Innovations

Diversity of Endophytic Fungi Isolated from *Pergulariadaemia* Pod

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Abstract : *Endophytic fungi is the richest source of many bioactive metabolites. It helps the host plant to improve the nutritional status, pest and disease resistance and physical stress tolerance. The aim of the present study was to separate fungal endophytes from the medicinal plant Pergularia daemiapod and isolate their metabolites. Totally 10 endophytic fungi were isolated and identified as;Alternaria alternate, Aspergillus sps, Mycospharella, Phomopsis, Cladosporium, Curvularia tuberculata, Fusarium graminaeram, Scytalidum acidophilum, Coelomycetes, Colletotrichum acutatum, Byssochlamys, Phanerochaeta chrysosporium. The highest frequency noted inColletotrichum acutatum(17.1%), the significant changes occur in the colony frequency Alternaria alternate (11%). The colonization frequency is high and it indicates that the diversity of fungal endophytes present in the Pergularia daemia pod. Thus the resultantpod extract possess the secondary metabolites such as alkaloids, steroids, saponins, taninsand flavonoids. These metabolites shows the anti-inflammatory, anti-viralproperties which are useful in pharmaceutical industry.*

Key words: *Colletotrichum acutatum,colonization frequency Fusarium graminaeram, Pergularia daemia, Pod extract and Phanerochaeta chrysosporium.*

Introduction

Endophytic fungi is the living fungi colonizing the internal tissues of the host plant, without causing any damage. Endophytic fungi are universally found in most of the medicinal plants in various parts such as roots, stems, leaves, flowers, fruits and seeds and also interact with their host plants Gouda *et al*. (2016). Most of the fungal endophytes are rich in novel metabolites and considered as beneficial role in their hosts in various ways Rudgers *et*

al.(2010).Some of the endophytic fungus enhance the host resistance against phytopathogens, insects and other biotic and abiotic stressesRodriguez *et al.* (2012). Endophytes secrete the valuable and active metabolites in the host after a long period and give the positive influences to the hostKumar and Kaushik (2013). Due to gene transfer, it produce a secondary metabolite and the biosynthetic pathway is similar to the host Soliman*et al.,* 2013.Subbulakshmi *et al.* (2012) Some of the endophytic fungi associated with their host plants provide all the support to the metabolic pathways and induce the metabolites with higher therapeutic potential in pharmaceutical industries. Endophytic fungi is a bioresource.It has the important novel bioactive metabolites,since a pool of metabolites isolated from the endophytic fungi are reported as potential agricultural, pharmaceutical including antimicrobial, anticancerous and anti-inflammatory and more such bioactivitiesKharwar*et al*. (2011).Maheshwari (2016) suggested that 5% of the fungi have been identified as new bioactive natural products. Nearly around 10,000 species are known as a weed plant and easily grows in the terrestrial land. However, only few studies focus on the isolation of endophytic fungi. In the present study we have selected *Pergulariadaemia* pod to investigate the preliminary phytochemical screening and GC-MS analysis of pod extract and fungal extracts. Finally, we have separated the active metabolites from *Colletotrichum acutatum*, *Fusarium graminearam*and *Phanerochaeta chrysosporium.*

Material and methods

Collection of samples

Pod samples of *Pergulariadaemia*podswere collected from Ayya Nadar Janaki Ammal College campus, Sivakasi, Tamil Nadu and India. Pods were cleaned in running tap water and the thorns were removed. The pods were cut into small pieces, labelled and placed separately in polythene bags after the removal of excess moisture. They were transferred to the laboratory and kept in a refrigerator at 4°C.

Isolation of endophytes

The pod samples were washed with running tap water and used to isolate the endophytic fungi by following the Devararajan *et al* (2002) protocol for entire isolation. All the pods were washed twice in dis.H_2O and then submerged into 70% ethanol for 1min and 4 min in Sodium hypochlorite and 30% ethanol for 30sec and then further washed three times in sterilized distilled water for 1min each time. Pod segments (5mm disc) were transferred to a petriplate containing potato dextrose agar medium with 50 μg/mL of streptomycin to suppress bacterial growth. After inoculation the petriplate were carefully incubated at 30°Cin dark period. The incubated petriplates were monitored everyday up to 30days.The fungal mycelial mats were transferred to fresh PDA petriplate and stored for future use.

Morphological characterization and identification

The isolated fungal endophytes were observed and identified for their morphological characters by using the protocol framed by Photita *et al*., (2004). Further, identification of fungal isolates was based on the standard taxonomic key including colony diameter, texture, colour, morphology of hyphae and conidia (Hyde *et al*., 2000).

Colonization frequency (CF %)

Single endophytic fungal species were calculated by a standard method colonization frequency (CF %) using the following formula (Suryanarayanan *et al*., 2003).

$$CF\ (\%) = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segments observed}} \times 100$$

Phytochemical Screening

The pod extracts of *Pergularia daemia*were screened to identify the main metabolites such as the confirmatory qualitative phytochemical screening of plant extracts was performed to identify the alkaloids, steroids, saponins, tanins and flavonoid followed by the method of Lawal *et al*. (2019).

Result

Phytochemical Screening

The phytochemicals were screened in the Pod extract of *Pergularia daemia*to confirm the presence of Steroids, alkaloids, flavonoids and tannins. The phytochemical constituents were tabulated (Table 1)

Test	***Pergularia daemia Pod***
Alkaloids	+
Flavonoids	+
Steroids	+
Saponins	+
Tannins	+

Fungal endophytes

Endophytic isolates were identified under light microscope by their sporulation structures on PDA growth medium such as*Alternaria alternate, Aspergillus sps, Mycospharella, Phomopsis, Cladosporium, Curvularia tuberculata, Fusarium graminaeram, Scytalidum acidophilum, Coelomycetes, Colletotrichum acutatum, Byssochlamys, Phanerochaeta chrysosporium* (Table 2).

Sl.No	Endophytic fungi from *Pergularia daemia* (pod)
1.	*Alternaria alternate*
2.	*Aspergillus sps*
3.	*Mycospharella*
4.	*Phomopsis*
5.	*Cladosporium*
6.	*Curvularia tuberculata*
7.	*Fusarium graminaeram*
8.	*Scytalidum acidophilum*
9.	*Colletotrichum acutatum*
10.	*Byssochlamys*
11.	*Phanerochaeta chrysosporium*

Colonization frequency (CF %)

The fungal species from *Pergularia daemia* pod tissue (Table 3).The Dominant fungal colonies are *Colletotrichum acutatum*(17.1%),*Phanerochaeta chrysosporium* (14.3%),*Alternaria alternate* (11.4%), *Aspergillus sps* (8.6%),*Phomopsis*(7.8%),*Fusarium graminaeram* (5.7%), *Scytalidum acidophilum* (2.8%) among these fungal colonies *Byssochlamys* (2.5%) has the low colony frequencies.

Sl.No	Endophytic fungi from *Pergularia daemia* (pod)	Colonization frequency (%)
1.	*Alternaria alternate*	11.4
2.	*Aspergillus sps*	8.6
3.	*Mycospharella*	5.5
4.	*Phomopsis*	7.8
5.	*Cladosporium*	5.7
6.	*Curvularia tuberculata*	2.8
7.	*Fusarium graminaeram*	5.7
8.	*Scytalidum acidophilum*	2.8
9.	*Colletotrichum acutatum*	17.1
10.	*Byssochlamys*	2.5
11.	*Phanerochaeta chrysosporium*	14.3
Total CF%		**7.65**

Discussion

In our present study, some of the fungal species were isolated in different plant parts. Likewise some of the new fungal endophytes were isolated from the *Pergularia* pod such as *Byssochlamys, Phanerochaeta chrysosporium,*

Mycospharella and *Scytalidum acidophilium.* The same results were obtained by Hawar S.N.(2022) to isolate different fungal strains from the leaves of the medicinal plant *Ziziphus spina*, including *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Cladosporium sp., Rhizopus sp., and Mucor sp*. Traditional cultivation and isolation of fungal-derived natural products is indeed time consuming, compound availability is very low and the structural complexity can be very high, amongst other disadvantages that make it unattractive for pharmaceutical industries, even if the starting biological material has great value. The rise of natural products research will not depend on funding, but in understanding the biology of microorganisms, which can increase the rate of isolated new molecules derived from microorganisms, for which a multidisciplinary approach is needed. Cruz *et al.* (2020) isolated the endophytes from *Rubiaceae* species. Patchett *et al.* (2021) isolated fungal endophytes, fungal metabolites and also studied the effect of the metabolome. Igiehon *et al.* (2021) The potential of other areas not commonly explored in this area should also be investigated, such as searching bioactive proteins from microorganisms. Currently, endophytic fungi are viewed as an outstanding source of bioactive natural products because there are so many of them occupying literally millions of unique biological niches growing in so many unusual environments. Kouipou and Boyom (2019) diversify the endophytes from the leaves of Terminalia. Nowadays the field is focusing in lead finding cytotoxic or antimicrobial new natural products. Though, if a new natural product does not have these biological properties, it must be seen not as a dead lead, but as the starting point for the remaining immense biological assays available. The future of the natural products research will be considered again economically valuable when the pharmaceutical industry regains the interest on the field. Fast, low cost, and working on biological samples with high probabilities of finding valuable natural products are in need.The same results were noted in Cruz *et al.*, (2020) isolated the endophytes form Rubiaceae species. Sana *et al.* (2019) isolated the fungal isolate *Aspergillus nidulans* from *Nyctanthes arbor-tristis,* which was used as the antibiotics of Cancer. Novel antibiotics, antimycotics, immunosuppressants, and anticancer compounds are only a few examples of compounds produced by endophytes. Mao*et al.* (2021) produced the exopolysaccharide and carried out the characterization and analysed the antioxidant activity of endophytic fungus *Aspergillus* sp from *Eucalyptus exserta*. A wide range of pharmaceutically significant compounds belonging to all structural classes were found to be produced by fungi (Abdou *et al*., 2020).

Rustamova *et al.*(2020)estimated the properties of novel metabolites and nematicidal activity of beauvericin which is produced by the endophyte fungi *Fusarium bulbicola*. Recently, endophytic fungi have received an increased attention because they can produce similar or same compounds as their host plant. Therefore, it can be used as potential source of novel natural products for food, industrial, medicinal and agricultural industries. Jin *et al.* (2021) believe the

reason, some endophytes produce certain phytochemicals, originally characteristic of the host, might be related to a genetic recombination of the endophyte with the host that occurred in evolutionary time. Recently, Dhakshinamoorthy *et al.* (2021) proved that the Plant-microbe interactions implicated in the production of camptotheci and anticancerous activity of fungal metabolites and isolated the fungal endophytes form *Phyllosticta elongata* MH458897 a novel endophytic strain isolated from medicinal plant of Western Ghats of India

Conclusion

The fungal endophytes is the one of the bioresource because most of the fungal endophytes are having the active metabolites. These metabolites express the various activities such as anti-inflammatory, antioxidant, antimicrobial, antimalarial, antiviral and anticancerous activity. In our present work,we have concentratedonly the isolation of endophytic fungi. In future studies, our team have planned to isolate the novel metabolites and proceed further.

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