

Review Article

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## Endophytes are Plant Helpers: An Overview

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### ABSTRACT

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Nearly all the plants that exist in the world were found to be associated with endophyte. Usually, interaction between plants and microbes leads to disease in plants. But only a few communities of microbes act as pathogens and information regarding their interaction is insufficient. A new term endophyte has been established to represent the microbes that are residing in plant tissues without causing harmful effects to the host plant. Since they have the ability to produce active biochemicals and genetic interaction with their host plant, more attention was paid towards the endophytes research. Many reports were published based on isolation and identification of endophytes, screening of bioactive compounds produced and their role in protecting host plant, etc. Based on the current reports, this review was framed to show how the endophytes interact with the host and promote its beneficial effects.

### Introduction

In natural ecosystem plants are associated with endophytes. Endophytes are capable of producing extreme secondary metabolites that are used as biocontrol agents, immunosuppressive compounds, etc. (Tan and Zou, 2001). Endophytes have provided many useful compounds that are necessary in developing fields such as medicine, agriculture, biotechnology, etc. (Strobel *et al.*, 2003). They may be seen in various habitats ranging from terrestrial to aquatic (Zhang *et al.*, 2006). More importance is given to endophytes research because of declining global biodiversity, disappearance of species and ecosystem, etc. (Gimenez *et al.*, 2007). Availability and composition of microbes present in the host plant depend on various factors.

Plant interaction with endophytes leads to plant productivity, enhance soil health, nutrients uptake by the plant (Mei and Flinn, 2010), and defence against plant stress, disease and insects (Gao *et al.*, 2010).

Endophytes also play a major role in other important characteristics of plants, such as greater resistance to stress conditions (i.e., water), alteration in physiological properties, production of phytohormones and other compounds of biotechnological interest (i.e., enzymes and pharmaceutical drugs) (Azevedo *et al.*, 2000). In addition to the economical aspects, the study of endophytic microorganisms has strong academic interests, concerning the discovery of new

microbial species, mainly when tropical hosts are investigated (Azevedo *et al.*, 2000). Researchers divide the activities of endophytes into direct and indirect methods. Direct method includes the influence of endophytes in plants under natural condition and indirect method refers to re-inoculation of isolated endophytes into the same or different host to enhance its particular character.

### **Role of Endophytes in Plant Physiology**

Usually microbial symbionts were rarely considered for plant physiology studies (Arnold *et al.*, 2007). For the past few years, studies on endophytes have increased because of their benefits provided to the host. Endophyte community structure (species diversity: richness and relative abundances) within the plant is dynamic and is influenced by biotic and abiotic factors such as plant species, microbe–microbe interactions, plant–microbe interactions, soil conditions and biogeography, both at local and larger scales (Gaiero *et al.*, 2013).

### **A Plant Growth Promoter**

Studies on interaction of endophytes with their host plants and their function within their hosts are important to deal with the biological significance of endophytes. The modulation of ethylene levels in plants by bacterially produced 1-aminocyclopropane-1-carboxylate deaminase is a key feature that enables interference with the physiology of the host plant. Endophytes with this capacity might profit from association with the plant, because colonization is enhanced. In turn, host plants benefit by stress reduction and increased root growth. This mechanism leads to the concept of ‘competent’ endophytes, defined as endophytes that are equipped with genes important for maintenance of plant–

endophyte associations (Hardoim *et al.*, 2008).

Under natural conditions, endophytes promote plant growth by using various mechanisms. These include phosphate solubilization activity (Verma *et al.*, 2001; Wakelin *et al.*, 2004), indole acetic acid (IAA) production (Lee *et al.*, 2004) and the production of a siderophore (Costa and Loper, 1994). Endophytic organisms can also supply essential vitamins to plants (Pirttila *et al.*, 2004). Moreover, a number of other beneficial effects on plant growth have been attributed to endophytes and it includes osmotic adjustment, stomatal regulation, modification of root morphology, enhanced uptake of minerals and alteration of nitrogen accumulation and metabolism (Compant *et al.*, 2005). The recent areas where these plant growth–promoting endophytes are being used in the developing areas of forest regeneration and phytoremediation of contaminated soils.

Associative bacteria as well as endophytic microbes use same mechanisms to influence plant growth. However, they differ in efficiency through which they exert their beneficial effect (Marella, 2014). The direct mechanisms are nitrogen (N<sub>2</sub>) fixation, phosphate solubilization, insoluble iron chelation through the production of siderophores and phytohormones, such as auxins, cytokinins and gibberellins (GAs) (Tsavkelova *et al.*, 2007; Jha *et al.*, 2012). Sturz *et al.* (1998) provided the evidence for plant growth–promoting activity by endophytic bacteria. The experiments with potato and clover showed 21 isolates of endophytic bacteria are responsible for plant growth.

Mostly endophytic bacteria promote plant growth by nitrogen fixation. Diazotrophy is usually used to study the plant growth.

Endophytic diazotrophs are assumed to have an advantage over root-associated diazotrophs, such as *Azospirillum* and *Azotobacter*, where they colonize the interior rather than the surface of the plants. Therefore, they exploit carbon substrates supplied by the plant. Moreover, they are located within the underground roots and dense plant tissues. The bacteria are likely to be growing within a low partial oxygen pressure environment, which is necessary for the expression and operation of nitrogenase (Baldani *et al.*, 1997).

Some important diazotrophic bacteria, such as *Acetobacter diazotrophicus*, *Herbaspirillum* sps. and *Azospirillum* sps., isolated from some agricultural crops showed evidence that these bacteria actually fix nitrogen in their host plants. The researchers suggested that the plant growth promotion might have been caused by enhanced plant mineral uptake and improved plant–water relationship and production of some plant growth regulators. All the above methods may be considered to be causal agents for altering plant growth and development. But the correct mechanism behind this was still not yet clear.

Many endophytic bacteria and fungi isolated from plants were tested for the production of important growth hormones because the endophytes have the capacity to produce metabolites same as their host plant. Some important growth hormones are IAA (Shin *et al.*, 2007; Sheng *et al.*, 2008; Luo *et al.*, 2012; Dalal and Kulkarni, 2013; Deshwal and Kumar, 2013; Jasim *et al.*, 2013; Syamsia *et al.*, 2015), GA (Hamayun *et al.*, 2009, 2010; Khan *et al.*, 2012), auxins and cytokinins (Dalal *et al.*, 2013). Some important plant growth–promoting activities are listed in Table 1.

Bandara *et al.* (2006) studied the effects and potential of endophytic bacterial and fungal interactions in *Oryza sativa* and found two types of interactions, biofilms (bacteria attached to mycelia) and mixed cultures (no such attachments). The experiment was mainly conducted to test the IAA production by bacteria and fungi (mixed/separated) and by its interaction (biofilms). The results revealed that biofilms showed high productivity than the mixed cultures which is essential for plant growth. Endophytic bacteria enhance plant growth by producing plant growth regulators such as GAs, cytokinins and IAA, which directly or indirectly promote plant growth and development (Holland, 1997; Barka *et al.*, 2002).

Various reports were produced based on *in vitro* experiments. *Paecilomyces formosus* (cucumber), *Penicillium minioluteum* (*Glycine max* L.), *Phoma glomerata* and *Penicillium* sp. (Waqas *et al.*, 2012) were inoculated into GA-deficient mutant rice cultivar Waito-C and normal GA biosynthesis rice cultivar Dongjinbyeo. Culture filtrate of isolated strain showed maximum growth and increased GA production (Khan *et al.*, 2011, 2012).

*Euphorbia pekinensis*, a medicinal plant, was inoculated *in vitro* with fungi isolated from the same plant and from other plant. Plant growth–promoting compounds were found to be higher in plants that were inoculated with fungus which were isolated from same plant (Dai *et al.*, 2008). For instance, seed treatment of maize with a culture of *Burkholderia cepacia*, isolated from the rhizosphere of maize, resulted in disease suppression and growth promotion (Bevivino *et al.*, 1998).

## A Plant Protector

The natural and biological control of pests and diseases affecting cultivated plants have gained much attention in the past decades as a way of reducing the use of chemical products in agriculture (Azevedo *et al.*, 2000). The use of agrochemicals, though decreased the attack of insects and phytopathogenic microorganisms, still represents a high risk to field workers and consumers. Many endophyte communities were isolated from agricultural crops. Not all of the endophyte–plant associations make plants more resistant to pests or diseases (Gimenez *et al.*, 2007). Endophytes are gaining attention as a subject for research and applications in plant pathology. This is because, in some cases, plants associated to endophytes have shown increased resistance to plant pathogens, particularly fungi and nematodes (Zabalgogezcoa, 2008)

## Against Diseases

Recently researchers focused on study of endophytes against some important diseases caused by several pathogens and insects. This includes some direct, indirect and ecological effects. Numerous plant-growth promoting endophytic strains have been isolated from the internal tissues of various crops and tested against effective diseases (Barka *et al.*, 2002). Researchers used to handle the *in vitro* co-culture techniques or compare the survival rate of plant inoculated with/without endophytes. By using these techniques, several potential mechanisms were framed to show endophytes which have the capacity to limit the pathogen damage. When pathogens and the plant interact directly, endophytes suppress the pathogen by producing some antibiotics (lytic enzymes). Endophytes have the capacity to produce antibiotics that act as antifungal, antibacterial and insecticidal

properties, which strongly inhibit the growth of other microorganisms, including plant pathogens (Gunatilaka, 2006). Endophytes which were isolated from various plants have the capacity to act against several pathogenic organisms such as *Streptomyces* sp. against *Alternaria brassicicola* (Igarashi *et al.*, 2000), *Cryptosporiopsis quercina* against *Candida albicans* (Strobel *et al.*, 1999), *Nigrospora* sp. against *Fusarium oxysporum* and *Phytophthora* spp. (Doley and Jha, 2010), *Talaromyces flavus* against the diseases produced by non-sesquiterpene peroxides (Talaperoxides AD (1–4)) (Li *et al.*, 2011).

Endophytes indirectly affect the pathogens by providing more strength to its host plant through secondary metabolite. Bhowmik *et al.* (2002) and Fu *et al.* (1999) reported that cotton seed and tissues were inoculated with bacterial endophytes for reducing cotyledonary infection with *Xanthomonas axonopodis* pv. *malvacearum* (Smith) Vauterin (*Xam*) and for effective seed germination and better control of cotton wilt caused by *Verticillium dahliae*. Many tropical plants living in highly humid environments (rainforest) were not affected by Oomycetes. Their associated endophytes produced active metabolites against *Phytophthora* and *Phytium* (Strobel *et al.*, 2003).

*Burkholderia cepacia* and *P. aeruginosa* isolated from symptomless oil palm root tissues showed potential to inhibit the spread of *Ganoderma boninense* (Basal stem rot disease). These two bacteria were re-inoculated into oil palm seedlings which resulted in inhibition of *G. boninense* (Sapak *et al.*, 2008). *Theobroma cacao* collected from lowlands of Panama was used for isolation of endophytic fungi. The isolated fungus were introduced into young seedlings of *T. cacao* and were found to inhibit leaf

necrosis and leaf mortality caused by *Phytophthora* sp. The results revealed the intrinsic activity against the pathogen and explained the mechanism of horizontal transmission of endophytes (Arnold *et al.*, 2003). The presence of compounds like IAA suggested a possible regulation of plant processes by endophyte metabolites (Gimenez *et al.*, 2007).

### **Against Insects**

The capacity of endophytic fungus to repel insects, induce weight loss, growth and development reduction and even to increase pest death rate was correlated with toxin production which act as protectors of host against different herbivores. Patterson *et al.* (1992) observed in *Lolium* and *Festuca* that the production of alkaloids by *Acremonium* reduced attacks of the Japanese beetle *Popilla japonica*. Members of *Acremonium* generally have the capacity to act against some insect pests. So they can be transferred to endophyte-free plant to resist against certain pests. Koga *et al.* (1997), by infecting *Festuca arundinacea* Shreb and *Lolium perenne* seeds with the endophytic fungus *Acremonium*, obtained plants resistant to the bluegrass webworm *Parapediasia teterrella*, *Chaetomium* and *Phoma* endophytes of wheat when inoculated were active against foliar diseases caused by *Puccinia* and *Pyrenophora* spp. (Dingle and McGee, 2003; Istifadah and McGee, 2006)

Bing and Lewis (1993) also reported that the entomopathogenic fungus *Beauveria bassiana* has been used for a long time in the control of the European corn borer and it was isolated as endophyte from corn. It has been discovered that grasses with high endophyte content were often resistant to attack by certain insects (Dutta *et al.*, 2014). *Phomopsis oblonga* against *Physocnemum brevilinenu* beetle (Webber, 1981),

*Muscodor vitigenus* against heat stem sawfly (Daisy *et al.*, 2002). Harley and Smith (1983) found that many orchids contain endophytic fungi of genus *Rhizoctonia*. Orchid seeds are small and lack sufficient nutrient for embryo development. The endophytic fungi grow out of the seeds after dispersal and enzymatically degrade the bark or other substrate to supply nutrient for developing orchid embryo (Dutta *et al.*, 2014).

### **Against Nematodes**

In some cases, the compounds responsible for the nematicidal activity observed in some plants was due to the production of 3-hydroxypropionic acid by the endophytes *Phomopsis phaseoli* and *Melanconium betulinum* Schumach & Kunze. However, other authors attributed these effects to 3-nitropropionic acid (3-NPA) (Gimenez *et al.*, 2007). Endophytic colonization of *F. oxysporum* showed resistance towards pests and diseases. Under *in vitro* condition *F. oxysporum* was tested against *Pratylenchus goodeyi* and *Helicotylenchus multicinctus*, plant-parasitic nematodes on banana (Waweru *et al.*, 2014). Bacterial endophytes like *Bacillus* spp., *Pseudomonads*, *Arthrobacter* spp., *Micrococcus* spp., *Curtobacterium* sp., *Serratia* isolated from *Piper nigrum* L. was used for *in vitro* bioassay against the nematode *Meloidogyne incognita* (Aravind *et al.*, 2009). Endophytes which act against some nematodes were listed in Table 2.

### **An Nutrient Enhancer**

Some endophytes appear to possess the ability to manipulate host plant metabolism to increase nutrient uptake and change nutrient homeostasis. *Neotyphodium coenophialum*, an endophytic fungus, was isolated from *F. arundinacea*. Under *in vitro* condition, two cloned tall fescue plants were

developed with and without the isolated strain in response to phosphorous deficiency. Relative growth of shoot and root, concentrations of potassium, iron and copper were found to be high in infected plants in response to phosphorous (Malinowski *et al.*, 2000); likewise when *Bacillus* sp. was inoculated to sweet sorghum, the uptake of heavy metals like cadmium and magnesium was increased in infected plants (Luo *et al.*, 2012).

### A Stress Controller

One of the effective ways of developing sustainable agriculture to ensure human and animal food production with a minimal disturbance to the environment is the exploration of microbe-based symbioses in plants (Marella, 2014). Plants are naturally subjected to drought, salt and cold stress.

**Table.1** Plant Growth Promoting Activity of Endophytes

Endophyte	Activity	Source	Reference
Fungi	Plant growth promotion	Halophytes	You <i>et al.</i> (2012)
Fungi	Plant growth promotion	<i>Vitex rotundifolia</i> , <i>Calystegia soldanella</i> , <i>Lathyrus littoralis</i> , <i>Polygonum convolvulus</i> , <i>Oxalis corniculata</i> , <i>Lathyrus japonica</i> , <i>Ixeris repenes</i> , <i>Glehnia littoralis</i> and <i>Salsola komarovi</i>	Khan <i>et al.</i> (2012)
Fungi	Plant growth promotion	<i>Rosa rugosa</i> , <i>Camellia japonica</i> , <i>Delonix regia</i> , <i>Dianthus caryophyllus</i> and <i>Rosa hybrid</i>	Zhou <i>et al.</i> (2014)
<i>Cladosporium sphaerospermum</i>	Plant growth promotion	<i>Glycine max</i> (L.) Merr.	Hamayun <i>et al.</i> (2009)
<i>Bacillus</i> sp.	Plant growth promoting	<i>Sorghum bicolor</i> L.	Luo <i>et al.</i> (2012)
Fungi	Plant growth promotion [Indole acetic acid (IAA)]	Aromatic rice	Syamsia <i>et al.</i> (2015)
<i>Pseudomonas aeruginosa</i> , <i>P. putida</i> , <i>P. cepacia</i> and <i>P. fluorescens</i>	IAA, hydrogen cyanide (HCN), siderophore and phosphorous solubilization	Rice crop	Deshwal and Kumar (2013)
<i>Bacillus</i> sp., <i>Sporosarcina aquimarina</i> and <i>Enterobacter</i> sp.	IAA and siderophore	<i>Avicennia marina</i> L.	Janarthan and Eganathan (2012)
<i>Enterobacter hormaechei</i>	IAA	<i>Shorea selanica</i>	Widowati <i>et al.</i> (2013)
<i>Endophytic diazotrophic bacteria</i>	Auxin, siderophore	<i>Korean rice cultivars</i>	Ji <i>et al.</i> (2014)

**Table.2** Endophytes Showing Nematicidal Activity

Plant species	Endophyte	Nematode species	Reference
Cucumber	Isolated species	Grey mold	An <i>et al.</i> (2005)
	<i>Pseudomonas fluorescens</i>	<i>Meloidogyne incognita</i>	Hallmann <i>et al.</i> (1998)
	<i>Fusarium</i> , <i>Trichoderma</i> , <i>Chaetomium</i> , <i>Acremonium</i> , <i>Paecilomyces</i> and <i>Phyllosticta</i>	<i>M. incognita</i>	Yan <i>et al.</i> (2011)
<i>Tripterygium wilfordii</i> Hook F.	<i>Cryptosporiopsis cf. quercina</i> Petr. (f)	<i>Pyricularia oryzae</i> Cav.	Li <i>et al.</i> (2000)
Bhendi	<i>Pseudomonas</i> sp., <i>Bacillus</i> sp. and <i>Methylobacterium</i> sp.	<i>M. incognita</i>	Vetrivelkalai <i>et al.</i> (2010)
Tomato	<i>F. oxysporum</i>	<i>Radopholus similis</i>	Shahasi <i>et al.</i> (2006)
	<i>P. aeruginosa</i> , <i>P. fluorescens</i> and <i>Phyllobacterizrm rubiacearum</i>	<i>Meloidogyne javatzica</i>	Siddiqui and Ehtesharnul-Haque (2000, 2001)
		Nematicidal	Schwarz <i>et al.</i> (2004)

*Piriformospora indica*, which is isolated from the roots of xerophytic woody shrubs such as *Prosopis juliflora* and *Zizyphus nummularia*, lacks host specificity and is cosmopolitan in nature. Positive interactions of *Pi. indica* were established for many important agricultural and horticultural plants, which allowed them to grow under extreme physical and nutrient stress. The fungus promoted plant growth especially in nutrient-deficient soils, conferred tolerance to abiotic (salinity, drought, water, cold, high temperature and heavy metals) and biotic (root and foliar pathogens) stresses, regulated plant growth and development, induced early flowering and enhanced seed production, stimulated the production of active ingredients in medicinal plants and helped in the hardening of micro-propagated or tissue-cultured plants. The interactions of *Pi. indica* also tested with the model plants *Arabidopsis thaliana* and *Hordeum vulgare* to understand the molecular basis of these beneficial plant–microbe interactions. The

current knowledge about the role of *Pi. indica* in improving the crop productivity and also in enhancing tolerance of the plant to biotic and abiotic stresses (Johnson *et al.*, 2014).

Bacterial strains such as *Arthrobacter* sp. and *Bacillus* sp. were isolated from pepper plant resulted in a significant reduction of upregulation and even downregulation of some stress-inducible genes when compared with gene expression in uninoculated plant (Sziderics *et al.*, 2007). *Phoma glomerata* and *Penicillium* sp. associated with cucumber plants significantly increased the plant biomass, related growth parameters, assimilation of essential nutrients such as potassium, calcium, magnesium and reduced the sodium toxicity under sodium chloride and polyethylene glycol–induced salinity and drought stress when compared with control plants. Under stress conditions, the endophyte infection significantly modulated stress through downregulated abscissic acid,

altered jasmonic acid and elevated salicylic acid contents when compared with control. In conclusion, the two endophytes significantly reprogrammed the growth of host plants during stress conditions (Waqas *et al.*, 2012).

The endophytic fungal symbiosis of *Penicillium minioluteum* also increased the Daidzein and Genistein contents in the soybean when compared with control plants, under salt stress. Thus, *P. minioluteum* showed the adverse effects of abiotic salinity stress and rescued soybean plant growth by influencing biosynthesis of the plant's hormone and flavonoids (Khan *et al.*, 2012).

In conclusion, nearly 300,000 plant species exist in this world. Every plant harbour one to hundreds of endophytes. Each endophyte, bacterium or fungus, has their own function and protects the plants from various factors. But still the mechanism behind its action on their host plant is not yet clear. Many unsolved problems are present in endophyte research, therefore, many workers are involved in endophyte research to show its potential to solve many problems faced by mankind. India being a tropical country with great variation in biodiversity offer more chances to chase more endophytes that are suitable to produce bioactive compounds.

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