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ENUMERATION OF ENDOPHYTIC FUNGI FROM *EICHHORNIA CRASSIPES* ROOT AND PRELIMINARY SCREENING FOR TANNASE ENZYME PRODUCTION

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Abstract – Endophytic fungi were isolated from root tissue of *Eichhornia crassipes* a true hydrophytic plant. Three hundred root segments were sampled. Six different endophytic fungal species could be isolated. The endophytic fungi were belonging to Ascomycete, Basidiomycete and Hyphomycete viz *Talaromyces aurantianum*, *Talaromyces minioluteus*, *Trametes versicolor*, *Trametes* sp, *Penicillium samsonii* and *Penicillium minioluteum*. The endophytes were identified using ITS primers. *Talaromyces aurantiacum, Trametes* sp and *Penicillium minioluteum* showed higher colonization frequency. All the 6 endophytic fungi were tested for production of extracellular enzyme tannase. *Talaromyces aurantianum*, *Trametes versicolor*, *Trametes* sp, and *Penicillium minioluteum* produced tannase in plate assay.

INTRODUCTION

In plant microbe interactions endophyte – host interaction was the least studied interactions. Endophytic fungi inhabit almost all varieties of plants and have been isolated from different parts of plants such as roots, stems, leaves, barks, floral organs and seeds. Plant roots are occupied by a range of microorganisms, including mycorrhizal, epiphytic and endophytic fungi. Tan *et al*., (2012) studied the endophytic fungi distribution in the roots of Holcoglossum plants belongs to (Orchidaceae). Cultivable endophytic fungi have been isolated from twelve plant species roots of Coastal Plants in South Korean East Coast (Hyun Kim *et al*., 2014). Bayman Paul *et al*., (1997) studied the endophytic fungi in roots and leaves of *Lepanthes* sp. Almeida *et al*., (2015) reported the endophytic fungal community of *Eichhornia azurea* and *Eichhornia crassipes* and their interaction with a host. Gan *et al*., (2017) explored root endophytic fungi and their interactions with insects. Jose *et al*., (2018) studied the root endophytic fungi for natural products discovery. However, endophytic fungi from true hydrophytic plants are very restricted yet little study was carried out on the enumeration endophytic fungi in root of aquatic plants (Li *et al*., 2010). Wang *et al*., (2016) considered endophytic fungi as microbial factories for the production of various bioactive metabolites and its sustainable applications in biotechnology. Rajagopal (1999); Suryanarayanan and Rajagopal, (1998) studied the endophytic fungi distribution in *Azadirachta indica* and the extracellular enzyme productions like laccase, tyrosinase, pectinase, cellulase etc. *Eichhornia crassipes* (water hyacinth), a hydrophyte is free floating aquatic plant that able to adapt to polluted environment and potential to clean up waste waters. The roots of *Eichhornia crassipes* naturally absorb pollutants, like lead, mercury, and strontium-90, as well as some organic compounds believed to be carcinogenic in the surrounding water. Water hyacinth can be cultivated for waste water treatment and it is efficient to remove about

60–80 % nitrogen, 69% of potassium and was found to remove particulate matter and nitrogen in a natural shallow eutrophicated wetland (Fox *et al*., 2008; Wenbing Zhou *et al*., 2007; Billore *et al*., 1998). Hence, in the current study *Eichhornia crassipes* root was screened for the endophytic fungi distribution and for tannase enzyme production.

MATERIALS AND METHODS

The *Eichhornia crassipes* was collected from a lake near Chennai in Tamilnadu. The plant species was a year old; healthy and disease free plants were collected after physical examination. The root tissues were washed in tap water. About 300 root segments (0.5 cm^2) were surface sterilized using 70% ethanol for 2 min and sodium hypochlorite 4% for 1 min. The surface sterilized root segments were inoculated in Petri plates. About 4 to 6 segments of root segments were placed on Potato Dextrose Agar (PDA) supplemented with Chloramphenicol (50 µg/mL), to arrest the growth of the bacteria. The inoculated petri plates were incubated at 27 °C ± 1°C for 3 weeks. The endophytic fungi grew out from root segments were isolated and sub cultured, periodically on PDA medium. The endophytic fungi which emerge from the root tissue were identified on the basis of conidial/fruit body structure (Ellis and Griffith, 1980). The endophytic fungi were also subjected to molecular level of identification by ITS primer sequencing method (Crous *et al*., 2004; Meenambiga, 2017). The Colonization Frequency (CF) was calculated as the number of root segments colonized by a single endophytic fungi divided by the total number of segments observed x 100 (Wipornpan Photita *et al*., 2001). The enzyme tannase was detected using the Tannic Acid Agar Media (TAA). The extra cellular tannase enzyme production was tested in Petri plates (90 mm diameter) with mycelial discs (5 mm diameter) taken from the growing edge of the endophytic fungi colony on PDA media. The petri plates were incubated at 25 °C ± 1°C for the production of tannase enzyme. The production enzyme was determined semi quantitatively by addition of substrates to the media. Addition of tannic acid to the medium produce clear zone around the colony indicates the tannase enzyme production. The appearance of clear zones was recorded in arbitrary units. Composition of Tannic Acid Agar Media: (Tannic Acid 4.0, Ammonium Chloride 3.0, Dipotassium Phosphate 0.5, Magnesium Sulphate 0.5, Glucose 0.1, Lyophilized bahera tannin, 1.0, Agar 30.0 in Gm/Litre and pH 5.0).

RESULTS

Endophyte researchers accept the fact that only handful plants and particularly true hydrophytes have been screened for their endophytic mycobiota. Hence, in the current study the root tissue of *Eichhornia crassipes* was screened for endophytic fungi. A total of 63 endophytic fungal isolates were obtained from 300 segments of the root tissue. Of these, each two belong to ascomycete, basidiomycete and hyphomycete (Table 1). Among two ascomycete *Talaromyces aurantianum* showed more CF than other ascomycete. Similarly, *Trametes* sp of basidiomycete and *Penicillium minioluteum* of hyphomycete showed more CF in their respective groups (Table 1 & Fig. 1). Out of the six endophytic fungi *Talaromyces aurantianum* with high CF% of (15.9), whereas it was least for *Penicillium samsonii* (6.0) (Fig 1). Endophytic fungi have the capacity to produce several enzymes in culture, including cell wall degrading and industrially important enzymes. Several endophytic fungi have been shown to produce cell wall degrading enzymes (Petrini *et al*., 1992). In the current study all the six root endophytic fungi were screened for the production of tannase enzyme. It was found that 4

Table 1. List of endophytic fungi isolated from *Eichhornia crassipes* root tissue

Name of the Endophytic fungi
Ascomycetes
Talaromyces aurantianum
Talaromyces minioluteus
Basidiomycetes
Trametes versicolor
Trametes sp
Hyphomycetes
Penicillium samsonii
Penicillium minioluteum

Table 2. Tannase enzyme production by endophytic fungi

Name of the Endophytic fungi	Tannase enzyme production
Talaromyces aurantianum	+
Talaromyces minioluteus	-
Trametes versicolor	+
Trametes sp	+
Penicillium samsonii	-
Penicillium minioluteum	+

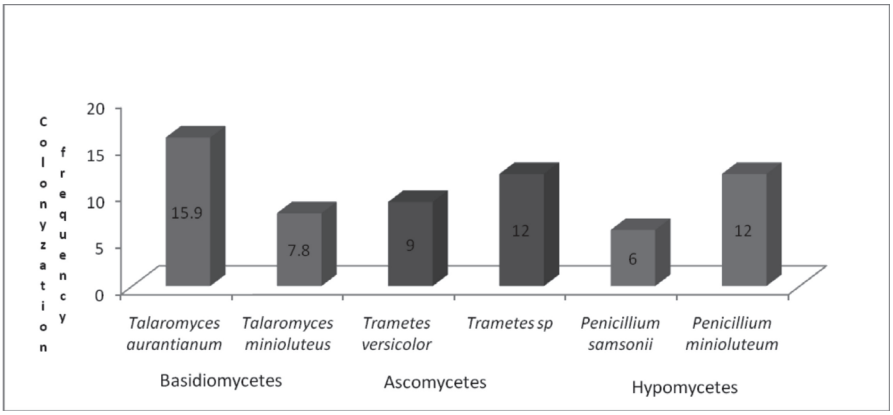

Fig. 1. Endophytic fungi isolated from *Eichhornia crassipes* root tissue and their Colonization frequency

endophytic fungi produced tannase in plate assay. Two endophytic fungi *Talaromyces minioluteus* and *Penicillium samsonii* have not produced tannase enzyme in plate assay (Table 2).

DISCUSSION

Every plant species are colonized internally by different microbial communities comprising archaea, protista, bacteria and fungi (Eyob Chukalo Chutulo and Raju Krishna Chalannavar, 2018). Endophytic fungi from different plants have been reported but very few true hydrophytes have been studied for endophytic fungi distribution particularly the root tissues. This paucity of research on root endophytes driven us to explore the root tissue of *Eichhornia crassipes*, a hydrophyte for the distribution of endophytic fungi and tannase enzyme production. The investigation showed that the root tissue colonized by the endophytic fungi. In the present investigation *Eichhornia crassipes* root harbored a total of 6 endophytic fungi of which two each belong Ascomycete, Basidiomycete and Hyphomycete. Ascomycete and Basidiomycete were the dominant group followed by Hyphomycete. Coelomycete and sterile forms were absent (Fig. 1). In previous studies on endophytic fungal research Hyphomycetes show more colonization frequency than other groups but in current study this group was equally distributed as other groups. Generally Basidiomycetes were either absent or typically isolated in especially low numbers as endophytic fungi (Petrini, 1986) however in this study two endophyte belong to basidiomycetes namely *Trametes versicolor* and *Trametes* sp were isolated and their CF were also higher (Fig. 1). Ranga *et al.,* (2016); Almeida *et al.,* (2015) reported only limited number of endophytic fungi from the leaves of *E. crassipes* and they mainly belong to Hyphomycete, Ascomycete and Coelomycete. It indicates that the distribution and diversity of endophytic fungi vary within the plant tissues. In other endophytic fungal diversity study one or few sterile forms appear but in this study it was absent. The restricted number of endophyte in the root tissue of *E. crassipes* could be due to the anatomy, host chemistry and habitat of the host. Of the 6 endophytic fungi isolated *Talaromyces aurantiacum, Trametes* sp and *Penicillium minioluteum* showed higher colonization frequency (Fig. 1). Even though six different endophytic fungal species were isolated, only 1 endophytic fungi *Talaromyces aurantiacum* showed noticeable colonization frequency above 5% (Fig. 1). *Trametes* sp and *Penicillium minioluteum* showed colonization frequency of 4% (Fig. 1). Petrini, (1986) and Kalyanaraman Rajagopal *et al*., (2018) stated that only one or a few endophytic fungi dominate single host species. Therefore, the variation in endophytic fungi colonization frequency indicated that competition among certain endophytic fungi.

Endophytic fungi have to invade the host tissue to lead an endophytic mode of life thus; endophytic fungi must have the capacity to generate cell wall degrading enzymes like cellulase and pectinases enzymes. Many of the endophytic fungi isolated from different hosts produced extracellular enzymes like laccase, pectinase, protease, amylase, cellulase and chitinase, etc (Petrini *et al*., 1992; Kumaresan and Suryanarayanan, 2002; Rajagopal, 1999). In the current study out of 6 endophytic fungi only 4 produced tannase enzyme in plate assay (Table 2). Sun *et al*., (2011) stated that endophytic fungi have to necessarily produce a variety of

enzymes to overcome the hostile environment, host barriers of plant to enter and counter the host defense mechanism. Numerous investigation on endophytic research have shown that endophytic fungi are the good source of enzymes and it was proved in this study also, four endophytic fungi namely *Talaromyces aurantianum*, *Trametes versicolor*, *Trametes* sp and *Penicillium minioluteum* from root of *Eichhornia crassipes* produced tannase (Table 2). Further, it indicates that this plant is widely used to treat water pollution probably the endophytic fungi help the host by producing the tannase enzyme that could degrade the dye in the polluted site. To end, the root of *Eichhornia crassipes* a hydrophytic plant harbor endophytic fungi and it was shown that they produce tannase enzyme one of the industrially important enzymes.

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