Science Forum (Journal of Pure and Applied Sciences)

Journal Homepage: https://atbuscienceforum.com.ng/index.php/jpas

A Review of the Diversity, Distribution, and Metagenomic Analysis of Endophytic Fungal Communities in Different Ecosystems

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Abstract

Endophytic fungi are a diverse group of microorganisms that inhabit internal plant tissues without inducing visible disease symptoms, thereby establishing symbiotic relationships that significantly benefit their hosts. They contribute to plant health and productivity by enhancing nutrient acquisition, promoting growth, improving tolerance to abiotic stresses, and strengthening resistance against pathogens and herbivores. In addition, they influence soil structure and fertility by mediating nutrient cycling, uptake, and translocation, ultimately shaping plant–soil interactions and ecosystem dynamics. Despite their ecological importance, endophytic fungi are difficult to study using traditional culture-based methods, as many species fail to grow under laboratory conditions or persist only as dormant spores. Consequently, their true diversity, ecological functions, and biotechnological potential have remained largely underestimated. The emergence of metagenomic technologies has transformed fungal research by enabling high-resolution profiling of fungal communities independent of cultivation. Through DNA sequencing, functional gene analysis, and bioinformatics, metagenomics provides unprecedented insights into the taxonomic diversity, metabolic capabilities, and ecological roles of endophytic fungi. This article synthesizes current knowledge on the diversity, distribution, and ecosystem functions of endophytic fungi, highlighting advances gained through metagenomic approaches. It also examines key methodological challenges—including biases in DNA extraction, sequencing limitations, and interpretation of functional data—that hinder a full understanding of fungal ecology. Beyond ecological significance, endophytic fungi present immense opportunities for applied sciences. In agriculture, they offer sustainable alternatives for crop improvement by enhancing nutrient efficiency, promoting stress tolerance, and providing natural disease control. In forestry, they contribute to tree health, resilience, and sustainable forest management. Biotechnologically, their bioactive metabolites hold potential for the development of pharmaceuticals, biofertilizers, and biopesticides, while their role in bioremediation underscores their utility in addressing environmental challenges. Metagenomic profiling thus not only advances our understanding of fungal diversity but also opens new frontiers for their utilization in sustainable agriculture, biotechnology, and ecosystem conservation.

Keywords
Agriculture,
Cultivation,
Exploration,
Potential,
Sporulating

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Introduction

Endophytic fungi, which form symbiotically in plant tissues, form the majority of plant microbiomes. The fungi infect almost all plants, from dense rainforests to deserts, and play a vital role in helping plants grow, stay healthy, and resist stress (Arnold 2007; Rodriguez et al. 2009; Aliyu et al. 2022). Their distribution, activity, and diversity are determined by the plant, environment, and location (Yakubu et al., 2022). Understanding this interplay, can enhance productivity in forestry, biotechnology, and agriculture by integrating advanced genomic tools, sustainable resource management, and precision agriculture, potentially increasing yields while reducing environmental impacts (Christian et al. 2015).

The intensity of diversity associated with endophytic fungi may be gauged from the diversity of functions they play at an ecological level. Their associations with plants are diverse, varying from symbiotic to commensal or neutral, and sometimes pathogenic depending on their interaction with the host plant and their environment (Stone et al., 2000; Stone et al., 2004; Rodriguez et al., 2009). Previously, research into these fungi had been focused on their identification through genetic and morphological features. The last decades have seen the establishment of this science with the identification of metagenomic technology, which has facilitated extensive exploration of such populations, both classificationally and functionally (Strobel et al., 2004; Jumpponen & Jones, 2009; Aliyu and Fidelis, 2021).

Endophytic fungi inhabit various different environments, and the resultant communities that they can form are highly variable according to the environment (Yakubu et al., 2022). There are more fungi in tropical ecosystems where there are greater numbers of plant species and diverse conditions than in temperate ecosystems (Arnold & Lutzoni, 2007). These fungal communities might also be controlled by soil, climate, elevation, and health and age of the plants (Osono, 2008; Isma'il et al., 2023).

One of the ways in which one understands the complexity of such endophytic fungal populations in real-world situations is through the application of metagenomic analysis. The technique allows scientists to ascertain the role of fungi in various ecological processes, such as nutrient cycling, plant defense, and stress coping. Additionally, it facilitates the identification of fungal species that are difficult or impossible to culture a laboratory setting (Bulgarelli et al., 2013; Nilsson et al., 2019; Langa-Lomba et al., 2023). This would be useful to researchers who would like to learn more about the genetic diversity and activities of endophytic fungi through the analysis of the combined genomes of a number of species (Bulgarelli et al., 2013; Aliyu and Fidelis, 2021; Isma'il et al., 2023).

In this study, we seek to synthesize current knowledge on the diversity, distribution, and ecosystem functions of endophytic fungi, highlighting advances gained through metagenomic approaches.

Diversity of Endophytic Fungal Communities Across Ecosystems

Endophytic fungal communities vary to a very large extent in terms of range in richness across different ecosystems. This is due to the reasons such as the environment, plant type, and species interactions of a particular area. The tropical rainforests, for instance, are among the most diversified endophytic fungi available. Most scientists, including Arnold and Lutzoni (2007), Yakubu *et al*. (2022), and Isma'il *et al*. (2023), have defined the biodiversity of such communities.

The forest consists of a mixture of vegetation and different ecological processes, hence a favorable environment for the growth of fungi. Such fungi are beneficial because they enhance ecosystem stability and health by promoting nutrient cycling, disease resistance, and initiation of plant growth (Rodriguez *et al*., 2009; Aliyu *et al*., 2022).

The arid and semi-arid conditions, however, support more specialized but richer endophytic fungal community but at higher richness. These are conditions of high temperature, low humidity, and poor soils (Porras-Alfaro and Bayman, 2011). The fungi in such conditions typically have some mechanisms by which they can withstand stressful conditions and defend host plants against environmental stresses. For instance, there are fungi that have the ability to synthesize osmoprotectants or help in the storage of secondary metabolites to induce plant resistance against water stress (Redman *et al*., 2011; Langa-Lomba *et al*., 2023).

The situation is the same in agricultural ecosystems as well. These areas typically comprise monocultures, and agricultural activities such as irrigation, tillage, and chemicals introduce disturbances (Schulz and Boyle, 2005; Yakubu *et al*., 2022). Therefore, agriculture's endophytic fungal communities are less diverse compared to nature. Nonetheless, field crops have high endophytic fungal loads, with most playing significant roles in helping crops survive adversity. They enhance nutrient acquisition, promote healthy growth, and provide pest and disease resistance, enabling sustainable agriculture (Gond *et al*., 2015; Isma'il *et al*., 2023).

Endophytic fungal community richness is determined by a range of factors including plant diversity, abiotic conditions, and ecosystem type. Following Arnold (2007), high plant diversity and high microhabitat diversity in the tropics enhance fungal community richness. Nevertheless, sites such as agricultural fields are less diverse since they are composed of fewer plant species and stable conditions (Christian *et al*., 2015; Langa-Lomba *et al*., 2023). There are some fungi with high host preference for certain vegetation, and their existence is in most cases found alongside some vegetation (Osono, 2008; Aliyu and Fidelis, 2021).

Due to molecular progress, researchers have separated varied sets of fungal taxa from numerous types of habitats, including plant-host-associated fungi and fungus-plant symbiotic organisms and fungi that cause organic matter decay (Nilsson *et al*., 2019; Aliyu *et al*., 2022). It highlights that in environments rich in fungi, reservoirs exist and additional research must be carried out to reveal endophytic communities of fungi and the roles played by them in the environment.

Distribution of Endophytic Fungi

The prevalence of endophytic fungi in ecosystems is regulated by several factors, such as the environment, the plant types, and the presence of suitable growing surfaces. These alone decide not just the location at which fungi can establish themselves but also the extent and volume of the species present in given situations (Arnold and Lutzoni, 2007; Rodriguez *et al.*, 2009). You may encounter endophytic fungi especially common in the tropical regions that are also known to be highly diversified and ecologically complexly linked (Isma'il *et al.*, 2023). Arnold *et al.* (2000) assert that it is natural for endophytic fungi to exist in an environment that consists of high organic material, such as marshes, forest streams, and leaf litter. Dense vegetation and humidity in such environments provide ideal conditions for the growth and propagation of endophytic fungi.

Furthermore, tropical rainforests are rich in plant diversity and provide a range of different environments that are conducive to a diverse number of different fungal species (Arnold, 2007). The interaction between endophytic fungi and their hosts is complex, and this needs to be interpreted in terms of where precisely these fungi develop in tropical ecosystems (Rodriguez et al., 2009). Availability of water and fluctuations in rainfall significantly influence the distribution of endophytic fungi.

In the tropics, the transition period from the wet to dry seasons can affect the conditions facilitating the colonization and development of the fungi (Arnold & Herre, 2003). Fungi will be more profusely and with higher diversity during the wet season because of higher moisture, organic debris, and leaf fall (Arnold *et al.*, 2000; Isma'il *et al.*, 2023).

Consequently, under drought periods, the population of fungi reduces or community organization might be altered by less water and more stress (Arnaldo and Herre, 2003; Thangavel *et al.*, 2022). Under these fluctuating environments to cope, certain endophytic fungi have adopted mechanisms for survival in the form of developing resistant spores and drought-resistant host plants with which to form association (Rodriguez *et al.*, 2009). The host plant with which these fungi are found to be associated largely determines where these fungi naturally occur.

Some endophytic fungi are host-specific towards specific host plants, in the sense that they are found in close association with some plant species or a specific subset of them (Arnold, 2007). Due to this specialization, most fungi have limited distribution patterns, and only occur where their respective host plants occur. Some of the fungi may be limited to tropical rain forests and some plant families, while others occur in a broader variety of host plants. The mode of spread of endophytic fungi and how they operate within an environment is therefore a function of the availability of the correct host plants in correct environments only (Isma'il *et al.*, 2023). The distribution of these fungi can also be determined by complex plant-fungal relationships, climatic seasons, and other environmental conditions. Detection of such patterns of distribution is important in understanding the ecological role of endophytic fungi and their contribution to the health of many ecosystems.

Overview of Endophytes and Their Secondary Metabolites

Endophytic fungi are becoming a rich source of bioactive metabolites that are useful to medicine, agriculture, and industry. The fungi develop innocuously inside plant tissues (Thangavel *et al.*, 2022; Strobel and Daisy, 2003). They produce a variety of secondary metabolites including flavonoids, alkaloids, phenolic acids, terpenoids, and saponins that display antimicrobial, anticancer, antioxidant, and anti-inflammatory properties (Kharwar *et al.*, 2011). Since they possess a variable chemical makeup, they can be utilized in drug discovery and other environments (Gundersen *et al.*, 2011).

The host plant-endophytic fungus interaction is central to how endophytic fungi can produce secondary metabolites. Rodriguez *et al.* (2009) explain this symbiotic connection that appears to be implicated in the exchange of nutrient and chemicals and influencing the kind and amount of metabolites that the fungi produce. For instance, under disease or environmental stress, the fungi are able to react by yielding specific chemicals that have the ability to assist the plants in developing resistance (Verma *et al.*, 2015). The process enables the synthesis of a variety of chemicals that are valuable for different applications (Schulz *et al.*, 2002).

Besides, the environment also plays a part in controlling the metabolic patterns of such fungi. Factors such as light, soil, temperature, and humidity in the environment can control the synthesis of secondary metabolites (Strobel and Daisy, 2003). The same species of fungus can form a variety of compounds depending on its biological environment (Gundersen *et al.*, 2011). The potential of endophytic fungus as a new source of bioactive compounds is also brought to the fore by this environmental factor, especially if used in different habitats (Kharwar *et al.*, 2011). Overall, endophytic fungi possess a treasure trove of secondary metabolites with diverse applications. They are abundant with potential for discovery and innovation since they rely on the interactions of these fungi with host plants as well as the external world (Rodriguez *et al.*, 2009).

Metagenomic Analysis of Endophytic Fungal Communities

Metagenomics is a thrilling and groundbreaking method for microbial community discovery by direct isolation of DNA from environmental samples and subsequent high-throughput sequencing and sophisticated bioinformatics. Metagenomics has revolutionized endophytic fungal science by avoiding culturing, which has unveiled gigantic and hitherto unknown diversity of plant tissue-inhabiting fungal species (Kumari *et al.*, 2023). Through ITS region analysis, researchers have uncovered striking differences in fungal community structure in various plant tissues and offered new insight into the interaction between fungi and hosts (Zhao *et al.*, 2023).

The method provides rich information on endophytic fungal community structure, as well as their ecological role and contribution to host plants and ecosystems. It is now well known that many endophytic fungi have biosynthetic gene clusters to biosynthesize secondary metabolites, enzymes, and bioactive compounds, with others having acute medicinal and agricultural applications (Rai *et al.*, 2022). Endophytic fungi participate in critical plant growth processes concerning stress tolerance, growth promotion, and disease resistance through mechanisms such as hormone production, nutrient solubilization, and immune system regulation (Ahmad *et al.*, 2023).

Metagenomics techniques employed in the study of endophytic fungi propel sustainable agriculture by isolating unique fungal taxon with plant growth-promoting potential. They have been shown by some studies to possess the capacity to stabilize atmospheric nitrogen, dissolve phosphorus, and secrete plant hormones like indole acetic acid, resulting in sustainable and enhanced cropping (Pan *et al.*, 2023). Metagenomics, therefore, not only improves science but also possesses valuable practical applications like the manufacture of bio-fertilizers and bio-pesticides. Aside from application in agriculture, the biotechnological and ecological significance of endophytic fungi is also extended to forestry and environmental conservation.

They help facilitate nutrient cycling, carbon sequestration, and ecosystem restoration, particularly regarding the threats of climate change. Through the mapping of variability and function of endophytic fungal communities among indigenous and threatened plant species, conservation initiatives can be integrated to confer ecosystem stability and biodiversity conservation (Nguyen *et al.*, 2024). The convergence of omics technology—metagenomics, metabolomics, and transcriptomics—has broadened the platform of knowledge of fungal-host interaction as well as for the discovery of new chemicals utilized in industry and drug production (Ali *et al.*, 2024). Generally, metagenomic analysis has been a rich asset in modern ecological science, that is able to conduct basic biology and applied applications.

Challenges and Future Directions in Endophytic Fungal Research

Despite all the gargantuan feats that have been achieved, there are still titanic challenges facing the research on endophytic fungi. How endophytic fungi are connected to their host plants and to the environmental microbes is very sophisticated and not so well understood. We understand that endophytic fungi are capable of affecting plant well-being and assisting the plants in stress tolerance, but we need to examine the interaction of the fungi with the host plants as well as other microorganisms also. In order to utilize endophytic fungi more effectively for agricultural, medicinal, and environmental applications, we need to understand more about how the interactions occur (Guo *et al.*, 2021).

Increased complexity is new fungal species discovered and described by Thangavel *et al.*, 2022. Methods that we are employing presently, culture-dependent among others, fail when one attempts to isolate and type endophytic fungi. Pluralities of such kinds of fungi cannot be cultured in the laboratory, thus the majority of the diversity is not being sensed. We need to employ more advanced methods, such as culture-independent methods like metagenomics and single-cell genomics, in an attempt to increase the insight into the activity and diversity of fungi (Kharwar *et al.*, 2022).

Subsequent research needs to consider the possible market value of secondary metabolites of endophytic fungi. Even though culturing, extraction, and up-scaling these bioactive compounds have been a bottleneck to the process, they can be of huge value to utilize commercially such as drugs and agriculture. If we are able to extract and produce them successfully, they can provide new renewable materials. We also need to determine what endophytic fungi do in the natural world, particularly how they interact with the organisms surrounding them. These fungi also increase plant resilience, fostering biodiversity and therefore are a fundamental component of reclamation and ecosystem management (Rodriguez *et al.*, 2023).

Conclusion

These organisms are highly diverse and of crucial importance in our biosphere, and they hold colossal potential worth to agriculture, forestry, and biotechnology. Metagenomic analysis has enabled us to understand further the diversity of the fungi, where they are located, and what roles they play. Everything is still in the process of being discovered with regard to the ecology and biology of the endophytic fungi, and there is much left to be unearthed with the help of subsequent studies about all the potentiality they possess.

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