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APPLICATION OF ENDOPHYTIC PLANT GROWTH-PROMOTING BACTERIA FOR SUSTAINABLE CROP PRODUCTION.

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ABSTRACT Endophytic bacteria are live inside their host throughout different stages of their life cycle and play crucial roles in the growth, development, fitness, and diversification of plants. These microbes help the host to combat a diverse array of biotic and abiotic stressful conditions. Endophytic microbes play a major role in the growth promotion of their host by solubilizing of macronutrients such as phosphorous, potassium, and zinc; fixing of atmospheric nitrogen, synthesizing of phytohormones, siderophores, hydrogen cyanide, ammonia, and act as a biocontrol agent against wide array of phytopathogens. Endophytic microbes belong to different phyla such as Actinobacteria, Acidobacteria, Bacteroidetes, Deinococcus–thermus, Firmicutes, Proteobacteria, and Verrucomicrobia. The most predominant and studied endophytic bacteria belonged to Proteobacteria followed by Firmicutes and then by Actinobacteria. The most dominant among reported genera in most of the leguminous and non-leguminous plants are Bacillus, Pseudomonas, Fusarium, Burkholderia, Rhizobium, and Klebsiella. In future, endophytic microbes have a wide range of potential for maintaining health of plant as well as environmental conditions for agricultural sustainability. The present review is focused on endophytic microbes, their diversity in leguminous as well as non-leguminous crops and ability to promote the growth of plant for agro-environmental sustainability.

KEYWORDS : Bacterial endophytes; Diversity · Plant growth promotion; Bioremediation; Biocontrol agents;

INTRODUCTION

Rhizobacteria, endophytic bacteria and phyllospheric bacteria are plant-associated bacteria. However, among the rhizospheric bacteria some of them enter into the plant tissue that rhizosphere bacteria capable of entering plant tissues are called endophytes (Azevedo et al. 2000). The diversity of microorganisms in the rhizosphere since the beginning of the twentieth century was considered only from the fungal groups Tervet and Hollis 1948). The techniques for culturing and advanced identification of bacterial endophytes were difficulties from the past two decades. Endophytic microorganisms reflect much attention for researcher for their potential of host protection against insects-pests and pathogens was recognized along with plant growth-promoting properties which associate with most of the plant species and seem ubiquitous in plant tissues. The place of endophytes are flowers, fruits, leaves, stems, roots and seeds of various plant species (Kobayashi and Palumbo 2000). Endophytes inhabitant in the host body and remain protected from environmental stress, face lesser competition and gain higher access for nutrients (Dutta et al. 2014). The rhizosphere microflora less stronger than endophytes due to the direct nature of interaction and endophytes multiply in plant apoplast, enriched with nutrients like calcium, carbohydrates, chlorine, phosphorous, potassium, sulphur (Canny and McCully 1988; Madore and Webb 1981), several amino acids and organic acids (Canny and Huang 1993).The plants are benefited by Endophytes either directly by stimulating growth or indirectly by decreasing disease incidences. Endophytes improve plant growth and survival by conferring host resistance against pests and drought and by improving host N assimilation to yield higher seed set (Fescue 1990). Endophytes defend plants from phytopathogen attack by manufacturing biofilm around roots (Rybakova et al. 2015). Rhizobia are may be the most effective example of plant-associated endobacteria as they facilitate N uptake in plants through Rhizobium-legume mutualism.With sure physiological variations, many species of bacteria genus were isolated from legume plants like alfalfa (Stajković et al. 2009), herb (Sturz et al. 1998) and pea (Saini et al. 2015) .

Diversity Of Endophytic Microbes

The various teams of microbes were reportable for their association with different host plants within the kind of plant life, endophytic, and rhizospheric (Yadav et al. 2018, 2020a). An enormous diversity of endophytic microbes as archaea belong to the phylum Euryarchaeota and fungi including to the phylum Ascomycota, division, and Mucoromycota, during which Ascomycota were the foremost dominant were reportable. Bacterium as endophytes are measure the foremost numerous and thick cluster of organisms on earth reportable from the phylum Actinobacteria,

Acidobacteria, Bacteroidetes, Deinococcus-thermus, Firmicutes, Proteobacteria, and Verrucomicrobia (Fig. 1).

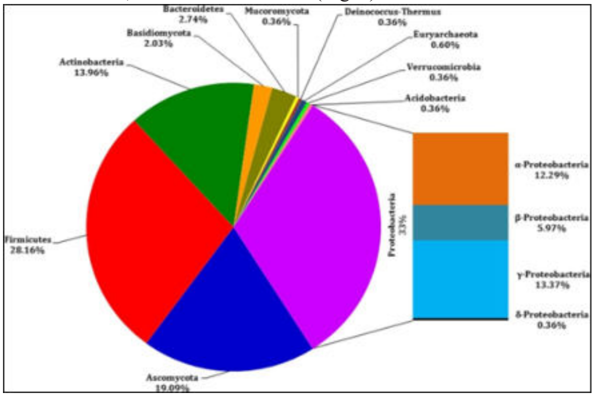

Fig. 1 Abundance Of Endophytic Microbes Belonging To Diverse Phylum (source- Rana Et Al; 2020)

The phylum Proteobacteria were again classified as a-, b-, c-, and d-proteobacteria. The foremost dominant phylum among bacterium was found to be Proteobac- teria, whereas phylum Acidobacteria, Bacteroidetes, and Deinococcus–thermus, have least range of endophytic bacterium was isolated (Fig. 2).

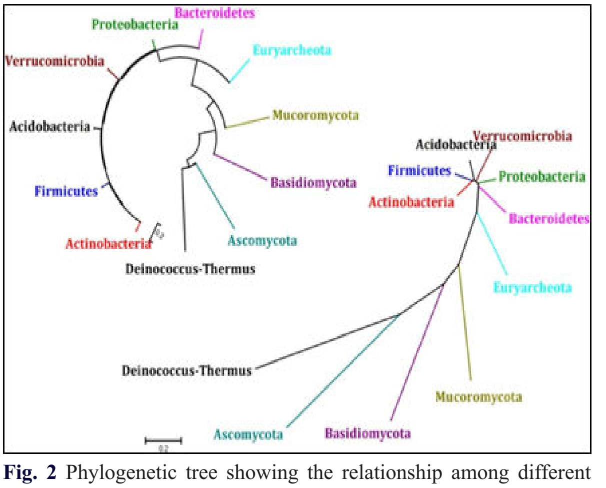

Fig. 2 Phylogenetic tree showing the relationship among different groups of endophytic microbiomes isolated from different host plant (source - Rana et al; 2020).

On review of endophytic analysis from totally different plants including to leguminous and non-leguminous, it's found that endophytic microbes belonging to phylum Proteobacteria, Firmicutes, Ascomycota, and Actinobacteria were most dominant. regarding 165 genera were reported from each leguminous and non-leguminous plants, out of that most predominant genera, includes *Acinetobacter, Alternaria, Azospirillum, Bacillus, Burkholderia, Bradyrhizobium, Cladosporium, Enterobacter, Epicoccum, Fusarium, Herbaspirillum, Klebsiella, Paenibacillus, Pantoea, Pseudomonas, Rhizobium, Staphylococcus* and *Streptomyces* (Fig. 3)

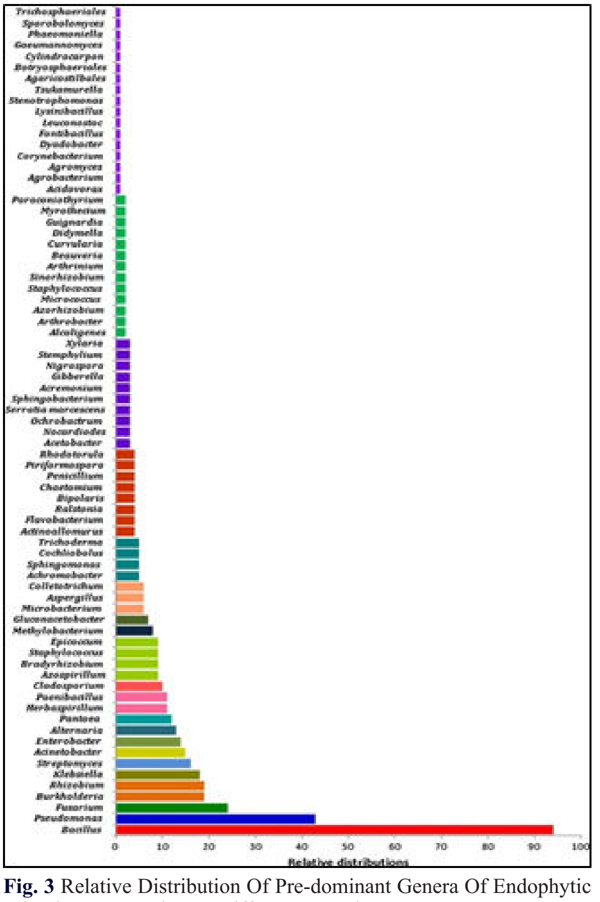

Fig. 3 Relative Distribution Of Pre-dominant Genera Of Endophytic Microbes Reported From Different Host Plants

The genera *Bacillus, Cladosporium, Herbaspirillum*, and *Pseudomonas* as an endophyte were found common in wheat, maize, rice, and sugarcane, whereas, the genera *Bacillus* is common in chick pea, peanut, soybean, and bean. Out of eight host plants, a number of the endophytic microbes were reported as niche-specific (Fig.4).

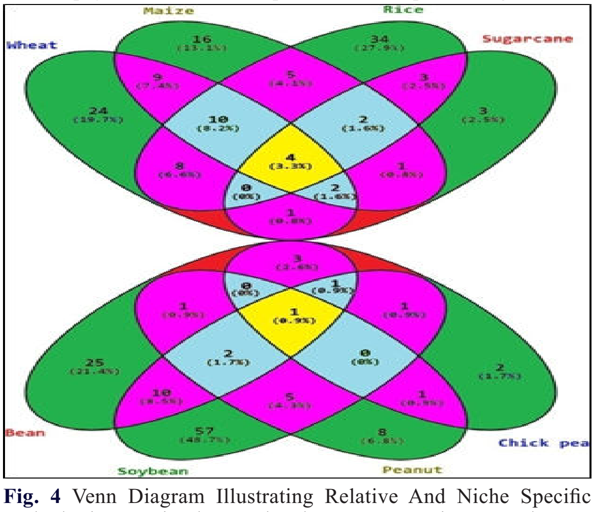

Fig. 4 Venn Diagram Illustrating Relative And Niche Specific Endophytic Microbes Isolated From Leguminous And Non-Leguminous Crops.

The most predominant genera *Bacillus* belongs to the phylum Firmicutes that was found to colonise wheat (Zhao et al. 2015), maize (Gond et al. 2015), rice (Ji et al. 2014), sugarcane (Manoel public prosecutor timber et al. 2015), chick pea (Joseph et al. 2012), peanut (Wang et al. 2013), soybean, and bean (Zhao et al. 2018). One of every species of *Bacillus* referred to as *Bacillus amyloliquefaciens* showed numerous plant growth promoting attributes as an example nitrogen fixation, phosphorous, potassium, and zinc solubilization, production of phytohormones (IAA) and siderophores act as bio- control agent (Verma et al. 2015. Additional novel species of genera *Bacillus* named as *Bacillus oryzicola* YC7010^{T} and *Bacillus endoradicis* CCBAU 05776^{T} were isolated as an endophyte from rice and soybean (Chung et al. 2015; Zhang et al. 2012b) (Table 1). The second most predominant genera of endophytic bacteria are *Pseudomonas* which belongs to the phylum Proteobacteria and class c- proteobacteria. The different species of genera *Pseudomonas* as *Pseudomonas aeruginosa, Pseudomonas fulva, Pseudomonas lini, Pseudomonas montelli, Pseudomonas putida, Pseudomonas thivervalensis* were reported from wheat (Verma et al. 2017), maize (Montan˜ez et al. 2009), rice (Mbai et al. 2013), sugarcane (Govindarajan et al. 2007), peanut (Wang et al. 2013), soybean (Zhao et al. 2018), and bean (Dinic´ et al. 2014), fixes atmospheric nitrogen, solubilizes phosphorous, synthesizes IAA, siderophores and also act as biological agent against various phytopathogens. The next most predominant genus is *Burkholderia* belongs to the phylum Proteobacteria and class b- proteobacteria. The different species of genus *Burkholderia* i.e. *Burkholderia* sp., *Burkholde- ria vietnamiensis, Burkholderia silvatlantica,* and *Burkholderia tropica* were reported from maize (Estrada et al. 2002), rice (Rangjaroen et al. 2015), sugarcane (Govindarajan et al. 2008), and soybean (Perin et al. 2006) (Table 1).

Table 1 Endophytic Novel Microbial Species Isolated From Different Crops

Novel endophytes	Host	References
Flavobacterium phragmitis BLN2^{T}	Bean	Liu et al. ()
Actinoallomurus oryzae GMKU 370^{T}	Rice	Indananda et al. ()
Acidovorax radicis N35^{T}	Wheat	Li et al. ()
Mesorhizobium muleiense CCBAU 83963^{T}	Chick pea	Zhang et al. ()
Acinetobacter oryzae B23^{T}	Rice	Chaudhary et al. ()
*Bacillus endoradicis*CCBAU 05776^{T}	Soybean	Zhang et al. ()
Streptomyces harbinensis NEAU-Da3^{T}	Soybean	Liu et al. ()
Enterobacter sacchari SP1^{T}	Sugarcane	Zhu et al. ()
Micromonospora zeae NEAU-gq9^{T}	Maize	Shen et al. ()
Sphaerisporangium rufum R10-82^{T}	Rice	Mingma et al. ()
Wickerhamiella siamensis DMKU-SE106T	Sugarcane	Khunnamwong et al. ()
Paenibacillus zeae 6R2^{T}	Maize	Liu et al. ()
Paenibacillus wenxiniae 373^{T}	Maize	Gao et al. ()
Flavobacterium endophyticum 522^{T}	Maize	Gao et al. ()
Micromonospora endophytica DCWR9-8-2^{T}	Rice	Thanaboripat et al. ()
Streptomyces oryzae S16–07^{T}	Rice	Mingma et al. ()
Novosphingobium oryzae ZYY112^{T}	Rice	Zhang et al. ()
Plantactinospora soyae NEAU-gxj3^{T}	Soybean	Guo et al. ()
Paenibacillus hispanicus AMTAE16^{T}	Wheat	Mene´ndez et al. ()
Filimonas zeae 772^{T}	Maize	Gao et al. ()
Paenibacillus radicis 694^{T}	Maize	Gao et al. ()
Chryseobacterium endophyticum CC-YTH209^{T}	Maize	Lin et al. ()
Sphingomonas zeicaulis 541^{T}	Maize	Gao et al. ()
*Dyadobacter endophyticus*65^{T}	Maize	Gao et al. ()
Nocardioides zeicaulis JM-601^{T}	Maize	Ka¨mpfer et al. ()

Agricultural Applications Of Endophytic Microorganisms

Plants mostly require nutrients such as nitrogen (N), phosphorous (P), and potassium (K) for their growth and development. These elements are available in complex compound form in soil. Endophytic microbes have the ability to solubilize micro and macro elements for plants and facilitate the mobilisation and uptake of macronutrients and micronutrients by solubilizing the complex compound present in the soil and transfer it to their host plant. The farmers mostly utilise chemical fertilizers for enhancing the yield production of crops. The utilisation of chemical fertilizers leads to leaching of nitrates which finally contaminate the underground and surface water further disturbing the fertility of soil. For increasing productivity of crops another option is the application of microbes with multifarious plant growth promoting attributes or biofertilizers (Table 2). The recent omics research offer information about endophytic microbes which are suitable for bioformulations/biofertilizers and can be used in agricultural fields (Garima and Nath 2015).

Table 2 Endophytic Microbe From Different Crops With Multifarious Plant Growth Promoting Attributes

Endophytic microbes	Host	N_2F	P	IAA	Sid	References
Acitenobacter braumalli	Maize			1		Sandhya et al. ()
Pseudomonas thivervalensis	Maize			1	1	Sandhya et al. ()
Flavobacterium anhuiense	Peanut			1		Wang et al. ()
Pantoea agglomerans	Peanut			1		Wang et al. ()
Klebsiella pneumoniae	Rice		1	1	1	Puri et al. ()
Bacillus amyloliquefaciens	Rice	1	1	1	1	Verma et al. ()
Enterobacter cloacae	Soybean	1		1	1	Zhao et al. ()
Pseudomonas putida	Soybean	1		1	1	Zhao et al. ()
Arthrobacter sulfonivorans	Wheat				1	Singh et al. ()
Bacillus amyloliquefaciens	Wheat	1	1	1	1	Verma et al. ()
Pseudomonas aeruginosa	soybean		1	1	1	Kumawat et al. ()
Bradyrhizobium sp.	soybean		1	1		Kumawat et al. ()
Burkholderia cepacia	chickpea			1		Shahid and Khan ()
Acinetobacter guillouiae	Wheat		1	1		Rana et al. ()

(Source -Rana et al; 2020)

Production Of Growth Hormone

Endophyte bacterial phytohormone-mediated plant growth promotion is a well recognized method that changes the morphology and structure of plants. These traits render endophytes as the best option for agricultural applications (Hallmann et al. 1997; Sturz et al. 2000). Endophytes enhance legume crop yield by producing indole acetic acid (Khan et al. 2014; Patel and Patel 2014), gibberellic acid (Khan et al. 2014), ethylene (Kang et al. 2012; Long et al. 2010; Straub et al. 2013) auxins (Dutta et al. 2014). Like rhizobacteria, endophytes produce phytohormones through similar mechanisms. Similarly, auxins induce rapid growth in plants by triggering cell elongation, division and differentiation (Taghavi et al. 2009).

Nitrogen Fixation

Nitrogen is one of the macro element required for increasing the yield and production of plants. In order to utilise atmospheric nitrogen the diazotrophs convert the atmospheric di-nitrogen into ammonia (form that can be easily assimilated by plants) by the producer of biological nitrogen fixation. Nitrogen fixing endophytic bacteria belonging to different genera *Azospirillum* (Montan˜ez et al. 2009), *Burkholderia* (Estrada et al. 2002), and *Pseudomonas* (Montan˜ez et al. 2009) have been reported from maize. *Bradyrhizobium* (Castro et al. 1999) from Peanut; *Alcaligenes* (You and Zhou 1989), *Azoarcus* (Hurek et al. 1994), *Methylobacterium* (Kumar et al. 2016a), *Pantoea* (Verma et al. 2001), and *Serratia* (Gyanesh- war et al. 2001) from rice; *Acinetobacter, Bacillus, Enterobacter* (Zhao et al. 2018), *Bradyrhizobium* (Subramanian et al. 2015), *Burkholderia* (Kuklinsky-Sobral et al. 2004), and *Klebsiella* (Ikeda et al. 2010) from Soybean; *Acetobacter* (Sevilla et al. 2001), *Azoarcus* (Hurek and Reinhold-Hurek 2003), *Glu- conacetobacter* (Suman et al. 2008), and *Herbaspir- illum* (Oliveira et al. 2002) from Sugarcane; *Achromobacter* (Jha and Kumar 2009), *Acinetobacter* (Verma et al. 2015), *Azorhizobium* (Sabry et al. 1997), *Azospirillum* (Saubidet et al. 2002), and *Klebsiella* (Iniguez et al. 2004) from wheat.

Phosphate Solubilization

Phosphorous is the third most essential macronutrient for plants. It is present in soil as mineral salts or lies incorporated in organic compounds. Due to the sparingly soluble nature, the major portion of soil P remain unavailable to plants (Miller et al. 2010). Certain bacteria that transform insoluble P into the soluble form to make it plant accessible are called phosphate solubilization bacteria (PSB). Rhizosphere bacteria are known to exude organic acids into soil that solubilize phosphate complexes that convert to ortho-phosphates. Phosphate solubilization is one of the common traits of endophytes. For example, the endophyte Pantoea sp. from the family Enterobacteriaceae shows P-solubilizing feature (Sulbaran et al. 2009). Literature also supports that bacteria from the genus Pantoea are efficient phosphate solubilizers (Rodríguez et al. 2006; Son et al. 2006). Apart from P solubilization, PSBs can facilitate plants in multiple other ways (Vassileva et al. 2010). PSBs help plant growth by improving their nutrient uptake, phytohormone production and by providing protection against phytopathogens (Singh et al. 2010).

Siderophore Production

Siderophores are iron-chelating agents produced by some microorganisms under iron deficiency. During the deficiency of this micronutrient, the siderophore complex provides Fe to plants and deprive the pathogen of it (Compant et al. 2005). Some endophytes produce siderophores like catacholate, hydroxymate and phenolate with biocontrol potential (Rajkumar et al. 2010). A siderophore-producing trait is commonly observed in endophytes because the bacteria face scarcity of free iron ions inside plant tissues (Sessitsch et al. 2004). Furthermore, siderophore after binding to heavy metals could lower their toxic effects. Siderophores indirectly help plants by presenting Fe and Mo factors to endophytic diazotrophs for nitrogenase synthesis and metabolic functioning (Kraepiel et al. 2009). Bacterial siderophores also enhance bioavailability of metals other than iron to induce better plant growth (Rajkumar et al. 2010).

Insecticidal Properties

A plethora of literature supports the insecticidal (Azevedo et al. 2000; Banerjee et al. 2005; Chanway 2002; Liarzi and Ezra 2014; Verma and Gange 2013) and nematicidal (Hallmann et al. 1997) properties of endophytes. For example, the insecticidal activity of the endophytes Streptomyces albus and Claviceps purpurea has been reported against cotton aphid (Aphis gossypii Glover) (Shi et al. 2013). Similarly, several species of Bacillus and Pseudomonas genus were shown to reduce cotton bollworm incidence (Rajendran et al. 2007). The insecticidal property of endophyte finds applicability as a biocontrol agent. The potential of bacterial endophytes in biocontrol is vast as they colonize the same ecological niche like phytopathogens and therefore impart direct effect in the endosphere (Berg et al. 2005). Endophyte-derived metabolites correspond to varied structural groups like terpenoids, steroids, xanthones, chinones, phenols, isocumarines, benzopyranones, tetralones, cytochalasines and enniatines (Schulz et al. 2002).

Phytoremediation

Phytoremediation refers to plant-based remediation against environmental and soil pollutants. The concept of phytoremediation is newer in agriculture and seems cheaper than available engineering solutions. This 'greener' and pragmatic approach is receiving wide attention from the scientific community. A better understanding of the plant-endophyte association could aid in remediating barren lands and groundwater. Endophytes could equip plants with required degradation pathways for improved biodegradation and reduced phytotoxicity (Weyens et al. 2009). They can improve phytoremediation and benefit plant by fixing nitrogen, solubilizing minerals, producing phytohormones, producing siderophores, transforming nutrients and administering ACC as the N source (Germaine et al. 2009; Germaine et al. 2006; Rajkumar et al. 2009; Stepniewska and Kuźniar 2013). In addition, endophytes decrease metal toxicity and modify its translocation and accumulation in plants. Some plants accumulate toxic end products in tissues, leading to stunted growth (Glick 2003).

Mechanism Of Action Of Microbial Biocontrol Agents

B. subtilis is a gram-positive bacterium that forms biofilms on inert surfaces and possesses many transcriptional factors (Stanley et al., 2003). Different strains of *B. subtilis* synthesize a variety of hydrolytic enzymes, including i.e. cellulases, proteases, and b- glucanases. Cazorla et al. (2007) suggested that since *B. subtilis* has the ability to secrete antibiotics and hydrolytic enzymes, it can modify its' environment in a self-beneficial manner and also produce resistant endospores to sustain itself under adverse condi- tions. The ability of *B. subtilis* to exhibit biocontrol activity is dependent upon three factors: (1). host vulnerability; (2). pathogen virulence; and (3). the environment.

Bacteria also produce the cell-wall-degrading enzymes and var- ious metabolites that can limit the growth or activity of other microorganisms (Shoda, 2000). Notably, *B. subtilis* strains are known to synthesize antibiotic lipopeptides, including fengycin, surfactin, and iturin. Lipopeptides are low molecular weight compounds with amphiphilic features. Surfactants and antimicrobial compounds produced by *B. subtilis* are receiving more attention. A recent study reported that *B. amyloliquefaciens* L-1 was a good biocontrol agent against pear ring rot (Pingping et al., 2017).

Conclusion And Future Prospects

Our current level of understanding about endophyte functioning is limited due to their unique microenvironment in endosphere. The proper endophyte study, which remained restricted due to non cultivability, is now gaining momentum from culture-independent microbial identification methods. Most of the previous plant-bacterial research focused on interaction of single endophyte with plants under controlled conditions. Therefore, future research should study field-level interaction of endophyte consortium with plant host using evolved statistical methods and tools. This approach would ensure reliability of results with better reproducibility under varied land and environmental conditions. The future research should focus on understanding molecular-based endophyte-host interaction as much of the current study is missing the involvement of host genotype in plant-microbe interaction. Thus it can be said that the exploration of host-endophyte interaction could pave path for low-input sustainable agriculture practices. Crops productivity could also be improved by gene modification of plant or associated microflora. Adoption of gene modification methods could equip crops with pesticide resistance, phytoremediation, etc. to suitably regulate metabolism. This newer microbial technology needs to prove its commercial viability to become successful. However, several hurdles impede the viability of endophytes in agriculture.

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