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ISOLATION OF ENDOPHYTIC FUNGI FROM *AGELE MARMELOS* LEAVES AND ITS CHARACTERIZATION

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ABSTRACT

Endophytic fungi have symbiotic association with the host plant and known to produce various bioactive compound similar to the host plant. Enzymes are one of group of bioactive compound produced. These enzymes help in the symbiotic association of endophytes. Among the two fungi isolated one was showing fair cellulolytic, amylolytic and lipolytic activity.

KEYWORDS: *Agele marmelos,* Endophytic fungi, cellulose, Amylase, Lypholytic.

INTRODUCTION

Endophytes are group of microbes which form colonies in living internal tissues of plants. Usually this endophytes will not cause any harmful effect on plants (Bacon, 2000). These endophytes are found in almost all vascular plants (Zhang, 2006). Distibution of endophyte in plants varies according to the type. Enophytes were known to secrete bioactive secondary metabolites which play a vital role in protecting the host plants (Azevedo, 2000; Carroll, 1978, Strobel, 2003).

Endophytes includes a variety of bacteria, fungi and actinomycetes which form association with wild (Brooks, 1994), cultivated crops (Liu, 1996) which can be either monocot (Fisher, 1992) or dicotyledon (El-Shanshoury, 1996). Among the varies microbial groups of entophytes, the frenquently identified group are fungi. Around one million species of endophytic fungi alone has been estimated by Dreyfuss & Chapela (Dreyfuss, 1994). The endophytic fungi are known to have physiological (Malinowski, 2006) and ecological (Tintjer, 2006; Malinowski, 2004) roles in the life of their host plants. Even though the endophyes are omnipresent their diversity, host range and geographical distributions are yet to be explored (Arnold, 2007). It is note that plant infected with endophytes are more healther than uninfected counter parts (Waller, 2005). Endophytes are ubiquity so as they occupy different biological niches of various kind of environment. So they can be considered as potent source of useful bioactive products. Endophytic fungi forming symbiotic association with medicinal plants are capable of producing pharmaceutically important products .This implies the importance of carrying out studies on endophytic fungi found in medicinal plants.

Endophytic fungi, as apt source for extraction of medically important metabolites has been gaining increasing interest (Knight, 2003). Even then endophytes remain as least explored group of microbes. There is a lack of information about endophytic diversity in this region. In the present study, an investigation is carried out to understand the endophytic fungi present in *Aegle marmelos*, an important medicinal plant, and to explore its exoenzyme producing potential.

MATERIALS AND METHODS

Collection of plant material: Endophytic fungi were isolated from *Aegle marmelos* by modified method described by Hallman, 2007. The samples were rinsed gently in running water to remove dust and debris. After proper washing, leaves were cut into 3-4 mm x 0.5-1 cm pieces under aseptic conditions. Surface sterilization was doneby 0.5% Mercuric chloride ($HgCl_2$). Then the plant material was treated with 75% ethanol for 1 min followed by immersion in Mercuric chloride and again in 75% ethanol for 30 s (Bills, 1996). They were finally rinsed with deionized sterile distilled water to remove the sterilants and blot dried on sterile tissue paper, sterilized leaf explants were cultured in petri dishes containing potato dextrose agar medium (PDA) supplemented with 100 µg/mL of streptomycin. The petri dishes were sealed with parafilm and incubated at 27±2°C for 15 days under dark conditions and monitored every day. Fungi growing out of the plant explants were subcultured on separate PDA plates at room temperature

Colony Characteristics: Isolates were incubated for 7 days at 27°C. The experimental design was completely randomized with 3 replicates. Colonies were analyzed with respect to their borders, the coloration of the mycelium, the coloration of the reverse of the petri dish also and the coloration of the medium

Enzyme Assay: The production of enzyme by fungal endopytes was qualitatively determined by using Hankin andAnagnostakis method

Amylase enzyme activity: Amylase enzyme activity wasassessed by growing the fungi on glucose yeast extractpeptone (GYP) agar medium (glucose-1g, yeast extract0.1g, peptone 0.5g, agar 16 g, distilled water 1000mL andpH 6) containing 1% soluble starch. After 5 days incubation, the plates with fungal colony were flooded with1% iodine in 2% potassium iodide. The appearance of clear zone surrounding the colony was considered positive for amylase enzyme.

Cellulase enzyme activitys: For cellulase, the fungi were culturedon yeast extract peptone agar medium (yeast extract, 0.1g; peptone 0 .5 g; agar,16 g and distilled water, 1000 mL) supplemented with 0.5% Na-carboxymethyl cellulose (CMC). After incubation, the plates were flooded with 0.2 aqueousCongo Red and destained with 1M NaCl for 15 minutes. The clear zone surrounding the colony indicated the cellulase activity.

Lipolytic enzymes activity: Lipolytic enzymes activity was performed by growing fungi on GYP medium supplement with 1% of Tween 20 after 5 days of incubation the production of lipolytic enzymes by the test strain i was measured by estimation of degradative capacity of Tween- 20 substrate of this enzyme. Lipolytic activity was confirmed by appearance of a transport zone around the radial colony.

RESULTS

Entophytes are noted for their ability to protect the host plant against pathogen and pest. Moreover endophytes produce beneficial natural compounds such as Taxol which is having antifungal and anticancer activity. In this context an attempt was done to isolate endophytic fungi from *A.marmelos* leaves and to carry out a morphological characterization and study on exoenzyme production of the same. In this study two endophytic fungi were isolated from the leaves of *A. marmelos* plant and their external morphology was viewed. Two enophytic fungi isolated from the *A. marmelos* leaves was shown in (Fig1).

Figure.1.PDA plate with endophytic fungi isolate from *A. marmelos* leaves

Figure.2.Pure culture of the isolate 2

The Pure colony of isolate 2 was shown in the fig 2 .The colony was showing a characteristic white cottony structure in the edges and orange slimy structure in the middle with a black edge. There was no visible exudates from the fungi and the colour of the media remained same. Among the two isolates, isolate 2 had good enzyme activity. This isolate showed positive for amylase, protease and lipolytic activity when compared with other isolate. In the Fig 3a, showing a discoloration around the fungal colony indicating the degradation of starch by the fungal amylase enzyme in Fig 3b represents the production of protease enzyme by endopytic fungi. The clear zone around the fungal colony after staining with congo red indicates degradation of CMC due to cellulose activity And Fig 3c indcate production of lipolytic enzyme by the isolated endophytic fungi.

Figure.3a.Amylase activity; 3b.Cellulase Activity; 3c.Lipolytic Activity

DISCUSSION

Like the medicinal plant endophytic fungi are known to produce various secondary metabolites which are responsible for number of bioactivity. Enzymes are important metabolites produced by endophytic fungi. These enzymes help in evade and colonization of fungi in the plant. The fungal enzymes are having application in medicine, industry and agriculture, moreover they are more stable in terms of high temperature and extreme pH when compared with plant and animal countrer part. Fungal enzymes are having application in industries such as food, beverage, textile and leather industries for processing of raw materials. Extracellular enzyme produced by foliar endophytic fungi of *Rhizophora apiculata* exhibited litter degradation. Fungi have proven themselves invaluable sources of natural products for agriculture as well as biomedical development for over a half century.

While much of the interest in endophyte bioactive compound is for medicinal use, compounds that may have industrial or agricultural applications are also gaining attention. In particular, amylase is an important enzyme that is used in numerous applications in a variety of industries and there is growing interest in amylases with a wider spectrum of biological properties that can function at diverse pH and temperature ranges. Proteases are used in clinical applications especially in the treatments like diabetes. It is known from the history tha textracts of Tulsi plant being used for the diabetic control. Proteases are one of the enzymes involved in controlling diabetes as reported by Wiest-Ladenburger *et al* whose administration delays Insulin-dependent diabetes mellitus (IDDM) onset in an animal model for autoimmune diabetes, in the non-obese diabetic mice.

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