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Distribution and diversity of foliar endophytic fungi in the mangroves of Andaman Islands, India

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ABSTRACT

Fungal endophytes represent a major component of plant microbiomes. Various aspects of these fungi such as their diversity and technological potential have been studied in detail. However, their distribution and diversity in a mangrove community has not been addressed. In this study, we report the presence of culturable fungal endophytes from 20 obligate mangrove hosts from south Andaman Islands. *Phomopsis/Diaporthe* was isolated from all the mangrove species studied while *Xylaria*, *Colletotrichum* and *Phyllosticta* were recorded from the majority of the mangroves studied. A phylogenetic analysis of representative *Phomopsis/Diaporthe* isolates clearly indicated the broad host range of this genus. Our study also highlighted the fact that leaf endophytes of mangroves are not unique with reference to their species diversity and frequency of occurrence when compared to those of terrestrial plants. These observations suggest that the extraordinary success of some fungal endophytes in colonizing taxonomically disparate hosts could be due to development of traits specific to their ecosystem.

1. Introduction

Endophytic fungi infect and live within plant tissues without inducing any disease. They are ubiquitous and constitute an integral component of the plant microbiome. Endophyte association increases plant host fitness by enhancing its tolerance to abiotic (Yamaji et al., 2016; Lata et al., 2018) and biotic (Estrada et al., 2015; Suryanarayanan et al., 2018) stressors; these filamentous fungi are thus being investigated for their potential to improve crop fitness (Vega et al., 2008; Raghavendra and Newcombe, 2013). Furthermore, endophytic fungi represent a source of novel bioactive molecules (Suryanarayanan et al., 2009; Kharwar et al., 2011; Kusari et al., 2012) and industrially important enzymes (Thirunavukkarasu et al., 2011, 2015; Suryanarayanan et al., 2012; Sengupta et al., 2017). There are many studies on endophytes residing in the leaves of individual angiosperm plants and a few of them address the status of the foliar endophyte of plant communities (Suryanarayanan et al., 2003, 2011; Arnold and Lutzoni, 2007; Sudhakara Reddy et al., 2016). Here, we report on the distribution and diversity of culturable foliar endophytes of mangroves of Andaman Islands, India.

Mangroves are plants of the tidal habitats and survive in the ecotone between the terrestrial and marine ecosystems. Mangroves constitute an unique ecosystem which provides crucial ecosystem services including fisheries, shoreline shield, carbon sequestration and bioremediation of wastes (Lee et al., 2014). Furthermore, mangrove forests support a wide biodiversity and constitute the most carbon rich forests of the tropics (Donato et al., 2011). Current satellite data confirm that anthropogenic activity is leading to the loss of mangrove forests globally (Thomas et al., 2017).

While macroscopic basidiomycetes (Gilbert and Sousa, 2002; Maekawa et al., 2003; Sakayaroj et al., 2012; Nogueira-Melo et al., 2014) and marine fungi (Sarma and Hyde, 2001) of mangroves have been studied for their diversity and distribution at the community level, investigations on endophytes of mangroves pertain only to a few mangrove species (Suryanarayanan et al., 1998; Kumaresan and Suryanarayanan, 2001; Costa et al., 2012; de Souza Sebastianes et al., 2013; Li et al., 2016) or to their ability to produce novel bioactive metabolites and extracellular enzymes (Maria et al., 2005; Aly et al., 2010; Debbab et al., 2013). To our knowledge, there are no studies involving simultaneous sampling of many mangrove species to address the diversity and distribution of foliar

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endophytes in them. Furthermore, according to Niranjan and Sarma (2018) there are no studies on the endophytes of Andaman and Nicobar Islands. We chose to investigate this facet of endophytes of Andaman Islands (in the Bay of Bengal, east of India, 11.7401° N, 92.6586° E) since 13% of the total mangrove cover of India is present here (Forest Survey of India, 2013), and the mangroves of this region exhibit the highest density and growth among the mangroves of the country (Dagar et al., 1991).

2. Materials and methods

2.1. Sample collection

We chose South Andaman for our study as it supports 23 of the 25 mangrove species and all the 10 mangrove families distributed in the Andaman and Nicobar Islands (Goutham-Bharathi et al., 2014). Mature and healthy leaves of 20 obligate mangrove species (Goutham-Bharathi et al., 2014) belonging to 10 families were collected from the following seven different locations in South Andaman Island (Fig. 1, Table 1) and sampled for their endophyte presence - Burmanallah, Chidiyatapu, Corbyn's Cove, Manjeri, Shippighat, Shoal Bay and Wright Myo. The leaves were transported to the laboratory in sterile bags and screened for endophyte presence within 48 h of collection, by surface sterilizing and plating them on nutrient agar medium. The study involved a one time sampling between May 2016 and February 2017.

2.2. Surface sterilization

For each mangrove species, a total of 40 leaves were collected from 4 individual plants (10 leaves/individual). From these, 120 tissue segments (0.5 cm^2 each) were cut from the midrib region (including the lamina portion) -one each from the apical, middle and the basal region of the leaf. Of these, 100 leaf segments were surface sterilized and screened. These leaf segments were surface sterilized by immersing them in 70% ethanol for 5 s, followed by treatment with sodium hypochlorite (4% available chlorine) for 90 s and rinsing in sterile distilled water for 10 s (Suryanarayanan et al., 1998). The tissue segments were then plated in Petri dishes (9 cm dia.) containing antibiotic-amended (Chloramphenicol, 150 mg/l) Potato Dextrose Agar (PDA) medium (20 ml). We had previously used this sampling design for studying leaf endophytes of trees of different forest types occurring in the Western Ghats (Suryanarayanan et al., 2002, 2011; Govinda rajulu et al., 2013; Sudhakara Reddy et al., 2016).

2.3. Incubation procedure and isolation of endophytes

The efficacy of the sterilization protocol in removing the surface microbes was confirmed (Schulz et al., 1998) and each Petri dish with ten leaf segments was incubated in a light chamber (12 h light: 12 h dark cycle, 2200 lux of light) at 26 °C for 4 weeks (Suryanarayanan, 1992). The endophytes which grew out of the tissue segments were isolated, cultured on PDA slants and identified using standard manuals (Barnett and Hunter, 1998; Ellis, 1971, 1976; Ellis and Ellis, 1988; Sutton, 1980; Onions et al., 1981). Isolates which failed to sporulate were given codes based on culture characteristics such as growth rate, colony surface texture and hyphal pigmentation (Suryanarayanan et al., 1998) and were assumed to be different taxonomic species (Bills and Polishook, 1994). Since the identification was done primarily based on spore morphology, the anamorph (asexual state) and teleomorph (sexual state) were enumerated separately.

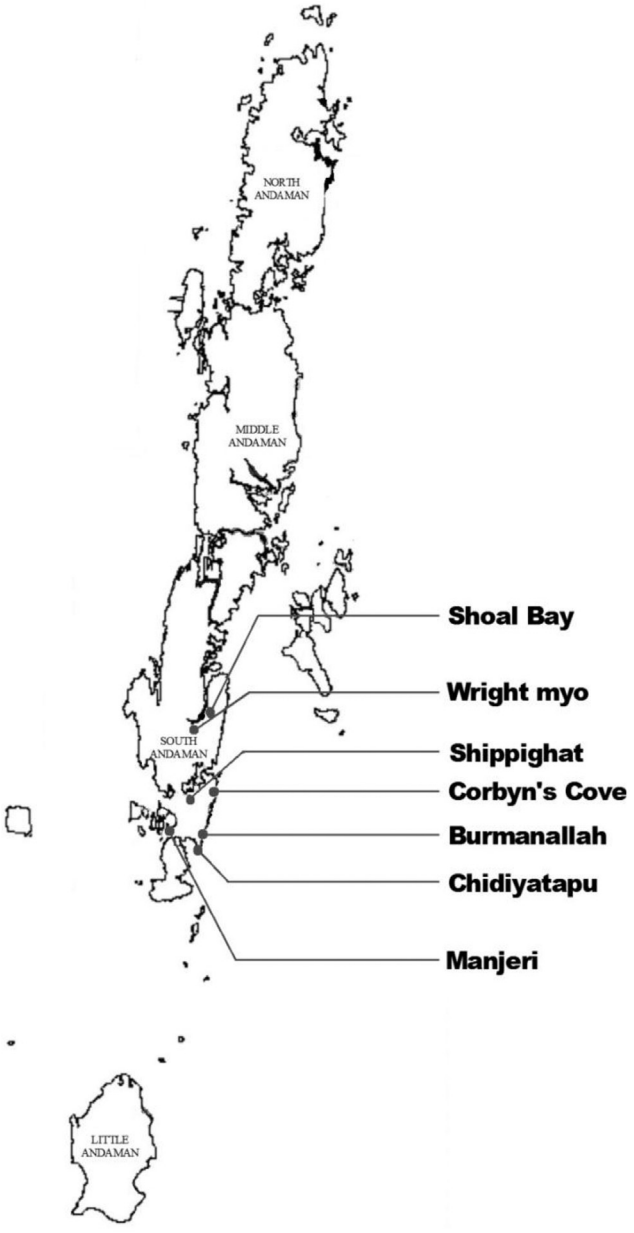

Fig. 1. Map of Andaman Islands showing the places of sample collection.

2.4. Genomic DNA extraction

The genomic DNA was extracted from fresh mycelia collected from 7 d old cultures growing in PDA medium. For this, the phenol-chloroform method was used (Sudhakara Reddy et al., 2016). The extracted DNA was resuspended in 50 μl of sterile distilled water and stored at −80 °C for further studies. For PCR amplification, the samples were thawed, concentration of the genomic DNA was checked in a 1% agarose gel and then used.

2.5. PCR amplification and sequencing of ITS region

A polymerase chain reaction was performed to amplify the ITS region and its flanking sequences. Fungal specific primers ITS4 and ITS5 were used for the reaction (White et al., 1990). The PCR reaction mix consisted of PCR buffer, forward and reverse primers, dNTPs, Taq Polymerase, DMSO, $MgCl_2$ and fungal DNA and was