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Secondary metabolite production by Endophytic Fungi isolated from *Andrographis paniculata*

Gajalakshmi S., Iswarya V., Ashwini R.,Bhuvaneshwari M., Mythili S. and Sathiavelu A.
School of Biosciences and Technology, Vellore Institute Technology, Vellore, TamilNadu, INDIA
asathiavelu@yahoo.in

ABSTRACT
Endophytic fungi are the one that resides inside the plant tissues. In the present study, five endophytic fungal strains were isolated from the medicinal plant *Andrographis paniculata.* The plant possess pharmacological activities such as vermicidal, analgesic, antibacterial, antipyretic, anti thrombotic ,antiviral ,cardio protective, hepatoprotective, hypoglycemic etc. Inspite of all the above properties, the plant was investigated to determine the presence of the endophytic fungi inside their inner tissues such as *Aspergillus niger, Aspergillus flavus, Fusarium species, Alternaria species* and *Yeast* like fungi. Endophytic fungi have the possibility of being both beneficial as well as pathogenic to the host plant. The cultures isolated in the present study revealed the beneficial effect to their host plant by producing certain secondary metabolites. The preliminary screening of the enzyme production by the isolated strains has given a positive result. Enzyme production from the plant source has a remarkable value in the present research world as they possess wide application in many fields.

Keywords: Endophytic, Secondary metabolites, Pathogen, Andrographolide, Cardioprotective, ethnobotanical.

INTRODUCTION

Endophytic fungi that resides in the living plants are the under-explored group of microorganisms[1].It is estimated that there could be a possible of at least one million species of endophytic fungi alone in the microbial world. They have attained a considerable attention in the present day due to its capacity to protect their host against insect pests, pathogens and even against some domestic herbivores [2,3,4]. Almost all the plant species (~400,000) contains one or more endophytic organisms within it[5].But, only few plants have been extensively studied for their endophytic biodiversity and their ability to produce the major bioactive secondary metabolites. But, sometimes these fungi would act as the facultative pathogen under diversified condition. The essential role of endophytic fungi is to begin the biological degradation of the dead host-plant, which is significant for the recycling of the nutrients .Medicinal plants are reported to possess endophytes [6] that in turn provides protection to their host against the infectious agents and provides adaptability nature in order to survive in the adverse environmental conditions. Therefore, it is significant to determine the endophytic diversity of the plants of medicinal value.

Fungi provides a source of bioactive compounds [7]. Especially, endophytic fungi isolated from the medicinal plants could be used for the development of new drug [8,9]. Endophytic fungi lives inside all kinds of plants which includes trees, grasses, algae and herbaceous plants. But, they do not cause any of the symptoms or any other apparent injury to the host plant [10]. Colonization of the endophytic fungi onto the host plant contains a array of steps which involves the host recognition by the fungus followed by the spore germination and then the penetration of the epidermis and finally the tissue colonization. Most of the endophytic fungi belong to the group of ascomycetes and fungi imperfecti. Certain chemical factors only control the equilibrium to be attained between the host and the fungi [11]. There are also many research reports which demonstrates that many of the antitumour agents such as taxol [12,13] and the antimicrobial agents [14,15,16] could be produced by these endophytic fungi.

Andrographis paniculata is one of the significant herbs with several medicinal properties. *Andrographis paniculata* belongs to the family of *Acanthaceae.* Andrographolide is a bicyclic diterpenoid lactone , found to be the major constituent that has been extracted from the leaves of the plant[17]. Immunostimulating agents were also present in *Andrographis paniculata* [18]. Andrographolide is found to be a pharmacophore with the anticancer and Immunomodulatory activities [19].The ent-labdene diterpenes showed the viricidal activity against herpes simplex virus 1(HSV-1) [20]. Antidiabetic effect of the crude extract of *Andrographis paniculata* leaves were reported on the normal and alloxan induced hyperglycemic rats[21]. The cardiovascular effects of the 14-deoxy-11,12-didehydro andrographolide (AP3) of the *Andrographis paniculata* extracts was also reported [22].

Molecules that are derived from the natural products provides a novel chemical structural compounds for the development of new pharmaceutical products [23]. Plant of ethnobotanical usage with different environmental setting provides a novel endophytic microorganisms [24]. Plants with ethnobotanical history, unusual longevity and growing in the areas of great bio diversity possess endophytes with great biodiversity [7].

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Table 1: The pharmacology of the medicinal plant *Andrographis paniculata*[28]:

S.NO.	Pharmacological activity	Description
1	Abortifacient	Have the ability to abort the pregnancy
2	Vermicidal	Intestinal worms would be killed
3	Analgesic (pain killer)	Reduces the swelling and also cuts down the exudation from the capillaries
4	Antibacterial	Fights against bacteria
5	Antiperiodic	Counteracts the periodic or the intermittent diseases like malaria
6	Antipyretic	Reduce the fever
7	Antithrombotic	Prevents the blood clot formation
8	Antiviral	Fights against viruses
9	Cancerolytic	Fights and even kills the cancer cells
10	Cardioprotective	Protects the heart muscles
11	Choleretic	Tends to alter the properties and flow of bile)
12	Depurative	Involves the cleaning and purification of the system, especially the blood
13	Digestive	Promotes the process of digestion
14	Expectorant	Mucus discharge is promoted from the respiratory system
15	Hepatoprotective	Protection of the liver and gall bladder
16	Hypoglycemic	Reduces the blood sugar level
17	Immune Enhancement	White cell phagocytosis is increased up , inhibition of the HIV-1 replication and improvement of the CD4 + and T lymphocyte counts
18	Laxative	Bowel elimination is promoted
19	Sedative	Relaxing effect
20	Thrombolytic	Bursts the blood clot

Secondary metabolites produced by the plant during their growth period are of great interest in the day to day life in the field of medicine, pharmaceuticals, cosmetics, food etc. The products might be the antimicrobial compounds, or the anticancer derivatives or simply the enzymes with wider applications. Considering this in mind, the fungal isolates were screened for the enzyme production in the present study.

MATERIALS AND METHODS

SAMPLE COLLECTION

For the isolation of endophytic fungi, healthy plants of *Andrographis paniculata* were collected from Vellore district. All the samples were collected in sterile plastic bags and transported aseptically to the laboratory.

SAMPLE PRETREATMENT

Fresh *Andrographis paniculata* plant parts were washed in running tap water and then washed in 70% ethanol for about 2 minutes and finally in 2% sodium hypochlorite containing 0.1% Tween 20 for a period of about 10 seconds. They were then washed with distilled water for about 2 minutes and then again in sterile distilled water for five times [25].

ISOLATION OF ENDOPHYTIC FUNGI

After drying, each of the plant parts was divided into three segments and was placed on potato dextrose agar (PDA) plates supplemented with chloramphenicol to suppress the bacterial growth. All the plates were then incubated at 27˚C for upto 3 weeks. Isolated fungi were then transferred to fresh PDA plates and was again incubated for 1 week and periodically checked for purity[26].

IDENTIFICATION OF ENDOPHYTIC FUNGI

The fresh slant cultures were subjected to the lactophenol cotton blue staining and were observed under the microscope. The fungal strains were identified based on the external morphology, spore formation and the hyphal structures.

PRELIMINARY SCREENING OF ENDOPHYTIC FUNGI FOR THE ENZYME PRPODUCTION

The plate assay was done to identify the enzyme production by the fungal strains. A fresh seven days culture of the fungal isolates was taken without any contamination. All the five strains were single streaked onto the Potato dextrose agar plates substituted with casein, starch, tributyrin oil as a substrate. The plates were incubated at 20˚c for about seven days and were

checked for the zone. Casein acts as a substrate for protease production, starch for amylase and tributyrin for lipase production. Thus, if the fungal strains produced the tested enzymes as a secondary metabolite, then the organism would exhibit a zone around its growth, which means that the enzymes produced by the organisms during the growth cycle has utilized the substrate. Based on this, the five fungal isolate were checked for the enzyme production at the preliminary level. Only lipase production would be visible directly in the incubated plate where as the amylase production could be seen after the addition of iodine solution for the starch plate and for protease production, 10% solution of mercuric chloride dissolved in 20% Hydro chloride solution should be flooded over the incubated casein plate.

RESULTS AND DISCUSSION

Plants were the ultimate source of the medicinal agents that are helpful in the formation of the drugs [27]. Though synthetic chemicals have been used as active agents in reducing the incidence of plant, animal and human diseases, they are costly, potentially harmful effect on the environment and may induce pathogen resistance. Thus, biological control or the use of microorganisms or their secretions to prevent diseases offers an attractive alternative or supplement to disease management without the negative impact of chemical control.

In natural product discovery programs, typical procedures included isolating microorganisms from samples, growing them at various temperatures in a variety of selective or nonselective media and testing the extracts in a spectrum of targeted activity for potential industrial or pharmaceutical applications. For a successful fungal screening, a varied and novel repertoire of either well-known or unexplored fungi is desirable. The most promising trend in isolating new fungi is the move towards investigation of novel endophytes, with the idea that unusual endophytes may produce untapped natural products [26].

In the present study, five endophytic fungi were isolated from various parts of the *Andrographis paniculata*. Two isolates from the root part of the plant were identified to be *Aspergillus niger* and *Aspergillus flavus*,other two isolates from the leaves were identified as *Alternaria* and *yeast* like fungal species. *Fusarium* species was isolated from the inner tissue of the stem of the plant.

The preliminary screening for the enzyme production has yielded the positive result. *Aspergillus niger* , *Aspergillus flavus* and *Alternaria* showed the clear zone for all the substrates and hence proves that it possess protease, lipase and amylase activity. *Fusarium* species showed positive only for amylase and lipase whereas the *yeast* like fungi showed the production of amylase alone in an increased level.

Enzymes that are isolated from the plant sources in terms of the endophytic organisms that resides within the plant is the new trend for the efficient researches to be carried out.

CONCLUSION

In the present study, an effort has been made to isolate the endophytic fungal strains from the medicinal plant *Andrographis paniculata.* The plant possesses many of the medicinal properties and it has been used from the traditional period. Secondary metabolite production of the microbes has attained a great interest in the present world . This study evidenced that *Andrographis paniculata* has potential endophytic fungi which showed promising secondary metabolite production. Detailed investigations on *Andrographis paniculata* plant endophytic fungi were needed to prove its potential further which will lead to the discovery of numerous high value metabolites.

Enzyme production from the microbial strains has attained a great interest due to possibility of large scale production with minimum expenses and easy maintenance that ultimately yields higher economic output. In the present research world, enzymes have attained a wide area of application in various fields.

Table 2:List of endophytic fungi isolated, site of isolation, and its designation.

S.No	IDENTIFIED ISOLATES	SITE OF ISOLATION	DESIGNATION
1	*Aspergillus niger*	Root	R_1
2	*Aspergillus flavus*	Root	R_2
3	*Fusarium*	Stem	S
4	*Alternaria*	Leaf	L_1
5	*yeast* like fungal species	Leaf	L_2

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Table 3: culture plate and microscopic view of the fungal isolates

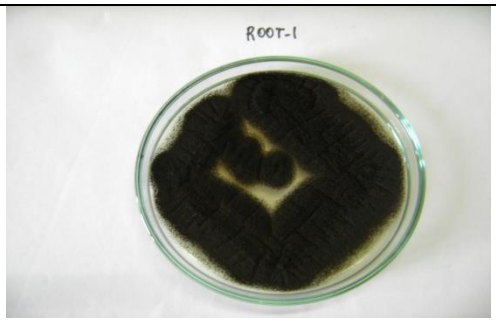
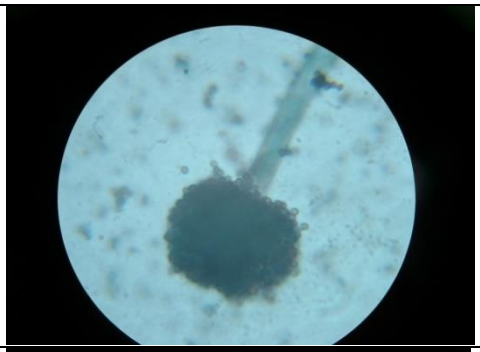
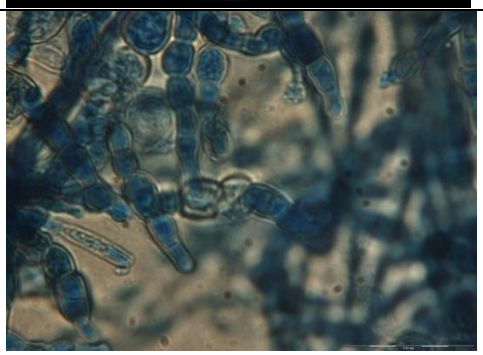
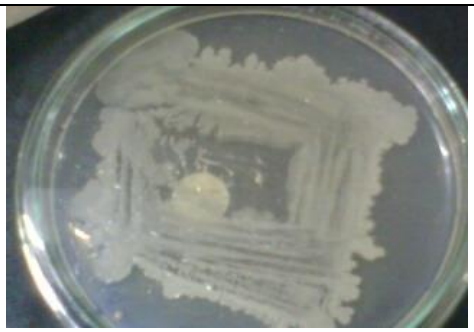
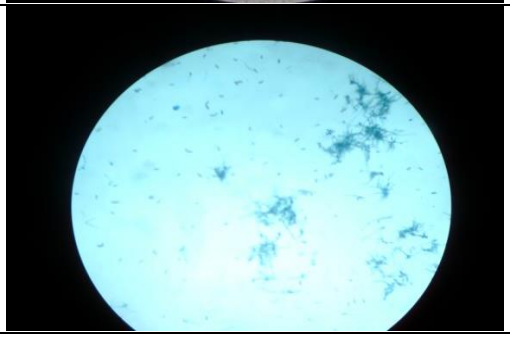
ISOLATE	PLATE VIEW	MICROSCOPE VIEW
R_1		
R_2		
S		
L_1		
L_2		

Table 4: Enzyme production by the fungal isolates.

FUNGAL ISOLATES	STARCH-AMYLASE ACTIVITY	LIPID-LIPASE ACTIVITY	CASEIN-PROTEASE ACTIVITY
R_1	+	+	+++
R_2	+	+	+
S	+	+	_
L_1	+	+	+
L_2	+++	_	_

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