Chapter 10
Harnessing Endophytes as Biocontrol Agents

Sakshi Tewari, Vijay Laxmi Shrivas, P. Hariprasad, and Shilpi Sharma

Abstract Microbial endophytes represent an endosymbiotic group that colonizes internal plant tissues. Endophytes are one of the least studied and unexplored groups of microbes that need attention, so as to provide comprehensive knowledge regarding beneficial plant-microbe interactions. One of the emerging issues in the area of agriculture is a gradual decrease in productivity (quality and quantity) of agroproducts because of various biotic and abiotic stresses. The problem pertaining to the rise of pesticide resistant phytopathogens and decreased soil fertility is linked with improper use of pesticides. Recent advancement in the area of endophytic microbes working as biocontrol agents could be a potential option to address the aforementioned problems. But the real challenge lies in taking these potential candidates from laboratory to land. In the present chapter, we have discussed different mechanisms through which endophytes suppress microbial diseases in host plant, the major steps involved in developing mechanism-based bioformulations from these endophytes, and their use in advanced agricultural system for future benefits.

Keywords Endophyte · Endorhizae · Plant-Microbe interaction · Bioformulation · Phytopathogens

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10.1 Introduction

Rapid rise in the world's population is becoming a common phenomenon with the head count of this populace predicted to reach around ~8 billion by 2020 (Scherbov et al. 2011). Feeding this gigantic population with limited resources is a big challenge for the world community. About 50 years back, "green revolution" was a much-talked topic, which led to tremendous increase in food production in India. During the time of green revolution, high-yielding varieties and enormous use of chemical fertilizers and pesticides have undeniably contributed to the terrific increase in food production. With time this has led to gradual loss of natural soil microbiota and soil fertility (Nicholson and Hirsch 1998). Incessant use of pesticides gave rise to many pest-resistant species, and huge risk to producers and consumers. In spite of adopting several agricultural advancement strategies, plant pathogens still account for more than 15% losses in the global harvest. Among these, fungal pathogens are a major threat to crops leading to 30% reduction in crop yield. Such a loss translates to nearly 200 billion rupees per annum in global market (Shaikh and Sayyed 2015). Most of the pesticides in use are recalcitrant to biodegradation resulting in long-term environmental concern and health problems. Thus, in order to resolve this burning issue of pest/pathogen control, more eco-friendly, green, and sustainable approach is required. The utilization of biological agents, especially beneficial microbes, is considered as potential alternative and safe way to protect plants from pathogens. Controlling these pathogens by diverse microorganisms acting as natural antagonists has been practiced routinely over the past century.

Biocontrol using antagonistic microbes offers a highly efficient, cost-effective, and eco-friendly substitute to the application of synthetic chemical pesticides. Plant-associated microbes (PAM) are effective competitors, which can establish and persist on diverse crop plants. Extensive literature unfolding possible roles of PAM as plant growth promoters and disease-suppressive agents is available (Singh et al. 2016; Odoh 2017; Shafi et al. 2017). However, one of the least studied and unmapped group of PAM that resides within the plant system and establishes in internal plant environment are known as endophytes. Most of them are able to surpass the endodermal barricade by passing root cortex to the vascular and consequently flourish as endophytes in leaves, tubers, seeds, stem, and other plant organs (Patriquin and Dobereiner 1978; Hallmann et al. 1997). Cryptic life of endophytes states that they are prime colonizers of dead plant tissues and chiefly act as decomposers in the ecosystem (Osono 2006). There are certain validations that suggest that few endophytes play uncommon roles in the ecosystem such as protecting plants from pathogens that cause diseases (Prieto et al. 2011). Also, endophytic colonization within plant system results in development of an intimate relation between the two and offers protection to plants against diverse pathogens. Endophytes have evolved a close relationship with their host plants during the time of evolution thereby affecting physiological route of plants. Additionally, their exclusive ability to persist and reside within plant system without any competition makes them suitable for biological control (Devi and Momota 2015).

"Harnessing the role of endophytes as biocontrol agent" is an interesting topic that will be covered in this chapter. The reason for selecting endophytes over non-endophytic population in this chapter is due to its innumerable advantages. First, as endophytes reside inside host plants, they can colonize very easily and remain protected throughout their life span. Second, application of endophytes is easy, as it enters the target site and does not require several field applications (booster doses) during crop development (Wani et al. 2015). Third, they have extraordinary capacity to tolerate abiotic and biotic stress factors. Additionally, they also find application in the fields of nanosciences, modern medicine, bioremediation, bio-augmentation, forest management, and industrial perspective (Devi and Momota 2015). In spite of several advantages, endophytes hold some disadvantages too; culture-dependent techniques, used for isolating absolute endophytes, sometimes give false results, and it is difficult to analyze the exact endophytic diversity in plant. Franks et al. (2006) reviewed innumerable molecular tactics for isolating and characterizing endophytic community, which include culture-independent methodologies to gain maximum information on endophytic diversity.

Though different stories of endophytes have been elucidated by different workers, the aim of this chapter is to precisely focus on the biocontrol attributes of endophytes along with their potent mechanisms. The chapter also focuses on the important criteria involved in taking this endophytic system from laboratory to land.

10.2 Endophytes

The term endophytes was first coined by a German botanist, Anton de Bary, in 1886 referring to those organisms that inhabit internal tissues of leaves and stems (Wilson 1995). The existence of endophytes was first documented by Vogl in 1898 revealing a mycelium residing in the seed of *Lolium temulentum* (Guerin 1898; Vogl 1898). Different definitions of endophytes are given by different researchers, but the most widely accepted one is "bacteria or fungi allocated within the plant tissues without causing any harm to the host" (Bressan and Borges 2004).

On the basis of functionality, endophytes are characterized into three main groups, viz., plant growth promoters, biocontrol agents, and plant stress homeo-regulating microbes (Bashan and Holgiun 1998; Cassan et al. 2009). On the basis of distribution, endophytes have been classified into three main groups: the first group includes obligate endophytes that can proliferate only inside the plant, and they fail to flourish outside; the second group includes facultative endophytes that are usually free-living, but, if opportunity ascends, they can exhibit massive colonization in plant through coordinated infection (Hardoim et al. 2008); and the third group includes passive endophytes, which do not show active colonization but do so as a result of stochastic events like wounds or abrasion in the root curls. Endophytes are generally host specific. Relationship of endophytes with its host partner could be described in terms of host selectivity, host recurrence, or host preference.

Several authors have elaborated on the microbiome present in pockets of rhizosphere and rhizoplane, but very few have focused on the microbial community residing within the plant root system. Microorganisms present inside plant root significantly differ from those residing in the rhizosphere and rhizoplane (Kloepper and Beauchamp 1992; Gottel et al. 2011). Hence, it is necessary to unravel this hidden and complex zone inside the plant roots, termed as endorhiza, for further exploration of microbial diversity. Endorhiza is broadly defined as root tissues below the epidermal layer including vascular and cortical tissues (Mahaffee and Kloepper 1997).

Rhizobium etli is a well-known endophyte that naturally occurs in maize plant, when maize-bean crops are grown in association with each other (Gutiérrez-Zamora and Martínez-Romero 2001). Sprouts and seeds of alfalfa mainly harbor endophyte *Salmonella enterica* and *Escherichia coli,* which have been detected by green fluorescent proteins (Cooley et al. 2003). *Rhizobium rhizogenes* and *R. leguminosarum* are normal red clover symbiont found in the root nodules of clover plants (Sturz et al. 1997). Xylem vessels and stomatal compartments of *Vitis vinifera* primarily contain endophyte *Burkholderia* within it (Compant et al. 2005a, b). Dong et al. (2003) observed clumping of *Klebsiella* strain Kp342 at lateral root joints of alfalfa and wheat plant. Thus, it seems that the endophytes, whether bacterial or fungal, best adapted for dwelling inside plants are naturally selected and recruited from soil to aboveground plant tissues.

Distribution of endophytes within plant is governed by two main factors: first is colonizing aptitude, and second is resource allocation throughout the plant. Root endophytes often colonize and enter the epidermis from the site of root cracks, lateral root emergence, and below the root hair zone (Compant et al. 2005a, b; Zakria et al. 2007). During initial colonization, few endophytes can enter aboveground plant parts by entering the vascular tissues and scatter systemically (Johnston-Monje and Raizada 2011). Johnston-Monje and Raizada (2011) confirmed the transport of the green fluorescent protein tagged endophytes from seeds into roots, roots into stem, and stems to roots and rhizosphere, suggesting a continuous movement of endophyte throughout the plant system.

The second factor influencing dispersion of endophytes is the allocation of plant resource. Chi et al. (2005) stated that different slices of plant tissues can harbor distinct endophytes, like *Pseudomonas* are more common in the stems than in the roots of potatoes after a month of growth (Garbeva et al. 2001). Higher endophytic population in crown region of carrot was observed compared to metaxylem tissues due to high level of photosynthate (Surette et al. 2003). Fisher et al. (1992) reported significant difference in the distribution of endophytes colonizing maize crop. Leaves of maize disclosed heavy colonization by bacterial endophytes in comparison to stem; however, more fungal endophytes were recovered from core and epidermis of stem in comparison to the leaves. Ji et al. (2010) documented the epiphytic and endophytic lifestyle of rhizobia in tobacco plant and suggested that endophytic rhizobia depart from the leaf interior through stomata and colonize the phyllosphere.

Additionally, rhizobia can also colonize roots and aerial plant tissues of rice, wheat, barley, canola, *Arabidopsis*, and lettuce (Stone et al. 2001; Luby-Phelps et al. 2003). Certain endophytes can colonize fruits, flowers, berries, and seeds. Patil (2013) reported plant growth-promoting endophytic bacteria *Asaia bogorensis* associated with mango fruit. Similarly *Bacillus*, *Acinetobacter*, and *Enterobacter* are common endophytes present in papaya fruit (Krishnan et al. 2012). Fruits belonging to the family Cucurbitaceae *Cucumis melo reticulatus*, commonly known as melon, usually contains endophytes α-, β-, and γ-*Proteobacteria*, *Firmicutes*, and *Actinobacteria* within it (Glassner et al. 2015). Endophytic genera, including *Acinetobacter*, *Methylococcus*, *Bacillus*, *Micrococcus*, and *Planococcus*, residing in rose (*Rosa damascena trigintipetala*) during flowering state, have growth-promoting and biocontrol attributes (El-Deeb et al. 2012). The involvement of these endophytes in development and maturation of reproductive segment of plants and their potential use as biocontrol agent is yet to be elucidated.

Hence, it could be concluded that different endophytes display diverse distribution on associated plants. Several molecular studies have been conducted to observe the distribution of endophytes within plant cell, but the exact mechanisms behind this establishment needs further elucidation. Further investigations related to transcriptomics of endophytes and host plants may serve as promising approaches to discover the drivers of plant–endophyte interactions.

10.2.1 Bacterial Endophytes

Bacterial endophytes have recently been in focus as biocontrol agents, as they provide additional benefits in comparison to rhizospheric colonizer (Hallmann 2001). Bacterial endophytes are recruited from the rhizosphere at the site of wound, cut, or lesion and colonize both vegetative and reproductive parts of plant like tuber, root, stem, leaf, flower, and fruits (Gray and Smith 2005; Compant et al. 2005a, b). Mechanisms by which they protect their host plant are more or less similar as described for PAM in the rhizosphere. Different workers have reviewed the elaborated mechanisms of these endophytes (Kloepper et al. 1999; Hardoim et al. 2015; Chaturvedi et al. 2016). Mechanisms by which endophytes enhance plant growth are categorized as direct and indirect (Long et al. 2008). Direct mechanisms include nitrogen (N_2) fixation, phosphate (P) solubilization, iron (Fe) chelation, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, and phytohormone production, whereas indirect modes include pathogen suppression by outcompeting them for macro- and micronutrients, siderophore production, antibiotic production, establishment of the plant's systemic resistance, secretion of lytic enzymes, and secondary metabolite production (Fig. 10.1).

Diseases of bacterial, fungal, and viral origin, and in some cases damage caused by nematodes and insects, can be decreased by endophytic inoculation (Berg and Hallmann 2006; Ryan et al. 2008). Few endophytic microbes elicit the phenomenon of induced systemic resistance (ISR). The role of bacterial endophytes in connection

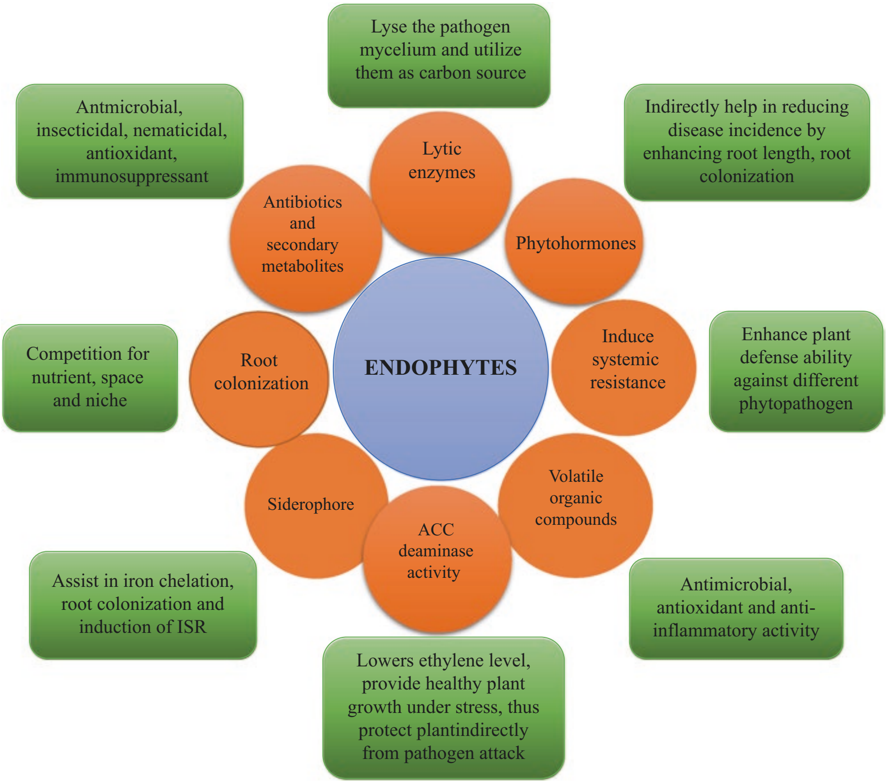

Fig. 10.1 Different mechanisms of disease suppression by endophytes

with ISR has been reviewed by Kloepper and Ryu (2006). Several examples of bacterial endophytes such as *Actinobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Streptomyces*, etc. are used nowadays as biocontrol agents against plant pathogens; few of them have been discussed below briefly. Table 10.1 summarizes details of endophytic bacteria along with their mechanisms/metabolites that validate their potential role in biocontrol of phytopathogens.

Bacillus pumilus INR7, an endophyte found in the stem of cucumber plant, is capable of suppressing cucurbit wilt disease caused by *Erwinia tracheiphila* under field conditions. There was noteworthy increase in plant growth parameters and disease suppression in sets receiving bacterial treatment in comparison to control sets under field conditions (Wei et al. 1996). *Pseudomonas fluorescens* PICF7 is a native olive (*Olea europaea* L.) root endophyte and active biocontrol agent against *Verticillium* wilt of olive. Strain PICF7 is an active root colonizer, and this rapid invasion not only triggers defense response in root system but also mounts an extensive range of systemic defense responses in aboveground aerial parts of plant like stems and leaves, thus explaining how ISR contributes to biocontrol.

Table 10.1 Diverse mechanisms and metabolites involved in inhibition of growth of phytopathogens by endophytes

Endophyte	Host plant	Biocontrol agent against	Mechanism/bioactive metabolites	References
Bacterial endophytes				
Ampelomyces	*Urospermum picroides*	*Enterococcus*, *Staphylococcus*	3-O-methylalaternin Altersolanol	Aly et al. (2008)
Bacillus cereus BT8	*Solanum lycopersicum*	*Phytophthora capsici*	ISR	Melnick et al. (2008)
Bacillus subtilis MJMP2	*Brassica campestris*	*Xanthomonas oryzae*, *Fusarium oxysporum*, *Rhizoctonia solani*	Iturin A	Cheng et al. (2016)
Bacillus subtilis CEN_3	*Brassica napus*	*Fusarium*, *Magnaporthe*	Siderophores, root colonization	Etesami and Alikhani (2016)
Bacillus amyloliquefaciens CEIZ-11	*Solanum lycopersicum*	*Alternaria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum*, *Pythium aphanidermatum*	Cyclic lipopeptide	Gao et al. (2015)
Bacillus pumilus INR7	*Cucumis sativus*	*Erwinia tracheiphila*	ISR	Yi et al. (2013)
Enterobacter HA01	*Gossypium*	*Verticillium dahliae*, *Fusarium oxysporum*	Siderophore, protease, root colonization	Li et al. (2012)
Pseudomonas fluorescens PICF7	*Olea europaea*	*Verticillium dahliae*	ISR	Lama Cabanás et al. (2014)
Pseudomonas viridiflava	*Poaceae*	*Cryptococcus neoformans*, *Candida albicans*	Ecomycins	Miller et al. (1998)
Paenibacillus polymyxa M1	*Triticum aestivum*	*Erwinia amylovora*, *Erwinia carotovora*	Polymixin	Niu et al. (2013)
Paenibacillus polymyxa PB71	*Cucurbita*	*Didymella bryoniae*	Unkown soluble and volatile metabolites	Fürnkranz et al. (2012)

(continued)

Table 10.1 (continued)

Endophyte	Host plant	Biocontrol agent against	Mechanism/bioactive metabolites	References
Paenibacillus polymyxa Wb2–3, Mc5Re-14	*Matricaria chamomilla*	*Fusarium culmorum*, *Rhizoctonia solani*, *Verticillium dahliae*	β-1,3-glucanase, siderophores	Köberl et al. (2013)
Rhizobium etli G12	*Solanumtuberosum*, *Arabidopsis*	*Meloidogyne incognita*,	Extensive root colonization, ISR	Hallman (2001)
Serratia plymuthica G3	*Triticum aestivum*	*Botrytis cinerea*, *Cryphonectria parasitica*, *Rhizoctonia cerealis*	Chitinase, protease, pyrrolnitrin, siderophores	Liu et al. (2010)
Stenotrophomonas maltophilia S37, *Bacillus mojavensis*	*Datura stramonium*	*Fusarium oxysporum*, *F. lycopersici*	Lytic enzymes (chitinase, protease, and pectinase) VOC	Abdallah et al. (2016)
Streptomyces somaliensis	*Glycine max*, *Citrus sinensis*	*Guignardia citricarpa*, *Rhizoctonia solani*, *Colletotrichum sublineolum* *Fusarium oxysporum*, *Phytophthora parasitica*	Chitinase	Quecine et al. 2008
Fungal endophytes				
Acremonium zeae	*Zea mays*	*Aspergillus flavus*, *Fusarium verticillioides*	Pyrrolidines	Wicklow et al. (2005)
Acremonium	*Gossypium herbaceum* *Meloidogyne incognita*	Root-knot nematode disease	Toxin production	Kim et al. (1988) and Goswami et al. (2008)
Beauveria bassiana ARSEF 3113	*Zea mays*	*Ostrinia nubilalis*	Reduction of larval tunneling	Bing and Lewis (1991)
B. bassiana G41	*Musa balbisiana*	*Cosmopolites sordidus*	Larvicidal	Akello et al. (2008)

(continued)

Table 10.1 (continued)

Endophyte	Host plant	Biocontrol agent against	Mechanism/bioactive metabolites	References
Clonostachys rosea	*Moniliophthora roreri*, *Theobroma gileri*	*Botrytis cinerea*	Antibiotic	Morandi et al. (2000), Berry and Deacon (1992), Evans (1999), and Hajlaoui et al. (2001)
Cladosporium	*Tinospora cordifolia*	*Spodoptera litura*	Larval and pupal mortality	Thakur et al. (2013)
Epicoccum nigrum P16	*Saccharum officinarum*	*Fusarium verticillioides*, *Colletotrichum falcatum*, *Ceratocystis paradoxa*, *Xanthomonas albilineans*	Epicorazines A–B, epirodines A–B, flavipin, epicoccines A–D, pipiridones	Fávaro et al. (2012)
Fusarium oxysporum EF119	*Capsicum*	*Pythium ultimum*, *Phytophthora infestans*, *Phytophthora capsici*	Fungal inhibitors	Benhamou et al. (2002)
Lasiodiplodia pseudotheobromae F2	*Camptotheca acuminate*	*Protozoa*	Lasiodipline 5	Wei et al. (2014)
Lasiodiplodia pseudotheobromae XSZ-3	*C. acuminate*	Human *promyelocytic* *Leukemia* cells	Palmarumycin LP1	Lu et al. (2014)
Lasiodiplodia pseudotheobromae	*Ilex cornuta*	*Blumeria graminis*	Antifungal substances	Xiang et al. (2016)
Leptosphaeria	*Gossypium*	*Arabidopsis thaliana*	Unknown	Yuan et al. (2017)
Nigrospora	*Tinospora cordifolia*	*Spodoptera litura*	Griseofulvin, dechlorogriseofulvin, 8-dihydroramulosin, mellein	Zhao et al. (2012)
Penicillium simplicissimum	*Gossypium*	*Pseudomonas syringae*	Unknown	Hossain et al. (2007)
Phomopsis cassiae	*Cassia spectabilis*	*Cladosporium sphaerospermum*, *Cladosporium cladosporioides*	Cadinane sesquiterpenes	Silva et al. (2006)

As reported, different species of endophytic *Paenibacillus* have been associated with diverse crop plants including *Arabidopsis*, *Coffea arabica*, potato, poplar, pinus, etc. (Rybakova et al. 2016). *Paenibacillus* strain PB71 was obtained from the spermosphere of the Styrian oil pumpkin (SOP), and could efficiently inhibit the phytopathogen *Didymella bryoniae*, causal organisms of SOP under greenhouse conditions (Fürnkranz et al. 2012). *Paenibacillus* is well known for producing large amount of diverse hydrolyzing enzymes that enable plant tissue colonization (El-Deeb et al. 2013). Excellent colonizing ability of *Paenibacillus* results in biofilm formation around the plant roots that act as a protective barrier and restrict the entry of pathogen (Timmusk et al. 2005). Additionally, these endophytes also release certain types of volatile metabolites that hinder the growth of pathogens and induce systemic resistance in plants (Timmusk and Wagner 1999). Currently, few species of *Paenibacillus* can produce antimicrobial compound known as polymyxins, which is active against gram-negative bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Stenotrophomonas maltophilia* (Landman et al. 2008; Niu et al. 2013).

A very good example of endophytic bacteria is *Rhizobium*. Several reports suggest *Rhizobium* as an efficient plant growth promoter, but limited data is available on its biocontrol potential. An endophytic strain of *R. etli* isolated from potato rhizosphere (and further re-isolated from the root interior) has been shown to be a potent antagonist against potato cyst nematode *Globodera pallida* and root-knot nematode *Meloidogyne incognita*, respectively. There are two mechanisms that have been proposed for this antagonism: first is the massive colonization of internal tissues of plants by *Rhizobium*, thereby suppressing the growth of invading pathogens by niche occupation, nutrient competition, and antibiosis (Hallmann et al. 1997). The second mechanism was believed to be stimulation of general plant defense resistance mechanism (ISR). This defense mechanism is activated due to lipopolysaccharides secreted by the strain (Reitz et al. 2001).

Bacillus amyloliquefaciens recorded strong antagonism against wide range of phytopathogens like *Aspergillus niger*, *Botrytis cinerea*, *Alternaria alternata*, *Fusarium oxysporum*, and *Pythium aphanidermatum,* which causes damping-off disease in tomato. The main mechanism responsible for this inhibition was production of metabolites like cyclic lipopeptide (CLP). In vivo field experiments were also carried out to check the efficacy of the strain in reducing damping-off disease in tomato. Metabolites of CLP were extracted, and active fractions were again tested against *P. aphanidermatum* by well diffusion method. Detailed analysis of CLP by liquid chromatography coupled with mass spectroscopy (LC/MS) showed compounds like iturin, fengycin, and surfactin (Zouari et al. 2016). Similarly another endophytic strain of *Bacillus subtilis* E1R-J proved to be a promising biocontrol agent against *Blumeria graminis*, causal organism of wheat powdery mildew (Gao et al. 2015). An endophytic bacterial strain, *B. subtilis* MJMP2, isolated from fermented *Brassica campestris* displayed strong antimicrobial activity against *Xanthomonas oryzae*, *Rhizoctonia solani*, and *Fusarium oxysporum*, pathogens responsible for causing blight disease, sheath blight, and root rot, respectively, in rice. The metabolite responsible for antagonism was identified as iturin A, which disturbs fungal cytoplasmic membrane by forming transmembrane channels, resulting in the leakage of K^+ ions from the fungal cells (Hsieh et al. 2009). Crude extract of the supernatant containing iturin A showed antagonistic activity against rice blight disease under in vivo pot assay (Arrebola et al. 2010).

Different strains of endophytic *Streptomyces* sp. including *S. somaliensis*, *S. cyaneus*, *S. purpurascens*, and *S. griseus* isolated from citrus and soybean plant were evaluated for their activity against fungal pathogens, viz., *Guignardia citricarpa*, *Rhizoctonia solani*, *Colletotrichum sublineolum*, *Fusarium oxysporum*, *Pythium* sp., and *Phytophthora parasitica*. High biocontrol activity of the strains was due to the secretion of cell wall-degrading enzyme like chitinase, which was further validated through electron microscopy (Quecine et al. 2008). Shekhar et al. (2006) stated *Streptomyces violaceusniger,* an endophytic bacteria, displayed high chitinase activity and strong antagonism against wood-rotting fungi. Hence, higher chitinase activity has direct corelation with pathogen inhibition. Hastuti et al. (2012) reported other endophytic strains of *Streptomyces*, AB131-1, AB131-2, and LBR02, to be efficient in reducing bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* in rice crop. Effectiveness of the strains was checked both under laboratory and field conditions. Strains AB131-1 and LBR02 displayed strong inhibition of *Xanthomonas* by producing enzymes like phosphatase, chitinase, cellulase, and siderophore. Other endophytes, *Streptomyces griseofuscus* and *S. hygroscopicus*, established 54.5% and 21.8% biocontrol against pathogen *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*), which attacks rice plant and causes disease incidence (Tian et al. 2004). Endophytic strain of *Serratia,* isolated from the stems of *Triticum aestivum,* exhibited antifungal activity against phytopathogens like *Cryphonectria parasitica*, *Rhizoctonia cerealis*, and *Botrytis cinerea*. Diverse mechanisms of biocontrol like chitinase, exoprotease, antibiotic pyrrolnitrin, and siderophore production were displayed by *Serratia* against these pathogens (Liu et al. 2010).

Endophytic strains belonging to the genus *Enterobacter* displayed antagonistic activity against *Verticillium dahliae* causing verticillium wilt in cotton. The strain was phylogenetically affiliated to *Enterobacter cancerogenus*. Its biocontrol efficacy was monitored in pots and further taken to the field with cotton as test crop. Field trials confirmed its antimicrobial activity against *V. dahliae* due to its excellent root-colonizing ability (Berg and Hallmann 2006). *Enterobacter cancerogenus* HA02 displayed extensive colonization and secretion of siderophores and protease that helped in controlling verticillium wilt (Li et al.2010).

10.2.2 Fungal Endophytes

Generally fungal endophytes are found in plant tissues like leaves, stems, and barks asymptomatically. Fungi growing inside vascular tissues play crucial role in protecting host plant by producing different metabolites or toxins that kill many plant pathogens. From the perspective of pest management and control, endophytic fungus appears to be one of the potential candidates. A list of some important fungal endophytes that have emerged as potent biocontrol agents has been compiled in Table 10.1.

Trichoderma is a filamentous, soilborne fungus that forms mutualistic relationship with different plant species and is capable of colonizing host plant. Different species of *Trichoderma* like *T. viren*, *T. atroviride*, and *T. harzianum* are well known for their biocontrol activity (Abdel-Moity et al. 1982; Elad et al. 1983; Fahim et al. 1989). *Trichoderma* inhibits growth of different phytopathogens like *Macrophomina phaseolina* (Larralde-Corona et al. 2008), *Phytophthora*, *Pythium* (Maisuria and Patel 2009), *Sclerotinia sclerotiorum* (Ibarra-Medina et al. 2010), *Fusarium*, *Sclerotinia rolfsii* (Suraiya et al. 2014), etc. The most common mechanisms for biocontrol by *Trichoderma* are host plant resistance, antibiosis, competition, and parasitism.

Fusarium is a filamentous fungi belonging to the group of hyphomycetes that is widely distributed in soil and plants. Usually it is known as phytopathaogenic fungus that affects majority of crops worldwide. *Fusarium* wilt and *Fusarium* root rots caused by different species of *F.oxysporum* are the most common fungal diseases that affect diverse crop plants. *F. oxysporum* is generally of three types, viz., saprophytic, pathogenic, and parasitic. Although endophytic activity is not well studied in case of *Fusarium*, there are some studies that report its biocontrol potential. Zibbermann et al. (2016) studied the biocontrol activity of *F. oxysporum* f. sp. *strigae* strain "Foxy-2" against parasitic weed *Striga hermonthica* in maize rhizosphere. Since then several workers reported that nonpathogenic *Fusarium* sp. can be used as biocontrol agent against pathogenic *Fusarium* (Park et al. 1988; Biles and Martyn 1989; Kroon et al. 1991; Minuto et al. 1995; Leeman et al. 1996; Fuchs et al. 1997). Mechanisms of action were also studied for the control of *Fusarium* wilt by *F. oxysporum*. ISR was found to be the reason for disease control (Biles and Martyn 1989; Kroon et al. 1991; Fuchs et al. 1997). Few strains of *F. oxysporum* displayed promising nematicidal activity against *Radopholus similis* nematode, causing disease in banana plant (Schuster et al. 1995).

Beauveria bassiana is a fungus that belongs to the family of Clavicipitaceae. It occurs in different forms such as entomopathogens, fungal parasites, plant pathogens, parasites of slime molds, and endophytes of grass (White et al. 2003). For instance, as a fungal parasite, it causes white muscardine disease in many arthopods worldwide. On the other hand, it is also used as a biological insecticide for the control of different pests like white flies, beetles, and bedbugs (Barbarin et al. 2012). *Beauveria bassiana* has a wide host range; however, it differs from strain to strain, which can be categorized into selective or nonselective host range. Members of this family are also known for their toxicogenic secondary metabolite production (White et al. 2003). In addition to this, *B. bassiana* showed their endophytic presence in many plant species helping them to combat different plant pathogens (Vega 2008). Campbell and Coe (1991) reported inhibitory activity of *B. bassiana* against soilborne and foliar plant pathogen *Gaeumannomyces graminis* var. *tritici*. Several studies supported the fact that *B. bassiana* has inhibitory spectrum against wide range of plant pathogens such as *Armillaria mellea*, *Rosellinia necatrix*, *Fusarium* *oxysporum*, *Botrytis cinerea*, *Pythium ultimum*, and *Septoria nodorum* due to lysis of cell wall (Vesely and Koubova 1994; Reisenzein and Tiefenbrunner 1997; Lee et al. 1999). Under field conditions, Flori and Roberti (1993) reported that *B. bassiana* not only enhanced plant growth parameters of onion crop but also reduced infection of *Fusarium oxysporum.*

Phoma is a well-known fungal genus that is globally present in soil, plants, air, animals, and human body. *Phoma* is commercially one of the most important fungi, as it produces various pigments and secondary metabolites owing antimicrobial potential. There are certain species of *Phoma* that showed significant biocontrol activity against different plant pathogens, for instance, recently Gupta et al. (2016) reported *P. herbarum* to show inhibitory activity against *C. gloeosporioides*. Endophytic species of *Phoma* are also helpful in controlling weeds by producing secondary metabolites such as anthraquinone and phytotoxin. Hoffman et al. (2008) isolated endophtytic strain of *Phoma*, from *Saurauias caberrinae*, which produced a metabolite called phomodione, an inhibitor of *Staphylococcus aureus*. *Phoma* also produces an antifungal compound known as cytochalasin that is effective against plant pathogens (Wagenaar et al. 2000). Many species of *Phoma* like *P. glomerata*, *P. tracheiphila*, *P. macdonaldii*, *P. sorghina*, *P. proboscis*, *P. herbarum*, *P. macrostoma*, *P. foveata*, and *P. multirostrata,* are well known for their antimicrobial activity against different pathogens, and metabolites from few of them could be used for the production of agrophytochemicals, dyes, and mycopesticides (Rai et al. 2009).

Genus *Cryptosporiopsis* belongs to family Dermateaceae. *Cryptosporiopsis quercina*, a synonym of *Pezicula cinnamomea*, earlier mentioned by Sutton in the 1980s as an imperfect stage of *Pezicula cinnamomea,* was found in association of hardwood species (Sutton 1980). In later year, Tscherter and Dreyfuss (1982) confirmed *Pezicula* sp. as a teleomorph state of the anamorphic fungus *Cryptosporiopsis,* which produces a secondary metabolite that belongs to a group, echinocandin of lipopeptides. After this Fisher et al. (1984) found that endophytic *Cryptosporiopsis* sp. from ericaceous plants showed biological activity against fungi such as *Aspergillus niger*, *Candida albicans*, *Mentagrophytes*, and *Trichophyton*. In further studies on the comparison of fungal endophytes found in xylem and in the whole stem of plants *Fagus sylvatica* and *Pinus sylvestris*, Petrini and Fisher (1988) discovered that fungal *Cryptosporiopsis* species strain P30A was found in the twigs of *Pinus sylvestris*, whereas other endophytic strain P47 of species *Pezicula* was isolated from *Fagus sylvatica*. Noble et al. (1991) reported that fungi P47 and P30 also produce a lipopeptide called L-671,329 which is known as novel antifungal agent. Li et al. (2000) isolated a peptide called cryptocin from endophytic *Cryptosporiopsis* cf. *quercina,* which showed inhibitory activity against pathogens like *Gaeumannomyces graminis*, *Rhizoctonia cerealis*, *Pyricularia oryzae*, and *Phytophthora capsici*. Recently Terhonen et al. (2016) also proclaimed the diversity of metabolites produced by endophytic *Cryptosporiopsis* and its promising biocontrol activity against plant pathogens.

Heteroconium chaetospira is a demantiaceous endophytic fungi. *H. chaetospira* was first reported as an encouraging biocontrol agent by Narisawa et al. (1998). This endophytic fungus was isolated from roots of Chinese cabbage grown in wheat field.

In his experiment, Chinese cabbage seeds were pretreated with an isolate of *H. chaetospira,* which showed reduction in clubroot disease that was caused by soilborne protozoan, *Plasmodiophora brassicae*. Morita et al. (2003) suggested that the isolate of *H. chaetospira* was helpful in suppressing diseases that were caused by *Alternaria brassicae* and *Pseudomonas syringae* due to induced systemic resistance (ISR).

10.3 Bioactive Metabolites from Endophytes

Bioactive metabolites or compounds can be defined as by-products obtained from plants, animals, and microbes (Baker et al. 2000). These bioactive metabolites halt the growth of disease-causing agents especially pathogens causing disease in plants. Few endophytes, which produce bioactive metabolites, belong to the genera *Bacillus*, *Burkholderia*, *Pseudomonas*, *Rhizobium*, *Trichoderma*, *Phoma*, etc. These genera are already known for their secondary metabolite products like antibacterial, antifungal, antiviral, antioxidant, anticancer, insecticidal, immunosuppressants, volatile organic compounds (VOCs), etc. (Strobel 2003). In addition, wide-ranging bioactive metabolites such as alkaloids, aliphatic compounds, benzopyranones, phenols, flavonoids, quinones, steroids, terpenoids, tetralones, xanthones, etc. have been associated with endophytes (Tan and Zou 2001). An endophytic *Pseudomonas viridiflava*, isolated from grass species, produces novel antimicrobial compound ecomycin that is effective against a wide range of microbes (Miller et al. 1998). VOCs obtained from endophytes also possess antibacterial, antifungal, and antiviral properties (Firakova et al. 2007). Group of phenolic acids were extracted from the culture broth of a *Phoma* sp. by Hoffman et al. (2008), displaying antagonistic activities against *Sclerotinia sclerotiorum*, *Pythium ultimum*, and *Rhizoctonia solani*. Further research highlighted the role of another bioactive metabolite pyrrocidines, an alkaloid derivative isolated from endophyte *Acremonium zeae* residing in maize plant, in antagonizing phytopathogen like *Aspergillus flavus* and *Fusarium verticillioides* (Wicklow et al. 2005). An endophyte *Ampelomyces* isolated from the medicinal plant *Urospermum picroides* synthesized quinolone-derived bioactive metabolites known as 3-O-methylalaternin and altersolanol. These compounds presented inhibitory spectrum against a wide range of pathogens such as *Staphylococcus aureus*, *S. epidermidis*, and *Enterococcus faecalis* at minimum inhibitory concentration (MIC) value ranging from 12.5 to 25 mg/ml (Aly et al. 2008). Phenolic compounds, like pestalachloride, were extracted from endophytic fungi *Pestalotiopsis adusta*, which established significant antifungal activity against plant pathogens *Gibberella zeae*, *Verticillium albo-atrum*, and *Fusarium culmorum* (Li et al. 2008). Ethyl 2, 4-dihydroxy-5,6-dimethylbenzoate and phomopsilactone are bioactive metabolites, isolated from an endophytic fungus *Phomopsis cassiae* that showed robust antifungal activity against phytopathogenic fungi *Cladosporium sphaerospermum* and C. *cladosporioides* (Silva et al. 2005).

The aliphatic compound, chaetomugilin, detected in the cell-free culture supernatant of an endophytic fungus *Chaetomium globosum* collected from *Ginkgo* *biloba* showed antifungal activity against diverse fungal pathogens (Qin et al. 2009). A unique tetramic acid cryptocin, which possesses biocontrol activity against rice pathogen *Pyricularia oryzae*, was extracted from endophytic fungus *Cryptosporiopsis quercina* (Li et al. 2000). Novel spiroketals, isolated from an endophytic fungi *Edenia gomezpompae*, displayed significant inhibition against *Alternaria solani*, *Fusarium oxysporum*, and *Phytophthora parasitica*. A naphthodianthrone-derived compound hypericin and esmodin revealed antimicrobial activity against *Pseudomonas aeruginosa*, *Salmonella enterica*, *Escherichia coli*, *Aspergillus niger*, *Candida albicans*, etc. (Kusari et al. 2009). Lactone-derived secondary metabolite known as brefeldin, produced by *Cladosporium* sp., demonstrated maximum antifungal activity against phytopathogens. Antifungal bioactive compound pumilacidin produced by *Bacillus pumilus* and compounds like 2-hexyl-3-methyl-butanodioic acid and cytochalasin were synthesized from the endophytic fungus *Xylaria* with strong antifungal activities (Cafeu et al. 2005). Recently, cyclohexanone derivatives have been extracted from endophytic fungus *Pestalotiopsis fici*, which is effective against *Aspergillus fumigatus* (Liu et al. 2009). Antifungal metabolite trichodermin gained from endophytic fungus *Trichoderma harzianum* showed inhibitory spectrum against pathogens causing early blight of tomato and damping-off disease on cucumber plants (Chen et al. 2008). These were the role of few bioactive metabolites that participate in inhibiting pathogens and protecting plant health from diseases.

10.4 Endophytes from Lab to Land

10.4.1 Bioformulations from Endophytes

The delivery of biocontrol agents under field conditions is often hindered by the vulnerability of viable cells due to extremities in environment. Thus, biocontrol agents showing impressive disease-suppressing ability in the laboratory stage or under control conditions like plant growth chamber or glass house study fail to convey similar results in natural field. Several studies have shown that biocontrol agents fail to deliver good results, due to their deprived cell number in the soil, which generally arises due to tough competition with the native microbial community. Formulating suitable bioformulation is an essential criterion for exploiting any microbe-based technology into field. Hence, to certify the viability of endophytic cells, they must be properly shielded and secured. This protection could be offered by formulating them with suitable carriers and developing bioformulations from them (Bashan et al. 2014).

10.4.2 Selecting Right Endophyte

Strain selection is one of the most important steps in bioformulation development. As most of the endophytes are host specific, depending on the type of crop sown, it is necessary to select the correct endophytes for formulation development. Selected endophytes should not be generalized, but it should be specific, so as to give targeted results. Obligate endophytes that colonize plant parts without altering common plant functioning are encouraged for formulation development (Berg et al. 2005). Moreover these obligate endophytes face less competition and remain safe inside plant cells (Hardoim et al. 2008; Gaiero et al. 2013). High temperature-tolerant and endospore-producing strains could also be selected as suitable candidate for developing perfect endophytic bioinoculant (Senthilkumar et al. 2007). Endospore-forming ability makes this strain easy to use, formulate, and commercialize as it has extended shelf life. This distinctive trait has constantly attracted the attention of major research groups attempting to develop biocontrol agents for practical applications as it shows continued existence in soil even when host is not available. Also, while selecting an endophyte, it is of prime importance to state the target disease and the host on which it will be used. Before subjecting them to bioformulation, their mechanism and interaction with plant and pathogen should be established well by using whole or part of endophytic organisms under laboratory, greenhouse, and field conditions.

10.4.3 Selecting Optimized Conditions for Mass Multiplication

Once the right strain has been selected, the next step is to optimize the protocol for its mass multiplication and metabolite production. Optimization of various parameters like concentration of cells, temperature, pH, oxygen, moisture content, and nutrients is considered while mass multiplying the microbes. Zahir et al. (2010) reported that high mass of *Rhizobium* could be obtained by supplementing tryptophan in medium. Formulation containing tryptophan plus *Rhizobium* delivered significant enhancement in improving yield of mung bean crop under field conditions in comparison with untreated sets. For some endophytes applying the same parameters may not work as they are from unique origin; they may require some specialized conditions/nutrients that have not been unraveled. Further to make the process and product economic, cheaper substrates (like egg shells, sawdust, bagasse, hay, soil, peat, charcoal, etc.) at optimum conditions coupled with innovative and competent multistep downstream methods should be explored (Muthusamy et al. 2008). The mass production of cells under optimized state should be cost effective that will not only enhance the applicability of the bioformulation in industries but will also create confidence among the farmers and the production houses.

10.4.4 Formulations and Shelf Life Analysis

Evaluation of different inorganic and organic carriers has been done for the preparation of bioformulations and shelf life analysis of endophytes (Bashan et al. 2014). Bazilah et al. (2011) stated that for commercialization of microbial formulation, it is important to have good viability for certain period of time. Inoculants containing CFU of 10^9 cells and extended shelf life of 1–2 years have successful distribution in fields (Deaker et al. 2004; Schulz and Thelen 2008). Talc-based formulations developed from *Trichoderma* showed growth-promoting effects on cantaloupe plants under greenhouse condition (Vidhyasekaran and Muthamilan 1995). Viability of *Bacillus subtilis* and *Pseudomonas corrugata* in wet alginate beads was recorded to be 3 years (Trivedi and Pandey 2008), whereas, in dry alginate beads, viability of *Azospirillum brasilense* and *Pseudomonas fluorescens* was found to be 14 years (Bashan and Gonzalez 1999). Liquid formulation of *Bradyrhizobium japonicum*, used for enhancing soybean production, could be stored up to 8 years (Bashan et al. 2014).

10.5 Mode of Application

As discussed above, entry point is specific for certain bacteria; hence, the mode of application acts as major detrimental factor in deciding the efficacy of endophytes for disease suppression. Endophytic formulations are available either in powdered form or liquid form and can be inoculated by diverse methods like seed pelleting, seed dressing, soils drench, and foliar spray (Ramyabharti et al. 2016). Seed coating is the most common technique of inoculation as it is very easy and requires small amount of inoculant. Soil drench is generally used while introducing large bacterial cells in the soil. Granules of marble combined with perlite, peat, charcoal, and soil are also in use for soil inoculation as they enhance inoculant to be in contact with plant roots (Bashan et al. 2014). Recently, spraying methods are gaining popularity in case of endophyte inoculation as they can very easily enter inside the plant system and deliver better results. Endophytes that reside within fruits and flowers could just be sprayed or sprinkled to get good results.

Ramyabharathi et al. (2016) observed the utility of liquid formulation developed from endophytic *Bacillus subtilis* strain for enhancing shelf life of strain and reducing wilting symptoms (caused by *Fusarium*) in tomato plant. Formulation of the endophytic fungus *Cladosporium oxysporum* prepared from culture filtrates and conidial suspensions was tested for its inhibitory activity against the black bean aphid *Aphis fabae* by micro-irrigation technique. Results showed that formulation developed from culture filtrate gave much better results in inhibiting aphid population in comparison to conidial suspension, hence suggesting that proteolytic activity plays much important role in inhibition than the chitinolytic activity of the fungus against the aphid (Bensaci et al. 2015). Gao et al. (2015) evaluated different biopreparations of endophytic *B. subtilis* strain using their cells, cell-free culture supernatant, crude proteins, and non-protein fermentation liquid against *Blumeria graminis* infection in wheat. Application of these formulations demonstrated significant reduction of disease incidence in wheat plant; however, best results were obtained when fermentation liquid of *B. subtilis* was applied on the leaves in comparison with other formulations/treatments. Talc-based bioformulation developed from the combination of rhizobacteria *P. fluorescens* (Pf1) and endophytic bacteria *Bacillus* sp. was quite effective in reducing the incidence of Banana bunchy top virus by 52% in field conditions and also enhancing growth attributes of host plant (Harish et al. 2009).

Apart from direct inhibition of pathogens, endophytes are also known to induce host resistance, which is evidenced by an upsurge in PR proteins, defense-related proteins, and phenolic compounds in host plants. Applications of consortia of beneficial microbes, which can occupy different niches, are considered advantageous over formulations with single microbes. Formulation developed from this combination was not only effective in suppressing banana bunchy top virus but also active in reducing panama wilt of banana caused by *Fusarium oxysporum* (Harish et al. 2009). Talc-based bioformulation developed from rhizobacterial strains of *Pseudomonas fluorescens* and endophytic fungus *B. bassiana* amended with chitin recorded an enhanced biocontrol activity against leaf miner insect and collar rot disease (Senthilraja et al. 2013). Chitin supplement augmented the antagonistic activity of the entomopathogenic fungal and bacterial bioformulation, thus assisting the fact that chitin may induce systemic resistance in plants against insect pests and pathogens (Senthilraja et al. 2010). Muthu and Sharma (2011) reported the potency of talc-based bioformulation developed from *Trichoderma viride* and endophytic *P. fluorescens* (EBL 20-PF) in inhibiting growth of *Pythium aphanidermatum* (causes damping-off disease in chili). Formulation of these co-inoculating bioagents displayed high elicitation of defense-related enzymes, PR proteins, and phenols, in comparison with their sole application (Muthu and Sharma 2011).

Alghuthaymi et al. (2015) reported a special type of formultions developed from nanoparticles (NPs) of different fungi like *Aspergillus*, *Fusarium*, *Verticillium*, and *Penicillium* known as nano-formulations. These diverse fungi have been used to synthesize gold, silver, platinum, tellurium, selenium, silica, quantum, magnetite, and zirconia NPs possessing antifungal activity. Recently, different nano-fungicides, nano-pesticides, and nano-herbicides are being used extensively in the area of agriculture sciences (Alghuthaymi et al. 2015). Park et al. (2006a, b) reported the antimicrobial activity of nano-sized silver particles in suppressing plant diseases. The use of silver NPs (Ag-SiO_2 NPs) as fungicides is safer than using any synthetic fungicides (Oh et al. 2006). Ag-SiO_2 NPs have strong biocontrol activity against *Botrytis cinerea.* Amalgamation of Ag-NPs with fluconazole showed good antifungal activity against *Aspergillus*, *Fusarium*, *Phoma*, *Trichoderma*, *Candida*, etc. (Gajbhiye et al. 2009). Application of nanotechnology in the field of plant pathology is still in its infancy and needs further exploration in the area of nano-delivery systems in natural field conditions.

10.6 Monitoring the Endophytes in Environment

Soil is a composite, heterogeneous, and nutrient-rich habitat where billions of indigenous microbes already exist. After releasing targeted endophytes embellished in a proper formulation, it is somewhat challenging to identify the exact population of bioinoculant, as the added inoculants have to compete with resident soil microorganisms for the nutrient and niche occupation. The probability of finally achieving successful endophytic inoculants is a gruesome task as these microbes enter inside the plant system from roots to stem and further on. So isolating the desired endophytes by crushing or macerating plant tissue is usually opted for to obtain the endophytic load or to check for root colonization. But again only cultivable endophytes could be obtained from plating, baiting, or macerating technique, while unculturable endophytes cannot be obtained. Hence more precise and consistent methods for monitoring the fate of introduced endophyte are required for monitoring its efficacy under field conditions. Conn and Franco (2004) described noteworthy decrease in the population of local actinobacterial endophytes when inoculated with a commercial consortial product. Devi and Momota (2015) reported that successful endophyte colonization can also be visualized by using β-glucuronidase reporter system as shown in case of *Herbaspirillum seropedicae* Z67 when inoculated onto rice seedlings. Apart from that, proteomics, genomics, trancriptomics, or metabolomics could be exploited as an influential tool to comprehend the complex design of genes, proteins, and metabolites with respect to different environmental niches in which the bacteria live (Trivedi et al. 2012).

10.7 Ecological Impact Assessment

It is well established that pesticides and chemicals used in agriculture are highly efficient but their excessive and unregulated use also leads to serious aftereffects on soil, environment, and human health. These concerns are well realized today, in all quarters of scientific community, and are gradually being acknowledged by various social groups and individuals. It must be realized by the policy makers and governments too that the time is ripe, to regulate the use of these chemicals and pesticides and make enabling provisions for replacing them with bioformulations which are more reliable, environment-friendly, and safe. Governments must also promote vigorous research into advanced agricultural systems where the use of chemicals and pesticides shall be completely prohibited and replaced with organic and biological products and compounds. Formulations developed from microbes like endophytes will be purely biological. Apart from their non-toxicity, these formulations will be purely biodegradable, nonpolluting, leaving no carbon footprints (Bashan et al. 2014), and non-disturbing toward the ecology of soil, human, or environmental health, together with helping in carbon sequestration, thereby increasing soil organic carbon. Sharma et al. (2017) performed comparative study by applying

Bradyrhizobium inoculants and chemical fertilizers in pigeonpea in field. The effect of bioinoculant gave promising results not only in terms of plant growth enhancement but in enhancing local microflora residing in the field, thus authenticating the nontarget effects contributing to the overall efficacy of such applications.

10.8 Conclusion and Future Prospects

After analyzing the available scientific literature, it can be concluded that studies on endophytes have opened a new avenue in the area of plant disease management. Endophytes are designated as future “plant probiotics” as they reside inside the plant host and leverage multiple beneficial effects without causing any harm to the host plant. The study of endophytes involves several challenges, the most common being its isolation process. The process of isolating true endophyte by surface sterilization and overlooking the rest of the microbes is somewhat difficult. Further, there are chances of hindering growth of endophytes due to the penetration of surface-sterilizing chemicals in the tissues. Hence, appropriate methods and precautions for isolation should be followed based on the plant type and tissues under consideration. In order to study the diversity of endophytes, more emphasis should be given on culture-independent approaches as they are quick, specific, and time-saving and can find large number of endophytes that could not be easily cultured in laboratory.

Culture-independent approaches concentrate on molecular methods including polymerase chain reaction (PCR) and quantitative PCR (Q-PCR), but they too have their own limitations as no pure culture of endophytes are obtained for field application using this methodology; besides there are some biases that are introduced when performing analysis using cultivation-independent techniques. Various other genomic approaches like denaturing gradient gel electrophoresis (DGGE), and ultrahigh-throughput sequencing methods such as pyrosequencing and microarray are used nowadays to understand endophytic diversity.

Exploring potent endophytes can pave way for rich source of bioactive and novel metabolites, which can find plethora of uses in various agricultural and industrial arenas. Diverse bioactive secondary metabolites produced by endophytes, exhibiting promising biocontrol activities, have been illustrated in the chapter, but much more research is needed to optimize and standardize the protocols for extracting many other unknown and unidentified compounds which might be useful at commercial level.

Though bioformulations derived from endophytic cells (either bacterial or fungal) deliver promising results (as cited with several examples in the chapter) in terms of suppressing disease incidence, efficacy and potency of these formulation can be further enhanced by exogenous application of bioactive secondary metabolites in combination with beneficial endophytes, as these formulations would be more target -specific. Bioformulations derived from pure bioactive ingredients or combination of endophytes plus metabolites is a novel topic that needs to be researched and worked upon for controlling phytopathogens.

Recently endophytes have been explored for synthesizing nanoparticles like gold and silver, which can treat dreadful diseases in the near future. These innovative technologies suggest boundless role of endophytes in upcoming years for producing more effective and economical nano-formulations that could be used for controlling plant and animal diseases.

Hence, the futuristic approach recommends encouraging research on bioprospecting of endophytes and isolating them from wild, untouched, and unexplored regions. Detailed knowledge on this topic will provide a better understanding of these endophytes and their application in diverse agricultural practices to ensure better food productivity and security in future.

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