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Endophytes: A Biocontrol Agent for Management of Plant Diseases

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ABSTRACT: Beneficial microbial endophytes that live inside the host plant and have the capacity to colonize plant roots without endangering the plant system. A wide variety of microbial associations, such as positive, negative, or neutral reactions to their host plant, attach plants together. Most of the study focuses on well-known epiphytic beneficial microbial associations with root zones, based on trends. However, one important aspect of plant microbiomes is that endophytic microbes that promote plant growth are found in the rhizospheric microbiome. Endophytic microorganisms communally inhabit and augment within the parts of the plants that include tissues of root, stem, leaf, bud, fruit and seed without causing any damage to the host plant. It is necessary to research microbial endophytes, a potent microbial resource, for use in sustainable agriculture. Association with a variety of bacterial, fungal, or actinomycete endophytes in addition to plant hosts, which may balance soil and plant health. Diversified plant growth regulating and promoting characteristics are directed by the association with endophytes and in this regard to microhabitat and the host metabolic activity; they synthesize several bioactive metabolic compounds emanating growth promoting and regulating parameters that similar to root zones microbes. They regulate substantial symbiosis by delivering several bioactive metabolic compounds providing to plant growth and microbial association. The characteristic features correlated with endophytes such as extracellular enzyme production, secondary metabolic synthesis against phytopathogens, bioremediation and induced systemic resistance.

Keywords: Microbial endophytes, Phytopathogens, Rhizhosphere associations, Plant growth promotion.

INTRODUCTION

Ensuring food security is one of the critical issues facing the growing global population. Projections suggest that by 2050, the world population could reach approximately 9.7 billion, resulting in a need to increase agricultural output by over 50% compared to current levels (Muthu Narayanan *et al*., 2022). Moreover, diminishing natural resources such as farmland, deforestation, shifting climate conditions, and decreased agricultural productivity caused by pre- or post-harvest pathogen invasions or plant diseases have intensified the challenges in ensuring food accessibility (Kumari *et al*., 2022). Plants encounter various biotic and abiotic stresses daily, which negatively impacts their growth and survival. Annually, around 15–30% of total agricultural output is lost due to diseases or attacks from pathogens. To address the issues related to plant diseases, many people depend on chemical pesticides; however, the ongoing and indiscriminate application of these chemicals has detrimental effects on food quality, soil composition, native microorganisms, and human health (Kumar A, 2022).

Strengthening the Agriculture is a major concern to the production, safety and quality of food for the increasing world population. By using chemical fertilizers affects the soil health and fertility by decline water holding capacity, decreasing soil fertility which results in losing soil nutrients and soil microflora and also damage the human health and ecosystem. Researchers are focused on replacing chemical fertilizers with microbial-based fertilizers by taking into account all of the problems. Application of biofertilizers as microbial endophytes which leads to effective approach to improvise better soil microorganism that encourages the natural soil microbiota, hence dominating nutrient availability and organic matter decomposition (Fasusi *et al*., 2021).

The term "endophyte" was coined in 1866 by Anton de Bary (endo = within, phyto = plant), he was the father of modern Plant Pathology as “any organism growing within plant tissues”. These endophytes have the ability to improve plant health, function as a biocontrol agent, protect the host against pests in a natural way, and maintain resistance to a variety of biotic and abiotic challenges. Microbial endophytes have ability to produce various growth hormones namely IAA, ACC deaminase, increased absorption of K ions in tissues of plant and decline the level of ethylene are the possible mechanisms to mitigate stress conditions for several plants (Agri *et al.,* 2022).

These endophytes also allow the host plant more room for improved survival and sustainability and are capable of better absorbing nutrients from the soil, particularly phosphorus, zinc, magnesium, sulfur, and nitrogen (Fan *et al.,* 2020). Endophytes, which include fungi and bacteria, have a great deal of potential for use as biocontrol agents. They exhibit antagonistic activity against phytopathogens that cause disease, which inhibit the harm that phytopathogens cause. These endophytes produce many antioxidants to combat pathogens in addition to a small number of bioactive bioactive antibacterial and antiviral biocompounds (Agri *et al.,* 2021). Diverse range of bacteria such as *Pseudomonas, Bacillus, Acidobacterium* a and *Pedobacter* participated in nitrogen fixation, mineral solubilization and metabolite production. Various fungal species such as *Beauveria bassiana, M. robertsii, B. metarhizium, Acremonium* spp. and *Chaetomium globosum* are involved in plant protection. With the diversified host range, endophytic fungus develops into beneficiaries compare with other biocontrol agents. Specifically, *Trichoderma viride* isolated from *Spilanthes paniculata* exhibit wide range that pursuit against *Colletotrichum capsici, Pythium aphanidermatum*and *Fusarium solani* (Qi *et al.,* 2019).

It is important to remain microbial endophytes and the novel biocompounds to be developed for their future exploitation in sustainable agriculture. In account of the microbial endophytes that induce systemic resistance in the host plants and deliver more resistance versus pathogenic infection, has lately gained more attention for effective management of diverse crop diseases (Chaudhary *et al.,* 2022). This chapter gives a brief statement on microbial endophytes that develop defense mechanisms within the host plants.

ENDOPHYTES

A. Endophyte Community in Root Zone Area

In microbial endophytes, roots are the main association and inhabitant in plant system. Root cracks, root hairs or wounds originated from activities of microbes and nematodes are the first level entry point for bacterial association in roots, cortical and epidermal intercellular gaps are among these relationships (Compant *et al.,* 2005). Nutrient-rich root-associated bacteria stimulate plant development through soil uptake and nutrient recycling. Overall endophytic root microorganisms is metagenomically broad most often falling under the following groups such as *Proteobacteria, Actinobacteria, Firmicutes,* and*Bacteriodetes.* In addition to, root associated microbes secrete various bioactive compounds which is useful for plant health.

Production of plant growth promoting hormones specifically Indole acetic acid (IAA), Cytokinins and gibberellins that enhance the overall plant development. And also, the enzyme called ACC deaminase (1-amino cyclopropane-1-carboxylic acid) used for nodulation in plants that will fix atmospheric nitrogen to plants. Along with, few are involved with plant mycorrhization for phosphorous solubilization and mobilization. Endophytes survive under unfavorable conditions are also investigated to enhance the crop productivity. Mycorrhizae are grow critically low in abundance under stressed condition, in which different fungal endophytes are capable of main root mutualistic symbionts. These symbionts improve the stimulation of growth-promoting processes, such as the synthesis of secondary metabolites, the mobilization and solubilization of phosphorus, and the upregulation of a small number of stress-regulating genes while under stress (Rat *et al.,* 2021).

The penetration of endophytic root microorganisms has various types are as follows: passive endophytes- the entry point of microbes by cracks present at root tips or root emerging area which is created by pathogens; obligate endophytes- they survive based on plant metabolism; facultative endophytes - they are found on the host plant's outer surface and are frequently connected to plants from the nearby soil; endofungal bacteria – bacterial symbionts of fungi occur inside the fungal spores and hyphae (Kukreti *et al.,* 2020; Kumari *et al.,* 2020).

B. Endophyte Community in Leaf Tissues (Phyllosphere)

Not all the endophytic microbes get into through root zones and travel into the xylem vessels; they harbor broad groups which enter the aerial tissues through above ground surfaces. Well studied endophytes that reside within the tissues of leaves and stems. Phyllosphere endophytes are the major component of microbial inhabitants which reside asymptomatically within the leaf tissues and useful for plant growth and development. The most abundant phyla allied with leaf endophytes are *Proteobacteria* constitutes 90%, *Actinobacteria* constitutes 2.5%, *Plancomycetes* constitutes 1.4%, *Verrucomicrobia* and *Acidobacteria* constitutes 1.1 and 0.5%. These leaf endophytes live inside the leaf and help the host plant form a symbiotic relationship (Lugtenberg and Kamilova 2009).

It is apparent to put forward that endophytes enter leaves and stem by opening that includes hydathodes and stomata by diffused with the help of soil and rain. These leaf endophytes has potential in synthesis of several metabolites promoting plant growth and development that includes indole-3 carboxylic acid and secondary metabolites such as 2-phenyl-ethanol (Qian *et al.,* 2014).

ENDOPHYTES IN PLANT DEFENSE SYSTEM

Plants have a broad range of defense system versus various pathogens. Among them, induced systemic resistance in plants is established to work against variety of phytopathogens. ISR is a process in which some plant beneficial endophytic microorganisms produce immunity, that can restore crop growth and flexible against several phytopathogens, parasites and insects. These endophytic microorganisms ameliorate plant health by regulate hormone signals such as ethylene (ET), Salicylic acid (SA) and jasmonic acid (JA) pathways that functionate the gene expression of ISR, the production of secondary metabolites, synthesis of several enzymes and volatile metabolites that eventually induce defense mechanism in plant system. For protecting plants against disease, they have several advance defense mechanisms that includes systemic gene silencing, local acquired resistance, systemic acquired resistance (SAR), induced systemic resistance (ISR) and systemic wound response are participated. Various endophytes activate the SA- involved SAR pathway by secreting SA at root zone surface. In other words, other endophytes can activate various signaling pathway independent of SA (SA- independent ISR pathways) includes those dependent on ET and JA signaling (Rabari *et al.*, 2023).

ENDOPHYTES AS ISR

This is another mechanism that endophytic microorganisms control pathogens. These endophytes perceive the characteristic to reduce disease susceptibility against pathogen attack through activating induced resistance to the host plant. At first, systemically acquired resistance (SAR), or resistance brought on by infections, was differentiated from immune system resistance (ISR) (Knoester *et al.*,1999). Through the communication of growth regulators, both inducers activate reactions from the plant's defense mechanism, increasing resistance against infections. On the other hand, SAR triggers a localized reaction that leads to an accumulation of salicylic acid and the synthesis of gene products associated with pathogenicity, which ultimately causes an overreaction. Visual signs appear at the infection site because of this. Conversely, ISR entails signaling via ethylene/salicylic acid, ethylene/jasmonic acid, or both combinations, activating genes related to pathogenicity that produce proteins that reduce damage from free radicals. Consequently, ISR shields plant cell walls without causing obvious symptoms. (Elhamouly *et al.*, 2022; Hemmati *et al.*, 2023; Salwan *et al.*, 2023). Even with our current understanding of ISR, there are still a lot of questions. Knowing which microbes cause ISR and how they affect responses of the plant defense system makes it easier to create biotechnological solutions that effectively manage illness (Salwan *et al.*, 2022).

The familiar signaling pathways are regulated by ISR through phytohormones includes ethylene or jasmonic acid and SAR through salicylic acid (Fig. 1) (Card *et al.*, 2016).

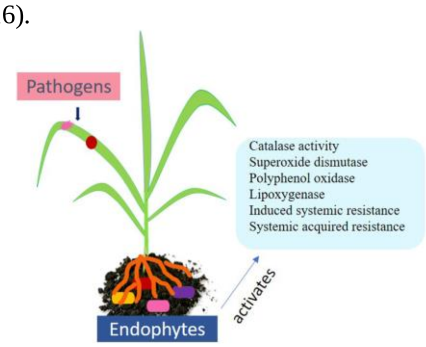

Fig. 1. Activation of plant defense system by Endophytes.

A. Bacterial endophytes as ISR

Based on latest studies, many similar plant diseases affect the plant system which resulted in declining of crop productivity. Among those, some of them cause serious illness such as wilt disease, leaf spot, root rot, leaf curl, powdery mildew and blight. To address these phytopathogens, endophytic bacteria are significant. By secreting proteins associated with pathogenesis (PRPs) and defense enzymes that inhibit the growth of phytopathogens which cause disease by production of siderophores, systemic resistance and antimicrobial compounds. Due to these properties bacterial endophytes act as biocontrol agents (Pandey *et al.*, 2019). Generally, chemical pesticides are used to control phytopathogens, however this strategy has elevated major concerns for environmental pollution and give raise to the expose of resistance to chemicals over time (Mushtaq *et al.*, 2023). *Arthrobacter, Bacillus, Pantoea, Burkholderia, Streptomyces,. Rhizobium, Enterobacter, Micrococcus, Pseudomonas, Serratia, etc.*, are the some of the examples of bacterial endophytes as biocontrol agents against phytopathogens (Prasad *et al.*, 2020).

B. Fungal endophytes as ISR

Fungal endophytes are act as most familiar bio-control agents for phytopathogens and are omnipresence in plant system. Majority of fungal species plays various participation against several plant pathogens. Fungal endophytes give various benefits to be used as disease control agents, by using these agents to enhance the plant growth and development and induced systemic such as inhibition of secreting variety of antifungal secondary metabolites lipopeptides, enzymes and antibiotics via colonization and fight with other disease causing pathogens for growth factors includes space and nutrients (Alam *et al.*, 2021; Jha *et al.*, 2023).

MECHANISM OF ACTION OF ENDOPHYTES

Endophytes are prominent to have capability for protecting plants against detrimental diseases that disturb plant health. These endophytic microorganisms can promote biocontrol by various mechanisms that include (1). Antagonism versus phytopathogens by synthesis of siderophore (2). Production of antibiotics (3). Production of lytic enzymes and (4). Capability to persuade ISR in plant system against phytopathogens (Latha *et al.*, 2019; Vázquez-Garcidueñas *et al.*, 1998)

A. Production of siderophores

For growth and development for all living organisms require iron, so it called as fourth important element in the earth's crust. Under aerobic circumstances, iron is not easily available even though it is abundant in the earth's crust because the free ferrous iron (Fe^{2+}) is oxidized to the ferric iron (Fe^{3+}) to form oxyhydroxide polymers that is not readily soluble. In Greek word siderophore represents "iron carriers" or "iron bearers". It is secreted by various PGPR, including endophytic microorganisms which has low in molecular weight is about 500-1500 Daltons. These iron molecules act as iron chelators, that made available form of iron to the root system, by that suppress the association of pathogens which is not supplying them adequate iron (Zhang *et al.*, 2013).

Most of the endophytes are described as siderophore producers, where *Pseudomonas* sp. is identified as one of the major groups. The bacterium *P. fluorescens* was first established the secretion of siderophore as a main strategy of bio-control agents on *Erwinia carotovora*, so that a direct correlation was settled in vitro of synthesis of siderophore by *P. fluorescens* versus their capability to control germinition of pathogens such as *Fusarium* sp. The synthesis of various siderophore includes hydroxymate, catecholets, and carboxylate by endophytic microorganisms which are capability to inhibit the various phytopathogens. So, it is referred as siderophore-producing biocontrol agents (Rajkumar *et al.*, 2010).

B. Antibiosis

Antibiosis defined as the competence of an endophytic microorganism to control disease causing pathogens by the synthesis of antibiotics or toxins. Antibiotic production is an effective mechanism occupied by several endophytes to inhibiting soil pathogens (Duffy *et al.*, 2003). This competence can correlate with induced systemic resistance in crops through basic antimicrobial mechanism by the presence of biosynthetic genes, due to this mechanism antibiotics act as a determinant that provoking the response. Antifungal compounds such iturin A synthesized by *Bacillus subtilis* and pyrrolnitrin synthesized by *Serratia plymuthica* are released by the endophytic bacteria.

Microbial antagonists employ diversified collection of strategies that influence the interactions of plant pathogens; the pathogen may have notably various responses to mitigate the antagonisms. These responses include active efflux of detoxification, antibiotics, repression of antibiotic resistance and biosynthetic genes (Duffy, 2006).

C. Production of lytic enzymes

The lytic enzymes namely celluloses, lipases, chitenases, proteases, glucanases and pectinases produced by endophytic microorganisms which is to infest disease causing fungal strains. Inhibition of germ tube elongation and spore germination by endophytic bacteria can destroy plant pathogenic fungi. The synthesis of degrading enzymes from extracellular cell wall has been correlated with biocontrol potential of the enzyme producing bacteria. This enzyme potentiality which activates the plant to sheltered from pathogens in plant system. Chitinases, beta 1-3 glucanases, and proteases released from *Trichoderma viride*and *Trichoderma harzianum* were found to be lytic enzymes that dramatically decreased the prevalence of *Aspergillus niger* caused collar rot disease (Gajera and Vakharia 2012). It facilitates the disintegration of glycosidic bonds. Likewise, the antagonistic action of beta -1, 3-glucanases produced from *Trichoderma harzianum* was demonstrated by their ability to hydrolyze the O-glycosidic bond of b-glucan chains in the parasitic fungus *Sclerotinia sclerotiorum*. White mold is a harmful disease that occurs in *Phaseolus vulgaris* (Whipps, 2001).

Table 1: Summary of mode of action of endophytic bacteria.

Activities	Mechanisms	References
Root colonization through competition	Distinct stage of development, adhering to the roots, mobility, efficient utilization of the organic acids found in root exudates, and the production of several components, such as amino acids, via the type III secretion system.	(Chaudhary *et al.*, 2021)
Antibiotics and antibiosis inhibitors	Biosynthesis of volatile HCN, pyoluteorin, pyrrolnitrin, and phenazines, among other antibiotics.	(Card *et al.*, 2016; Constantin *et al.*, 2019)
	Generation of volatiles such as 2, 3-butanediol, 6-pentyl-α-pyrone, and DMDS, as well as antibiotics such as D-gluconic acid and 2-hexyl-5-propyl resorcinol.	
	Various lipopeptides, including surfactin, fengycin, polymyxin, bacitracin, and the iturin group, have the potential to exhibit significant effects on disease control.	
	Phloroglucinol, pyrrolnitrin, phenols, and volatile organic compounds such as benzothiazole, pyrazine (2,5-dimethyl), and phenolic derivatives are produced.	
Interference between signals	AHL molecule inactivation, which is necessary for the synthesis of exoenzymes.	(Dandurishvili *et al.*, 2011)
Conflict for ions of ferric iron	Formation of siderophores to bind to ferric ions and starve the infections of iron.	(Zeng *et al.*, 2018)
Competence for nutrients and niches (CNN)	The competitive root colonization method also applies to CNN.	(Malfanova *et al.*, 2013)
Detoxification and virulence factor degradation	Detoxification of toxins by fusaric acid synthesized by pathogens.	(Cho *et al.*, 2003)
	Ability of quorum sensing by destroying autoinducer signals and preventing the production of certain virulence genes.	
Induced systemic resistance (ISR)	Induction of resistance by the synthesis of siderophores, salicylic acid, pyocyanins, c-LPs, etc.	(Elhamouly *et al.*, 2022; Hemmati *et al.*, 2023; Salwan 2022; Salwan, 2023)
	Co-application of endophytic bacteria with chemical elicitors such as derivatives of chitin, chitosan will increase the ability of ISR.	
	Increased formation of peroxidases, PAL and PPO enhances ISR.	
	Phytoalexins induction will increase the activity of lipoxygenase.	

D. Production of secondary Metabolites

It is well known that most endophytes generate secondary metabolites with potent antibacterial and antifungal properties that inhibit the growth of pathogenic microbes. Numerous metabolites from endophytic bacterial and fungal strains have been found, including phenols, flavonoids, peptides, steroids, and terpenoids. Alkaloids have a strong ability to prevent the growth of microorganisms. According to Panaccione *et al*. (2014), fungi endophytes such *Clavicitaceae* sp. that was isolated from the grass family produced alkaloids that are toxic to aphids. Alkaloids have been found to contaminate specific hosts and to slightly harm species that are not their intended targets. Strong antibacterial activity against pathogenic bacteria was demonstrated by the alkaloid Altersetin, which was isolated from *Alternaria* spp. (Akutse *et al*., 2013; Hellwig *et al*., 2002). Using GS-MS analysis, it was discovered that *A. alternate* AE1 isolated from neem leaves produced thermostable metabolites such d-norandrostane and longifolenaldehyde. According to Chatterjee *et al*. (2019), both compounds exhibit zone of inhibition against a variety of gram-positive and gram-negative bacteria in addition to their bactericidal and antioxidant qualities. In addition, it is commonly known that many endophytes have antibiotic action. Numerous endophytes produce lipopeptides, which are widely known for their antibacterial properties and may also exhibit surfactant and antimicrobial properties. The *Bacillus amyloliquefaciens* strain generates lipopeptides that have biocontrol properties towards the fungal pathogen *Erysiphe cichoracearum*. *Bacillus* sp. generated fengycin, iturin, and surfactin, which all assisted in preventing the growth of the fungal infection. Additionally, the capacity of infections to colonize was impacted by the production of pellicle biofilms (Jiao *et al*., 2021).

CONCLUSIONS AND FUTURE SCOPE

Recently, endophytic microorganisms have become increasingly recognized due to their ability to effectively colonize and adapt. Besides their role in managing phytopathogens, endophytes also promote growth through mechanisms such as modulating phytohormones, solubilizing phosphates, and producing siderophores. Moreover, they have the added advantage of infiltrating plant tissues, establishing a presence within the host, and successfully passing to the subsequent generation of plants through seeds, unlike rhizospheric microbiota. Identifying compatible microbes capable of colonizing within host tissues and being transferred to future plant generations via seeds could offer a novel strategy to maximize the benefits of endophytes in controlling plant diseases. In addition, it is essential to thoroughly examine the interactions between selected endophytes and plant microbiota to gain a comprehensive understanding of both the positive and negative implications of utilizing endophytes within plant microbiomes.

The criteria used for screening play a crucial role in identifying potential endophytes. Endophytes like *Trichoderma* sp., *Bacillus* sp., and *Pseudomonas* sp. are frequently identified through standardized isolation methods. Nonetheless, it is essential to adjust the screening parameters to extract endophytes capable of withstanding biotic and abiotic stress conditions and subsequently assess them in various environmental settings. Additionally, recent advancements in NGS technology and omics methodologies can aid in the discovery and screening of non-culturable endophytes, as well as in exploring plant-pathogen-endophyte interactions at a molecular level. Furthermore, creating a global, country-specific database of isolated endophytes and their metabolites could provide a strategic framework for further screening, identification, and utilization of endophytes in managing plant diseases.

Fungi and bacteria are the most examined endophytes in managing plant diseases; however, recent research has highlighted the significant contribution of endophytic actinomycetes and yeasts in this area. Additional research on non-traditional microbial types as endophytes is needed to identify more effective candidates that can help plants withstand biotic stress.

Although endophytes offer several advantages in managing plant diseases, their application in field settings remains restricted. Greater attention should be directed towards identifying stress-resistant endophytes and facilitating their application from laboratory research to agricultural practice. Additionally, efforts must be made to inform end users about this technology to promote effective and eco-friendly plant disease management.

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Conflicts of Interest. None.

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