic particularly mangrove derived. Endophytic fungi were reported by many Authors For example mangrove endophytic fungi *Phomopsis* sp from *Hibiscus tiliaceus* derived a new substance namely A - Seco – Oleane – Type triterpenes. Cytospornes, coumating and alkaloid compounds derived from endophytic fungi of *Pestalotiopsis* sp isolated from *Rhizophora mucronata* Isofalvones isolated from mangrove endophytic fungi *Penicillium chermesinum* exhibited cytotoxicity activity against the cancer cell lines. Conversely, toxin and acids such as Paeciloxocins were isolated from the mangrove fungus *Paecilomyces* sp. Paeciloxocin exhibits strong cytotoxicity against the hepG2 cell line (*Cehm et al*., 2010)

References

1. Bacon,C.W. and white, J.F., 2000 Microbial endophytes, Marcel Dekkarinc. New York.
2. Tan.R.X., and W.X.ZOU.2001. Edophytes: arich source of functional metabolites. Nat prod Rep.,**18**:448-459.
3. Saikkoen, K., Faeth, S.H., Helander, M. and Sullivan, T.J. (1998) A Continum of interactions with lost plants. Annual Reviews of Ecology and systematics**29**:319-343.
4. Saikkonen,K., Wali, P., Helander,M. and Faeth, S.H.(2004) Evolution of endophyte – plant symbiosis. Trends in plant science. **9**:275-280.
5. Dreyfuss, M.M. and Chapela, I.H.(1994). Potential at fungi in the discovery of novel low – molecular weight pharmaceuticals. In: the discovery of natural products with therapeutic potential (Ed. Guno V.P) Butterworth – Heinemann London, UK : 49-80.
6. Song, J.H., 2008 what's new on the antimicrobial horizons. Int.J.Antimicrob. Agents**324**:2-207-213.
7. Yu.H.,Zhang, L., Li, L., Zheng C., Guo, L., W., Sun, P. and Qin.L., 2010 Recent developments and future prospects of antimicrobial metabolites produced by entophytes. Microbial Res., (**165**):(6):437-449.
8. Wain,C., Taylor, H.L., Wall, M.E., Coggon, P. and Mcphail.A.T., 1971 plant antitumor agent. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolla*.J. Amer. Chem. Soc, 93 (9) 2325 -2327.
9. Tan,R.X., and W.X.ZOU.2001.Engophytes: a rich source of functional metabolites. Nat.Prod.Rep.**18**:448-459
10. PetRini,O., Stone, J. and carroll, F.E., 1982 endophytic fungi in evergreen shrubs in wester ororegon: A preliminary study. Can J. Botany, **60**:789-796
11. Gillman,J.C.,1957 A manual of soil fungi. Revised 2nd edition oxford and IBH publishing company (India reprint) Calcutta, Bombay, New Delhi.
12. Cuomo,V., Palomba,I., Perretti,A., Guerriro.A., D Ambrosio, M and Pietra,F., 1995. Antimicrobial activities from marine fungi J.Mar Biotechnology., 2:199-204.
13. Wagner, 1995. Analysis of quantitative phytochemicals for bioactive compounds in TLC methods.221
14. Rajendran, N. and Kathiresan, K., 2007 Microbial flora associated with submerged mangrove leaf litter in India. Rev.Biol.Trop., **55**(2) 393-400
15. Maria, G.L., Sridhar, K.R and Raviraja, N.S., 2005. Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest cost of India.J.Agricult.technol.,1: 67–80.
16. Liv,A.R., WV, X.P and XV,T., 2007. Research advances in endophytic fungi of mangrove. Ying yong sheng TaixveBai.**18**(40:912-918
17. Rosell, K.G. and Sirvastava, L.M., 1987. Fatty acids as antimicrobial substances in brown algae. Hydrobiologia, **152**:471-475.
    Chen,G., She, Z., Wen,L.,Yan,C., Cai,J. and
18. Mu,L.,2010. Two new paeciloxocins from a mangrove endophytic fungus *Paecilomyces* sp. Russian Chemical Bulletin., **59**(8):1656-1659.