



## Bacterial Endophytes: diversity, colonization and importance as plant growth promoters

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### Abstract

Plant helpful bacteria called endophytic bacteria survive inside plants and can help them grow better in both common and complex environments. They can directly advantage host plants by enhancing nutrient absorption and regulating phytohormones that regulate development and exertion. Indirectly, endophytic bacteria can promote plant health by using antibiotics, hydrolytic enzymes, nutrient restriction, and priming plant defences to target pests and diseases. Endophytes penetrate plant tissues through the root zone or aerial regions, as well as by germination radicles, secondary roots, stomata, and foliar routes. Endophyte-plant polymer degrading enzymes like cellulases and pectinases are involved in internal colonisation and may be identified via immunological or *in situ* hybridization or reporter gene tagging. Endophytes interact with their host plant biochemically and genetically, synthesising osmolytes, osmoprotectants, and antioxidants that help the plants cope with abiotic stress. Few endophytic bacteria have a wide host category and can be exploited as bioinoculants in the development of a secure and long-term farming system. The variables that influence the variety of bacterial endophytes, their host specificity, and plant growth promotion processes are discussed in this study. In addition, the review highlights diverse methodologies for studying endophytic populations, wild plants as a resource of new endophytic bacteria, and emerging tactics that may improve plant endophyte relationship. Furthermore, the obstacles of studying bacterial genes expressed in plants are emphasised. Some endophytes are able to promote the growth of plants. For those strains the mechanisms of plant growth-promotion known to be employed by bacterial endophytes are similar to the mechanisms used by rhizospheric bacteria, e.g., the acquisition of resources needed for plant growth and modulation of plant growth and development.

**Keywords:** endophytes, antibiotics, antioxidants, plant growth promoters, rhizospheric bacteria

### Introduction

Microorganisms are found all over the world and are unrivalled in their potential to develop new bioactive substances with biological properties. Endophyte (Gr. Endon= within; Phyton= plant) the term was first coined by de Bary in 1866. Nowadays, endophytic organisms are defined as “microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects and are reported to perform myriad biological roles.”

Microorganisms and plants develop a symbiotic relationship that is beneficial to both organisms (host and symbiont). The presence of the microbe in the plant has an effect on the plant's growth and health performance, which increases agricultural attributes such as root length and fresh and dry weight of the root and shoot, as well as crop production, soil quality, and the nutrient cycling [1]. Endophytes are prevalent in all plant species, living both inter and intracellularly. They interact biochemically and genetically to provide favourable effects on plant development and defence while avoiding harmful symptoms [2, 3, 4, 5].

Bacterial endophytes inhabit an ecological niche comparable to phytopathogens, making them good candidates for biocontrol agent [6]. Endophytes in bacteria can be either obligatory or facultative [3]. Obligate endophytes are transported vertically from parental tissue into the young plant before germination, but optional endophytes can be transferred horizontally or vertically from the host's environment. The rhizosphere or stomata (pores on the stem, cotyledons, wounds, flower, leaves and abrasionsetc) are the most common entrance points [3, 7].

Many endophytes belong to Bacillus, Burkholderia, and Pseudomonas, which are typical soil bacterial species [8].

Volatile chemical compounds, anticancer chemicals, antibiotics, antifungal substances, antiviral substances, insecticidal substances, and immunosuppressive substances are among the secondary metabolic products produced by these species. While endophytic organisms have produced a vast spectrum of physiologically active chemicals, they are still a largely unexplored source of new natural products.

### Sources of endophytes

Endophytes are found in all plants on Earth, including: ferns [9], mosses [10], shrubs [11], grasses [12], deciduous and coniferous trees and even lichens [13]. Most known endophytes are bacteria and fungi, but some endophytic algae and oomycetes are also reported. Endophytes are widespread and have been present in all plant species studied to date; however, much of the relationship between the endophytes and plants is not well known. Endophytic species varies in plant to plant and according to the environment conditions.

Endophyted pollen, stems, buds, tubers, seeds, fruits, roots, leaves, flower, and seed tissues all contain endophytes. They survive as non-pathogens in the intercellular gaps of plants, feasting on apoplastic nutrients and generating no defence responses [2, 7]. Although endophytic communities change with tissue type, plant age, and host genotype [14]. The root tissue of a few plants contains the largest concentration of endophytes [15].

Emerging points of lateral roots and zones of differentiation and elongation at the root tip, where somewhat disturbed or not totally differentiated tissues promote penetration, are prominent locations for active entry into the roots.

## Diversification of endophytes

According to recent estimates, there are over 300,000 plant species on the earth, the great multitude of which include endophytes<sup>[16]</sup>. Microbial endophytes (fungus and bacteria) have been discovered in each plant species studied so far. An endophyte-free plant, according to Partida-Martnez and Heil (2011)<sup>[17]</sup>, is a rare exclusion to what is generally observed in nature. A plant without endophytes would be less able to fight with infections and more vulnerable to environmental exertion conditions, according to findings of rhizospheric PGPB distribution in nature<sup>[18]</sup>.

Endophytic microorganisms have been proven in various studies to have the ability to suppress plant pathogen<sup>[19, 20, 21]</sup>, insects<sup>[22]</sup> and nematodes<sup>[23, 24]</sup>. The capacity of various bacterial endophytes to support plant development is the result of direct or indirect methods. When a bacteria either improves the uptake of vital nutrients or regulates the amount of hormones within a plant, it promotes direct plant development.

Henning and Villforth reported the existence of bacteria in healthy stems, roots and leaves in 1940, which sparked suspicion and sparked a huge zest in studying these plant micro symbionts. It should be emphasised that, despite their abundance, bacterial endophytes are isolated from plants less frequently than fungal endophytes<sup>[25]</sup>.

### 1. Elements affecting a plant's endophytic bacterial diversification

Apart from the ability of bacteria to colonise plants as endophytes, the host plant and environmental conditions can have a significant impact on the endophytic multiformity of a given plant. The sort of endophytic bacteria that a host plant has is determined by its age, genotype, geographical location, and climatic condition<sup>[26]</sup> even the tissue being studied<sup>[27]</sup>.

The type, concentration, and even length of occupational therapy time for a sterilising agent used to protect bacteria can all affect the range of bacteria recovered from a plant<sup>[23, 27]</sup>.

Endophytic diversity varies significantly across plant species growing in the common soil. More intriguingly, the endophytic body of a plant is effected by the type of soil it is grown in. As a result, endophytic bacteria from the same plant cultivar growing in various agricultural soils might be highly diverse. The variation in endophytic communities can be caused by a variety of factors.

In response to soil and stress, the plant host imposes a preference. circumstances Plants growing in petroleum hydrocarbon-contaminated soil acquired endophytic bacteria with the essential contaminant-degrading genes, according to<sup>[28]</sup>. Endophytic bacterial communities are selected by the host plant in a dynamic process<sup>[29, 30]</sup>, where bacteria that favour the plant host in a given condition are favoured by the plant over other bacterial endophytes.

### 2. Endophytic Bacterial Diversity Research Methods

The isolation approach should be susceptible enough to recover the majority of culturable endophytic bacteria while also being powerful enough to remove epiphytes and other contaminating bacteria from the plant tissues being processed. Surface sterilisation of plant tissues is usually followed by maceration, serial dilution, and plating on bacterial growth medium in most isolation protocols<sup>[31]</sup>.

Sodium hypochlorite, ethanol and hydrogen peroxide are commonly used as surface sterilizing agent<sup>[8, 32, 33]</sup>. After sterilization to eliminate any remaining chemicals, the sterilised tissue is rinsed numerous times with sterile distilled water.

### 3. Endophytic bacteria colonise aerial plant tissues-

The endophytic bacteria can spread systemically after entering the roots and colonise above-ground tissues. Despite this, due to the physiological necessity required to inhabit these plant niches, only a few bacteria can colonise the aerial vegetative sections of their host plants<sup>[34]</sup>. As a result, bacteria that move to above-ground plant tissue have evolved successfully to this endophytic habitat. Bacterial flagella and plant transpiration assist bacterial mobility within the plant<sup>[35, 36]</sup>.

### Colonization of plants by the endophytic bacteria-

A variety of bacterial characteristics influence endophytic colonisation of the host by the bacterium. The whole process of plant colonisation is regulated by these features, which are collectively referred to as colonisation characteristics. The procedure of colonisation necessitates extensive connection between the two spouses. The process usually begins at the roots and requires endophytic bacteria to recognise specific compounds in the root exudates<sup>[7, 37]</sup>. These root exudates are produced by plants to interact with profitable bacteria for their own ecological benefit<sup>[35]</sup>. Furthermore, endophytic bacteria have been observed colonising the plant interior in a series of events similar to rhizosphere colonisation by rhizobacteria<sup>[23]</sup>.

Methods based on AFP (autofluorescent protein) are now widely used to examine microbe-plant interactions and biofilm development<sup>[38]</sup>. Microorganisms have been detected and counted in situ on plant surfaces and in planta using these approaches<sup>[39, 40, 41]</sup>. One of these AFP (autofluorescent protein) strategies uses a marker system, which encodes the GFP (green fluorescent protein).

Intercellular gaps in the epidermis and cortical areas with high cell densities of up to 1010 cells-1cm<sup>3</sup> are the main sites of colonisation<sup>[3]</sup>. Invasion of vascular tissues and xylem cells is also possible, though at a lesser density. When endophytes colonise vascular tissue, they propagate throughout the body and into the shoots<sup>[35]</sup>. Endophytic colonisation is not as specialised as Rhizobia colonisation, but it does require a suitable host plant as well as defence systems<sup>[7, 42]</sup>. Endophytes' establishment and internal colonisation are influenced by surface features of bacteria and plant-polymer degrading enzymes such as cellulases and pectinases<sup>[35]</sup>.

### 1. Rhizosphere colonization by the endophytic bacteria-

The colonisation of the rhizosphere by endophytic bacteria is a very emulative challenge for them to occupy areas and get nutrients<sup>[43]</sup>. Only helpful or pathogenic bacteria that can colonise the plant rhizosphere competitively will flourish in this environment and have an impact on plant growth and development<sup>[44]</sup>. Bacterial characteristics such as mobility and polysaccharide synthesis are significant in plant rhizosphere colonisation.

The bacteria must colonise the plant rhizosphere and rhizoplane proficiently to provide positive effects on the host plant<sup>[35]</sup>. While colonising the host plant, they must also compete with other rhizospheric members<sup>[45]</sup>.

Furthermore, the bacteria do not infiltrate the root system of the host plant in a consistent mode. Different variables affecting the process of root colonisation result in non-uniform bacterial colonisation of plant roots. Root secretion patterns, bacterial adhesion and mobility, bacterial quorum sensing, bacterial growth rate, reducing emulsion by creating opposite chemicals, and effectively collecting nutrients are only a few of these characteristics <sup>[46]</sup>. Furthermore, the bacteria must metabolically adjust to the variety of nutrients available in the plant root exudates in order to be effective.

## 2. Endophytic Bacteria Colonise the Roots.

Bacterial endophytes are known to find their path within the plant root after establishing themselves in the rhizosphere and rhizoplane, colonising themselves with sub-populations ranging from 105-107 cfu/g fresh weight <sup>[34]</sup>. Polysaccharides, pili, and bacterial adhesins are involved in bacterial adherence to cell surface structures <sup>[47]</sup>. Nonetheless, each endophytic bacteria has its unique colonisation pattern and preferred colonisation sites <sup>[48]</sup>. Once these bacteria have located themselves on the root surfaces, they use specific processes to enter into the root core.

Penetration into the host might take place in a passive or active manner. Cracks in root emerging sites, root tips, or those caused by harmful creature might allow passive penetration <sup>[49]</sup>. Active entrance by adequate endophytic bacteria is accomplished by specialised attachment and growth machinery. This includes the presence of pili, flagella, twitching motility, lipopolysaccharides, and quorum sensing, all of which can influence endophytic colonisation and bacterial movement inside host plants <sup>[50,51,20,52]</sup>. Furthermore, bacterial penetration and dissemination within the plant are known to be aided by the production of cell-wall destroying enzymes, namely pectinases and cellulases <sup>[35]</sup>. Leaf tissue is the last sink for these specialised endophytic bacteria.

Endophytic bacteria primarily colonise leaf tissues via plant roots, although they can also enter the leaves from the phyllosphere via leaf stomata, exactly as phytopathogenic bacteria <sup>[53]</sup>.

## Plant Growth Promoting Endophytes-

Agricultural operations in the twentieth century were mostly carried out with the help of agricultural machinery, high-yielding crop varieties, extensive tillage, irrigation, fertilisers, herbicides, and other manufactured inputs <sup>[54]</sup>. These applications have the potential to degrade soil quality and contribute to environmental contamination <sup>[55]</sup>. Microbial inoculations, such as biofertilizers, are gaining popularity as a way to mitigate the detrimental effects of conventional farming practises. Microorganisms and plants develop a symbiotic relationship that is profitable to both follows. The presence of the microbe in the plant has an effect on the plant's health and growth performance, which increases agricultural attributes such as root length and fresh and dry weight of the shoot and root, as well as crop output, soil quality, and nutrient cycling <sup>[56, 57, 58]</sup>.

In addition to producing exo-enzymes, which may help plant colonisation, endospheric microorganisms create secondary active compounds that protect plants from phytopathogens. Endophytes may promote plant development by producing phytohormones and assisting

plant development in the face of biotic and abiotic stress <sup>[59]</sup>. Endophytic microorganisms create a variety of bioactive substances with various biological activities that can either directly or indirectly promote plant development.

Endophytes promote plant development by producing chemicals such as cytokinins, indole acetic acid (IAA), siderophores, gibberellins, atmospheric nitrogen fixation, phosphorus solubilization, delivering important enzymes and vitamins, and assisting plants in nutrient acquisition <sup>[5,15,60]</sup>. Endophytes increase plant growth by releasing active metabolites by using nutrients produced by the plants <sup>[61]</sup>.

Due to PGP effects, bacterial endophytes from maize, tomato, melon, and pepper shoot, root, and seed tissues enhanced growth and biomass accumulation <sup>[60]</sup>. As evidenced by the identification of nitrogenase with antibodies in roots, inside cell walls of stems and rhizomes, endophytes *Pseudomonas*, *Stenotrophomonas*, and *Burkholderia* provided N to grasses growing in nutrient-poor sand dunes <sup>[62]</sup>. Endophytes also assist symbiotic rhizobia in forming nodules with non-specific hosts, promoting plant development. Wheat, pigeon pea, kudzu, and soybean nodulation and growth were aided by endophytic *Bacillus* species <sup>[63]</sup>.

Endophytes also manufacture the ACC deaminase enzyme, which break out ACC, a plant precursor of ethylene, and lowers ethylene levels, alleviating drought stress and promoting plant growth <sup>[64]</sup>. Other favourable impacts of endophytes on plant development include osmotic adjustment, stomatal modulation, root shape modification, mineral absorption enhancement, and nitrogen buildup and metabolism change <sup>[35, 42]</sup>.

In theory, PGPB might have a direct or indirect effect on plant development. The PGPB either (i) aids the acquisition of nutrients from the atmosphere, such as phosphorous, nitrogen, and iron; or (ii) controls plant development by delivering or regulating different plant hormones, such as cytokinin, auxin, or ethylene.

Bacterial endophytes hasten seedling emersion, aid plant emplacement under harsh circumstances <sup>[65]</sup> and boost plant development in some situations <sup>[66]</sup>. Endophyte mediated de novo synthesis of new chemicals and antifungal metabolites has been proven to reduce disease development in bacteria. The search for novel metabolites among endophytic strains could proceed to the discovery of new drugs for the cure of sickness in plants, humans, and animals <sup>[16]</sup>.

## Conclusion

Plants have a strong relationship with nonpathogenic endophytes, which have the potential to increase agricultural yields, suppress diseases, and produce new chemicals. Understanding endophytic biodiversity improves our understanding of plant physiology as well as endophytes. Managing beneficial microbial populations for plant colonisation and development is the challenge and objective. This will be achievable if we have a better understanding of endophyte ecology and plant interactions. Endophytic life inside plants may be studied using *in vivo* gene expression technologies in different atmosphere, such as the rhizosphere and root. Furthermore, functional genomics can aid in the development of colonisation and plant growth features. Exploiting endophyte-plant interactions can lead to the development of effective plant endophyte partnerships for the development of endophytes

(and rhizobacteria) to improve sustained yield generation under adverse environmental circumstances.

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