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## Review

# The need to study the holobiome for gainful uses of endophytes

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### ABSTRACT

It is established that endophytes (bacteria and fungi) affect positively several traits of their host plants including growth, nutrient acquisition, and tolerance to abiotic and biotic stressors. Owing to this, endophytes are envisaged as a route to improve sustainable agricultural production. However, such effects of endophytes on crops although evident under lab conditions, are devoid of consistency under field conditions. The main reason for this could be the lack of our understanding of the functioning of the endophytes as a consortium within the plant (holobiome). It is essential to study the complex web of interactions which operate in the inner tissues of plants harboring endophytes. I highlight here, alluding to the work on foliar fungal endophytes, the importance of studying endophytes as a consortium rather than merely as one species in isolation to better appreciate their role in the plant holobiome. Such an approach should aid in harnessing the technological potential of endophytes.

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## 1. Introduction

Among the microbes associated with plants, some fungi, bacteria, including actinomycetes colonize symbiotically the internal tissues of plants. Termed the endophytes, their association with plants is not casual and inconsequential, but could be mutualistic or commensalistic. Furthermore, some fungi are known to exist as both pathogens and symptomless endophytes in plants (Wheeler *et al.*, 2019). Recent studies show that endophytes influence the traits of their host plants such that plant fitness and performance is a reflection of both the host and its microbes. The use of endophytes, which affect plant traits positively, is envisaged as a route to improve sustainable agricultural production (Li *et al.*, 2019;

White *et al.*, 2019). However, most of the studies pertaining to this aspect are concerned with one candidate endophyte microbe isolated from a given plant species. It is becoming clear that studying endophytes as a consortium rather than merely as one species in isolation would help us better appreciate their role in the plant holobiome and aid in a more effective technological use of their desirable traits. In this paper, I emphasize this by referring to the results obtained on studies of foliar fungal endophytes (FFE) which belong to the class 3 type of fungal endophytes.

Based on their symbiotic criteria, the fungal endophytes are divided into four classes (Rodriguez *et al.*, 2009). Class 1 or Clavicipitaceous endophytes cause systemic intercellular infections in the shoots of some cool season grasses and are

transmitted vertically through seeds. All the other three classes of endophytes are non-Clavicipitaceous fungi. The Class 2 type endophytes colonize extensively the roots, stems and leaves and are transmitted via seed coats and rhizomes. The Class 3 endophytes infect locally the above ground tissues of vascular and non-vascular plants. They are hyperdiverse, ubiquitous and are transmitted horizontally. The Class 4 endophytes are root dwelling fungi and are characterized by the presence of dark septa in their mycelium.

With regard to the endobiome (different fungal and bacterial species surviving inside the tissues) of plants, there are more studies on the prokaryotes than on fungi (Rho *et al.*, 2018; Hassani *et al.*, 2018; White *et al.*, 2018; Kim *et al.*, 2020). With fungal associates of plants, more information is available on the influence of mycorrhizal, pathogenic, and saprotrophic fungi on the ecology and behavior of associated plants (Busby *et al.*, 2016) than on endophytic fungi. The association of fungal endophytes with leaves existed even as early as the Late Pennsylvanian period (ca 300 Ma ago) (Krings *et al.*, 2009). This long association and the invariable and universal presence of fungal endophytes in leaves of all groups of plants go to show that endophytism is a successful life style among fungi (mainly the Ascomycotina). One study shows that FFE infection upregulates several defense-related genes of the host plant (Mejía *et al.*, 2014). It enhances the plant's defense against herbivores or pathogens by inducing chemical responses similar to those produced by these biotic stressors (Hartley *et al.*, 2015). Such desirable traits and the non-pathogenic nature of Class 3 FFE have motivated attempts to employ them as an alternative for plant breeding to improve agronomic traits in crops (Khan *et al.*, 2017; Vega, 2018). Although such a FFE-mediated crop improvement appears attractive, results obtained so far lack reliability and consistency (Murphy *et al.*, 2018; Compant *et al.*, 2019). One of the reasons for this could be the general practice of selecting an endophyte for desirable traits and inoculating it in a crop without considering its interaction with the native endobiome of the crop (Rho *et al.*, 2018). It is imperative to elucidate the dynamics of the plant endobiome to improve the prospects of using endophytes for crop improvement. However, this is not a straightforward task. To appreciate the complexity of the endobiome, the probable roles of the plant host, the associated microbes and the environment are dissected and treated here.

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## 2. Plant host as a factor

The outcomes of plant–microbe interactions are influenced by environmental factors, the interacting microbes and the genotype of the host (Brader *et al.*, 2017). In plant–fungal pathogen interactions, the host and pathogen recognize each other almost immediately after they come in contact leading to the activation of signal pathways culminating in disease or resistance (Shen *et al.*, 2017). Indeed, advances in the field of plant immunity to fungal pathogens with reference to PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) have opened up ways to improve sustained disease resistance in crops (Miller *et al.*, 2017). With reference to Class 3 FFE, such fungus–plant interaction or even a direct mechanism of

plant driven selection of fungi has not been described as is the case with pathogens or the rhizosphere microbial community (Lundberg *et al.*, 2012; Duhamel *et al.*, 2018; Oberholster *et al.*, 2018; Schroeder *et al.*, 2019). However, indirect observations suggest the operation of such a mechanism. For instance, a comparison of the phylloplane and endophytic fungi of the leaves of 12 species of *Calamus* showed that the overlap in species composition between these two ecological groups of fungi was low (Girivasan and Suryanarayanan, 2004). A few species of culturable fungal endophytes exhibit a broad host and geographic range infecting taxonomically distantly related plants occurring in various locations (Suryanarayanan *et al.*, 2011a; Griffin *et al.*, 2019a). Among the FFE, the following situation is most common. A leaf usually harbors 15–25 species of fungal endophytes and their occurrence is always highly tilted with one species dominating the assemblage. Screening 224 angiosperm plant species belonging to 60 families revealed that a leaf supports one or two dominant fungal endophyte species (core species) with many other species unrelated to the core species exhibiting very low frequencies of occurrence (satellite species) (Suryanarayanan *et al.*, 2011a, 2018a). This is similar to the results obtained by Griffin *et al.* (2019b) for bacterial endophytes; they observed that host identity does not play a role in the constitution of bacterial endophyte communities and that a core microbiome is present in phylogenetically diverse plants. Interestingly, unlike with some pathogenic fungi where host sharing reduces with increasing phylogenetic distance between plant hosts (Gilbert and Webb, 2007; Webb *et al.*, 2008), FFE genera such as *Colletotrichum*, *Pestalotiopsis*, *Phomopsis*, *Phyllosticta* and *Xylaria* are cosmopolitan and dominant in taxonomically distantly related and even geographically separated plants and constitute the core species (Suryanarayanan *et al.*, 2018a). While it is not known how the wide host range of some FFE overcomes the associated functional trade-offs which could limit their fitness in a given habitat (Woolhouse *et al.*, 2001), it is obvious that their phenotypic plasticity and competitive ability is high. The work of Cord-Landwehr *et al.* (2016) exemplifies one mechanism of how such multi host endophytes are adapted to counter or escape the different defense reactions of taxonomically unrelated host plants. Upon infection, the chitin cell walls of fungi are immediately detected by the plant and triggers its immune response. To avoid detection by the host, a *Pestalotiopsis* endophyte while infecting the plant produces chitin deacetylase enzymes which alter its cell wall chitin by partial deacetylation such that is not recognized by the plant immune system (Cord-Landwehr *et al.*, 2016). Furthermore, transcriptomics and proteomics reveal that infection by the endophyte *Gilmaniella* sp. reduces the immune response of the host plant *Atractylodes lancea* probably aiding in its effective colonization of the host plant (Yuan *et al.*, 2019). More studies with different endophyte–host combinations in this line could solve the puzzle of long time survival of the core species as endophytes in many plants. Shefferson *et al.* (2019), based on their investigation on mycorrhizal fungal associations of orchids, opine that the robust dominance of particular fungal species in plant associations implies important ecological roles for them. This could also be true for the generalist FFE, considering their extraordinary success in colonizing different species of plants. If these fungi are able

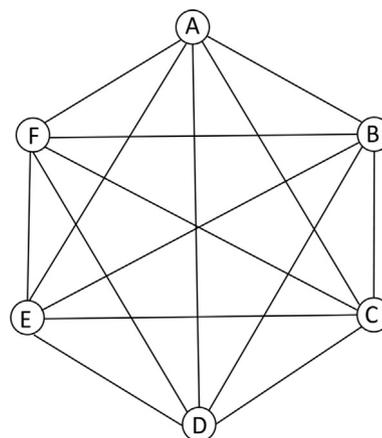
to enhance the stress resistance of their host plants, they could be excellent candidates for crop improvement. However, it is important to realize that some species of generalist FFE could be latent pathogens (Suryanarayanan and Murali, 2006; Photita et al., 2004). It is observed that a fungus could be endophytic in a plant species and pathogenic in another. For example, *Verticillium dahliae* is a symptomless endophyte in mustard and barley while it is pathogenic in potato (Wheeler et al., 2019). Thus, it is imperative that host selection, virulence factors and environmental signals which trigger expression of pathogenesis in endophytes are addressed before employing them in crop improvement. Additionally, the host genotype can also determine its FFE status. A non-native endophyte when inoculated in a high dose can infect and survive in a plant only for a short period. A *Trichoderma harzianum* endophyte of a seaweed when inoculated in crop plants for use as biocontrol agent of insect pests, was eliminated by the crops within 30 d suggesting host control of its endophyte assembly (Suryanarayanan et al., 2018b). Here, the tripartite interaction between the plants, endophytes and plant-associated insects need to be considered (Raman and Suryanarayanan, 2017). For instance, the density of endophyte infection appears to be directly correlated with the insect visitation of plants. For example, although the species diversity of endophytes in Bt plant and wild type cotton plant tissues does not differ, Bt plant tissues had significantly lower densities of endophyte infection compared to the wild type probably owing to reduced insect visitation (Suryanarayanan et al., 2011b). Furthermore, the ability of phytophagous insects to transmit FFE inoculum through their grass needs to be studied (Devarajan and Suryanarayanan, 2006).

### 3. Microbes as a factor

Leaves of taxonomically unrelated plants harbour similar core fungal endophyte species indicating complex interactions between them and the satellite fungal endophyte species which may vary with the plant host (Suryanarayanan et al. 2018a). In line with this argument, one study has confirmed the absence of competitive exclusion and the operation of robust positive interactions among fungal endophytes (Lee et al., 2019). The long evolutionary history and the universal existence of endophyte-plant association (Krings et al., 2009) should have resulted in strong mutual interactions between the two associates such that the survival and performance of plants is influenced by the endophytes they harbor (Rodriguez and Redman, 2008; Khare et al., 2018). Although robust experimental proofs for such a syllogistic conclusion are needed, the limited studies on endophytes and photosynthesis point to this. The diversity and richness of FFE correlates with leaf chlorophyll content (Sanchez-Azofeifa et al., 2012). Furthermore, eaves support heavy density of endophytes leading to the speculation that the respiratory CO<sub>2</sub> of the endophytes could locally enhance photosynthesis in leaf tissues by reducing photorespiration (Suryanarayanan, 2013). Although the secondary metabolites of a plant could influence its endophyte species composition (Voges et al., 2019), a reciprocal situation is also possible whereby the endophytes alter the secondary metabolome of the plant by biotransforming its

metabolites or by producing novel metabolites (Qin et al., 2018). For example, the FFE *Paraconiothyrium variabile* deglycosylates the host flavonoids to aglycones which enhance the growth of the endophyte (Tian et al., 2014). Infection by fungal endophytes induces several novel secondary metabolites in cells of grape fruits (Huang et al., 2018). The production of lipopeptides by bacterial endophytes enhances the defense of the host against its pathogens (Gond et al., 2015). The incidence of different fungal species in a leaf at various densities, the occurrence of different prokaryotes in the same tissue and the response of the host plant to colonization result in a complex web of interactions (Fig. 1). The community of FFE of *Mangifera indica* leaves is different after recovery from a systemic fungicide treatment when compared with the untreated leaves; this suggests the operation of resource-mediated competition whereby only upon elimination of the native endophytes the new ones competing for the same resource could colonize the leaf (Mohandoss and Suryanarayanan, 2009). *In vitro* results, showing the production of antibiotics by some resident endophytes imply that they could maintain the composition of the existing FFE community (Schulz et al., 2015). A situation similar to antibiosis existing in soils could be envisaged inside the leaf with antibiotic secreting endophytes having an edge over nonproducers competing for the same resources. However, the species composition of FFE can change significantly with time in a non-crop plant (Suryanarayanan and Thennarasan, 2004). Attendant changes in the interactions in the leaf endobiome with such successional changes in endophyte composition is expected and needs to be investigated for the application of FFE to improve crop production. These insights reiterate the need to understand the complex interactions and succession of species operating in the plant endobiome for effective technological use of endophytes.

Endophyte assemblages in leaves are made up of a few core species and many satellite species (Harrison et al., 2019). Given that the core fungal endophyte genera also include pathogens,



**Fig. 1 – An unweighted and undirected ecological network depicting possible interactions in a leaf. A) plant host, B) dominant or core FFE, C) satellite FFE D) keystone FFE, E) FFE with endophyphal bacterium, F) endophytic bacterium. The nature of interactions (antagonistic, competitive, or mutualistic) and the role of environment are not considered.**

it is possible that some of these endophytes are latent pathogens awaiting conducive environmental conditions to initiate disease or avirulent strains incapable of inciting disease. Thus, the distinctions between a true endophyte and a pathogen are blurred in some cases. The satellite endophytes occur in very low frequencies and are usually considered as casual residents whose presence is inconsequential to the host. However, the possible influence of satellite endophytes on the plant host traits, though not addressed yet, should not be ignored (Harrison et al., 2019). Similarly, Thirumalai et al. (2013) reported that the density of endophytic satellite *Fusarium* species in the leaves of *Piper betle* increased upon storage and produced different mycotoxins. While the core endophyte influences the leaf micro ecosystem by its abundance, the possibility of a satellite species functioning as a keystone species at least under some conditions, and influencing the constitution and functioning of the endobiome cannot be ruled out (Vandenkoornhuysen et al., 2015; Banerjee et al., 2018). Despite this, the current practice is not to include the low abundant microbial species while addressing the role of plant-associated microbes in selecting, constituting and functioning of the plant microbiome (Jones et al., 2019). Keystone species in different environments increase (Herren and McMahon, 2018) or decrease (Agler et al., 2016) the diversity of microbes and suppress establishment of plant pathogenic fungi in soils (Trivedi et al., 2017). The identity and functions of a keystone species could change depending on many factors thus posing difficulties in identifying it in a given community. Studies similar to that of Niu et al. (2017) are needed to identify the keystone species among the leaf endophytes. These authors created a simple assemblage of seven strains of bacteria associated with maize root and by repeated removal of each one of them from an assembly in axenic maize roots, identified *Enterobacter cloacae* as the keystone species.

The role of other microbes besides keystone species, present in the plant endobiome, in determining its species composition and host trait expression should also be considered. The interplay of the different fungal and bacterial species that occupy the endobiome within the plant host is little understood (Jones et al., 2019) though a few studies point to the operation of complex interactions. Furtado et al. (2019) showed that the fungal community in the endobiome is dependent on its bacterial community. Resource-mediated competition among plant-associated microbes involving the production of siderophores, antibiotics or volatile compounds also determine the endobiome homeostasis (Hassani et al., 2018). For instance, a Taxol insensitive endophyte induces the production of this antifungal metabolite by its plant host thereby avoiding fungal competitors (Soliman et al., 2013). Different endophyte species occurring in plant tissue could complement each other to enhance the production of the plant's metabolites or increase its stress tolerance (Ray et al., 2019; Bilal et al., 2018). Another less studied facet of fungal endophytes is the endosymbiotic bacteria that they may carry. Fungal endophytes carry associated bacteria (called endophthal bacteria) inside their hyphae (Hoffman and Arnold, 2010; Pakvaz and Soltani, 2016). This association endows certain traits to the fungal host (Arendt et al., 2016) including increasing the breadth of substrate utilization capacity

(Shaffer et al., 2017) and dominance over co-occurring endophytes through antibiosis (Pakvaz and Soltani, 2016). We are barely beginning to understand these bacteria-endophyte-plant interactions and their outcome on plant host performance.

#### 4. Environment as a factor

The natural environment plays a seminal role in structuring endophyte assemblies in different tissue types of plants (Neal et al., 2012). Our study on FFE of 75 tree hosts belonging to 33 families growing in three different tropical forest types revealed that environment has a larger role in determining the endophyte assemblage than the plant taxonomy (Suryanarayanan et al., 2011a). For example, mesophilic FFE of trees of forests experiencing frequent forest fires produce thermotolerant spores which survive exposure to dry heat for 2 h to 110 °C (Suryanarayanan et al., 2011c) and metabolize toxic furaldehydes which are the most abundant and toxic volatiles released during biomass burning (Govinda Rajulu et al., 2014). FFE isolated from mangrove leaves which are rich in tannins and accumulate salt in their tissues, grow in tannin or salt amended medium (Kumaresan et al., 2002). Leaf endophytes from plants of Baima Snow Mountain, China are adapted to low temperatures (Li et al., 2012). In some cases, environmental factors such as temperature and rainfall (Hashizume et al., 2010; Zimmerman and Vitousek, 2012), and not the host ecotype (Whitaker et al., 2018), determine the FFE community of plants. For instance, although *Phyllosticta* and *Colletotrichum* are multi-host and dominant leaf endophytes, the former dominates in the endophyte assemblage of trees dry tropical forests while the latter dominates in the endophyte community of wetter montane evergreen forests (Suryanarayanan et al., 2011a). These representative studies underscore the role of environment functioning as a filter in constituting the endophyte community in natural conditions.

Under agricultural conditions, the alterations in the environment due to agricultural practices affect the endophyte-plant association in many ways. Exposure to agrochemicals including fungicides (Mohandoss and Suryanarayanan, 2009), pesticides (da Costa Stuart et al., 2018) and herbicides (Stuart et al., 2010) alters the endophyte community in plants. Glyphosate herbicide treatment eliminates some endophytic bacteria in soybean and selects those which utilize the chemical as nutrient (Kryuchkova et al., 2014). According to Seghers et al. (2004), long-term use of herbicides and different fertilizers affects significantly the root endophyte community in *Zea mays*. Similarly, a plant can also alter its microbial composition by preferential recruitment of microbes depending on the presence or absence of environmental stress (Neal et al., 2012). One study shows that the effect of climate change on the occurrence of virus in grasslands could depend on presence of fungal endophytes and aphid vectors (Rúa et al., 2014). The influence of domestication of crops by selection and breeding for crop improvement on the endobiome is hardly known (Kim et al., 2020). These practices could alter or eliminate some members the endobiome of wild crop varieties during domestication.

## 5. The way ahead

We have just begun to appreciate that the expression of traits and the performance of a plant including its growth, nutrient status, and ecological fitness are the result of a net reaction between the plant and its microbiome, and between the holobiont (plant and its microbiome) and the environment. It is clear that the character of any endophyte species observed in isolation *in vitro*, could be altered or even lost when it is inoculated in the plant due to the web of interaction operating in the endobiont (Fig. 1). The FFE community is, like any other microbial community, a complex system due to the entanglement of the different types of interactions they have with the abiotic and biotic components of their ecosystem. These interactions are dynamic and change with alterations of their community composition, plant host and environment. A possible way to appreciate the complex behavior of FFE is by *in vitro* reconstruction of the microbial community of a leaf and studying it for perturbations in the interaction networks induced by different factors. Studies involving synthetic ecological approaches with constructed microbial communities along with mathematical modeling and relevant computational algorithms would provide more information about endobiont interactions (Zomorodi and Segrè, 2016). Although such efforts are fraught with shortfalls including not being robust enough in 'identifying indirect edges, understanding, biological implications, and describing network evolution' (Lv *et al.*, 2019), they may aid in predicting a suitable community composition for crop improvement. Network-based methods of analyzing ecological interactions have provided valuable information about the cross talk between hosts and parasites, and bacteria and phage (Delmas *et al.*, 2019). Models can be created consisting of core and satellite endophytes of a leaf and the influence of individual microbe in this created community could be determined by iterative elimination of a given species. Given its randomness, a systems biology approach including stochastic models would be worthwhile to identify various drivers of the leaf endobiont. High-throughput sequencing is another method that could add valuable information about the complex architecture of species-rich ecosystems (Toju *et al.*, 2014). More insights could be gained by resorting to interactome network analysis (protein, RNA and signal molecules) of the system as affected by the microbes (Rodriguez *et al.*, 2019). Modern genetic tools may be used to decipher endophyte-mediated host trait alterations. For e.g., using a CRISPR/Cas9 system, Huang *et al.* (2020) identified a gene in the fungal endophyte *Phomopsis liquidambaris* of rice plant as vital for its interaction with the plant. Although such approaches may not be robust enough to report the exact functioning of the endobiont, they could be used to understand better and predict the composition and functionality of the microbiome. Information gleaned from such approaches could aid in designing a functionally robust microbial consortium to improve crop production.

### Declaration of Competing Interest

The author declares no conflict of interest.

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