Diversity of Fungal Endophytes with Antibacterial Activity from five Medicinal Plants of Tripura

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SHORT COMMUNICATION

Diversity of fungal endophytes with antibacterial activity from five medicinal plants of Tripura

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Fungi are heterotrophic eukaryotes with unique characteristics that set them well apart from both plants and animals. Fungi are so versatile in adapting themselves that they could occupy a variety of ecological habitats. The role of symbiosis between plant and microorganism is considered a key element for eukaryotes colonization of the land. In the present study, a preliminary work has been carried out to check the assemblage of fungal endophytes from different parts of five medicinal plants of Tripura. It was found that a variety of fungal endophytes have been recorded from the different plant samples. Among them *Fusarium oxysporum, Diaporthe phaseolorum* were found to be dominant. Further the antibacterial activity of some selected endophytes were also screened against gram positive and gram negative bacteria. All the endophytes showed differential inhibition zone against the tested bacteria.

Key words: Fungal **e**ndophytes, diversity, antibacterial activity

INTRODUCTION

Plants serve as a reservoir of large numbers of different microorganisms which includes both epiphytes and endophytes. Epiphytes are mycota which resides on the surface of plant whereas endophytes colonize the internal tissues of the plant. Endophytes may be categorized into endophytic fungi, endophytic bacteria and endophytic actinomycetes.

Fungi are eukaryotic and they set them apart from the usual habitats due to their heterotrophic nature with unique characteristics. Fungi are also very eligible in adapting themselves in a wide variety of ecological habitats.

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Individual plant may be host to one or more endophytes suggesting that there may be many undiscovered endophyte species (Huang *et al,* 2007). The composition of the fungal community usually differs between host species (Arnold and Lutzoni, 2007; Saikkonen, 2007) and also differs within the various tissues of a host plant (Kumar and Hyde, 2004; Santamaria and Diez, 2005). Variation in the diversity of fungi may be associated with location, climate and leaf age. Sampling and characterizing fungal endophyte diversity is an emerging challenge which leads to the discovery of new species, novel compounds and a better understanding of their role in eco-systems (Arnold and Lutzoni, 2007; Saikkonen, 2007).

There are many reports of antibacterial activity of endophytic fungi against plant and human patho-

genic microorganisms. Chareprasert *et al,* (2006) reported on antimicrobial activity exhibited by endophytic fungi isolated from teak and rain tree and they were found to produce some metabolites active against bacteria and yeast. New bioactive metabolites (e.g. asperfumin) produced by *Aspergillus fumigatus* CY018, an endophytic fungus, has been shown to inhibit *Candida albicans* (Liu *et al,* 2004). The antimicrobial properties of both endophyte and host may be exploited as a tool to formulate new generation drug in pharmaceutical industries.

Tripura, a small state in the Northeastern region represents an area with large biodiversity where medicinal plants are yet to be explored for endophytic mycoflora. The use of endophytes as producers of bioactive agents will help in conservation of medicinal plants and maintenance of environmental biodiversity.

Leaf, bark and root from the healthy plant samples were collected from the selected plants in sterile polythene bags and were used for isolation of endophytic fungi.

Collected samples were thoroughly washed in running tap water followed by sterile water and the samples were dipped in 70% ethanol for 30 sec, 5%NaOCl for 2 mins and 70% ethanol for 30 sec, followed by rinsing with sterile distilled water. The samples were then cut into 0.5 cm each with the help of a sterile scissor and dried on sterile blotting paper then transferred onto plates containing MEA (Malt Extract Agar supplemented with antibiotic streptomycin 10mg/ml and incubated at 25±2^{O}C until the fungal growth was observed. The colonies were maintained in glass tubes with MEA for further use.

The endophytic fungal species were identified on the basis of morphological characteristics of fruiting bodies, spore and cultural characteristics by using the identification manuals (Ellis, 1993; Domsch *et al,* 1980; Watanabe, 2002). Isolates which do not sporulate were represented as sterile.

Fresh mycelia from seven days old culture of selected endophytic fungi isolated from medicinal host plants were inoculated in 100ml Malt Extract Broth MEB in 250 ml Erlenmeyer flasks and were incubated for 30 days at 25°C in stationary condition. The broth of each fungal endophyte was filtered and the filtrate was extracted three times with ethyl acetate at room temperature. Evaporation of the extracted solution was done in a rotary evaporator.

Table 1 : Selected medicinal plants used for the isolation of fungal endophytes

Plant species	Family
Terminalia arjuna (Roxb)Wight and Arn.	Combretaceae
Phlogacanthus thyrsiflorus Nees.	Acanthaceae
Boerhavia sp Linn.	Nyctaginaceae
Holarrhena antidysenterica Linn.	Apocynaceae
Ficus hispida Linn.	Moraceae

Extracts from endophytic fungus were screened for antibacterial activity against bacteria by disc diffusion method. Test bacterial strains were procured from IMTECH Chandigarh, India which include one gram positive bacteria and two gram negative bacteria viz., *Bacillus subtilis* (MTCC 619), *Pseudomonas aeruginosa* (MTCC 424) and *Erwinia* sp(MTCC 2760) respectively. 0.1 ml of the inoculum of the test bacteria was spread into nutrient agar plates. The paper discs immersed in the fungal extract and then were placed onto the bacterial plates in triplicate manner. The plates were incubated at 37^{O}C for 24 – 48 hrs. After the incubation, the inhibition zone was measured, recorded and expressed in millimeter.

At present medicinal plants are in the threshold of extinction and much interest is gaining in preserving this medicinal plants. In this regard fungal endophytes can play an important role as they have several applications such as providing resistance to plant against several biotic and abiotic factors, improving plant growth, producing several secondary metabolites, antibacterial activity against pathogenic bacteria. Diversity of fungal endophytes may contribute in maintaining the biodiversity of medicinal plants and fungi in Tripura. In the present study five different medicinal plants were selected for isolation of fungal endophytes from different tissues (Leaf, Bark and Root) of the plant species (Table 1).

A total of seven (7) types of endophytic fungi were isolated from five different plants of Tripura (Table 1 and 2). Sterile forms were also recovered. Occurrence of sterile mycelium as endophytes is not unusual . Of these 22 fungal isolates, it was found that *Fusarium oxysporum* was found to be present

Table 2 : Showing the Isolates of Fungal endophytes in different plants species of Tripura

	T a			*P t*			*B* sp			*Ha*			*F h*		
Names of Endophytes	L	B	R	L	B	R	L	B	R	L	B	R	L	B	R
Alternaria sp.	1	1	8	0	0	0	0	0	0	0	0	0	0	0	0
Curvularia sp.	0	0	0	0	0	0	0	0	0	2	0	0	0	3	0
Fusarium oxysporum	0	6	2	4	2	0	0	0	3	2	0	4	3	0	2
Fusarium equiseti	0	4	6	0	0	2	0	0	0	0	0	0	0	0	0
Penicillium sp.	0	2	2	0	0	0	0	0	2	0	0	0	0	0	2
Penicillium oxalicum	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0
Pestalotiopsis sp.	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Syncephalastrum monosporum	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0
Diaporthe phaseolorum	12	0	3	2	0	0	0	0	0	2	0	0	0	0	0
White sterile	0	2	0	0	0	3	0	0	0	2	0	0	0	0	0
Dark sterile	1	0	0	0	0	0	2	0	9	1	0	0	0	0	1

Ta-*Terminalia arjuna*, Pt-*Phlogacanthus thyrsiflorus*, B sp-*Boerhavia* sp, Ha- *Holarrhena antidysenterica*, Fh- *Ficus hispida ; L- leaf, B-Bark, R- Root*

in all the 5 plants whereas *Penicillium* sp and *Diaporthe phaseolorum* are present in three plants. Among all the plants the highest numbers of endophytes were obtained from *Terminalia arjuna* and the least was found in *Boerhavia* sp. It was also found that among the leaves the highest number of endophytes obtained from *Holarrhena antidysenterica*. Further higher number of endophytes recovered from leaves may be due to movement of water and food which are conducive for fungal growth. In case of bark and root the highest numbers of endophytes were obtained from *Terminalia arjuna. Fusarium oxysporum*, *Penicillium* sp, *Diaporthe phaseolorum* were found to be the dominant species. Mycelia sterilia have been often isolated as leaf endophytes from many host plants (Rajagopal *et al,* 2000). Presence of sterile mycelia in leaves of host plants was also observed in the present study. *Alternaria* sp are reported as endophytes in wide range of plant species (Rajagopal, 2004) Normally *Alternaria* and *Curvularia* occur as phylloplane fungi but they are capable of penetrating the superficial layers of leaf and grow as endophyte, suggesting that phylloplane fungi has report to an endophytic mode of life to overcome adverse environmental conditions. Fungi like *Alternaria* and *Curvularia* have been found to be well adopted for endophytic mode of life in wide varieties of plants.

Table 3 : Showing the antibacterial activity of different fungal endophytic isolates from five different medicinal plants species of Tripura

Alternaria sp.	0	0	0
Curvularia sp.	0	0	0
Pestalotiopsis sp.	8	0	0
Diaporthe phaseolorum	8.5	0	13
Fusarium oxysporum	20	2	0
Fusarium equiseti	8.8	8	9.5
Syncephalastrum monosporum	12	0	20
Penicillium oxalicum	9	Nil	Nil

All the endophytic fungal extracts, from the host plants displayed differential antibacterial activity against the test bacterial strains (Table 3) The extract of *Fusarium equiseti* showed inhibition zone against all the treated bacteria whereas *Fusarium oxysporum* showed highest activity against *Bacillus subtilis* and *Syncephalastrum* showed highest activity against *Erwinia* sp. Whereas*, Alternaria sp, Curvularia* sp showed negative response against all the bacteria. Some of the endophytes like *Pestalotiopsis* sp and *Penicillium oxalicum* showed activity against only one bacteria *Bacillus subtilis*. Some of the endophytes isolated from the present study matched with report from Biodiversity and antimicrobial activity of endophytes associated with Egyptian medicinal plants (Selim *et al,* 2011).

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