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RESEARCH ARTICLES

Distribution of endophytic fungi associated with *Meriandra bengalensis* Benth. and assessment of their bioactive potential in vitro

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Abstract
Fungal endophytes are known to be diverse and establish a mutualistic relationship with the host plant and protect them from the soil borne pathogens. In this study, we isolated a total of 283 fungal strains belonging to 8 different genera and 16 morphotypes from 450 tissue segments (Leaf, stems and flowers) of bengle sage (*Meriandra bengalensis* Benth.) plants during different (winter, summer and rainy) seasons, grown under natural conditions of Manipur and identified them on the basis of cultural morphology. The percentage of isolation rate (IR %) and colonization rates (CR %) of endophtic fungi communities were high in leaf segments (51% and 66%) and winter season (47% and 75%), respectively. The highest relative abundance (RA %) and isolation frequency (IF %) were recorded with *Colletotrichum* sp.2 and diversity indices of fungal endophytes were also calculated. The bioactive potential of *Phoma* sp. MBS13 was evaluated in vitro by dual culture tests against *Aspergillus flavus*, *Aspergillus niger*, *Alternaria solani* and *Fusarium oxysporum* isolates, which showed strong (Class 1and Class 2) antagonistic activity. In addition, the spectral (FTIR and GC–MS) analysis of ethyl acetate extracted metabolites of *Phoma* sp. MBS13 revealed the presence of various volatile compounds, and showed a good inhibitory activity against some human pathogenic bacteria i.e. *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*. Maximum inhibitory zone (15.3 ± 0.15 mm) was recorded with *E. coli*. Thus, the present findings reveal the importance of endophytic fungi for their role in antimicrobial activity and biocontrol potential against pathogenic microorganisms that could be exploited by the biotechnological and agricultural industries.

Keywords Fungal endophytes · Seasonal variation · Biocontrol · GC–MS analysis · Antibacterial activity

Introduction

Endophytic fungi have either symbiotic or asymbiotic association with the majority of the plant species exist in the environment without causing any discernible infectious symptom (Suryanarayanan et al. 2002). Further, endophytic fungi are beneficial to the host plants by modulating a variety of basic functions like primary productivity, alleviating abiotic stress tolerance, disease resistance and absorption of nutrients by promoting plant growth (Rodriguez et al. 2009). In recent years, the endophytic fungi have attracted increasing attention due to its potential secondary metabolites productions (Palanichamy et al. 2018) and that can be used as promising substance for antifungal and anticancer activity as a biological control agents in modern medicines, agriculture and industry such as novel antibiotics, antimycotics, immunosuppressants, and anticancer compounds (Strobel and Daisy 2003). Several studies revealed that around 3 lakhs plant species are colonized by endophytic fungi in almost every organ (leaves, stem, flower, root, rhizome and seed) of host plants (Wearn et al. 2012; Gautam 2014), and among them very few were studied to analyze the production of bioactive metabolites (Palanichamy et al. 2018; Kim et al. 2019) from these associated fungi. The diversity and distribution of endophytic fungal communities in medicinal plant species were outlined by different researchers in various ecosystems and niches (Krishnamurthy et al. 2008; Huang et al. 2008; Gautam 2014). However, the seasonal and organal diversity of endophytic fungi in ethanomedicinal

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ORIGINAL PAPER

Characterization and biological activities of melanin pigment from root endophytic fungus, *Phoma* sp. RDSE17

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Abstract
Melanins are high molecular weight hydrophobic pigments which have gained popularity for their role in virulence against different pathogens. In the present study, we isolated and characterized the melanin pigment produced by a dark septate endophyte fungus *Phoma* sp. RDSE17, which was associated with the roots of an indigenous *Oryza sativa* cv. 'Chakhao amubi' in Manipur, Northeast India. The biological properties of purified melanin from the fungus were evaluated for their antioxidant, antimicrobial and anticancerous activities. The pigment was extracted from *Phoma* sp. by alkaline–acid hydrolysis method and confirmed as melanin through physico-chemical tests and spectral (UV, FTIR, and EPR) analysis. The analyses of the elemental composition indicated that the pigment possessed a low percentage of nitrogen (N) contents, and therefore, would not fall under DOPA class of melanin. Exposure of the fungus to melanin pathway inhibitors revealed a positive melanin inhibition by tricyclazole, but not by kojic acid. Thus, the melanin from *Phoma* sp. may be a member of the DHN family. Moreover, the purified melanin showed high DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical-scavenging activity with an EC_{50} of 69 µg/mL and inhibited human lung cancer cell (A549 cells) proliferation at 80 µg/mL. The present study demonstrates that melanin from *Phoma* sp. RDSE17 could be employed as a potential biological (antioxidant) and antimicrobial agent for inhibiting the growth of humans and phytopathogens.

Keyword Antimicrobial properties · Antioxidant activity · Endophytic fungus · Identification · Inhibitors · Pigments · Rice

Introduction

Endophytic fungi have been reported to have a symbiotic or mutualistic association with the majority of plant species existing in different environments (Addy et al. 2005). These fungi are found to alleviate abiotic stress, improve disease resistance, and aid absorption of nutrients by plants (Mandyam and Jumpponen 2005; Vergara et al. 2018). The dark septate endophytic (DSE) fungal associations are common in the plant root systems of various habitats, viz., semiarid grasslands, high altitude forest, agroecosystems, arctic, tropical, and temperate regions, as well as heavy metal-polluted environments (Newsham et al. 2009). In recent years, the exploration and utility of endophytic fungi have made a huge impact due to their capability of producing extracellular enzymes (Uzma et al. 2016), and secondary metabolites (Kusari et al. 2012), with wide applications in agrochemicals industries (Giménez et al. 2007).

Root-colonizing DSE fungi are commonly known to accumulate melanin pigments in the hyphae and their abundance usually increases in stressed environments (Zhan et al. 2011; Berthelot et al. 2017). Melanins are natural or synthetic pigments with many functional characteristics and are widely distributed among animals, plants, and microorganisms (Nosanchuk et al. 2015). They are dark brown pigments, negatively charged, high molecular weight

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THE GENUS *OPHIOGLOSSUM* (OPHIOGLOSSACEAE) IN PUDUKKOTTAI DISTRICT, TAMIL NADU, INDIA

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Abstract

The survey of Snake tongue ferns in Pudukkottai district of Tamil Nadu, India, revealed the presence of five species of the genus *Ophioglossum*. A detailed description, illustrations, SEM image of spores, and key to the species of the genus in Pudukkotai district are provided.

Keywords: Pteridophytes; Adder's tongue ferns; Enumeration; spore SEM images; Key

Introduction

The genus *Ophioglossum* L. (Ophioglossaceae) is eusporangiate, monophyletic and cosmopolitan and represented by 50 accepted species (Hauk *et al.*, 2003; Hassler & Schmitt, 2020; POWO, 2022). Adder's/Snake tongue ferns are the species of *Ophioglossum* which were recognized greatly based on simple morphological features though at times confused due to complex morphological variations (Burrows & Johns, 2001). The variations within the genus are due to the large number of chromosomes owing to polyploidy, and consequently, several morphological variants were reported.

The species of *Ophioglossum* in India were first documented by Beddome (1883) and then followed Chakravarty (1951), Balakrishnan *et al.* (1960), Mahabale (1962), Panigrahi & Dixit (1969), Dixit (1984, 2000), Vasudeva & Bir (1993), Khullar (1994), Yadav & Tripathi (2002), Goswami (2007), Goswami *et al.* (2008), Singh *et al.* (2009), Patil & Dongare (2014), Kachhiyapatel *et al.* (2018), Patil & Rajput (2020), Mandal *et al.* (2020) and Jash & De (2020). Several new species of *Ophioglossum* were described from India in the last decade of which *O. chaloneri* H.K.Goswami *et al.*, *O. indicum* B.L.Yadav & H.K.Goswami, *O. raphaelianum* Anto *et al.*, *O. malviae* M.Patel & M.N.Reddy, *O. aleatum* M.Patel *et al.* and *O. hitkishorei* M.Patel & M.N.Reddy were not accepted by POWO (2022). The novelties accepted are *O. gujaratense* S.M.Patil *et al.* and *O. jaykrishane* S.M.Patil *et al.* So, the total number of species of *Ophioglossum* in India varies from 12–20 (Jash & De, 2020) of which, only 14 species were accepted by Fraser-Jenkins *et al.* (2017).

The pteridophytes of Tamil Nadu were studied by Matthew (1970), Bir & Vasudeva, (1971), Manickam, (1986), Manickam & Irudayaraj, (1992), Anandhan & Komalavalli (2011), Raja (2017), Kavitha *et al.* (2017), Alagesaboopathi *et al.* (2018) and Sureshkumar *et al.* (2020). However, there is no comprehensive work on the genus *Ophioglossum*. Hence, the present study was undertaken to study the morphology, taxonomy, distribution, and spore morphology of the genus in the Pudukkottai district.

Study area

The Pudukkottai district was once a princely states (Raju, 1985). It is situated (9°50′ and 10°40′ N, 78°25′ and 79°15′ E) covering an area of 4663.29 sq km in the eastern part of Tamil Nadu in Peninsular India (Fig. 1). The district has a coastline of 42 km, bordered on the south by Sivagangai and Ramanathapuram districts, east by Bay of Bengal, north and west by Tiruchirappalli district and in the north by Thanjavur district (Raja, 2017).

Field Survey and Documentation

The field surveys were conducted throughout the Pudukkottai district during 2020–2021 to collect *Ophioglossum* specimens. During post-monsoon seasons,