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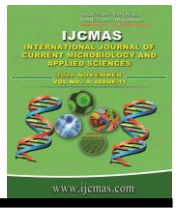

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Endophytic Fungi in Bamboo: A Review

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A B S T R A C T

The Plants are reservoir of large numbers of microorganisms known as endophytes that resides inside the plants with inconspicuous symptoms. The association of grasses with microorganisms, including fungal endophytes, is abundant and is vital to maintain the grasses' ecological health and the species diversity. The Bamboo is an essential plant, intertwining with native people indicates the opportunity to find new strains of endophytic fungi and as potential sources of novel natural products. Arunachal Pradesh, one of the world's biodiversity hotspots with 80% of the total state area under forest cover, will provide a vast potential for the research on endophytic fungi.

Introduction

Since the emergence of the term "symbiosis", which has been described as the living together of different organisms by De Bary in 1879, an array of symbiotic lifestyles has been defined based on benefits and impacts to macroscopic hosts microscopic symbionts (Lewis, 1985). Plants are a reservoir of large numbers of microorganisms known as endophytes (Bacon and White, 2000). De Bary (1866) originally introduced the word "endophyte", with 'endo' meaning inside and 'phyte' meaning plants. So, endophyte refers to the organisms that live within the plants (Wilson, 1995).

There have been diverse ways of defining the word 'endophytes'. Still, the most accepted definition was given by Stone *et al.*, (2000), which defines endophytes as those organisms whose "infections are inconspicuous, the infected host tissues are at least transiently symptomless, and the microbial colonization can be demonstrated to be internal". The researchers used this definition to describe the fungal endophytes. The same definition, however, is equally valid for the bacterial endophytes (Stone *et al.*, 2000).

The fossil record supported the plants' symbiotic relationships with the endophytic or the mycorrhizal fungi for about 400 million

years. It was likely to be associated when plants colonized the land, thus playing a crucial role in driving the evolution of life on earth (Krings *et al.,* 2007; Redecker *et al.,* 2000). The endophytes are very diverse and are termed as ubiquitous (Stone *et al.,* 2000; Petrini, 1991). They are also known to produce various functional metabolites (Tan and Zou, 2001). Their relationship with its host ranges from the latent phytopathogenic to a mutualism (Saikkonen *et al.,* 1998; Azevedo *et al.,* 2000).

More than a hundred years of research suggests that most plants in natural ecosystems have a symbiotic relationship with the endophytes (Petrini, 1986). They infect the tissue of the healthy plants for all or nearly all their lives without any symptoms for years and become parasitic only when their host is stressed (Firakova *et al.,* 2007; Limsuwan *et al.,* 2009).

Bamboo and their endophytes

Documentation of the endophyte and its association with the bamboo as a host has been undermined. Very negligible research has been reported to study the relationship between the endophytic fungi and the bamboo species. The bamboo, belonging to the family of grass, Poaceae, lacks the biosynthetic capacity to synthesize the secondary metabolites compared to the dicotyledonous plants, which are useful in the long-term survival strategy of each species. This lack of secondary metabolites' production, however, is accounted by notorious microorganisms living in a cohabitate condition with the grasses.

This association of grasses with microorganisms, including fungal endophytes, is abundant and is vital to maintain the grasses' ecological fitness and the species diversity (Kuldau *et al.,* 2008).

More than 1100 species of bambusicolous fungi are recorded, including the endophytic fungi with very limited known from bamboo seeds (Mohanan, 1997; Hyde *et al.,* 2002; Shukla *et al.,* 1998; Cai *et al.,* 2003). The infection of grasses by the endophytic fungi has been recorded over a century now (Freeman, 1904; Schardl *et al.,* 2004); still, its hyphal morphology and the growth concerning the cells of the host continue to evolve the questions on the mechanism of colonization (Tan *et al.,* 2001). The fungal growth paradigm prevails to explain that the vegetative hyphae grow exclusively by division and increased hyphal compartments at the apex (Bartnicki-Garcia, 2002; Harold, 2002). Until recently, the transmission of endophytic fungi in bamboo is described to be non-systemic and horizontally transmitted. Even then, systemic and the vertically transmitted endophytes cannot be ruled out in bamboos because they are commonly found in other grasses (Saikkonen *et al.,* 2004, 2007).

Compared to the other perennial grasses, the fast growth, age of the sexual maturity, and semelparity of the bamboo offer a significantly minimized systemic growth opportunities for the endophytic fungi (Saikkonen *et al.,* 2004). The statement was further supported by Helander *et al.,* (2013), stating that the endophytes are found only in the local horizontal transmission colonizing their bamboo samples.

Diversity of endophytic fungi in bamboo

The diverse range of the bamboo species in an ecosystem ranging from the temperate evergreen valleys to the high mountains presumably vary in its endophytic colonization, which is primarily defined by the host plant's altitude and environment. The current statement was supported by Doungporn *et al.,* (2006), where different

fungi species were isolated from a separate area of collection of the host plant, suggesting that the diversity of the endophytic fungi are influenced by the habitat, the region, and the range of their hosts. So, it would be interesting to investigate the ecological community.

Hyde *et al.,* (2002) recorded about 500 species of bamboo endophyte in Asia, of which 38% were recorded only from Japan. All the data related to the fungi associated with the bamboo have been recorded by Hino and Katumoto (1961). Doungporn *et al.,* (2006) isolated some more endophytic fungi that were not recorded by Hino and Katumoto in 1961. A list of new fungi species isolated were *Peziza*, *Diaporthe*, *Sordaria*, *Alternaria*, and *Microdochium*. Much of the research work had been done on the calvicipitalean endophytes (Clay, 1990), and a new genus *Heteroepichloe* was included by Tanaka *et al.,* (2002).

White (1997) included the seed transmitted endophytic fungi such as *Neotyphodium lolii*, *N. coenophialum*, and *Epichloe festucae* that were biotrophs and displayed stable symbiosis with the grass family (Schardl *et al.,* 2004; Schardl and Phillips, 1997). Further, Clay and Holah (1999) described their presence in host meristems throughout the leaves and the reproductive structures, evidently enhancing their hosts' survival.

The endophytic fungi associated with bamboo mostly belong to the phylum Ascomycotina and are primarily categorized under the class Dothideomycetes and Sordariomycetes. Both Morakotkarn *et al.,* (2007) and Shen *et al.,* (2012) supported that most of the endophytic fungi from the tissues of bamboos have been identified from the class Dothideomycetes and Sordariomycetes. Similar findings were reported by Shen *et al.,* (2014), while attempting to isolate the endophytic fungi from the Moso bamboo seeds based on their ITS region. They were able to isolate 350 fungal isolates distributed in 69 morphotypes based on the cultural characteristics and obtained at least ten genera from Dothideomycetes, seven genera under Sordariomycetes, and two and one genera in Agaricomycetes and Eurotiomycetes, respectively. Further, the sequence alignment depicted that the isolates belonged to the phylum Ascomycota (343 isolates) and Basidiomycota (7 isolates) with colonization frequency of 98.0% and 2.0%, respectively. In their study, they also reported some genera like *Cladosporium*, *Phoma*, and *Curvularia* as endophytes for the first time, which have previously been considered as pathogens for some bamboo species and its seeds, excluding Moso bamboo (Mohanan, 1997). Other genus including *Leptosphaerulina* (1.71%), *Simplicillium* (0.57%), *Sebacina* (1.71%), and an undetermined genus (2.86) were also a new addition as bambusicolous fungi, which were previously considered as the pathogens (Chen *et al.,* 2008; Kharkwal *et al.,* 2007; Thal *et al.,* 1986).

Recent studies have unveiled several new species of Endophytes in Bamboo. Arzanlou and Khodaei (2012) identified a new bamboo endophyte, *Aureobasidium iranianum* (Deuteromycetes), associated with the healthy bamboo stem. Khan and Uniyal (1999) isolated and recorded Ambispora, a dark septate fungus, for the first time from the bamboo species, the Dark septate endophytes (DSE) mainly comprise of a variety of root-inhabiting group of fungi. The DSE as sterile fungi belonging to the phylum Ascomycota colonize the living plant roots without causing any apparent adverse effects (Jumpponen and Trappe, 1998). Recently, Parkash and his co-worker in 2019 investigated on diversity and distribution of endomychorriza and the DSE on bamboo species, *Bambusa bamboos*, *B. tulda*, *B. pallida* and *B. nutans* of Assam,

India. They have observed 100% colonization of ectomycorrhiza and infection of hyphal DSE in the roots of all the bamboo species.

Significance of the bamboo endophytes

Most of the study related to the bamboo endophytes have been focused on a variety of aspects such as increasing the silica content for the growth of panda (Helander, 2013), enumerating the diversity of the DSE (Parkash *et al.*, 2019; Das and Kayang, 2010), focusing on a particular tissues of a particular bamboo species (Shen *et al.,* 2014), Witche's symptoms on bamboo due to endophytic fungi (Tanaka, 2009) and the more general association of the endophytic fungi with grass family.

The importance of the endophytic fungi associated with the bamboo as a source of novel bioactive products in the agriculture and the food industry has already been proven and established (Verma *et al.,* 2009; Gunatilaka, 2006; Strobel *et al.,* 2004). Shen *et al.,* (2014) demonstrated 69 representative endophytes' antimicrobial activities from Moso bamboo seeds against the clinical pathogens. They selected the bacteria and the yeast as model microorganisms for antimicrobial analysis and found that the endophytic strain *Cladosporium* spp. Depicted the inhibitory action against the growth of the two human pathogenic bacteria, *Straphylococcus aureus* and *Bacillus subtilis*, whereas *Pencillium* spp. displayed the most comprehensive spectrum of anti-microorganism against *S. aureus, B. subtilis, Listeria monocytogenes*, *Salmonella* spp., *Candida albicans* and *Rhodotorula rubra*.

The endophytic fungi of the bamboo have also been found to be an essential component for the production of hormones in their plant host. For instance, the endophytic fungi found in the meristematic tissues were responsible for the continuous primordium initiation in the shoot apex via auxin production (Tanaka *et al.,* 2003, 2009). The colonized shoot may not have the ability to produce a sufficient quantity of the endogenous free IAA for the leaves and stem expansion, which can be tackled by *Aciculosporium take* hyphae found within the apical meristem, which may be producing the exogenous free IAA for inducing primordium initiation and maintaining the apical dominance.

Based on the histological study, they postulated that *A. take* stroma formation destroys the shoot apical meristem. As a result, the endophytic hyphae pass through the epidermis to form stroma. Christensen *et al.,* (2008) postulated a novel growth mechanism via infection in the grasses by the vegetative hyphae of *Neotyphodium* and *Epichloe* species, followed by the intercalary division and expansion. The attached hyphae to the enlarging host cells and the collective growth along the filaments' length help the fungi extend at the same rate as their host. The mentioned study was the first evidence of the intercalary growth in the fungi, which challenges the century-old model describing the fungal growth in exclusive hyphal tips.

The endophytic fungi associated with the bamboo also enhance the contiguous composition and increasing the crude protein content of corn cob, hence giving an alternative to expensive animal feeds (Paynor, 2016). Besides being potent and versatile in its production for secondary metabolite, the endophytic fungi isolated from the bamboo also encourage input in bambusicolous fungi' diversity. As the previous studies have mentioned, the bambusicolous fungi also display some significant medicinal effects similar to their host or are even more useful. Ingredients like Cytochalasin-C and neoengleromycin from *Engleromyces sinensis* (Ma *et al.,* 2004) and hypocrellins from

Hypocrella bambusae and *Shiraia bambusicola*, are active ingredients from the medicinal macrofungi related with the bamboo species and displaying broad-spectrum activity against clinical microorganism and virus (Liu *et al.,* 2002; Zhan *et al.,* 2003; Ma *et al.,* 2004; Ali *et al.,* 2002; Wan *et al.,* 1981).

Shen *et al.,* (2014) unravelled macrofungi named *Shiraia bambusicola* as endophytic fungi from Moso bamboo seeds. Their strain coded zzz816 was closely related with *S. bambusicola* in ITS sequence analysis. *S. bambusicola* is mainly found in *Brachystachyum densiflorum* and its related species in China and *Bambusa* species in Japan (Li *et al.,* 2009; Hino, 1961). This fungal fruiting body has been used in traditional medicine in China and found it's compound to have antitumor and antiangiogenetic properties (Li *et al.,* 2009). Its principle ompound, hypocrellins, has attracted a great deal of attention because of its light-induced antifungal, antitumor, and antiviral activities (Cai *et al.,* 2011; Yang *et al.,* 2009). Shen and co-workers in 2014, while working on Moso bamboo seeds, isolated *S. bambusicola.* Their preliminary test for antimicrobial activity found that *S. bambusicola* exhibited the highest content of hypocrellins among all other isolates. There is a possibility of improving the compound by breeding novel industrial mutants and optimizing the fermentation process.

In conclusion research on endophytic fungi and its association with the bamboo species has been minimal. Until recently, their investigation had been taken into consideration in only some parts of Asia. There has been negligible literature recorded so far in the Eastern Himalaya, especially from Arunachal Pradesh. The bamboo plays a central role in the lifestyle among the rural folk of the state, from construction to medicine to their local cuisine, and mostly in the wild condition. Arunachal Pradesh, one of the world's biodiversity hotspots with about 80% of the state's total area under forest cover, will provide a vast potential for the documentation of the endophytic fungi based on the region of the collection of the host plant. The bamboo is an essential plant, intertwining with indigenous folk, indicating the opportunity to find new strains of endophytic fungi and as potential sources of novel natural products. The detailed understanding of the diversity of endophytes on bamboo species will help to chart and document the primary data, fill the gap, and establish future research. Furthermore, the microorganisms may be screened for a wide range of biological activities and explored for useful chemical entities produced continuously by them, including antimicrobial and extracellular enzymatic activities. Thus, an extensive study on endophytic fungal diversity from diverse bamboo species and also the in-depth research on their secondary metabolites is crucial.

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