

14. Microbes in Management of Plant Disease for Sustainable Food Production in Tuber Crop Production

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Abstract:

*Plant disease management is crucial in modern agriculture, and leveraging phyllosphere microorganisms has emerged as a promising strategy. The Phyllosphere, encompassing the above-ground plant parts, especially leaves, hosts a diverse microbial community influencing plant health. Interactions within this community provide sustainable and eco-friendly solutions for disease control. Phyllosphere microorganisms act as biological control agents, inhibiting plant pathogens. Bacteria like *Bacillus subtilis* produce antimicrobial compounds suppressing harmful fungi, while *Trichoderma* species combat pathogens on leaf surfaces. These microorganisms contribute to disease management through induced systemic resistance (ISR). Beneficial microbes, such as *Pseudomonas fluorescens*, stimulate plant defense mechanisms, enhancing resistance against pathogens. ISR primes the plant's immune system, reducing disease severity upon subsequent attacks. Understanding the phyllosphere microbiome dynamics is crucial for effective disease management. The leaf surface microbial community acts as a biological barrier, preventing pathogenic establishment. Studying these interactions allows identifying key microbial players for targeted disease control. Endophytic microorganisms within plant tissues produce antimicrobial secondary metabolites, protecting against pathogens.*

Exploring and utilizing endophytes offer potential for biocontrol agents targeting above-ground plant diseases. The phyllosphere hosts microorganisms capable of biological nitrogen fixation, contributing to plant nutrition and overall health. Nitrogen-fixing bacteria associated with leaves convert atmospheric nitrogen, enhancing plant resilience against diseases. Phyllosphere microorganisms indirectly contribute to disease management by promoting plant growth. They improve nutrient uptake, produce growth-promoting hormones, and enhance stress tolerance, creating an unfavourable environment for pathogens. The balance established by beneficial microorganisms' limits disease opportunities.

Keywords:

Microbes, plant disease, Biocontrol, Sustainable management

14.1 Introduction:

Soil, a critical component of the environment, not only serves as a valuable natural resource in agriculture but also sustains numerous life processes. Agricultural productivity is intricately linked, either directly or indirectly, to the diverse microbial communities inhabiting the soil. In recent years, plant growth-promoting bacteria (PGPB), especially endophytic bacteria, have emerged as key contributors to sustainable agriculture (Basu *et al.*, 2017; Riederer and Muller, 2008). Various forms of beneficial relationships exist between plants and microorganisms, including mutualism, commensalism, symbiosis, cohabitation, biofilms, and more. Among these, endophytes constitute a group of microorganisms residing within the host microenvironment, receiving protection from environmental stresses, gaining access to nutrients, and consequently facing reduced competition from other microbes. Microorganisms play a crucial role in plant disease management through various mechanisms that can either directly inhibit pathogens or enhance the plant's defense mechanisms (Dogra *et al.*, 2019). The Phyllosphere refers to the above-ground parts of plants, including leaves, stems, and flowers. The Phyllosphere is a dynamic environment that supports a diverse community of microorganisms, including bacteria, fungi, and viruses.

These microorganisms play crucial roles in plant health, development, and ecology. Here are some key points about Phyllosphere microorganisms. Phyllosphere microorganisms are crucial for plant health and ecosystem functioning (Singha *et al.*, 2019). Understanding their classification and role in plant-microbe interactions is essential for developing sustainable agricultural practices and disease management strategies. The Phyllosphere refers to the above-ground parts of plants, particularly the leaves, where a diverse community of microorganisms, including fungi and bacteria, can be found. Classifying Phyllosphere fungi and bacteria involves categorizing them based on various characteristics such as morphology, physiology, and genetic traits (Gupta *et al.*, 2010). Recent cultivation-independent studies have provided valuable insights into the composition of microbial Phyllosphere communities. It is evident that these communities do not form randomly but undergo a selection process, resulting in relatively predictable microbial communities characterized by a limited number of dominant (sub-) phyla. This observation is based on assessments of community composition concerning plant host species, as well as temporal and spatial factors (Ravishankar *et al.*, 2023). While most research on the Phyllosphere has

concentrated on bacteria and, to a lesser extent, fungi, it appears that archaea are not notably abundant in this environment. Endophytes play a more crucial role in the plant defense mechanism as they engage directly with the plant system compared to rhizosphere and Phyllosphere bacteria (Sturz *et al.*, 2000) within plant systems, the intercellular spaces serve as optimal sites for the growth and multiplication of endophytic bacteria due to the abundance of essential nutrients such as potassium, calcium, sulfur, carbohydrates, as well as various amino acids and organic acids (Maurya *et al.*, 2018).

Various common plant diseases have a significant impact on global crop productivity, including blight, canker, leaf curl, leaf spot, powdery mildew, root rot, and wilt disease. Endophytes, comprising bacteria, fungi, and actinomycetes, play a crucial role in combatting phytopathogens. Endophytic bacteria, for example, produce antimicrobial compounds, siderophores, and induce systemic resistance through the synthesis of pathogenesis-related proteins (PRPs) and defense enzymes, inhibiting disease development by phytopathogens bacterial endophytes also exhibit promise as biocontrol agents. Plant microorganisms causing diseases adversely affect crop yields by significantly reducing plant performance and crop quality (Gupta *et al.*, 2014; Simon *et al.*, 2021; Maurya *et al.*, 2023).

Endophytic bacterial strains such as *Lysinibacillus* sp., *Bacillus subtilis*, nitrogen-fixing bacteria (*Azotobacter chroococcum*), and phosphate-solubilizing bacteria (PSB) like *Pseudomonas cepacea*, as well as their combination as bio-fertilizers, have been demonstrated to inhibit bacterial wilt disease incidence in chili plants by up to 80% (Istifadah *et al.*, 2017; John *et al.*, 2019). The endophytic bacterial strain *B. subtilis* exhibited the highest (80%) disease suppression and significantly promoted chili growth. Traditional methods for controlling phytopathogens involve chemical pesticides, but concerns about environmental contamination and the development of resistance over time have led to a search for alternative solutions. Endophytic bacteria, acting as biocontrol agents or bioinsecticides (Beattie, 2006), present an effective alternative to chemical pesticides. Numerous mechanisms contribute to the biocontrol of plant diseases, including direct antagonism through the production of antibiotics, siderophores, hydrogen cyanide, hydrolytic enzymes (chitinases, proteases, and lipases), and more (Vincent *et al.*, 2022; Pant *et al.*, 2023). In a study conducted in Iran, endophytic bacteria isolated from healthy wild pistachio trees demonstrated biocontrol potential against bacterial plant pathogens. For instance, the *Pseudomonas protegens* Pb78 strain exhibited the highest inhibitory effects on bacterial plant pathogens, with *Pseudomonas syringae* pv. *syringae* Pss 20 and *Pseudomonas tolaasii* Pt18 showing a lesser effect under in vitro conditions (Fu *et al.*, 2016). Plant diseases caused by microorganisms can significantly reduce crop yields and impact plant performance and quality (Pandey *et al.*, 2022 b; John *et al.*, 2020).

Bio-fertilizers comprising endophytic bacterial strains such as *Lysinibacillus* sp., *Bacillus subtilis*, nitrogen-fixing bacteria (*Azotobacter chroococcum*), and phosphate-solubilizing bacteria (PSB) like *Pseudomonas cepacea* have demonstrated efficacy in inhibiting bacterial wilt disease in chili plants by up to 80%. The most effective strain, *B. subtilis*, not only exhibited an 80% disease suppression but also significantly enhanced chili plant growth. traditional control methods involving chemical pesticides have raised environmental contamination concerns and led to the development of resistance.

Consequently, there is a growing interest in using endophytic bacteria as biocontrol agents or bio-insecticides, offering an environmentally friendly alternative to chemical pesticides. Various mechanisms contribute to the biocontrol of plant diseases, including direct antagonism through the production of antibiotics, siderophores, hydrogen cyanide, and hydrolytic enzymes such as chitinases, proteases, and lipases. Endophytic bacteria isolated from healthy wild pistachio trees in Iran, including genera like *Pseudomonas*, *Stenotrophomonas*, *Bacillus*, *Pantoea*, and *Serratia*, exhibited promising plant growth-promoting potential and biocontrol activity (Pandey *et al.*, 2022 a). For instance, the *Pseudomonas protegens* Pb78 strain displayed the highest inhibitory effects on bacterial plant pathogens, with *Pseudomonas syringae* pv. *Syringae* Pss 20 and *Pseudomonas tolaasii* Pt18 showing lesser effects under in vitro conditions. The terms "Phyllosphere endophytes" and "epiphytes" refer to microorganisms that inhabit the surfaces of plants, particularly on leaves. However, there are distinctions between these two concepts:

14.2 Phyllosphere Endophytes:

Definition: Phyllosphere endophytes are microorganisms that live within the internal tissues of plant leaves without causing any apparent harm to the host.

Location: These endophytes reside inside the leaf tissues, making them distinct from epiphytes, which are found on the external surfaces of leaves.

Relationship: Phyllosphere endophytes can form symbiotic relationships with plants, providing benefits such as improved resistance to pathogens or enhanced tolerance to environmental stress.

Epiphytes:

Definition: Epiphytes are microorganisms, including fungi and bacteria, that live on the external surfaces of plant tissues, such as leaves.

Location: Unlike Phyllosphere endophytes, epiphytes do not invade the internal tissues of the plant; instead, they colonize the outer surfaces.

Relationship: Epiphytes can have various relationships with their host plants. Some may be commensal, where they neither benefit nor harm the plant, while others may be pathogenic, causing diseases. Some epiphytes may also have mutualistic relationships with plants.

Both Phyllosphere endophytes and epiphytes play important roles in plant health and ecology:

Phyllosphere Endophytes: They can contribute to plant defense mechanisms, produce bioactive compounds that deter herbivores or pathogens, and enhance the overall fitness of the host plant.

Epiphytes: While some epiphytes can be harmful and cause diseases, others may contribute to nutrient cycling, influence plant physiology, or have mutualistic relationships that benefit both the plant and the microorganism.

14.3 Role of the Endophytes in Plant Disease Management:

Endophytes play a crucial role in enhancing plant defense mechanisms and managing stress in host plants, with various direct and indirect beneficial mechanisms. These microorganisms are associated with phytoremediation, bio-prospecting, phytochrome synthesis, and siderophore production. Additionally, endophytic bacteria exhibit a range of biocontrol mechanisms to directly counter plant pathogens and insects. They are known to promote plant growth and manage diseases through the production of phytohormones like abscisic acid (ABA), auxin, cytokinin, and the enzyme ACC deaminase. Moreover, they prevent infections through antifungal or antibacterial pathogens, siderophore production, nanoparticles synthesis, and by establishing the plant's systemic resistance. Endophytic bacteria, particularly as plant growth-promoting bacteria (PGPB), offer advantages over conventional chemical control methods due to their eco-friendly and non-toxic nature.

Their application is sustainable for both the environment and human health. Furthermore, PGPB endophytes act against pathogens in ways that enhance crop growth and yields. For instance, four endophytic bacterial strains-*Ochrobactrum* sp. CB36/1, *Pantoea agglomerans* CC37/2, *Bacillus thuringiensis* CA41/1, and *Pseudomonas fluorescens* CC44-successfully suppressed bacterial spot disease effects on tomato and pepper plants caused by *Xanthomonas euvesicatoria* (Xe). *Bacillus* genus members are recognized as producers of biomolecules inhibiting the growth of multiple phytopathogens. Lipopeptides, particularly, have shown efficacy as versatile weapons against various phytopathogens. *Bacillus* spp. isolated from different maize varieties produce antifungal lipopeptides, with extracts showing inhibitory action against *Fusarium moniliforme*.

The endophytic bacteria in maize varieties may contribute to host protection by secreting antifungal lipopeptides that inhibit pathogens and induce the upregulation of pathogenesis-related genes in host plants. Additionally, endophytic bacteria from spinach roots and Agave tequilana plants demonstrated effective control against *Fusarium* wilt and antagonism against *Fusarium oxysporum* AC132, respectively.

Endophytic bacterial strain *Enterobacter cloacae* SM 10, isolated from spinach roots, significantly suppressed disease incidence by inhabiting xylem vessels. Similarly, isolates from healthy Agave tequilana plants, identified as *Acinetobacter* sp., *A. baumannii*, *A. bereziniae*, and others, exhibited plant growth-promoting activities and antagonism. Moreover, bacterial strains like *Bacillus cereus* and *B. subtilis*, isolated from different plant sources, demonstrated inhibition of various pathogens' growth and chitinase activity. These findings highlight the potential of endophytic bacteria as biocontrol agents for diverse plant diseases, contributing to sustainable agriculture and environmental health. Endophytes are microorganisms, primarily fungi and bacteria, that live within the tissues of plants without causing any apparent harm to the host. These symbiotic relationships can be beneficial for both the plant and the endophyte. The classification of endophytes is primarily based on the type of organism and the specific plant host they inhabit (Maurya *et al.*, 2019 b) Endophytes

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14.4 Fungal Endophytes:

Ascomycetes: These are a major group of fungi that form symbiotic relationships with plants. Examples include species of Clavicipitaceae, which are known for producing alkaloids that can protect the host plant from herbivores.

Basidiomycetes: Another group of fungi that includes endophytes like *Cryptococcus* and *Rhizoctonia*.

Bacterial Endophytes:

Actinobacteria: Some endophytic actinobacteria are known for their production of bioactive compounds with potential agricultural and pharmaceutical applications.

Proteobacteria: Certain members of the Proteobacteria, such as nitrogen-fixing bacteria, can form endophytic associations with plants.

Based on the plant host:

Grass Endophytes: Many endophytic fungi are associated with grass species. For example, Epichloë species form endophytic relationships with grasses like ryegrass and fescue.

Tree Endophytes: Endophytes can also be found in trees, forming associations with various types of woody plants. Examples include Colletotrichum species in eucalyptus trees.

Herbaceous Plant Endophytes: Endophytes are found in various herbaceous plants, forming symbiotic relationships with the host. Examples include Fusarium species in various herbaceous plants.

Crop Endophytes: Some endophytes are associated with important crop plants. For instance, Azospirillum species can form endophytic associations with crops like wheat and rice.

14.4.1 Fungal Endophytes in Plant Disease Management:

The devastating impact of fungal pathogens on crops, leading to plant death, reduced yield, and diminished quality, as well as the production of harmful mycotoxins, poses significant challenges to global agriculture. In response, synthetic chemical fungicides have become a staple in agricultural practices, aiming to control these fungal threats. However, the use of such fungicides raises environmental concerns due to their non-target impacts, affecting beneficial fungi crucial for crop health, including mutualistic fungi like arbuscular mycorrhizae.

Arbuscular mycorrhizae play a pivotal role in enhancing plant fitness, and their loss due to extensive fungicide application can result in dramatic declines in plant health. Moreover, chemical fungicides often exhibit selectivity, harming non-target beneficial microorganisms more than the targeted pests. To address these ecological concerns, biocontrol endophytes, exemplified by *Ampelomyces*, offer environmentally friendly alternatives. Biocontrol endophytes effectively decrease the prevalence of pathogenic fungi while preserving mutualistic fungal relationships, presenting a sustainable approach to agricultural pest management (Shubhransu Nayak *et al.*, 2017) (Misci *et al.*, 2022; Maurya *et al.*, 2019 a)

The integration of biocontrol agents into pest management strategies aligns with the principles of integrated pest management (IPM), contributing to improved sustainability in agriculture and the maintenance or enhancement of soil health. This holistic approach also has the potential to mitigate or manage chemical pesticide resistance, a growing concern in modern agriculture. Secondary metabolites produced by endophytes have garnered considerable attention for their potential as agrochemicals. Studies have employed top-down approaches to extract and isolate diverse compounds from specific taxonomic orders of fungi, such as Xylariales, revealing a wealth of bioactive metabolites. These compounds include glucosides, cytochalasans, azaphilones, terpenoids, non-ribosomal peptides, macrolide polyketides, benzenoids, and lactones. The identification and characterization of these compounds contribute to the exploration of natural products with agricultural applications. Researchers adopt various methods to identify specific antifungal compounds produced by endophytes.

This includes characterizing fungal endophytic diversity within host plant species, selecting endophyte cultures for dual-culture assays to assess antagonism against known plant pathogens, and subsequent extraction and analysis of compounds. Liquid chromatography and gas spectrometry coupled with mass spectrometry provide valuable insights into the chemical composition of these compounds. The results aid in identifying candidate endophyte species with biocontrol potential, underscoring the intrinsic role of antagonists within the plant microbiome. Moreover, endophytes contribute to host plant resistance against fungal pathogens by inducing a systemic response upon colonization (Maurya *et al.*, 2020 b.)

This response involves the initiation of a defensive strategy by the plant, reinforcing cell walls through the deposition of defensive compounds. Although endophytes possess mechanisms, such as exoenzymes, to access these strengthened cells, the deposits act as a barrier, preventing pathogens from penetrating. Understanding these mechanisms sheds light on how endophytes enhance plant resistance and underscores their multifaceted role in promoting overall plant health.

In summary, the intricate interactions between endophytes, plants, and fungal pathogens unveil a promising avenue for sustainable agricultural practices. The exploration of biocontrol endophytes, the identification of bioactive compounds, and the understanding of their mechanisms contribute to the development of environmentally friendly alternatives to synthetic chemical fungicides, fostering agricultural sustainability and ecosystem health (Abo-Elyousr *et al.*, 2014).

14.5 Endophytes of Selected Tubers:

Yam endophytes in tubers namely *Erwinia pyrifoliae* and *Erwinia pantoaea* have been isolated from tubers by Zhang *et al.* (2010) though, bacterial strains namely; *Erwinia pyrifoliae* and *Erwinia pantoaea* complex have been isolated from yam tubers by Zhang *et al.* (2010) and Omoregie *et al.* (2019), in some cases simultaneous inhabitation of dark septate endophyte and arbuscular mycorrhizae fungi which are good symbionts have also been observed in Yam tuber (Maggirwar *et al.*, 2013).

Sweet potato endophytes: Quite a number of endophytic bacteria, fungi and actinomycetes from sweet potato have been isolated, identified and their biological importance in agriculture documented (Adachi *et al.*, 2012).

For instance, eleven bacterial endophytes of the genera *Phyllobacterium*, *Enterobacter*, *Rhodanobacter*, *Pseudomonas*, *Rahnel-la*, *Xanthomonas* and *Stenotrophomonas* have been isolated from sweet potato stems. Among these endophytes, *Pseudomonas*, *Rahnella* and *Enterobacter* produced higher amount of Indole acetic acid (IAA) which have plant growth promoting effect.

Yam Bean endophytes: The presence of some endophytic bacteria and actinomycetes has been documented to exhibit symbiotic association with Yam bean. For instance, twenty-five isolates belonging to the genera *Rhizobium* and *Bradyrhizobium* isolated from root nodules of yam bean tuber, established effective nodules containing bacteroid cells in their peribacteroid membrane which confer nitrogen-fixing potential to the plant (Fuentes *et al.*, 2012, Sorensen, 2016).

Cassava endophytes: The existence of endophytes in cassava have long been suspected before now due to the fact that asymptomatic cassava plants grown in the same field with symptomatic cassava plants frequently showed a wide range of variation in root yield. Also, root yield of low-yielding virus-free cassava plants of traditional clones may be increased by meristem culture, furthermore, the root yield of meristem culture-derived cassava plants decreased uniformly sharply under field conditions coupled with the long growing cycle of cassava allowed infection and dissemination of diseases (Shubhransu Nayak *et al.*, 2017; Maurya *et al.*, 2023 a).

The bacterial isolates include; *Hyphomicrobium*, *Bacillus*, *Psuedomonas*, *Paenibacillus* among others. The fungal isolates include; *Septoria nodurum*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides*, *C. graminicola*, *Alternaria termissima*, *Trichoderma* sp., *Botrytis* sp., *Torula* sp., *Nigrospora* sp., among others, while the actinomycetes isolates include *Streptomyces malaysiensis*, *Streptomyces avermitilis* and *Streptomyces griseus*, etc (Rivera *et al.*, 2013; Khucharoenphalsan *et al.*, 2016; Maurya *et al.*, 2023 b).

Both detrimental and beneficial effects of endophytes have been observed in cassava plant depending on the method of inoculation. For example, a fungus *Curvularia* sp. was found to be detrimental when inoculated by spraying, but beneficial when inoculated by immersion or puncturing on plantlets and callus tissues of cassava variety M Col 2215 (Shubhransu *et al.*, 2017).

14.6 Conclusion:

The intricate relationships between plants and microbes play a pivotal role in the dynamic field of plant disease management. Endophytes association with tuber crops confer enormous benefits, as they have been shown to induce systemic resistance to biotic and abiotic stresses, increase in biomass, nitrogen fixation, protection against pathogens, among others in these crops. A couple of endophytic fungi, actinomycetes and bacteria may be applied as potential biofertilizers due to their nitrogen-fixing ability, and they can be used as antibacterial agents and fungicides in tuber crops with minimal environmental risks due to their ability to produce secondary metabolites. The devastating impact of fungal pathogens on crops and the associated risks of chemical fungicides have spurred a growing interest in harnessing the potential of plant-associated microbes for sustainable and eco-friendly solutions (Saxena *et al.*, 2016). Biocontrol endophytes, exemplified by fungi like *Ampelomyces*, emerge as promising alternatives to synthetic chemical fungicides. These microbes demonstrate the ability to reduce pathogen prevalence while preserving essential mutualistic relationships within the plant microbiome. The integration of biocontrol agents into integrated pest management approaches not only mitigates the environmental impact of chemical pesticides but also contributes to the overall sustainability of agricultural practices. Exploration into the secondary metabolites produced by endophytes adds another layer to the potential applications of plant-associated microbes. The identification of diverse and bioactive compounds holds promise for developing natural products that can serve as effective agrochemicals. This avenue of research not only expands our understanding of microbial diversity but also offers practical solutions for enhancing crop health and resilience against fungal pathogens. Moreover, the mechanisms through which endophytes enhance host plant resistance add depth to our comprehension of plant-microbe interactions. The induction of systemic responses, such as the reinforcement of cell walls, provides insights into the sophisticated strategies employed by plants and their associated microbes in the ongoing battle against pathogens. Understanding these mechanisms is crucial for optimizing the use of plant-associated microbes in disease management strategies. As we navigate the challenges of global agriculture, the holistic approach of integrated pest management, coupled with the judicious use of plant-associated microbes, stands out as a beacon for sustainable practices. The promotion of soil health, the reduction of chemical pesticide resistance, and the preservation of essential microbial communities within the plant ecosystem all contribute to the overarching goal of achieving agricultural sustainability.

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