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Harnessing untapped potential of endophytes for disease management

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ABSTRACT

Microbes are the key placers in ecosystem having tremendous influence in agricultural production system. While some microbes are pathogenic, some beneficial microbes sustain soil and crop health through multiple actions. Among different groups of microbes, rhizopsheric microbes are extensively studied. Endophytes have garnered significance in recent times owing to their multifaceted activities. Endophytes, the microbes residing inside plant tissues without causing apparent harm to plants can contribute to plant health through suppression of pathogens with varied mode of action. Endophytes can survive host defense and add onto overall plant health. Many fungi and bacteria are reported to spend a part of their life cycle or remain as endophytes through vertical transmission. Endophytes can colonize host tissues from seed to flower and many antagonsists and entompathogens such as Trichoderma, Metarhizium, Bacillus, Pseudomonads etc. have been isolated from plant endopshere. Many of these organisms are screened in vitro and in planta have significantly reduced pathogen and increased nutrient uptake by plants in many crops. The cross talk between endophyte and plant is still a subject of through study as it is still inconclusive what triggers a microbe to choose endophytic lifestyle.

Key words: Bioagent, disease. Endophytic diversity, host defense,

INTRODUCTION

Plant beneficial microbes are an integral part of agro-ecosystem, contributing immensely towards plant-soil health management. Plants are always accompanied by a wide range of fungi, bacteria, virus etc. majority of which are unculturable. The microbial niches including rhizosphere, endopshere, phyllosphere hosts many beneficial microbes and even air microbiomes are known to interact with plants. Although majority of microbes (98-99%) are unculturable, the culturable microbes have been hugely explored for disease and pest management and for nutrient management (Srivastava *et al.,* 2022). Of the different microbes, rhizospheric communities being predominantly associated with plants, are mostly identified and used for disease management programmes (Srivasta and Bora, 2023) Rhizopsheric fungal bioagents of different genera, such as *Trichoderma, Aspergillus, Chetomium, Metarhizium, Beauveria* etc. have long been used as biofagent or entomopathogen gainst a wide range of diseases and pests (Bora and Rahman, 2022; Damodaran *et al.,* 2022). While looking into phytomicrobiome as a green strategy for crop protection, another group of microbes known as endophytes have caught researcher's attention world wide. Recent advances in microbial ecology driven by the high-throughput sequencing techniques have unlid the treasure chest of microbial remained unknown to scientific community. Way back in 1866, Anton de Bary, one of the pioneers in plant pathology research, introduced a new group of microbes residing inside plant system, which he termed as "Endophytes". The word 'Endophyte' was derived from the combination of two Greek words, *endon* and *phyton*, which literally mean within and plant, respectively (de Bary, 1866). Subsequently many definitions of endophytes were given by different group of researchers. Endophytes are defined as endosymbiotic microorganisms colonizing the intra/and inter cellular spaces within a plant. Most widely accepted current definition of endophytes describe it as, microbes that can ubiquitously colonize in all parts of a plant and maintain a symbiotic existence without harming the plants. A comprehensive definition of endophyte describes it as diverse microorgansisms living within healthy tissue of living plants entirely or for atleast a part of their life cycle without causing harmful effects on the host plant (Fadiji & Babalola, 2020).

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Among different group of microbes fungi and bacteria are the most predominant and frequently isolated groups from plants. According to fossil record study, plants have been associated with fungal endophytes for more than 400 million years. They are capable of surviving in intra- or inter-cellular spaces of a plant both in above and below ground tissues. Some genera of endophytic fungi include *Aspergillus, , Chaetomium, Cladosporium, Diaporthe, Fusarium, Alternaria, Mucor, Nigrospora, Paecilomyces, Bipolaris Penicillium, Trichoderma, Porostereum* (Bamisile *et al.,* 2018; Bora and Bora*,* 2020). Many fungi as insect pathogens such as *Metarhizium* spp , *Beauveria bassiana* have been isolated as endophytes from plant tissues and can be used as enomopathogenic bioagent (Orole and Adejumo, 2009, Bora *et al.,* 2016, 2021; Erla *et al.,* 2022). Endophytic *Fuarium oxysporum* are often encountered in different plants. Among bacteria, four bacterial phyla viz., Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes consists majority of endophytic bacteria, and among Proteobacteria, 26% belong to the Gammaproteobacteria. Endophytic Gammaproteobacteria includes major genera like Pseudomonas, Acinetobacter, Pantoea, Stenotrophomonas, and Serratia. Among different gram-positive endophytic bacteria, the Actinobacteria are dominant endophytes (Bora *et al.,* 2019).

Based on endophytic lifestyles endophytes may be grouped as systemic and non-systemic endophytes (Wani *et al.,* 2015). Systemic endophytes have closely evolved with the host plants (coevolution), and these do not cause harm to plants and establish a symbiosis. On the other hand, non-systemic endophytes are facultative, and for which their population size and species richness varies depending on host and environmental factors, and other biotic factor.

Switching over to endophytic lifestyle

Host–microbe interactions are manifested in different forms including mutualism to commensalism and parasitism. Mutualism is defined as an interaction which is beneficial for both the partners, while, commensalism provides benefit to one partner as disturbed existence without affecting the other partner. In endophyte-plant interaction study, there is always a concern as to how and why a microbe chooses to have endophytic lifestyle, while they can acquire essential nutrients from soil. It might be due to coevoltionary relationship or due to other triggering elements. Endophytes are basically a subset of rhizospheric microbe which can switch over to the endosphytism or occasionally remain as endophytes (Hardoim *et al.,* 2015). Thus, commensalism and mutualism are observed in plant–endophytic microbe interactions, where the plant can get improved supply of nutrients, host defense by the action of endophyte, as in case of the arbuscular mycorrhizal symbiosis plant–fungal interaction (Harrison, 2005, Cheng *et al.,* 2021). In return the endophytes can get nutrient and shelter within the plant. It is very interesting to note, how the endophytes can breach or alter the host defense being a foreign entity. Probably, there is a sophisticated mechanism to balance between the host defence response in plant and the demands of the endophyic microbes. The imbalance in favour of the endophyte makes it a pathogen and host defense responses are eliviated. Further, colonization of endophytes inside the host can benefit the host by releasing antimicrobial/pesticidal metabolites and growth hormones combating both abiotic and biotic stress (Lata *et al.,* 2018). The plant in return provides the bacteria fungi with nutrients. Mutualism requires a sophisticated balance between the defence responses of the plant and the nutrient demand of the endophyte. Hence, a mutualistic interaction does not indicate absence of host defence. In fact, it is a well orchaestrated balance that keeps the plant - endophyte-interaction in a mutuality stable without causing apparent harm to the partners.

Despite the soil serving as nutrient factory for microbes, some rhizospheric microbe migrates inside the plant system probably in a nutrient low condition, to avoid antagonism by other soil microbes. However, whether it is governed by some specific genes or not, is still not clear. Recent studies suggests that plant can recruit and reframe their microbiome while challenged by a pathogen and the role of plant metabolites is certainly undeniable and root exhudates are reported to attract plant beneficial microbial genera (Handique *et al.,* 2023).

Diversity of endophytes in different plant parts

The diversity of endophytes in plants varies depending on several parameters with time and space including the age and type of plant, soil and environmental conditions etc. (Ek-Ramos *et al.,* 2013). Endophytes are found in all parts of plants including root, leaf, stem, seed and flower, however, plant part wise diversity may vary. Further, distribution also varies according to geographic locations. For example, studies showed that with change in latitude diversity and host range of endophytes may increase (Arnold & Lutzoni, 2007). Wani *et al.* (2015) reported that endophyte communities from plants of high latitude were characterized by few fungal species. Even, the different plants growing in similar environmental situations also do not harbor same endophytes. Endophytic diversity is expected to be high in tropical forests as they have diverse plant hosts (Wani *et al.,* 2015). It is often found that population of endophytes are always greater in roots than other plant parts (Mercado-Blanco *et al.,* 2004). This might be due to the fact that the rhizosphere microbes moving to root as endophyte prefer to be localized in the roots and bacterial endophytes typically enter through wounds in the roots or root hairs (Saikia *et al.,* 2022a). The average density of root endophytes was found to be 10^5 cfu/g fresh weight, whereas average values of 10^3 and 10^4 were recorded in leaf and stem, respectively (Hardoim *et al.,* 2015). Successful endophytic colonization is governed by factors like the plant genotype, plant age, , and soil physical and biochemical parameters, environmental conditions etc (Bamisile *et al.,* 2018). In another study by Saikia *et al.* (2022a), spatio-temporal distribution of endophytic microbes was assessed in tomato, wherein, 143, 51 and 40 isolates were obtained from roots, stems and leaves, respectively, showing dominance in root. Seasonal diversity was observed to be varied and the maximum (112 numbers) isolates were recorded during monsoon season followed by pre-monsoon (64 isolates) and post monsoon (58 isolates) seasons.

Role of endophytes in disease management

Both rhizospheric and endophytic microbes hold greater promise as sustainable option for plant health management. They have multipronged effect on plant health and play a major role in disease suppression, plant growth promotion, bio remediation of organic pollutants, and phytohormone signaling that could open unexplored dimensions. Endophytic BCA like Bacillus sp., Pseudomonas sp., Trichoderma sp., Verticillium sp., etc. have been established as green solutionst many phytopathogens that and benefit the crops in multiple ways (Bora and Bora, 2008 a, Bora and Bora, 2020). Some of the plant beneficial microbes used as bioagent facilitate crop production through disease suppression as well as enhanced plant growth. The microbes have diverse mechanisms to inhibit the pathogens. Bioagents can produce HCN, antibiotics, bacteriocins, lytic enzymes, VOCs, endotoxins against the pathogens and insect (Bora *et al.,* 2022; Saikia *et al.,* 2022b). Additionally they can trigger ISR in plants and activates their defense system to help combating phytopathogens (Sharma *et al.,* 2020; Bora and Bora, 2021, 22.). Also, some antagonistic bacteria release biosurfactants CLPs (cyclic lipopeptides) and exopolysaccharides forming a protective biofilm layer across the plants and microbes to protect them from pathogens and chelate nutrients. Another group of microbes excludes availability of nutrients (N, P, K, and Fe) and increase niche competition inhibiting the growth of phytopathogens. A single microbe may undergo different mechanisms to combat the pathogens depending upon the pathogen and host-pathogen interaction. Endophytic microbes have been shown to obtain nutrients in soils and transfer nutrients to plant, increase plant growth and development, reduce oxidative stress of hosts, protect plants from disease, deter feeding by herbivores and suppress growth of competitor plant species (Waqas *et al.,* 2012).

Endophytic fungi of some genera isolated from plant tissues can elicit induced systemic resistance against the soil borne and foliar pathogens (Baruah *et al.,* 2024). *Trichoderma* spp (*T. viride, T. harzianum, T. koningi, T. hamatum, T. polysporum* and *T.*

longibrachiatum) are reported successful against many soil borne diseases, employing an array of mechanisms, viz. hyperparasitism, competition for nutrients, antibiosis and induction of host defense mechanism in plants (Rahman *et al.,* 2021; 2022, Bora *et al.,* 2024). In a study, Baruah *et al* (2024) recorded that endophytic *Trichoderma harzianum* could inhibit mycelia growth of Fusarium wilt pathogen by 60-72%. *Trichoderma* as rhizosperic and endophytic bioagent can be effective against fungal pathogens like *Rhizoctonia solani, Fusarium* spp (Khan *et al.,* 2018). *Colletotrichum* spp. , *Pestalotiospsis* spp (Bora *et al.,* 2021); bacterial pathogens such as *Xanthomonas citri* (Nasreen *et al.,* 2020; Saikia *et al.,* 2021), *R. Solanacearum* (Bora and Bora, 2008b). Another Endophytic fungus *Metarhizium anisopliae* from rice showed anibaterial effect against *Xanthomonas oryzae* pv. *oryzae* (Saikia *et al.,* 2020). *Beauveria bassiana* as entomopathogen also live as endophyte and found effective against many insect pests without disturbing native insect flora (Bharadwaz *et al.,* 2023)

Endophytic bacteria have been proven to have a wide range of plant responses due to their ability to promote plant growth and development, reduce environmental stresses that have an antagonistic effect and contribute in phytoremediation. Previous research found that endophytic bacteria colonize inside the plants without causing any adverse effects on the host plant. Exploring the extensive range of endophytes may yield new strains, contributing to the precious supply of biological agents for long-term plant disease management. Among different groups, *Bacillus* spp are predominantly encountered a sendophytes in fdifferent palnt parts. Studies demonstrated that *Bacillus* spp. can manufacture a significant variety of secondary metabolites, which play an important role in antibiosis against various pathogenic microbes (Bora *et al.,* 2023, 2024,). *Bacillus* spp. produce lytic enzymes such as chitinase and β-1,3-glucanase, which help break down fungal cell walls. Xiang *et al.* (2023) studied the endophytic bacterial strain *B. velezensis* EB1against Foc TR4in banana and strong colonization ability was reported in banana tissue culture plantlets at the end of rooting stage. Bio priming of micro-propagated banana plantlets with endophytic *Bacillus subtilis* has been shown to improve plant growth, resulting in enhanced plant height, leaf number, area, and pseudostem girth (Rajamanickam *et al.,* 2018). Besides Bacillus, other bacteria such as *Pseudomonas fluorescens*, and *Streptomyces luteogriseus* have also been shown to have biocontrol efficacy and boost plant efficiency in solubilizing and mobilizing inorganic phosphate (Matsuoka *et al.,* 2013). *Pseudomonas fluorescens* have been reported to elicit systemic resistance against Fusarium wilt of radish (Saravanan *et al.,* 2004). The bacteria can act as antagonist against diverse range of pathogens in rice and vegetable crops (Sharma *et al.,* 2022 Bora *et al.,* 2016). Endophytes isolated from *Cicer arietinum* plants have been identified and characterized due to their ability to induce resistance in plants by producing higher levels of defense compounds, antioxidant, and phenolic enzymes, in addition to solubilizing P and Zn, and reducing infection by *B. cinerea* in plant tissues. The moderate and constant activation of these enzymes can be a key mechanism for plant resistance (Vijayabharathi *et al.,* 2018)

The endophytes adopts several molecular mechanisms for increasing stress tolerance in plants which include expression of stress-responsive genes, synthesis of antistress metabolites and generation of molecules like ROS (Lata *et al.,* 2018). The plant hormone abscisic acid (ABA)-mediated stomatal closure and plant growth regulation contributes to combat osmotic and other abiotic stresses in the plant and ABA biosynthesis get modulated by the presence of beneficial microbes in the plant endosphere contributing to the plant growth enhancement. Plant growth promoting endophytes may affect plant growth either directly or indirectly (Waqas *et al.,* 2012). Direct promotion of plant growth occurs when either the microbes facilitate the acquisition of resources from the environment including nitrogen, phosphorous and iron; or modulates plant growth by providing or regulating various plant hormones including auxin, cytokinin or ethylene. Indirect promotion of plant growth by endophytes occurs when a bacterium limits or prevents the damage to plants that might otherwise be caused by various pathogenic agents including bacteria, fungi, and nematodes. There are many common mechanisms that endophytes use to indirectly promote plant growth including the production of antibiotics, cell wall-degrading enzymes, lowering plant ethylene levels, induced systemic resistance, decreasing the amount of iron available to pathogens, and the synthesis of pathogen-inhibiting volatile compounds (Bora and Bora, 2020, Wani *et al.,* 2016).

CONCLUSION

Endophytic microbes are the new research interest to researchers across the globe and significant progress has been made to harnress their potential in agricultural production system in terms of disease suppression and growth promotion. Yet, further investigation is required to study the behavior of endophytes while switching over from rhizospheric to endophytic lifestyle and biochemical pathways triggering such shift. Development endophytic bioinoculant, colonization behaviour of introduced microbes, production of microbial metabolites in plant system and interaction study needs further investigation. A no. of plant associates microbes are known for bioremediation of organic and inorganic pollutants, heavy metals. This could be better explored by identification of such endophytes and further use for phytoremediation purpose. In coclusion, endophytes are the treasure house of lot of plant beneficial traits and with advanced omic tools the potential should be harnessed for development of cost effective green technologies to be delivered to different stakeholders, more particularly to the farming communities for better and safer crop production with high encomic outputs.

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