Biocommercial aspects of microbial endophytes for sustainable agriculture

13

H.C. Yashavantha Rao[a], *N. Chandra Mohana*[b], *Sreedharamurthy Satish*[b]
[a]Department of Biochemistry, Indian Institute of Science, Bengaluru, Karnataka, India;
[b]Microbial Drugs Laboratory, Department of Studies in Microbiology, Manasagangotri, University of Mysore, Mysuru, Karnataka, India

Chapter outline head

13.1 Introduction 324
13.2 Incidence of microbial endophytes diversity 325
13.3 Comparison of native and alien endophyte inoculants 326
13.4 Growth promotional aspects due to symbiosis 327
13.4.1 Direct mechanisms of plant growth promotion 327
13.4.2 ACC deaminase activity 328
13.4.3 Micro and macro nutrient 328
13.4.4 Abiotic stress 328
13.4.5 Phytoremediation 329
13.5 Deciphering disease suppressive mechanisms 329
13.5.1 Indirect mechanisms of plant growth promotion 329
13.5.2 Competition for niche and nutrition 329
13.5.3 Antagonism 329
13.5.4 Induced systemic resistance 330
13.5.5 Endophytes as biocontrol agents against pests 331
13.5.6 Mode of action by entomopathogenic fungi 331
13.5.7 Host range and host specificity of entomopathogenic endophytes 332
13.5.8 Development of endophyte inoculants 332
13.5.9 Types of inoculation for delivery 333
13.5.10 Foliar inoculation 333
13.5.11 Stem inoculation 333
13.5.12 Seed dipping 334
13.5.13 Root dipping 334
13.5.14 Soil spray 334
13.5.15 TwinN 334
13.6 Commercialization of endophyte products for sustainable agriculture 334
13.6.1 Bio vaccine 335
13.6.2 Biofertilizers 335
13.6.3 Biocontrol products 336
13.7 Bio market 336
13.7.1 Registration of product 337
13.7.2 Quality control 337

Microbial Endophytes. http://dx.doi.org/10.1016/B978-0-12-819654-0.00013-2

13.7.3 Efficacy of the products 337
13.7.4 Regulation of endophyte-based bioproducts market 337
13.7.5 Challenges in endophyte commercialization 337
13.7.6 Possible toxicity assessment 338
13.8 Recent developments and applications of microbial endophytes 338
13.8.1 Auto fluorescent protein (AFP) technique 338
13.8.2 Genome studies 338
13.8.3 Genetic engineering 338
13.9 Conclusion and future perspectives 339
References 339

13.1 Introduction

Microbial endophytes have been evolved over the period of time from being just defined as the microorganisms living inside the host plants indicating their location and type of association with their host. They are ubiquitous in higher plant species which live asymptomatically in the internal tissues and exhibit various relationships with their host. Microbial endophytes enter into the plants through wounds, root hairs, epidermal conjunctions, and naturally occurs as a result of plant growth (Shah et al., 2019). Besides entering to the plants through wounds or natural openings, endophytic microorganisms actively penetrate the plant internal tissues using cellulase and pectinase hydrolytic enzymes (Paramanantham et al., 2019). They are well-known to increase plant growth and yield by nitrogen fixation, solubilization of potassium, zinc, and phosphorus; production of phytohormones like gibberellins, cytokinin, and auxin and having antagonistic activities as well as by reducing the stress ethylene in host plants (Patle et al., 2018; Rao et al., 2015a). Several studies have been documented for understanding the structure and composition of plant-associated endophytes, which indicates plant-microbe, microbe-microbe interactions as well as abiotic factors that lead to plant endobiome composition and structure (Rakshith et al., 2016; Rao et al., 2017a). The plant endobiome is well-known to increase plant defense ability against several invading pathogens and insect herbivores. Several reports on the plant-microbe interactions involved in endobiome provide an alternative way for different biosynthetic metabolic pathways which are responsible for the biosynthesis of several bioactive and novel biomolecules of commercial significance (Rao and Satish, 2015; Rao et al., 2017b; Sheik and Chandrashekar, 2018). Indeed, plant endobiome is an important factor in global biogeochemical cycles. Hence, the use of plant endobiome is considered to bear the potential to promote production of plant secondary metabolites, plant disease protection, and chemical inputs, leads to more sustainable agricultural applications with enhanced productivity (Singh et al., 2017).

Plant-microbe interactions have been extensively studied and explored since decades (Chisholm et al., 2006; Rao et al. 2015b; Strobel and Daisy, 2003; Zhang et al., 2006). However, cognizance of plant–microbe interactions and their relationship is highly complex (Rosenblueth and Martínez-Romero, 2006; Saikkonen et al., 1998; Saikkonen et al., 2004). But it has established certain microbial interactions which could exert a positive effect, with respect to endophytes applications. Endophytes have been previously defined as microbes inhabiting internal tissues of host plant without expending any negative effects (Breen, 1994; Faeth, 2002; Hardoim et al., 2008; Mack and Rudgers, 2008). Endophytes extend advantages to the host plant like plant growth promotion and defense against invading pathogens (Clay, 1988; Conn et al., 2008; Gao et al., 2010; Saikkonen et al., 2010). Endophytic behavior is often related to a set of genes, however, there is no definite understanding to specify the genes involved (Card et al., 2016; Sevilla and Kennedy, 2000). The direct benefits of endophytes to host plant include phytohormone production (Khan et al., 2011; Shi et al., 2009; Waqas et al., 2014), biocontrol against phytopathogens and pests due to antimicrobial secondary metabolites (Clay, 1989; Downing and Thomson, 2000; Mejía et al., 2008), iron chelators, due to siderophore production (Bartholdy et al., 2001; Lacava et al., 2008; Loaces et al., 2011), phosphate solubilizing compounds (Otieno et al., 2015; Taurian et al., 2010), nitrogen fixation (Cocking, 2003; Hurek and Reinhold-Hurek, 2003; James, 2000), induced systemic tolerance (Kloepper and Ryu, 2006; Vu et al., 2006), and antagonism (Clay, 1991; Coombs et al., 2004; Ramesh et al., 2009; Schulz et al., 1999).

Plant growth promotion due to phytohormones produced by endophytes results in changes in the morphology and structure of host plant (Gaiero et al., 2013; Hardoim et al., 2008; Santoyo et al., 2016). The production of phytohormones such as indole acetic acid (De Battista et al., 1990), cytokinins (Frugier et al., 2008), gibberellic acid (Waqas et al., 2012), ethylene (Camehl et al., 2010), and auxins (Merzaeva and Shirokikh, 2010) would enable endophytes for their applications in sustainable agriculture. The ability of endophytes reducing the atmospheric nitrogen, which is limited for plants would be addressed biologically as an alternate to chemical fertilizers. The insoluble phosphate could reduce from endophytes by expulsion of organic acids converting them to soluble orthophosphate for plant uptake and utilization. Endophytes are well-known produced low-molecular weights called siderophore, which chelate iron for plant uptake and utilization. Some of them include catacholate, hydroxymate, and phenolate (Das et al., 2007). Indirect mechanism of benefits of endophytes confers tolerance to stresses like drought, cold, and hypersaline condition through mechanism, such as induced systemic resistance (ISR) and pathogenesis (Rodriguez and Redman, 2008; Waller et al., 2005).

13.2 Incidence of microbial endophytes diversity

The term biodiversity is used to describe the variation in population associated with organisms or between their populations. The understanding of biodiversity could open many avenues at different scientific levels. The taxonomic studies with systematics along with population biology could give insights for evolution, their conservation and most importantly for their efficient utilization for various applications. Microbial endophytes association with the plant is ubiquitous; the existence of endophyte-free plant is a rare exception (Arnold, 2007; Porras-Alfaro and Bayman, 2011). Microorganisms in an ecosystem will directly influence the functioning and biodiversity of ecosystems.

In this regard, the incidence of endophytes in plants can influence the potential factors to determine the diversity in ecosystems and also they could alter the structure and functioning of plants, which is highly complex and unpredictable. The changes in a shift of incidence in endophyte population could trigger changes in plant community composition due to various factors such as survivorship, competition (Rodriguez et al., 2009). Abiotic conditions could modify plants, which may have consequences in dynamics and diversity of the endophyte community (Bulgarelli et al., 2013). Hence, knowledge of different factors is imperative for environmental conservation and sustainable agriculture.

Over the past decades, sufficient collective efforts from all over the globe have been concentrated for the diversity and role of microbial endophytes for sustainable agriculture. Endophytic assemblages with respect to endophytic associations have shed considerable knowledge in this regard (FROeHLICH and Petrini, 2000; Rao et al., 2015c). Several reports regarding diversity data have been published from actinobacteria, bacteria, and fungi (Arnold, 2007; Rao et al., 2015a). Most of the reports are based on culture-dependent studies and very fewer reports on culture-independent techniques are available. The major disadvantage of the culture-dependent technique is that it does not reflect the aspect and functionality of unculturable microbiota, which could cause hindrance in understanding the complex phenomenon of host-endophyte relationship. The diversity incidences of bacteria and fungi have been reported more when compared with actinobacteria due to their slower growth rate. The endophytic bacteria have been reported from the following phylum Firmicutes, Proteobacteria, and Bacteroidetes with a varying distribution where the highest incidence was reported from Proteobacteria whereas least from Bacteroides (Andreote et al., 2009). The incidences of fungal endophytes are reported in phylums Ascomycota and Basidiomycota where Ascomycota is the most dominant (Rodriguez et al., 2009).

Tian et al. (2004) reported the population diversity of four rice cultivars. The results revealed that *Fusarium* and *Streptomyces* genera to be predominant. The study also revealed the incidence of endophytic fungi to be more diverse in leaf and actinomycetes in roots. The diversity study by Naik et al., (2009) in rice reported *Streptomyces* sp., *Chaetomium globosum, Penicillium chrysogenum, Fusarium oxysporum,* and *Cladosporium cladosporioides* as dominant species. Endophytes diversity was found to be lower during summer and high in winter suggesting the effect of climate on endophyte colonization.

13.3 Comparison of native and alien endophyte inoculants

Introduction of alien microbes imposes the threat for existing native endophytes, in turn leading to unforeseen disturbance. The disturbance could be due to elaborate interactions exerted due to the presence of alien endophytes or absence of native endophytes (Bonnardeaux et al., 2007). The extent of invasion studies is more been focused on macroorganisms than microbes. The invasion risk associated with microbes is poorly understood. The general focus on pathogenic microbes found to be invasive is prioritized for control and elimination, but the invasion of nonpathogenic microbes should also be considered even though there is low risk as it is not been detected (Pringle et al., 2009). A recent comparison study on indigenous mycorrhizal fungi *Rhizophagus irregularis* from yam and *Acaulospora* from cassava with commercial *Rhizophagus intraradices* revealed a high rate of colonization by native flora. The overall growth and yield of the plant was also found to be increased. One significant observation from the study was that the *Rhizophagus irregularis* from yam induce highest colonization between two indigenous microflora indicating the selective importance of native microflora from target plant species would be advantageous (Kouadio et al., 2017).

13.4 Growth promotional aspects due to symbiosis

Microbial endophytes colonization in host plant is considered as a symbiotic association. Microbial endophytes enhance their immune response and protection in the host plant and get benefited from secondary metabolites produced from these microbes aiding in plant growth (Berg, 2009). The symbiotic association is a coevolution of endophytes with host plant changes in microbial diversity that occurs depending on genotype, stage of growth, physiology, plant organs, and other ecological parameters. The evolution of endophytes brings in changes in cellular and molecular levels, which is highly complex. The intimate relationship occurring between endophyte and host plant is a positive selection process for evolution, which confers beneficial for successful propagation and survival. They accord plants with negative hinderance of biotic and abiotic factors. These interactions have not only beneficial in terms of plant health, growth, development, and production but also soil quality, which could stabilize adverse ecological conditions due to various anthropological activities (Choudhary, 2012). The positive effects of significantly increased plant biomass, dry matter yield, and grain yield would also produce higher income from agronomically with sustainability.

13.4.1 Direct mechanisms of plant growth promotion

13.4.1.1 Production of phytohormones

Endophytes produce phytohormones like indole-3-acetic acid (IAA), cytokinins, and gibberellins, which can stimulate the growth, reproduction, and germination. It also has a major role in conferring to biotic and abiotic stress. Endophytes have a crucial role in physiological changes in plants. Reddy et al., (2014) demonstrated when wheat was treated with *Metarhizium robertsii, M. brunneum*, and *Beauveria bassiana*, there was a substantial increase in overall plant yield and stand counts. Cotton plants inoculated with *B. bassiana* and *Purpureocillium lilacinum* resulted in improved growth of plants along with biomass (Lopez and Sword, 2015). Artificial inoculation of the endophytic bacterium *Pseudomonas* spp. strains in cotton-improved plant height and number of nodes on the stem (Erdogan and Benlioglu, 2010).

13.4.2 ACC deaminase activity

One of the major aspects in this regard is enzymatic hydrolysis of ACC (1-Aminocyclopropane-1-Carboxylate). ACC has involved in the ethylene biosynthetic pathway, its intermediary between methionine to ethylene conversion. Endophytes utilize ACC exudate from plants before the oxidation occurs due to ACC oxidase in the plant. These endosymbionts cleave the ACC deaminase to α-ketobutyrate and ammonia, followed by utilization of ammonia decreasing ACC with simultaneous reduction of ethylene in the plant system. This phenomenon helps the host plants in stress tolerance along with the plant growth. Extensively studied phytohormone IAA which aid in cell division and elongation contributes indirectly in plant growth as well as plant defense response (Ali et al., 2014; Glick, 2014; Sun et al., 2009). Other than IAA, several plant hormones such as cytokinins and gibberellin can stimulate plant growth and modify plant morphology based on environmental conditions.

13.4.3 Micro and macro nutrient

Micro and macronutrients are necessary for the entire microorganisms, as they act as a cofactor for numerous enzymatic reactions occurring in the biological system. For example, iron which exists in ferric state (Fe^{3+}) under aerobic conditions forms hydroxides and oxyhydroxides, which are insoluble. Siderophores could also trigger IAA biosynthesis, which is a beneficial aspect as mentioned earlier. For growth and development of biological system phosphorous is very much required. In nature, soluble phosphorous exists in two forms, that is, monobasic and dibasic soluble forms. The available natural soluble phosphorous is limiting and in heavy metal concentration, P-uptake is highly affected leading to retardation in plant growth. Endophytes aid in conversion insoluble phosphorous to soluble forms by acidification, chelation, exchange reactions, and release of organic acids (Jog et al., 2014). Endophytes could also solve phytotoxicity imposed by high metal concentrations by biosorption and bioaccumulation mechanisms.

13.4.4 Abiotic stress

Microbial-endophytes interaction with host plants has been proved to bestow abiotic stress tolerance and to minimize the obstructive ecological impacts on native as well-cultivated plant communities. The intrinsic associations help the host plant in the acquisition of nutrients during stress. Symbiosis with endophytes confer a variety of tolerance to hosts such as heat tolerance in high temperate regions; salt tolerance in plant communities present in coastal regions (Choudhary, 2012). Other mechanisms include biological nitrogen fixation and also the release of PGP factors facilitating the vegetation. When wheat was artificially inoculated with *Azospirillum brasilense* provided the host plant to mitigate water stress with better grain yield (Furlan et al., 2017). Induction of systemic tolerance against water and salt stress was observed in tomato and pepper plants by *Achromobacter piechaudii* (Paul et al., 2017).

13.4.5 Phytoremediation

Certain plants have the ability to strive on contaminated soil, this is associated with genetic as well as physiological changes employed to cope up with soil contaminants. One of the mechanisms includes harboring of endophytes especially bacteria for phytoremediation. These endophytes have evolved to tolerate high concentrations of pollutants and simultaneously exhibit plant growth promotion. Endophytes in such cases are involved in various activities like synthesis of siderophores, phosphate solubilization, ACC deaminase activity, production of IAA, cytokinins, and gibberellins (Kumar et al., 2015; 2016). Studies have revealed that endophyte resistance to heavy metals such as Cd, Zn, and Pb (Ma et al., 2015; Ullah et al., 2015). When *Sedum plumbizincicola* inoculated with endophytic bacterium *Bacillus pumilus* strain elevated the uptake of Cd, also increase in the root and shoot length of plants were observed. Thus, overall phyto-extraction capacity of the plant was observed along with plant growth promotion indicating the potential role of endophytes in phytoremediation.

13.5 Deciphering disease suppressive mechanisms

13.5.1 Indirect mechanisms of plant growth promotion

The indirect mechanism of plant growth promotion is due to suppression of pathogens on plants by inhibitory substances or by ISR in the host. Biocontrol of phytopathogens using endophytes was first described by Timothy (Paulitz and Bélanger, 2001). Endophytes as biocontrol agents have been interesting, but have not been received considerable attention in this regard. Considering the present situation and challenges imposed by phytopathogens, employing endophytic microorganisms would surely create greater demand as well as the market. The isolation of native endophytes of particular species in their respective geographical location and assessment of its effects on phytopathogens as well implications on plant growth could provide dual benefits for sustainable agriculture.

13.5.2 Competition for niche and nutrition

Endophytes could essentially deprive the space and nutrition against phytopathogens. Due to intrinsic properties and better adaptation to host as well as its environment, endophytes contend against invasive phytopathogens. The ability of native endophytes is relatively high on colonization in plant tissues when compared with invading pathogens (Backman and Sikora, 2008).

13.5.3 Antagonism

Antagonism refers to hostility toward microorganism to another; it can be either by parasitism or antibiosis. Parasitism is inhibition of another organism, with respect to fungus it can be termed as mycoparasitism. Mycoparasitism involves penetration of the parasite into the host hyphae with specialized structures such as haustoria and release of secondary metabolites resulting degradation of fungal structure enabling nutrient uptake from host fungus (Clay, 1991). The widely studied *Trichoderma* spp. could be the best example while explaining mycoparsitism. *Trichoderma* spp. can secrete a wide range of cell wall-degrading enzymes which is crucial for mycoparasitism. Enzymes like cellulase, xylanase, pectinase, glucanase, lipase, amylase, protease, endochitinases, β-glucanases, and proteases can degrade fungal cell walls (Adams, 2004). The hyphae of *Trichoderma* spp. coils around pathogens forming specialized structures like hooks, appressorium, haustoria breaking the cell wall, and release of antimicrobial compounds leading to death (Markovich and Kononova, 2003; Verma et al., 2007). Four *Pseudomonas* strains isolated from the roots of *Xanthium strumarium, Portulaca* sp., *Gossypium hirsitum*, and *Convolvulus* arvensis showed suppression of disease incidence due to *Verticillium* wilt (Erdogan and Benlioglu, 2010). Antagonism against *Ralstonia solanacearum*, causative agent bacterial wilt was exhibited by two bacterial strains belonging to *Streptomyces* genera isolated from a tomato plant, mode of action was observed due to the production of siderophores and ACC deaminase activity Tan et al. (2011). The endophytic bacterium strain HA02 had significant inhibition against *Verticillium dahlia* which is the causative agent of *Verticillium* wilt of cotton (Li et al., 2012). A study carried out by Ramesh and Phadke, (2012) using bacterial endophytes from the grape, watermelon, and papaya against *Ralstonia solanacearum* revealed the antagonistic activity of isolates. The results showed maximum inhibition by *Enterobacter cloacae* from papaya followed by *Bacillus subtilis* (EB-06) and *B. flexus* from watermelon and least by *B. pumilus* from the grape.

Antibiosis interaction by secretion of various secondary metabolites having antimicrobial properties or biostatic can suppress or reduce the growth of phytopathogens. Biocontrol of phytopathogens such as *Phytoptera infestans* and *Phytoptera capsica* by *Purpureocillium lilacinum* which are documented to produced antibiotic leucinostatins (Wang et al., 2016). The culture-based study carried out by Mohan et al. (2015) revealed antagonistic potential of eight ectomycorrhizal fungal isolates *Alnicola* sp., *Laccariafraterna* sp., *Lycoperdonperlatum* sp., *Pisolithusalbus* sp., *Russulaparazurea* sp. *Scleroderma citrinum*, *Suillusbrevipes* sp., and *Suillussubluteus* sp., against *Alternaria solani*, *Botrytis* sp., *Fusarium oxysporum*, *Lasiodiplodia theobromae*, *Phytophthora* sp., *Pythium* sp., *Rhizoctonia solani*, *Sclerotium rolfsii,* and *Subramanio sporavesiculosa*. The highest inhibition was shown against *Suillusbre vipes* while *P. albus* being least. The effective biocontrol of *Capsicum* bacterial wilt by *Ralstonia solanacearum* was observed by bacterial endophyte from *Bacillus amyloliquefaciens* due to production of antimicrobial protein LCI (Hu et al., 2010).

13.5.4 Induced systemic resistance

Plants have enabled themselves with diverse defense mechanisms to intercept and counter adverse negative impacts posed by invading pathogens. These pathways are triggered when the invasion of pathogens and pests occur. They involve specific pattern-recognition receptors, which could be either pathogen or microbe-associated molecular patterns (PAMPs or MAMPs) based on signaling molecules from microbes or damage-associated molecular patterns (DAMPs), which are plant-based. Endophytes could trigger MAMPs prior to infection stage by phytopathogens rendering ISR to host plant. Disease by *Verticillium dahlia* in oilseed rape was found to be suppressed by *Serratia plymuthica* along with plant growth stimulation, the role of AHL-mediated signaling for disease suppression was found to be in crucial in this regard (Müller et al., 2009). Olive root endophyte of *Pseudomonas fluorescens* was found to be effective against *Verticillium* wilt of olive. The isolate induced a broad range of defense response along with induction of systemic defense responses (Cabanás et al., 2014).

13.5.5 Endophytes as biocontrol agents against pests

Entomopathogenic fungi as a biocontrol agent against pests have been an emerging area of interest. Various genera of fungal endophytes have been reported to be entomopathogenic. The instigation of entomopathogenic fungi was studied using soil fungus *Beauveria bassiana* (Ascomycota) which was found to be effective against more than 700 insect pests, also being first commercial biopesticide. Many reports have been available in this regard from 1980s. Biocontrol using endophytes can also be employed for management of invertebrates' pests (Jaber and Salem, 2014). *Metarhizium, Beauveria, Lecanicillium,* and *Isaria* are commercially available as biopesticides. Protection against both phytopathogens and arthropod pests render endophytes to be dual biocontrol agent. Biocontrol of *Thripstabaci* was found to be effective in onion with *Fusarium* sp., *Hyprocrealixii,* and *Trichoderma* sp. (Muvea et al., 2014). Artificial inoculation of *B. bassiana* for the control of *H. armigera* was successful in tomato plants (Qayyum et al., 2015a). Application of *B. bassiana* and *M. brunneum* in melon was effective in inducing a significant mortality rate of *Bemisia tabaci* for the pest management of *Cucumis melo* L (Garrido-Jurado et al., 2017).

Even though endophytes have developed strong antagonism against phytopathogens, aiding in plant growth and induction of tolerance, there are several challenges need to be addressed for employing them as biocontrol agents. The artificial inoculation could essentially face strong competition from microbial diversity which is already established in host plant and can also be influenced by the effect of ecological changes. The effectiveness could vary from lab trials and field trials. This may be attributed to a variety of factors such as less viability during storage, toxicity to untargeted organisms or may have poor colonization rate.

13.5.6 Mode of action by entomopathogenic fungi

The mechanism of infection in insects occurs through adhesion, penetration, proliferation, and death. Adhesion of entomopathogenic fungi to insects is due to adhesion genes or hydrophobicity exerted by conidia. The hydrophobic lipid layer aid in attachment of propagules. In specific adhesion by genes include adhesion proteins such as hydrophobins, some of the genes involved in this regard includes Mad1, ssgA, HYD1, HYD2, HYD3 (Moonjely et al., 2016). The conidia adhered to the cuticle germinate with aid of insect components such as lipids, chitins, and proteins leading degradation of the cuticle. The lipids in the cuticle provide the major carbon source for conidial germination. After germination, the hyphae protrudes into cuticle releasing various proteolytic enzymes such as proteases, esterases, *N*-acetylglucosaminidases, chitinases, and lipases. The specialized hyphal structures formed appressoria confirms the successful penetration. After successful penetration, yeast-like bodies is formed in hemolymph now called blastopores ultimately resulting in death. In addition to these mechanisms entomopathogenic fungi such as *Beauveria* and *Metarhizium* also produce insecticidal metabolites such as beauvericin and destruxins (Strasser et al., 2000). A study conducted by Kim et al., (2016) reported suppression of powdery mildew by Zucchini Yellow Mosaic Virus (ZYMV) and aphids in cucurbits.

13.5.7 Host range and host specificity of entomopathogenic endophytes

The endophyte employed as controlling pests should be able to survive and maintain a symbiotic relationship with the host plant along with nontoxicity for nontarget organisms. Therefore, the target range and target specificity of entomopathogenic endophyte of interest should be determined to avoid detrimental changes. *Metarhizium robertsii* species is often considered as a potential candidate due to narrow host specificity and also it does not cause any time-lapse problems (Wang and Leger, 2005).

13.5.8 Development of endophyte inoculants

The endophyte inoculants for sustainable agriculture have a potential market of interest. It also has several challenges associated with the solutions which can develop the various industry for large-scale agronomical practices. The high uneconomical use of chemical and its adverse negative impacts could essentially be addressed using endophyte technology. The native endophyte diversity with related crop wild relatives is more suitable for tangible benefits like improved colonization and adaptability, thus reducing the chances of the impact of alien species for the environment which could be detrimental. The establishment of endophyte diversity in particular genera from the diverse environment or more endemic to that region would provide significant correlations on diversity dominance. To evaluate the diversity dominance for selecting endophytes, the most crucial factors to focused are sampling sites, plants should be disease-free, ecological parameters of sampling site (temperature, pH, water conditions, and salinity), sites selected should be free from external influence of anthropological activities, that is, undisturbed natural habitat, should not consist of any invasive species, plant growth stages (Busby et al., 2017). After establishing dominant species, the second step is to screen for potential endophytes as inoculants could be assessed. The endophyte to be screened for application should not be pathogen for either human or plant, genetic variation between the host plant and endophyte should be minimum, the ability of potential isolate should grow on a range of substrates, purity of culture should be considered along with its ability to produce spore for artificial inoculation. Efficacy of single endophyte along with a mixture of endophyte inoculants in the host

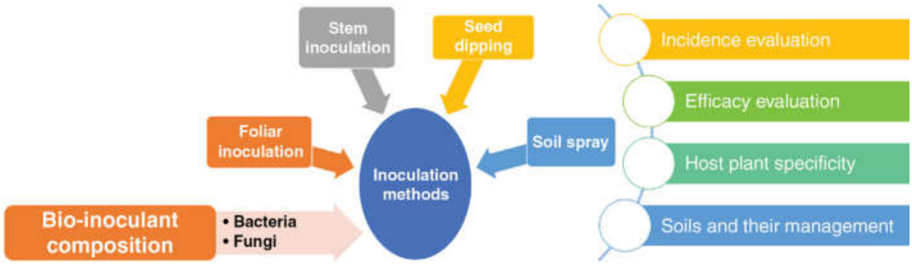

Figure 13.1 Different artificial inoculation methods for the colonization of microbial endophytes and their applications.

plant could also be considered for efficient plant improvement and yield. The tolerance assessment for various biotic and abiotic stresses should be validated for at least three generations with significant statistical results would be required (Faye et al., 2013). The successful translation from controlled environments to field trials would establish the successful commercialization. With global climate change and negative impacts by chemicals used in agriculture the employment of endophyte could provide a vision for sustainable agriculture.

13.5.9 Types of inoculation for delivery

The effect of artificial inoculation for colonization of endophytes depends on various biotic and abiotic factors, along with inoculation methods, growth media, and the density of inoculum. There are several inoculation strategies described such as foliar (micro-slit method, spraying conidial suspension), stem (micro-slit method), seed dipping, root dipping, soil spray, and callus culture. Some of these techniques have been observed to show a high success rate which is briefly discussed in Fig. 13.1.

13.5.10 Foliar inoculation

Foliar inoculation has been found to be successful in the majority of crops. Artificial foliar inoculation of *Beauveria bassiana* suppressed disease severity of downy mildew by *Plasmopara viticola* in grapevine (Jaber, 2015). Similarly, this method has been successful efficient in colonization in wheat, corn, bean, tomato, and pumpkin (Gurulingappa et al., 2010), soybean (Russo et al., 2015), tomato (Resquín-Romero et al., 2016), bean (Jaber and Enkerli, 2017), and grapevine (Rondot and Reineke, 2018).

13.5.11 Stem inoculation

Stem inoculation has also been found to be efficient in colonization of endophyte inoculants could be attributed to direct injection of conidia inside plant tissues by passing the external defense barriers. The stem inoculation has been successful in cacao (Rubini et al., 2005) and coffee (biocontrol of pest berry borer) (Posada et al., 2007).

13.5.12 Seed dipping

The presoaking of seeds in conidial suspension has been reported by Powell et al. (2009) in tomotoes (*B. bassiana*); bean (Akutse et al., 2013), and tobacco (Russo et al., 2015).

13.5.13 Root dipping

The dipping young seedling roots in conidial suspensions have been found most effective in banana when compared with other techniques (Akello et al., 2007). Also, the method had in successful in the recovery of *B. bassiana* in tomato (Qayyum et al., 2015), tobacco, corn, wheat, and soybean (Russo et al., 2015).

13.5.14 Soil spray

Application of conidial suspensions around root seedlings has also been found suitable for endophytes colonization. Reports of soil drenching in sorghum (Tefera and Vidal, 2009), tomato (Elena et al. 2011); common bean (Parsa et al., 2016), cassava (Greenfield et al., 2016) has been found to be successful. Soil drenching by the conidial suspension of *B. bassiana* and *M. brunneum* resulted in improved growth of sweet pepper plants.

13.5.15 TwinN

TwinN is a dried microbial inoculum packed with vacuum which provides prospicient shelf life period. For usage, first dissolved in less water and later in large amount of water. TwinN contains a consortium of microorganisms including growth hormones, nitrogen fixation, and phosphate solubilizers. These microorganisms can inhabit in the root, shoot, rhizosphere, and leaf as endophytes. It can be used as an inoculant for crops as well as trees. This TwinN can be well applied in irrigation, sprinkler, and spray depends on crops.

13.6 Commercialization of endophyte products for sustainable agriculture

Over the past 60 years, agriculture has been dependent on synthetic chemical pesticides and fertilizers resulting in the evolution of resistance. Endophytic microbes as an alternate to the traditional agricultural practices have been recently focused and have found wide interest globally by researcher communities (Fig. 13.2). These endophytes are desired as they can host a wide range of benefits from plant growth to plant protection. But due to the legislations imposed on biopesticides based on regulations for synthetic substances, it has brought barriers for biomarket of endophytes. The major bioinoculants employed and marketed globally includes rhizobium that helps in nitrogen fixation and phosphate solubilizers which is likely to be increased in demand.

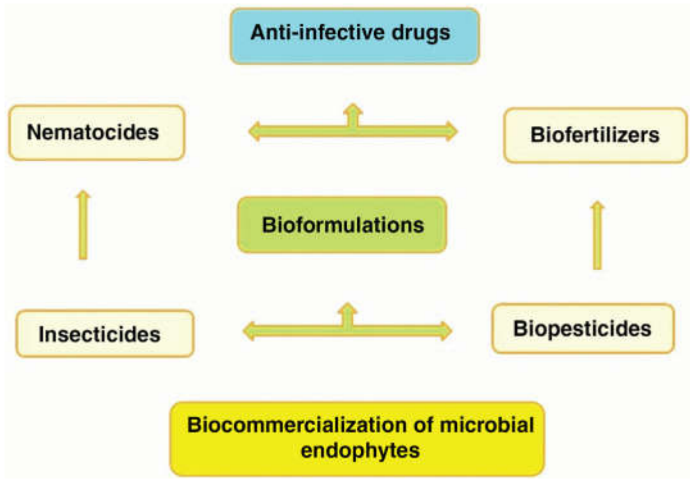

Figure 13.2 Biocommercial beneficial applications of microbial endophytes.

Most of the bioinoculants producers were from the United States and around 20–30 nations globally (Olson, 2015; Ravensberg, 2015). The use of general standards has hindered performance and viability of bioinoculants. Therefore, employment of native endophytes to region specific could solve wide issues such as performance, viability, endophyte adaptability, colonization efficacy, and storage efficiency. However, the need of strict regulations and quality control should not be neglected in this regard. The regulatory regimes and economic implications would be discussed below with product market aspects, product evaluation, and risk factors involved. As the endophyte application in agriculture is still in infancy the following evaluation would be based on available bioproducts in existence.

13.6.1 Bio vaccine

Bio Vaccine is a fungicide which contains *Trichoderma viride* and provide protection to the plant against rot and wilt diseases. It destroys fungal pathogens like *Fusarium* spp., *Pythium*, and *Rhizoctonia* which causes various rot and wilt diseases. *Trichoderma viride* emerges as coils around pathogens which degrades cell wall synthesis of fungal pathogens by producing various enzymes like celluloses and chitinases. This process is also called as mycoparasitism where one fungus kills other fungus by reducing their growth and metabolic functionality. It also enhances systemic resistant to destroy plant pathogens. It helps to enhance nutrients and moisture in the root system and also increases the stress tolerance.

13.6.2 Biofertilizers

The term "biofertilizer" is a product which are not chemically synthesized, biodegradable, and can be used as a fertilizer. However, biofertilizer entail as a fertilizer containing living organisms which is classified as phosphate-solubilizing and nitrogen-fixing biofertilizers which contains fungi or bacteria. They have been employed in many kinds of formulations.

AgriLife has launched fifteen biofertilizer products based on nitrogen fixers, phosphate solubilizers, potassium, ferrous, sulfur, manganese solubilizers, and zinc-mobilizing microbes in the market. Each biofertilizers contains one bacterial strain and for each nutrient suppliment, specific biofertilizers are available (Mehnaz, 2016).

JumpStart contains *Penicillium bilaii*, a fungus which provides phosphate availability to plants. *P. bilaii* colonizes the plant root and helps to release various organic compounds to the soil which breaks the bond between phosphate and elements. Plants get more access to phosphate whereas the fungus gets their nutrient supplements from the plant, establishing a symbiotic relationship. It is mainly suitable for canola, wheat as well as legume crops.

TagTeam is a multi-action inoculant that is suitable for legume crops. It provides more usage of phosphate and helps to fix more nitrogen. It comes with a combination of a fungus *Penicillium bilaii* and rhizobia strains. This natural product is available in the form of granular and liquid formulations to use on soybean, pea, dry bean, lentil, and chickpea.

RhizoMyco is a biofertilizer that contains eighteen species of endo as well as ectomycorrhizae and growth-promoting agents. RhizoMyco is available in the form of soluble or injectable form to give broad-spectrum benefits for enhanced nutrient supplements and increases root systems.

RhizoMyx is a well-known endomycorrhiza inoculant which is designed to increase the plant performance by enhancing root nodules development and providing nutrient suppliments more available.

13.6.3 Biocontrol products

Met52 is one of the potent bioinsecticides which contains spores of soil fungus *Metarhizium anisopliae*. The suspended spores of *Metarhizium sp.* attaches to the over surface of target insects which germinates, penetrates to the exoskeleton, and grows inside the pest. Later the death of target pest/insects will take place in a few days. **Taegro** is a bacteria-based biofungicides/bactericides which is used to suppress the selected soilborne and foliar diseases.

13.7 Bio market

Bioproducts in sustainable agriculture include biopesticides and biofertilizers which are derived naturally from animals, plants, and microbes for the plant growth promotion and protection. The current bioproduct market includes around 3 billion US dollars. The major market is located in the United States where more than 200 products are available followed by the European Union (EU) around 60. The rate of demand has been increasing around 10% global market annually. The growing concerns related to synthetic analogues due to their negative effects on human health and environment, the bioproduct market would gradually over take in the future. Also, the unavailability of new chemical substitutes with new stringent regulations has reduced the chemical product market. The global attention in this regard has shifted to the inclination scientific community and general population toward ecofriendly and safer technologies like bioproducts. The revenue for the North American biofertilizer market has risen over 72.5% (Dunham and Trimmer, 2015). The market price in the EU of bioproducts is around 25% more than synthetic analogues (Czaja et al., 2015; Olson, 2015).

13.7.1 Registration of product

The technical data required for bioproduct registration are quality, purity, and stability; efficacy; crop safety and maximum residue levels (Snyder, 2015).

13.7.2 Quality control

The qualities of endophytes bioproduct are impeded due to natural microflora and varying their functions in a new host. There is no available standard for endophytes in this regard for their performance and availability (INVAM, 2008).

13.7.3 Efficacy of the products

The overall yield gain through the application of endophyte bioinoculants could be ascertained as a success. But there is always the possibility of end results to be varied or inconsistent sometimes may become contradictory. The use of native endophytes would ascertain that the above complications to be minimized. Also, collective soil dynamics with native microflora could also affect the performance of bioproduct (Verbruggen et al., 2013).

13.7.4 Regulation of endophyte-based bioproducts market

The bio market includes scientists, regulators, marketers, and end-users in commercialization. The scientists are involved in the earlier stages of product development followed by regulators then marketers with the final end-users. If there is disagreements between them, that needs to be resolved by mutual agreements. Assessment of risks needs to be evaluated which needs new regulation as there is no existing market in this regard to endophytes. Tailoring of these new regulations is most important. The elimination of tiresome lengthy process is one of the major concerns need to focus. Product development already involves a good amount of time, so it should not delay the new product commercialization.

13.7.5 Challenges in endophyte commercialization

The limited companies in bioproduct-based industries are one of the major problems. The lack of initial investment is the main lacuna. The government and private funding for the development of these products are crucial. The government should provide improved regulations and should emphasize small and medium scale startups. The strategic investment in infrastructure is also a need for large-scale production. The regulatory frameworks for registration of bioproducts should be implemented with the view of faster commercialization for the products to enter the market.

13.7.6 Possible toxicity assessment

The beneficial aspects of microbial endophytes are intriguing, but toxin production potential of these microbes should not be neglected. It should be regulated as the prohibition of toxin-producing endophytes for agricultural and industrial applications. The critical assessment and complete characterization of endophytes should be employed to carry out the assessment of genomic data with lab and field investigation.

13.8 Recent developments and applications of microbial endophytes

13.8.1 Auto fluorescent protein (AFP) technique

Auto fluorescent protein technique has been employed to study the plant-microbe interaction and their colonization. AFP as a marker system, coding for green fluorescent protein has been successful in the monitoring of colonized endophytes in root tissues (Tombolini and Jansson, 1998). Green fluorescent protein has an advantage of fluorescing without additional requirement of substrate or cofactor. This method has been using poplar plants using different artificial inoculation techniques used in the b-glucuronidase (GUS) reporter system (Germaine et al., 2009).

13.8.2 Genome studies

With the advancement of genome technologies whole genome of several endophytic bacteria like *Enterobacter* spp., *Stenotrophomonas maltophilia, Pseudomonas putida* are available (www.jgi.doe.gov). The valuable insights of mechanisms at molecular level would enable better understanding along with base for experimental design (Rao et al., 2016; Rao and Satish, 2015).

13.8.3 Genetic engineering

The genetic modulation of *Metarhizium robertsii* was carried out by Wang and St Leger, (2007) for expressions neurotoxin from scorpion, reduced the survival of the tobacco hornworm by 28%. Four insect toxins were engineered in *Metarhizium acridum*, the synergistic effect of the combination of toxins reduced the incidence of acridids by 48% when compared with wild type (Fang et al., 2014). The whole genome analysis might enable the exploitation of gene clusters and depicting mechanisms of complex interactions associated with endophyte-plant host relationship (Mohana et al., 2018). The genetic engineering of GRAS endophytes can enable tailored benefits without affecting nontarget organisms should be more explored.

13.9 Conclusion and future perspectives

The depth of scientific knowledge about microbial endophytes and their mechanism is highly in its infancy with published research literature is either scarce or not fully understood. No microbial technology can be considered a successful technique until its commercial availability is proved. The endophytes specificity with the host plants is a major hurdle for large-scale production. The host-specific studies of endophyte are much required before initiating the large-scale production, which involves microbial technology-based advance in research. More efforts are needed in the formulation of host plant specific inoculum doses of microbial endophytes. The optimized and enhanced host specific inoculants will certainly reduce the cost for production of bulk inoculum with their applications and thus might increase productivity. New strategies of exploration like the discovery of novel endophytic strains or endophyte gene alterations are on the apparent horizon of replacing the need for host-specific targets. Instead, new endophytic microbes can be screened for suitable traits from medicinal plants growing under unique niche and extreme environment conditions. The alternative strategy of genetic manipulation can fit host plants with new traits like disease resistance, phytoremediation and other applications could more suitably regulate the metabolism.

Acknowledgments

Authors thank University Grants Commission (UGC), New Delhi for the award of Dr. D.S. Kothari Post-Doctoral Fellowship to the first author (No.F.4-2/2006 (BSR)/BL/17-18/0234). N. Chandra Mohan thanks ICMR for the award of Senior Research Fellowship (Award no. 45/69/2018-PHA/BMS/OL).

References

Adams, D.J., 2004. Fungal cell wall chitinases and glucanases. Microbiology 150 (7), 2029–2035.

Akello, J., Dubois, T., Gold, C.S., Coyne, D., Nakavuma, J., Paparu, P., 2007. *Beauveria bassiana* (Balsamo) Vuillemin as an endophyte in tissue culture banana (*Musa* spp.). J. Invertebr. Pathol. 96 (1), 34–42.

Akutse, K.S., Maniania, N.K., Fiaboe, K.K., Van den Berg, J., Ekesi, S., 2013. Endophytic colonization of *Vicia faba* and *Phaseolus vulgaris* (*Fabaceae*) by fungal pathogens and their effects on the life-history parameters of *Liriomyza huidobrensis* (Diptera: *Agromyzidae*). Fungal Ecol. 6 (4), 293–301.

Ali, S., Charles, T.C., Glick, B.R., 2014. Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol. Biochem. 80, 160–167.

Andreote, F.D., de Araújo, W.L., de Azevedo, J.L., van Elsas, J.D., da Rocha, U.N., van Overbeek, L.S., 2009. Endophytic colonization of potato (*Solanum tuberosum* L.) by a novel competent bacterial endophyte, *Pseudomonas putida* strain P9, and its effect on associated bacterial communities. Appl. Environ. Microbiol. 75 (11), 3396–3406.

Arnold, A.E., 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biol. Rev. 21 (2–3), 51–66.

Backman, P.A., Sikora, R.A., 2008. Endophytes: an emerging tool for biological control. Biol. Control 46 (1), 1–3.

Bartholdy, B.A., Berreck, M., Haselwandter, K., 2001. Hydroxamate siderophore synthesis by *Phialocephala fortinii*, a typical dark septate fungal root endophyte. Biometals 14 (1), 33–42.

Berg, G., 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biotechnol. 84 (1), 11–18.

Bonnardeaux, Y., Brundrett, M., Batty, A., Dixon, K., Koch, J., Sivasithamparam, K., 2007. Diversity of mycorrhizal fungi of terrestrial orchids: compatibility webs, brief encounters, lasting relationships and alien invasions. Mycol. Res. 111 (1), 51–61.

Breen, J.P., 1994. Acremonium endophyte interactions with enhanced plant resistance to insects. Ann. Rev. Entomol. 1, 401–423.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E.V., Schulze-Lefert, P., 2013. Structure and functions of the bacterial microbiota of plants. Ann. Rev. Plant Biol. 64, 807–838.

Busby, P.E., Soman, C., Wagner, M.R., Friesen, M.L., Kremer, J., Bennett, A., Morsy, M., Eisen, J.A., Leach, J.E., Dangl, J.L., 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. PLoS Biol. 15 (3), e2001793.

Cabanás, C.G., Schilirò, E., Valverde-Corredor, A., Mercado-Blanco, J., 2014. The biocontrol endophytic bacterium *Pseudomonas fluorescens* PICF7 induces systemic defense responses in aerial tissues upon colonization of olive roots. Front. Microbiol. 5, 427.

Camehl, I., Sherameti, I., Venus, Y., Bethke, G., Varma, A., Lee, J., Oelmüller, R., 2010. Ethylene signalling and ethylene-targeted transcription factors are required to balance beneficial and nonbeneficial traits in the sybiosis between the endophytic fungus *Piriformospora indica* and *Arabidopsis thaliana*. New Phytol 5 (4), 1062–1073.

Card, S., Johnson, L., Teasdale, S., Caradus, J., 2016. Deciphering endophyte behaviour: the link between endophyte biology and efficacious biological control agents. FEMS Microbiol. Ecol. 92 (8), fiw114.

Chisholm, S.T., Coaker, G., Day, B., Staskawicz, B.J., 2006. Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124 (4), 803–814.

Choudhary, D.K., 2012. Microbial rescue to plant under habitat-imposed abiotic and biotic stresses. Appl. Microbiol. Biotechnol. 96 (5), 1137–1155.

Clay, K., 1988. Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecol 9 (1), 10–16.

Clay, K., 1989. Clavicipitaceous endophytes of grasses: their potential as biocontrol agents. Mycol. Res. 92 (1), 1–2.

Clay, K., 1991. Endophytes as antagonists of plant pests. Microbial Ecology of Leaves. Springer, New York, NY, pp. 331–357.

Cocking, E.C., 2003. Endophytic colonization of plant roots by nitrogen-fixing bacteria. Plant Soil 2003 (2521), 169–175.

Conn, V.M., Walker, A.R., Franco, C.M., 2008. Endophytic actinobacteria induce defense pathways in *Arabidopsis thaliana*. Mol. Plant-Microbe Interact. 21 (2), 208–218.

Coombs, J.T., Michelsen, P.P., Franco, C.M., 2004. Evaluation of endophytic actinobacteria as antagonists of *Gaeumannomyces graminis* var. tritici in wheat. Biol. Cont. 29 (3), 359–366.

Czaja, K., Góralczyk, K., Struciński, P., Hernik, A., Korcz, W., Minorczyk, M., Łyczewska, M., Ludwicki, J.K., 2015. Biopesticides–towards increased consumer safety in the European Union. Pest Manag. Sci. 71 (1), 3–6.

Das, A., Prasad, R., Srivastava, A., Giang, P.H., Bhatnagar, K., Varma, A., 2007. Fungal siderophores: structure, functions and regulation. Microbial Siderophores. Springer, Berlin, Heidelberg, pp. 1–42.

De Battista, J.P., Bacon, C.W., Severson, R., Plattner, R.D., Bouton, J.H., 1990. Indole acetic acid production by the fungal endophyte of tall fescue. Agro. J. 82 (5), 878–880.

Downing, K.J., Thomson, J.A., 2000. Introduction of the *Serratia marcescens* chiA gene into an endophytic *Pseudomonas fluorescens* for the biocontrol of phytopathogenic fungi. Can. J. Microbiol. 46 (4), 363–369.

Dunham, W.C., Trimmer, D.L.L., 2015. Evolution and future of biocontrol. In 10th Annual Biocontrol Industry Meeting (ABIM), Basel, Switzerland.

Elena, G.J., Beatriz, P.J., Alejandro, P., Lecuona, R.E., 2011. Metarhiziumanisopliae (Metschnikoff) Sorokin promotes growth and has endophytic activity in tomato plants. Adv. Biol. Res. 5 (1), 22–27.

Erdogan, O., Benlioglu, K., 2010. Biological control of *Verticillium* wilt on cotton by the use of fluorescent *Pseudomonas* spp. under field conditions. Biol. Control. 53 (1), 39–45.

Faeth, S.H., 2002. Are endophytic fungi defensive plant mutualists? Oikos, 2002, 981, 25–36.

Fang, W., Lu, H.L., King, G.F., Leger, R.J., 2014. Construction of a hypervirulent and specific mycoinsecticide for locust control. Sci. Rep. 4, 7345.

Faye, O., Faye, O., Diallo, D., Diallo, M., Weidmann, M., 2013. Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. Virol. J. 10 (1), 311.

FROeHLICH, J., Petrini, O., 2000. Endophytic fungi associated with palms. Mycol. Res. 4 (10), 1202–1212.

Frugier, F., Kosuta, S., Murray, J.D., Crespi, M., Szczyglowski, K., 2008. Cytokinin: secret agent of symbiosis. Trends Plant Sci. 13 (3), 115–120.

Furlan, F., Saatkamp, K., Volpiano, C.G., de Assis Franco, F., dos Santos, M.F., Vendruscolo, E.C., Guimarães, V.F., da Costa, A.C., 2017. Plant growth-promoting bacteria effect in withstanding drought in wheat cultivars. Sci. Agraria 18 (2), 104–113.

Gaiero, J.R., McCall, C.A., Thompson, K.A., Day, N.J., Best, A.S., Dunfield, K.E., 2013. Inside the root microbiome: bacterial root endophytes and plant growth promotion. Am. J. Bot. 100 (9), 1738–1750, doi: 10.3732/ajb.1200572.

Gao, F.K., Dai, C.C., Liu, X.Z., 2010. Mechanisms of fungal endophytes in plant protection against pathogens. Afr. J. Microb. Res. 4 (13), 1346–1351.

Garrido-Jurado, I., Resquín-Romero, G., Amarilla, S.P., Ríos-Moreno, A., Carrasco, L., Quesada-Moraga, E., 2017. Transient endophytic colonization of melon plants by entomopathogenic fungi after foliar application for the control of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). J. Pest Sci. 90 (1), 319–330.

Germaine, K.J., Keogh, E., Ryan, D., Dowling, D.N., 2009. Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiol. Lett. 296 (2), 226–234.

Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol. Res. 169 (1), 30–39.

Greenfield, M., Gómez-Jiménez, M.I., Ortiz, V., Vega, F.E., Kramer, M., Parsa, S., 2016. *Beauveria bassiana* and *Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation. Biol. Control 95, 40–48.

Gurulingappa, P., Sword, G.A., Murdoch, G., McGee, P.A., 2010. Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when in planta. Biol. Control 55 (1), 34–41.

Hardoim, P.R., Overbeek, .L.S., Elsas, J.D., 2008. Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol. 16 (10), 463–471.

Hu, H.Q., Li, X.S., He, H., 2010. Characterization of an antimicrobial material from a newly isolated *Bacillus amyloliquefaciens* from mangrove for biocontrol of Capsicum bacterial wilt. Biol. Control 54 (3), 359–365.

Hurek, T., Reinhold-Hurek, B., 2003. *Azoarcus* sp. strain BH72 as a model for nitrogen-fixing grass endophytes. J. Biotechnol. 106 (2–3), 169–178.

INVAM, 2008. Results of Infectivity (MIP), Assays of Commercial Inoculants, [Online]. Available from:http://invam.caf.wvu.edu/otherinfo/commercial/commercial_MIPs.pdf.

Jaber, L.R., 2015. Grapevine leaf tissue colonization by the fungal entomopathogen *Beauveria bassianasl* and its effect against downy mildew. Biocontrol 60 (1), 103–112.

Jaber, L.R., Enkerli, J., 2017. Fungal entomopathogens as endophytes: can they promote plant growth? Biocontrol Sci. Techn. 27 (1), 28–41.

Jaber, L.R., Salem, N.M., 2016. Endophytic colonisation of squash by the fungal entomopathogen *Beauveria bassiana* (*Ascomycota: Hypocreales*) for managing Zucchini yellow mosaic virus in cucurbits. Biocontrol Sci. Techn. 24 (10), 1096–1109.

James, E.K., 2000. Nitrogen fixation in endophytic and associative symbiosis. Field Crops Res. 65 (2–3), 197–209.

Jog, R., Pandya, M., Naresh kumar, G., Rajkumar, S., 2014. Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. Microbiol. 160 (4), 778–788.

Khan, A.L., Hamayun, M., Kim, Y.H., Kang, S.M., Lee, J.H., Lee, I.J., 2011. Gibberellins producing endophytic *Aspergillus fumigatus* sp. LH02 influenced endogenous phytohormonal levels, isoflavonoids production and plant growth in salinity stress. Process Biochem. 46 (2), 440–447.

Kim, D.K., Seo, S.G., Kwon, S.B., Park, Y.D., 2016. Development of RAPD and SCAR markers related to watermelon mosaic virus and zucchini yellow mosaic virus resistance in *Cucurbita moschata*. Hortic. Environ. Biotechnol. 57 (1), 61–68.

Kloepper, J.W., Ryu, C.M., 2006. Bacterial endophytes as elicitors of induced systemic resistance. Microbial Root Endophytes. Springer, Berlin, Heidelberg, pp. 33–52.

Kouadio, A.N.M.S., Nandjui, J., Krou, S.M., Séry, D.J.M., Nelson, P.N., Zézé, A., 2017. A native arbuscular mycorrhizal fungus inoculant out competes an exotic commercial species under two contrasting yam field conditions. Rhizosphere 4, 112–118.

Kumar, A., Singh, R., Yadav, A., Giri, D.D., Singh, P.K., Pandey, K.D., 2016. Isolation and characterization of bacterial endophytes of *Curcuma longa* L.3. Biotech, 6, 60.

Kumar, V., Kumar, A., Pandey, K.D., Roy, B.K., 2015. Isolation and characterization of bacterial endophytes from the roots of *Cassia tora* L. Ann. Microbiol. 65, 1391–1399.

Lacava, P.T., Silva-Stenico, M.E., Araújo, W.L., Simionato, A.V., Carrilho, E., Tsai, S.M., Azevedo, J.L., 2008. Detection of siderophores in endophytic bacteria *Methylo bacterium* spp. associated with *Xylella fastidiosa* subsp. pauca. Pesqui. Agropecuária Bras. 43 (4), 521–528.

Li, C.H., Shi, L., Han, Q., Hu, H.L., Zhao, M.W., Tang, C.M., Li, S.P., 2012. Biocontrol of *Verticillium* wilt and colonization of cotton plants by an endophytic bacterial isolate. J. Appl. Microbiol. 113 (3), 641–651.

Loaces, I., Ferrando, L., Scavino, A.F., 2011. Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. Microbial. Ecol. 61 (3), 606–618.

Lopez, D.C., Sword, G.A., 2015. The endophytic fungal entomopathogens *Beauveria bassiana* and *Purpureocillium lilacinum* enhance the growth of cultivated cotton (*Gossypium hirsutum*) and negatively affect survival of the cotton bollworm (Helicoverpa zea). Biol. Control 89, 53–60.

Ma, Y., Oliveira, R.S., Nai, F., Rajkumar, M., Luo, Y., Rocha, I., Freitas, H., 2015. The hyperaccumulator *Sedum plumbizincicola* harbors metal-resistant endophytic bacteria that improve its phytoextraction capacity in multi-metal contaminated soil. J. Environ. Manag. 156, 62–69.

Mack, K.M., Rudgers, J.A., 2008. Balancing multiple mutualists: asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. Oikos 117 (2), 310–320.

Markovich, N.A., Kononova, G.L., 2003. Lytic enzymes of *Trichoderma* and their role in plant defense from fungal diseases: a review. Appl. Biochem. Microbiol. 39 (4), 341–351.

Mehnaz, S., 2019. An overview of globally available bioformulations. In: N.K. Arora et al. (eds.), Bioformulations: for Sustainable Agriculture. Springer. DOI 10.1007/978-81-322-2779-3_15.

Mejía, L.C., Rojas, E.I., Maynard, Z., Van Bael, S., Arnold, A.E., Hebbar, P., Herre, E.A., 2008. Endophytic fungi as biocontrol agents of *Theobroma cacao* pathogens. Biol. Control 46 (1), 4–14.

Merzaeva, O.V., Shirokikh, I.G., 2010. The production of auxins by the endophytic bacteria of winter rye. Appl. Biochem. Microbiol. 46 (1), 44–50.

Mohan, V., Nivea, R., Menon, S., 2015. Evaluation of ectomycorrhizal fungi as potential biocontrol agents against selected plant pathogenic fungi. JAIR 3 (9), 408–412.

Mohana, N.C., Rao, H.C.Y., Rakshith, D., Mithun, P.R., Nuthan, B.R., Satish, S., 2018. Omics based approach for biodiscovery of microbial natural products in antibiotic resistance era. J Genetic Eng. Biotechnol., 10.1016/j.jgeb.2018.01.006.

Moonjely, S., Barelli, L., Bidochka, M.J., 2016. Insect pathogenic fungi as endophytes. Adv. Genet. 94, 107–135.

Müller, H., Westendorf, C., Leitner, E., Chernin, L., Riedel, K., Schmidt, S., Berg, G., 2009. Quorum-sensing effects in the antagonistic rhizosphere bacterium Serratia plymuthica HRO-C48. FEMS Microbiol. Ecol. 67 (3), 468–478.

Muvea, A.M., Meyhöfer, R., Subramanian, S., Poehling, H.M., Ekesi, S., Maniania, N.K., 2014. Colonization of onions by endophytic fungi and their impacts on the biology of *Thrips tabaci*. PLoS ONE 9 (9), e108242.

Naik, B.S., Shashikala, J., Krishnamurthy, Y.L., 2009. Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities in vitro. Microbiol. Res. 164 (3), 290–296.

Olson, S., 2015. An analysis of the biopesticide market now and where it is going. Outlooks Pest Manag. 26 (5), 203–206.

Otieno, N., Lally, R.D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K.J., Dowling, D.N., 2015. Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. Front. Microbiol. 6, 745.

Paramanantham, P., Pattnaik, S., Siddhardha, B., 2019. Natural products from endophytic fungi: Synthesis and applications. Advances in Endophytic Fungal Research. Springer, Cham, pp. 83–103.

Parsa, S., García-Lemos, A.M., Castillo, K., Ortiz, V., López-Lavalle, L.A.B., Braun, J., Vega, F.E., 2016. Fungal endophytes in germinated seeds of the common bean, *Phaseolus vulgaris*. Fung. Biol. 120 (5), 783–790.

Patle, P.N., Navnage, N.P., Ramteke, P.R., 2018. Endophytes in plant system: roles in growth promotion, mechanism and their potentiality in achieving agriculture sustainability. Int. J. Chem. Stud. 6, 270–274.

Paul, S., Dukare, A.S., Manjunatha, B.S., Annapurna, K., 2017. Plant growth-promoting rhizobacteria for abiotic stress alleviation in crops. Advances in Soil Microbiology: Recent Trends and Future Prospects. Springer, Singapore, pp. 57–79.

Paulitz, T.C., Bélanger, R.R., 2001. Biological control in greenhouse systems. Ann. Rev. Phytopathol. 39 (1), 103–133.

Porras-Alfaro, A., Bayman, P., 2011. Hidden fungi, emergent properties: endophytes and microbiomes. Ann. Rev. Phytopathol 49, 291–315.

Posada, F., Aime, M.C., Peterson, S.W., Rehner, S.A., Vega, F.E., 2007. Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). Mycol. Res. 111 (6), 748–757.

Powell, W.A., Klingeman, W.E., Ownley, B.H., Gwinn, K.D., 2009. Evidence of endophytic *Beauveria bassiana* in seed-treated tomato plants acting as a systemic entomopathogen to larval *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Entomol. Sci. 44 (4), 391–396.

Pringle, A., Bever, J.D., Gardes, M., Parrent, J.L., Rillig, M.C., Klironomos, J.N., 2009. Mycorrhizal symbioses and plant invasions. Ann. Rev. Ecol. Evol. Syst. 40, 699–715.

Qayyum, M.A., Wakil, W., Arif, M.J., Sahi, S.T., Dunlap, C.A., 2015a. Infection of *Helicoverpa armigera* by endophytic *Beauveria bassiana* colonizing tomato plants. Biol. Control 90, 200–207.

Qayyum, M.A., Wakil, W., Arif, M.J., Sahi, S.T., Saeed, N.A., Russell, D.A., 2015b. Multiple resistances against formulated organophosphates, pyrethroids, and newer-chemistry insecticides in populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Pakistan. J. Econ. Entomol. 108 (1), 286–293.

Rakshith, D., Santosh, P., Pradeep, T.P., Gurudatt, D.M., Baker, S., Rao, H.C.Y., Satish, S., 2016. Application of bioassay-guided fractionation coupled with a molecular approach for the dereplication of antimicrobial metabolites. Chromatographia 79 (23–24), 1625–1642.

Ramesh, R., Phadke, G.S., 2012. Rhizosphere and endophytic bacteria for the suppression of eggplant wilt caused by *Ralstonia solanacearum*. Crop Prot. 37, 35–41.

Ramesh, R., Joshi, A.A., Ghanekar, M.P., 2009. *Pseudomonas*: major antagonistic endophytic bacteria to suppress bacterial wilt pathogen, *Ralstonia solanacearum* in the eggplant (*Solanum melongena* L.). World J. Microbiol. Biotechnol. 25 (1), 47–55.

Rao, H.C.Y., Rakshith, D., Harini, B.P., Gurudatt, D.M., Satish, S., 2017b. Chemogenomics driven discovery of endogenous polyketide anti-infective compounds from endosymbiotic *Emericella* variecolor CLB38 and their RNA secondary structure analysis. PloS one 12 (2), e0172848.

Rao, H.C.Y., Baker, S., Rakshith, D., Satish, S., 2015c. Molecular profiling and antimicrobial potential of endophytic *Gliomastix polychroma* CLB32 inhabiting *Combretum latifolium* Blume. Mycol 6 (3–4), 176–181.

Rao, H.C.Y., Rakshith, D., Gurudatt, D.M., Satish, S., 2016. Implication of PKS type I gene and chromatographic strategy for the biodiscovery of antimicrobial polyketide metabolites from endosymbiotic *Nocardiopsis prasina* CLA68. Sci. Nat. 103 (5–6), 45.

Rao, H.C.Y., Rakshith, D., Harini, B.P., Satish, S., 2017a. Antimicrobial profiling and molecular identification of *Alternaria arborescens* CLB12, a myco-endosymbiont inhabiting *Combretum latifolium* Blume. J. Biol. Act. Prod. Nat. 7 (1), 1–9.

Rao, H.C.Y., Rakshith, D., Satish, S., 2015b. Antimicrobial properties of endophytic actinomycetes isolated from *Combretum latifolium* Blume, a medicinal shrub from Western Ghats of India. Front. Biol. 10 (6), 528–536.

Rao, H.C.Y., Santosh, P., Rakshith, D., Satish, S., 2015a. Molecular characterization of an endophytic *Phomopsis liquidambaris* CBR-15 from *Cryptolepis buchanani* Roem. and impact of culture media on biosynthesis of antimicrobial metabolites. 3Biotech 5 (2), 165–173.
Rao, H.C.Y., Satish, S., 2015. Genomic and chromatographic approach for the discovery of polyketide antimicrobial metabolites from an endophytic *Phomopsis liquidambaris* CBR-18. Front. Life Sci. 8 (2), 200–207.
Ravensberg, W.J., 2015. Commercialisation of microbes: present situation and future prospects. Principles of Plant-Microbe Interactions. Springer, Cham, pp. 309–317.
Reddy, S.K., Liu, S., Rudd, J.C., Xue, Q., Payton, P., Finlayson, S.A., Lu, N., 2014. Physiology and transcriptomics of water-deficit stress responses in wheat cultivars TAM 111 and TAM 112. J. Plant Physiol. 171 (14), 1289–1298.
Resquín-Romero, G., Garrido-Jurado, I., Delso, C., Ríos-Moreno, A., Quesada-Moraga, E., 2016. Transient endophytic colonizations of plants improve the outcome of foliar applications of mycoinsecticides against chewing insects. J. Invert. Pathol. 136, 23–31.
Rodriguez, R., Redman, R., 2008. More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. J. Exp. Bot. 59 (5), 1109–1114.
Rodriguez, R.J., White, Jr., J.F., Arnold, A.E., Redman, R.S., 2009. Fungal endophytes: diversity and functional roles. New Phytol. 182 (2), 314–330.
Rondot, Y., Reineke, A., 2018. Endophytic *Beauveria bassiana* in grapevine *Vitis vinifera* (L.) reduces infestation with piercing-sucking insects. Biol. Control 116, 82–89.
Rosenblueth, M., Martínez-Romero, E., 2006. Bacterial endophytes and their interactions with hosts. Mol. Plant-Microb. Interact. 19 (8), 827–837.
Rubini, M.R., Silva-Ribeiro, R.T., Pomella, A.W., Maki, C.S., Araújo, W.L., Dos Santos, D.R., Azevedo, J.L., 2005. Diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipellis perniciosa*, causal agent of Witches' Broom Disease. Int. J. Biol. Sci. 1 (1), 24.
Russo, M.L., Pelizza, S.A., Cabello, M.N., Stenglein, S.A., Scorsetti, A.C., 2015. Endophytic colonisation of tobacco, corn, wheat and soybeans by the fungal entomopathogen *Beauveria bassiana* (Ascomycota, Hypocreales). Biocont. Sci. Technol. 25 (4), 475–480.
Saikkonen, K., Faeth, S.H., Helander, M., Sullivan, T.J., 1998. Fungal endophytes: a continuum of interactions with host plants. Ann. Rev. Ecol. Sys. 29 (1), 319–343.
Saikkonen, K., Saari, S., Helander, M., 2010. Defensive mutualism between plants and endophytic fungi. Fungal Divers 41 (1), 101–113.
Saikkonen, K., Wäli, P., Helander, M., Faeth, S.H., 2004. Evolution of endophyte–plant symbioses. Trends Plant Sci. 9 (6), 275–280.
Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M., Glick, B.R., 2016. Plant growth-promoting bacterial endophytes. Microbiol. Res. 183, 92–99.
Schulz, B., Römmert, A.K., Dammann, U., Aust, H.J., Strack, D., 1999. The endophyte-host interaction: a balanced antagonism. Mycol. Res. 103 (10), 1275–1283.
Sevilla, M., Kennedy, C, 2000. Genetic analysis of nitrogen fixation and plant-growth stimulating properties of *Acetobacter diazotrophicus*, an endophyte of sugarcane. In: Prokaryotic nitrogen fixation: a model system for the analysis of a biological process., Horizon Scientific Press, Wymondham, UK, pp. 737–760.
Shah, S., Shrestha, R., Maharjan, S., Selosse, M.A., Pant, B., 2019. Isolation and characterization of plant growth-promoting endophytic fungi from the roots of *Dendrobium moniliforme*. Plants 8 (1), 5.

Sheik, S., Chandrashekar, K.R., 2018. Fungal endophytes of an endemic plant *Humboldtia brunonis* wall. of Western Ghats (India) and their antimicrobial and DPPH radical scavenging potentiality. Ori. Pharm. Exp. Med. 18 (2), 115–125.

Shi, Y., Lou, K., Li, C., 2009. Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. Biol. Fertil. Soils 45 (6), 645–653.

Singh, M, Kumar, A, Singh, R. and Pandey, K.D., 2017. Endophytic bacteria: a new source of bioactive compounds, 3 Biotech, 7: 315.

Snyder, S.W. (Ed.), 2015. Commercializing Biobased Products: Opportunities, Challenges, Benefits, and Risks. Royal Society Chem. Publisher: Royal Society of Chemistry.

Strasser, H., Vey, A., Butt, T.M., 2000. Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? Biocontr. Sci. Technol. 10 (6), 717–735.

Strobel, G., Daisy, B., 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol. Mol. Biol. Rev. 67 (4), 491–502.

Sun, Y., Cheng, Z., Glick, B.R., 2009. The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN. FEMS Microbiol. Lett. 296 (1), 131–136.

Tan, H., Zhou, S., Deng, Z., He, M., Cao, L., 2011. Ribosomal-sequence-directed selection for endophytic streptomycete strains antagonistic to *Ralstonia solanacearum* to control tomato bacterial wilt. Biol. Control 59 (2), 245–254.

Taurian, T., Anzuay, M.S., Angelini, J.G., Tonelli, M.L., Ludueña, L., Pena, D., Fabra, A., 2010. Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. Plant Soil 329 (1–2), 421–431.

Tefera, T., Vidal, S., 2009. Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus *Beauveria bassiana*. BioControl 54 (5), 663–669.

Tian, X.L., Cao, L.X., Tan, H.M., Zeng, Q.G., Jia, Y.Y., Han, W.Q., Zhou, S.N., 2004. Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their antipathogenic activities in vitro. World J. Microbiol. Biotechnol. 20 (3), 303–309.

Tombolini, R., Jansson, J.K., 1998. Monitoring of GFP-tagged bacterial cells. Bioluminescence Methods and Protocols. Humana Press, pp. 285–298.

Ullah, A., Heng, S., Munis, M.F.H., Fahad, S., Yang, X., 2015. Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. Environ. Exp. Bot. 117, 28–40.

Verbruggen, E., van der Heijden, M.G., Rillig, M.C., Kiers, E.T., 2013. Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytol 197 (4), 1104–1109.

Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.Y., Valero, J.R., 2007. Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. Biochem. Eng. J. 37 (1), 1–20.

Vu, T., Sikora, R., Hauschild, R., 2006. *Fusarium oxysporum* endophytes induced systemic resistance against *Radopholus similis* on banana. Nematology 8 (6), 847–852.

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Franken, P., 2005. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc. National Acad. Sci. 102 (38), 13386–13391.

Wang, C., Leger, R.J.S., 2005. Developmental and transcriptional responses to host and nonhost cuticles by the specific locust pathogen *Metarhizium anisopliae* var. acridum. Eukaryotic Cell 4 (5), 937–947.

Wang, C., St Leger, R.J., 2007. A scorpion neurotoxin increases the potency of a fungal insecticide. Nat. Biotechnol. 25 (12), 1455.

Wang, G., Liu, Z., Lin, R., Li, E., Mao, Z., Ling, J., Xie, B., 2016. Biosynthesis of antibiotic leucinostatins in bio-control fungus *Purpureocillium lilacinum* and their inhibition on Phytophthora revealed by genome mining. PLoS Pathog. 12 (7), e1005685.

Waqas, M., Khan, A.L., Kamran, M., Hamayun, M., Kang, S.M., Kim, Y.H., Lee, I.J., 2012. Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. Molecules 17 (9), 10754–10773.

Waqas, M., Khan, A.L., Kang, S.M., Kim, Y.H., Lee, I.J., 2014. Phytohormone-producing fungal endophytes and hardwood-derived biochar interact to ameliorate heavy metal stress in soybeans. Biol. Fertil. Soils 50 (7), 1155–1167.

Zhang, H.W., Song, Y.C., Tan, R.X., 2006. Biology and chemistry of endophytes. Natural. Prod. Rep. 23 (5), 753–771.