Chapter 16
Enhanced Nutrient Accumulation in Non-leguminous Crop Plants by the Application of Endophytic Bacteria *Bacillus* Species

Gazi Md. Akram Hossain, Amir Hamzah Ghazali, Tofazzal Islam, and M. A. Baset Mia

Abstract Endophytic bacteria exert beneficial effects on various crop plants especially the non-legumes, and the effects are carried out in multidimensional mechanisms unlike the biological N_2 fixation (BNF) process. They create a conducive environment in the apoplastic area of root tissues as well as in shoot for providing benefits to the host plant. Among the endophytic bacteria, recently, *Bacillus* spp. are gaining prominence as a biofertilizer and bioenhancer for crop production. A large number of species and strains of this genera have been isolated and identified from the diversified crop plants such as rice, wheat, maize, alfalfa, banana, black pepper, canola, cucumber, clover, oil palm, and apple. Inoculation of plants with these endophytic Bacilli resulted in various beneficial effects on the colonization including better nutrition, improvement of growth, yield, and quality of crop plants. A significant amount of atmospheric N_2 is fixed and incorporated into a good number of non-legumes like rice, wheat, maize, banana, oil palm etc. that are confirmed by the ^{15}N isotopic dilution technique. The *Bacillus* spp. are found in the apoplastic area, produce phytohormone especially auxin, and excrete their fixed N_2 as ammonium to

G. M. A. Hossain
Soils and Nutrition Division, Bangladesh Sugarcrop Research Institute, Ishurdi, Pabna, Bangladesh

A. H. Ghazali
School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia

T. Islam
Institute of Biotechnology and Genetic Engineering (IBGE), Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

M. A. B. Mia (✉)
Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh
e-mail: miabaset@bsmrau.edu.bd

M. T. Islam et al. (eds.), *Bacilli in Agrobiotechnology*, Bacilli in Climate Resilient Agriculture and Bioprospecting,
https://doi.org/10.1007/978-3-030-85465-2_16

the host plant cells. Additionally, these endophytes are also able to enhance the accumulation of P, K, and Ca through the stimulation of cell membrane-ATP-ase activity. The *Bacillus* spp. are also capable to solubilize complex rock phosphate to a simpler form of phosphate, i.e. dihydrogen orthophosphate and monohydrogen orthophosphate, and help plant phosphorus nutrition. Besides, they produce siderophores, which are very much effective in iron uptake in diversified crop plants. Therefore, the endophytic Bacilli are considered as microbial agents for enhancing uptake and better utilisation of nutrients in different crop plants under normal and harsh environmental conditions. This chapter updates our understanding of nutrient accumulation in non-legumes crop plants by endophytic *Bacillu*s spp.

Keywords ^{15}N isotopic dilution · Phytohormones · Non-legumes · Endophytes · ATP-ase activity; · Cell membrane

16.1 Introduction

Endophytic bacteria thrive and colonize in the internal tissue of the plant showing no external sign of infection or adverse effect on the host plant (Afzal et al. 2019; Holliday 1989; Schulz and Boyle 2006). Generally, they complete their life cycle partially or wholly inside the plant and inhabited in the apoplastic area, i.e. intercellular space or middle lamella of epiblema, cortical tissue of roots, or in the xylem vessel of stem and even in the leaf as well (Xia et al. 2015). They can be regarded either as obligate or facultative endophytes where obligates are not culturable and require a more specific environment for their growth. On the contrary, the facultative endophytes are able to survive in soil, rhizosphere, artificial medium, and inside the plants. The facultative endophytes are widely distributed throughout the plant biota for their advantage of growth potentiality, higher adaptability under adverse conditions. The most common endophytes were isolated from wild or cultivated crops of monocotyledonous and dicotyledonous plants. They may be classified as actinomyces, bacteria, and fungi depending on the microorganism, with bacterial and fungal endophytes being the most studied organisms. The interactions between endophytes and the host plants are complex which involve mutualism and antagonism, and the association might be obligate or facultative. Nearly 300,000 plant species are present on earth, and each individual plant is host to one or more endophytes (Strobel et al. 2004). Only a few of these plants have ever been completely studied in relation to their endophytic biology. Hence, the potentiality to explore this arena is crucial, in order to boost up the biofertilizer development in a sustainable approach. Plant growth-promoting bacterial endophytes (PGPBE) have also been identified which promote plant growth and development in versatile approaches (Mia et al. 2016). Their identification as PGPEBE is attributed to their role in enhancing plant growth using various traits namely production of ammonia, indole-3-acetic acid (IAA), siderophores, N_2 fixation, and accumulation of essential nutrient elements viz. P, K, Ca, Mg, and Fe (Mia et al. 2009). Several rhizospheric or endophytic bacteria belonging to the genera *Azospirillum, Azotobacter, Burkholderia, Bacillus, Enterobacter, Pseudomonas, Rhizobium*, and *Agrobacterium* have been noted as plant growth-promoting (PGP) microorganisms (Vessey 2003). Among them, *Bacillus* is frequently reported as a potential biofertilizer due to its multifunctional PGP traits namely, phosphate solubilisation, IAA production accumulation of nutrients, siderophore (iron chelator) production, and biopesticidal activity (Bahadir et al. 2018; Bjelić et al. 2018; Mohite 2013; Wahyudi et al. 2011, 2020). In addition, biofertilizers containing *Bacillus* strains are considered important because of their spore-forming capacity, allowing their adaptation to extreme abiotic conditions, like extreme temperatures, pH, or pesticide exposure. These species have been shown to have positive effects on soybean seed germination by enhancing the root and shoot length or the number of lateral roots of the seedling, which is related to the production of phytohormone and siderophore, and the capability of these bacteria to solubilize P complex. It has also been reported that *Bacillus* promoted seed germination and growth of tomato, pepper, eggplant, and rice (Mia and Shamsuddin 2013; Beneduzi et al. 2008). Other studies also revealed that *Bacillus* have improved plant growth under drought stress and produced a variety of compounds that can be used for the management of a wide array of plant pathogens. Bacteria viz. *B. megaterium, B. cereus, B. pumilus, B. circulans, B. licheniformis, B. subtilis, B. brevis,* and *B. firmus* have been recognised as N_2 fixers based on their nitrogenase activity (Radhakrishnan et al. 2017). It is the most abundant genus in the rhizosphere, and the PGP activity of some of these strains has been known for many years. There are several metabolites that are released by these strains, which strongly affected the environment by increasing nutrient availability in the plants (Barriuso and Solano 2008).

Currently, one of the major challenges of agriculture is to boost up crop productivity under adverse environmental conditions, especially poor fertile soils having less availability of nutrients. It is interesting to note that microbes that are beneficial to plants are used to enhance crop yield and are alternatives to chemical fertilizers. Additionally, several endophytic *Bacillus* species have emerged as a complementary, efficient, and safe alternative to current crop management practices. It is reported that endophytic *Bacillus* is not host-specific, can colonize a wide array of crop plants, and this gives it a great potential tool for increasing crop productivity in a sustainable manner. This chapter reviews current knowledge on the roles of endophytic *Bacilli* in nutrient accumulation in non-legume crop plants.

16.2 Beneficial Effects of Endophytes on Accumulation of Nutrients in Non-legumes

The major contributions of beneficial and biofertilizer endophytes are to supply nutrients like fixed N_2, enhanced uptake of other essential nutrients like P, K, Zn, and Fe. However, limited information are available on the direct contribution of endophytes to host plant like legume-*Rhizobium* symbiosis process (Hardoim et al. 2008). Nevertheless, endophytes mediate plant growth promotion through direct and indirect mechanisms. Since they start their journey as rhizosphere bacteria, it is assumable that they may retain their attributes inside the plant. Their mechanisms of beneficial effects seem related to rhizosphere bacteria because most endophytes can be cultured and can survive outside of the host in the rhizosphere. A list of endophytic *Bacilli* performs beneficial effects has been presented in Table 16.1.

Table 16.1 Performance of endophytic *Bacillus* spp. inoculation on growth and development of different crop plants

Host plant	Name of endophytic *Bacillus*	Mode of beneficial effect	References
Rice (*Oryza sativa* L.)	*Bacillus aryabhattai* strain E7, *B. aryabhattai* MN1, *B. fortis* strain T9. *B. aryabhattai* strain HS-S05; *B. megaterium* strain KW7-R08; *B. subtilis* strain CB-R05	Increase rice plant growth Synthesis of indoleacetic acid (IAA)	Shen et al. (2019) Ji et al. (2014)
Sweet sorghum (*Sorghum bicolor*)	*B.* spp.	Increase plant growth	Mareque et al. (2015)
Banana (*Musa* spp.)	*B. sphaericus* strain UPMB10	Increase growth and nutrient uptake	Mia et al. (2007)
Beet (*Beta vulgaris* L.)	*B. pumilus*	Increased concentration of carbohydrates	Shi et al. (2010)
Sugar cane (*Saccharum officinarum* L.)	*Bacillus* sp. strain H15	Increase plant growth	Chauhan et al. (2012); Wang et al. (2020)
Cocco (*Theobroma cacao* L.)	*B. subtilis*	Promote plant growth	Leite et al. (2013)
Sunflower (*Helianthus annus* L.)	*B. pumilus*	Improve plant growth	Forchetti et al. (2007)
Maize (*Zea mays* L.)	*B. subtilis, B. lentimorbus*	Nitrogen fixation; IAA synthesis; growth promotion	Wang et al. (2010); Szilagyi-Zecchin et al. (2014)
Soybean (*Glycin max* L.)	*B. amyloliquefaciens, B. japonicum*	Production of siderophores; IAA synthesis; ACC-deaminase; antifungal activity; phytases; N_2 fixation	Sharma et al. (2013) and Hungria et al. (2013)
Wheat (*Triticum aestivum* L.)	*B. subtilis, Bacillus* spp.	IAA synthesis; phosphate solubilisation; growth promotion; increase in grain yield	Wang et al. (2010); Hungria (2011) and Upadhyay et al. (2012) Zhao et al. (2015)

16.2.1 Mechanism of Beneficial Effects of **Bacillus** *Spp. on Plants*

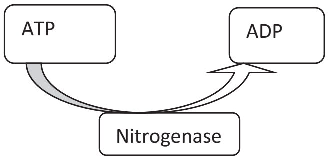
Unlike nutrient accumulation, they can directly benefit plants by providing nitrogen supply via N_2 fixing abilities iron chelators and siderophore production (Long et al. 2008), P complex solubilizing compounds and (Knoth 2014). In addition, they influence on plant growth through the production of phytohormones especially auxin which increased root growth through hair formation, increased lateral roots, volume, and surface area. Also, several S oxidizing endophytes are known which can oxidize elemental S into SO_4^{2+} to be used by plants (Banerjee and Yesmin 2009). Moreover, endophytes are prolific sources of phytochemicals (Nisa 2015) which impede plant hygiene (Chen 2011; Benhamou et al. 1998). They are also excellent sources of biologically active secondary metabolites and contributing to the production of metabolites (Brader 2014; Schulz and Boyle 2002).

16.2.2 Phytohormone Production in Relation to More Root Growth for Higher Nutrient Uptake

Plant growth promotion by endophytic *Bacillus* spp., through phytohormone production, is perhaps the well-agreed method which causes morphological and anatomical changes in roots of the plant. The mechanism of phytohormones production by endophytic *Bacillus* in host plants is similar to plant growth-promoting rhizobacteria. But here, the roots get extra benefits for proper utilisation of synthesized auxin, since the endophytes remain in the apoplastic areas. There are several pathways to produce auxin where endophytes mainly follow the tryptophan precursor–mediated pathways. Whatever the pathways followed for auxin synthesis, the products are released in the apoplastic area of root cortical zone transported to endodermis through apoplastic and symplastic pathways via downhill process. The endodermis releases auxin to the pericycle, located just beneath it; the auxin stimulates the pericycle to convert into meristem consequently form the lateral roots primordia and finally, produced the lateral roots as well as the root hair (Péret et al. 2009; Waidmann et al. 2020). The increased root growth facilitated the plants to absorb more nutrients through root interception, which is one of the mechanisms of nutrient uptake (Marschner 1995).

16.2.3 Atmospheric Nitrogen (N_2) Fixation

Nitrogen is one of the most important nutrient elements which limits crop yield under deficient conditions. The main source of N is the atmospheric N_2, which is unavailable to crop plants due to strong triple bond ($N \equiv N$), which must be reduced by the fixation process. Biological N_2 fixation is the most significant where a large amount of N_2 is fixed by various types of symbiotic associative free-living and endophytic bacteria in association with plants, both of dicotyledon and monocotyledon. The process is mediated by the activity of enzyme nitrogenase using ATP and the overall reaction is shown as follows:



$$N_2 + 8e^- + 8H^+ 16\ ATP \longrightarrow 2NH_3 + H_2 + 16\ ADP + 16Pi$$

Diazotrophic endophytes can fix a handsome amount of N_2, which have been recorded in rice, wheat, maize, sugarcane, oil palm, and bananas, and have been documented by various researchers (Mia et al. 2007, 2010a; Amir et al. 2001; Bashan and Holguin 1997). The mechanism of fixing N_2 by nitrogenase enzyme present in the endophytes are very much similar to *Rhizobium* species symbiosis with legumes, and their genomes possess an *nifHDK* operon, encoding both nitrogenase components: the nitrogenase protein (MoFe protein, *NifDK*), which contains a molybdenum–iron cofactor in the prosthetic group (Carvalho et al. 2014). The nitrogenase enzyme is encoded by ~20 genes which are termed as *nif* genes (N-fixation genes) that are organised in seven operons (*nif* cluster) spanning over 24 kb. The *nifH* gene encodes the Fe protein and *nif D* and *nifK* genes encode the Mo-Fe protein of the nitrogenase enzyme (Rubio and Ludden 2008; Seefedt et al. 2009). The fixed N_2 could be confirmed and measured by several methods like acetylene reduction assay (ARA), ^{15}N isotopic both enrichment and dilution techniques. The ARA technique is a qualitative measure for the activity of enzyme nitrogenase and can be used under certain cases and conditions (Mia and Shamsuddin 2010; Danso 1985). The ^{15}N isotopic technique is used to provide a direct method for detecting BNF (Danso 1995), which comprises enrichment and dilution techniques. The ^{15}N isotope dilution technique was used to quantify the contribution of BNF to clover legume by McAuliffe et al. (1958) which has the capacity to separate out any plant-associated contribution of BNF to plants and is recognised as a more accurate method (Boddey et al. 1996; Roger and Ladha 1992).

The microbial bioassay where isolated endophytes could be cultured specific N-free cultural media and specific conditions and can be quantified by the degree of growth of endophytic bacteria (Das and De 2018). Cell sap analysis is another technique of estimating N_2-fixation where analysis of N solute in xylem exudates and plant parts is based on the determination of the composition of N compounds in plant tissues or N flowing through the xylem sap to the shoot, although the method has some limitation as only a small proportion of known N_2 fixing plants are ureide exporters (Mia and Shamsuddin 2010). A list of N_2-fixing *Bacilli* spp. has been presented in Table 16.2.

Table 16.2 List of N_2-fixing endophytic *Bacillus* spp. beneficial to different non-legumes

Endophytic *Bacillus* sp.	Isolated from crop plant	Colonized into crop	Method used to confirm N_2-fixing ability	References
B. aryabhattai strain HS-S05 *B. megaterium* *B. subtilis* strain CB-R05	Rice (*Oryza sativa* sub sp. *japonica*)	Rice (*Oryza sativa* sub sp. *japonica*)	Amplification of *nifH* genes	Ji et al. (2014)
B. subtilis strain EB-04; *B. pumilus* strain EB-64, EB-169; *Paenibacillus* sp. strain EB-144	Banana cultivar 'Prata Anã' (*Musa acuminata* × *balbisiana*)	–	Amplification of *nifH* genes; acetylene reduction assay (ARA)	Andrade et al. (2013)
B. sp. strain CNPSo 2476, CNPSo 2477, CNPSo 2478;	Corn (*Zea mays* L.)	Corn (*Zea mays* L.)	Amplification of *nifH* genes; ARA	Szilagyi-Zecchin et al. (2014)
B. cereus strain KU097330	Sugarcane (*Saccharum officinarum* L.)	Sugarcane	ARA	Hossain et al. (2020)
B. sphaericus strain UPMB10	Oilpalm roots (*Elaeis guineensis*)	Oil palm, banana, soybean, sweet potato	ARA, ^{15}N isotopic dilution technique. ARA	Amir et al. (2001) Mia et al. (2007, 2010a, 2010b), Saad et al. (1999)

A good number of endophytic *Bacillus* spp. have been identified based on the nitrogenase activity like *B. megaterium, B. cereus, B. pumilus, B. circulans, B. licheniformis, B. subtilis, B. brevis,* and *B. firmus* (Xie et al. 1998). One of the plant-associated members, viz. *B. sphaericus* strain UPMB10, fixes a substantial amount of atmospheric N_2 in association with various non-legumes, including, banana, oil palms, and sweet potato (Mia et al. 2007, 2010a; Mia and Shamsuddin 2010; Mia et al. 2013). The strain UPMB10 has been evaluated for their N_2-fixing capacities in association with oil palm seedlings, banana, rice, and vegetable soybean through ARA and ^{15}N isotopic dilution technique (Mia et al. 2007). Inoculation with *B. spharecus* strain UPMB10 could fix 37–39%N_2 in banana tissue cultured plantlets enumerated by ^{15}N isotopic dilution technique. The highest % Ndfa (nitrogen derived from atmosphere) was recorded at the lowest fertilizer-N applied plant with strain UPMB10. Generally, % Ndfa declined with an increase in fertilizer N application, making a lower contribution of N_2 fixed to the total plant N. The confirmation of N_2 fixation was supported by higher ARA values (129 ηmole plant^{-1} hour^{-1}) in roots of inoculated plants (Mia et al. 2007**)**. In field conditions, rice plants could obtain 20% of their total N requirement and 25–50% of N for oil palm in nursery condition by rhizobacterial inoculation (Shrestha and Ladha 1996; Amir et al. 2001). In a greenhouse study on sugarbeet, three different *Bacillus* isolates fixed N_2 and increased growth (Çakmakçi et al. 2006). Enhanced accumulation of N in sweet sorghum by inoculation of *Bacillus* spp. were recorded by Ribeiro et al. (2018). In another study, Hafeez et al. (2006) noted that selected *Bacillus* sp. used as bioinoculants on wheat resulted in increased root length, plant biomass, and higher accumulation of N and P. Generally, they are widely used in crop production system and are important P-solubilizing inocula, resulting in improved growth and yield of crops (Prakash and Arora 2019).

The production of plant growth regulators such as auxin, cytokinin, and gibberellin by these bacteria may also give additional support to the growth and development of host plant species (Joo et al. 2009; Kang et al. 2009). Recent literature indicates that *Bacillus* sp. have a positive role in plant growth enhancement and biologically active metabolites production (Indiragandhi et al. 2008; Kang et al. 2009). Shen et al. (2019) isolated *B. aryabhattai* E7, *B. aryabhattai* MN1, and *B. fortis* T9 from rice seedling roots and found that these strains have both N_2 fixation potential and IAA production abilities. Sequence analyses of endophytic *Bacilli* in banana cv. 'Prata Anã' roots revealed that PCR amplification of the *nifH* gene was detected in 24 of the 102 bacterial isolates. Seven species of *Bacillus* were *nifH*-positive, including: *B. amyloliquefaciens*, *B. cereus*, *B. flexus*, *B. licheniformis*, *B. pumilus*, *B. subtilis,* and *B. tequilenses* (Suzane et al. 2013). Çakmakçi et al. (2006) observed that three different *Bacilli* isolates fixed N_2 and increased growth in sugar beet in a greenhouse study. A good number of endophytic and associative *Bacillus* spp. have been commercialized as bioinoculants for the growth of crop plants (Islam and Hossain 2013).

16.2.4 Transfer of Fixed N_2 to Host Cell

Whatever the amount of N_2 fixed by endophytes, the release and transportation of fixed N_2 to the host tissues is of the greatest importance. The release or excretion of NH_4^+ and subsequent transportation to the host cell is not clearly identified in the case of associative bacteria since very little amount of N_2 is fixed, which is most probably being utilized by the bacteria itself. Nevertheless, several strains of endophytic bacteria have a unique N regulation system where the NH_4^+ is excreted out of the cell through simple diffusion despite accumulation in its own cell (Brewin et al. 1999; Castorph and Kleiner 1984; Day et al. 2001; Kleiner 1982). The excreted NH_4^+ are transported to cytoplasm via ion channel or plasmodesmata either by downhill or uphill process thereafter being utilized via GS-GOGATT pathway for the synthesis of amino acid and subsequently, other amino acids through transamination process (Mia 2015).

16.2.5 Solubilisation of Soil Insoluble Phosphates

Apart from N_2 fixation, several endophytes release organic acids into the soil which solubilize the phosphate complexes and convert them into ortho-phosphate for plant uptake and utilisation. Although phosphorous exist abundantly in soils, most of it remains unavailable as an insoluble form (Miller 2010). Endophytic *Bacillus* is capable to solubilize the complex unavailable rock phosphate to an available form of P where inoculation of *Bacillus* spp. could solubilize the P-Fe minerals and enhanced uptake of P and Fe in pearl millet (Ribeiro et al. 2018). A good number of *Bacillus* spp. have been isolated from bananas and aerobic rice by Matos et al. (2017) and Panhwar et al. (2009), which are very much effective in the solubilisation of rock phosphate. Rhizobacteria *B. methylotrophicus* CKAM isolated from apple roots showed higher P- solubilisation (Mehta et al. 2014). Similarly, the application of *B. megatorium* in combination with fish bone produced the highest amount of available P for crop plants (Saeid et al. 2018). One of the most important mechanisms is the production of organic acid and release to the soil to solubilize monocalcium, bicalcium, and tricalcium complex phosphate to simpler phosphate as well as increase the activity of phosphatase. The concentration of organic acid is released through root exudates is decreased with increasing the presence of complex phosphate in the rhizosphere.

16.2.6 Enhancement of K Uptake by Plants

Potassium is an essential macronutrient element that contributes to the growth and development of crop plants by influencing the activities of various enzymes system. This element has non-specific functions not directly involved in any reaction system in the cell and do physiological functions in the various arena through osmoregulatory, enzymetic regulation, charge balance in the cytosole (Mia 2015). The greatest amount of K is found in the plant body which is highly mobile in the plant system. Although higher quantities of K present in the soil plant cannot take this element as those are fixed and unavailable for the plants. Recently, the use of potassium solubilizing bacteria (KSB) viz. *Acidothiobacillus ferrooxidans, Paenibacillus* spp., *B. mucilaginosus, B. edaphicus,* and *B. circulans* have shown to solubilize K- bearing minerals, like biotite, feldspar, illite, muscovite, orthoclase, and mica. This type of bacteria can dissolve silicate minerals and release K through the production of organic and inorganic acids, acidolysis, polysaccharides, and complexolysis, chelation, and exchange reactions. The application of *B. sphaericus* strain UPMB10 could not increase the K concentration but its total accumulation was greatly increased due to higher (36%) dry matter production in tissue-cultured banana plantlets growth under a hydroponic condition where dry matter production is directly related to K accumulation. The higher accumulation of K might be due to enhanced root proliferation from the inoculation process (Mia et al. 2010a). Anyway, the KSB have a greater impact on the release of K by various mechanisms. Inoculation of KSB *Bacillus* strain B2084 and B2088 increased the higher accumulation K in a shoot of pearl millet (Ribeiro et al. 2018). The endophytes may also have functions in providing K for a plant by increasing root exudates through supplying different chemicals which can lower the pH by proton efflux, enhancing chelation of the cation bonds to K, and acidolysis of the fixed K area of the soil (Etesami et al. 2017; Meena et al. 2014).

16.2.7 Improvement of Ca Uptake and Absorption by Plants

The endophytic PGPB inoculation greatly increased Ca concentration (nearly 14%) in root especially in plants inoculated along with supplemental 33% fertilizer-N in banana. However, inoculation without fertilizer-N, which could not show any increment, indicated the requirement of starter inorganic N. As Ca is a non-mobile element in the plant system, a higher concentration is found in the root and no influence has been observed in the pesudostem and leaf. Similarly, inoculated plants provided with 33% fertilizer-N also showed lower concentrations when compared to inoculated plants without fertilizer-N. However, in leaf, application of 100% fertilizer-N showed lower Ca concentration (Mia et al. 2009).

The higher Ca uptake by the inoculated plants might not only be due to higher plant growth but also higher uptake capacity, which is induced by bacterial interaction through the acceleration of proton efflux, which results in the acidification of the rhizosphere. It is one of the important mechanisms in cation uptake by the roots, which is the consequence of stimulation of root membrane ATP-ase activity. The endophyte could increase this enzyme activity through bacteria-root interaction as both the strain successfully colonized banana roots. Higher activity of this enzyme resulted in higher Ca concentration in roots (Marschner 1995). Similarly, Bashan et al. (1989) concluded that *Azospirillum* could increase the proton efflux by stimulating ATP-ase activity in the root of wheat seedlings. Increased accumulation of Ca in the cytosole may be the stimulation of the Ca^{2+} ion channel consequently greater influx of Ca^{2+} from the apoplastic area.

16.2.8 Influence of Endophytic Bacilli on Uptake and Absorption of Mg by Plants

Magnesium is an essential macronutrient element which is mobile in the plant system. It plays an important role in the synthesis of chlorophyll molecule as it is the central atom of chlorophyll. Application of endophytic bacteria *B. sphericus* strain UPMB10 could not increase the concentration of Mg rather enhanced the total accumulation which is due to increased root growth not to general uptake rate as in Ca.

16.2.9 *Influence of* **Bacillus** *Spp. on Siderophores Production and Iron Uptake*

Iron is an important micronutrient element that performs many metabolic activities in the plant through the activation of many enzymes taking part as a prosthetic group. However, it is a problematic element in case of uptake and absorption by the plants because the ferric form (Fe^{3+}) readily becomes insoluble in the soil. Therefore, crop plants suffer deficiency despite having sufficiency in the soil. Recently, the microbial siderophore technique in the uptake of iron is gaining prominence for boosting up crop productivity. It is a smaller molecule organic compound synthesize by microorganisms and is capable of absorbing ferric even under Fe-limited soil conditions thereby solving iron nutrition problems in crop plants. The Fe^{3+} siderophore complex can easily enter into cytosole and release the Fe^{3+} where it reduces as $Fe^{2+}$for further utilisation (Saha et al. 2016). Similarly, endophytes also synthesize siderophore which can absorb and utilize Fe^{3+}as like as rhizosphere bacteria. Endophytes produce small molecular compounds called siderophores, which are iron-chelating compounds that can make available iron to plants and deprive pathogens of iron (Compant et al. 2005). Out of the range of siderophore produced by endophytes, one with biocontrol properties are catecholate, hydroxymate, and/or phenolate types (Rajkumar 2010). Also, siderophores specifically help iron-deficient plants in fixing $N_{2,}$ since diazotrophs require Fe^{2+} and Mo factors for nitrogenase synthesis and functioning (Kraepiel 2009). The interaction between plants and beneficial bacteria can have a profound effect on plant health, growth, development, production, and soil quality.

Ribeiro et al. (2018) reported the capacity of four endophytic *Bacillus* strains to solubilize iron phosphate (Fe-P), produce siderophores and indole-acetic acid (IAA) in vitro, and evaluate their plant growth promotion ability in greenhouse conditions by inoculation into pearl millet cultivated in a P-deficient soil. All strains solubilized Fe-P, and three of them produced carboxylate-type siderophores and high levels of IAA in the presence of tryptophan.

16.3 Conclusion and Future Perspectives

Endophytic *Bacillus* spp. promote better nutrition and increase the yield of crop plants. These biologicals are highly potential tools for boosting crop productivity in a sustainable agricultural system. They exert beneficial effects on plants through multifaceted mechanisms. Recently, *Bacillus* spp. gaining prominence as potential candidates for endophytic biofertilisation. This chapter reviews the effect of these bacteria on the nutrient accumulation of non-legumes and discusses their mode of action. Our knowledge of molecular crosstalks between endophytic *Bacillus* spp. and host plants are limited (Alvin et al. 2014). Recent progress in genomics and post-genomics analytical methods enhances our understanding about the underlying molecular mechanisms of the beneficial effects of endophytic Bacilli on plants. Exploration of elite strains of endophytic Bacilli and their genes involved in exerting beneficial effects on plants would help us to apply them in sustainable and climate-smart agricultural systems. Furthermore, the application of the recently developed genome editing by the CRISPR-Cas toolkit would be helpful to design industrially powerful biofertilizers and growth promoters from the genus of plant endophytic *Bacillus*.

References

Afzal I, Shinwari ZK, Sikandar S, Shahzad S (2019) Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. Microbiol Res 221:36–49

Alvin A, Miller KI, Neilan BA (2014) Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. Microbiol Res 169(2014):483–495

Amir HG, Shamsuddin ZH, Halimi MS, Ramlan MF, Marziah M (2001) Effects of *Azospirillum* inoculation on N_2 fixation and growth of oil palm plantlets at nursery stage. J Oil Palm Res 13:42–49

Andrade LF, De Souza GL, Nietsche S, Xavier AA, Costa MR, Cardoso AM, Pereira MC, Pereira DF (2013) Analysis of the abilities of endophytic bacteria associated with banana tree roots to promote plant growth. J Microbiol 52(1):27–34

Bahadir PS, Liaqat F, Eltem R (2018) Plant growth promoting properties of phosphate solubilizing *Bacillus* species isolated from the Aegean region of Turkey. Turk J Bot 42:1–14

Banerjee MR, Yesmin L (2009) Sulfur–oxidizing plant growth promoting rhizobacteria for enhanced canola performance. Google Patents

Barriuso J, Solano BR (2008) Ecology, genetic diversity and screening strategies of plant growth promoting Rhizobacteria (PGPR). J Plant Nutr:1–17

Bashan Y, Holguin G (1997) *Azospireillum*/plant relationship: environmental and physiological advances (1990–1996). Can J Microbiol 43:103–121

Bashan Y, Levanony H, Mitiku G (1989) Changes in proton efflux in intact wheat roots inoculated by *A. brasilense* Cd. Can J Microbiol 35:691–697

Beneduzi A, Peres D, Vargas LK, Bodanese-Zanettini MH, Passaglia LMP (2008) Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing *Bacilli* isolated from rice fields in South Brazil. Appl Soil Ecol 39:311–320

Benhamou N, Kloepper JW, Tuzun S (1998) Induction of resistance against fusarium wilt of tomato by combination of chitosan with an endophytic bacterial strain:ultrastructure and cytochemistry of the host response. Planta 204(2):153–168

Bjelić D, Ignjatov M, Marinković J, Milošević D, Nikolić Z, Gvozdanović-Varga J, Karaman M (2018) *Bacillus* isolates as potential biocontrol agents of *Fusarium* clove rot of garlic. Zemdirbyste-Agriculture 105(4):369–376

Boddey RM, Alves BJR, Urquiaga S (1996) Nitrogen cycling and sustainability of improved pastures in the Brazilian cerrados. In: Pereira RC, Nasser LCB (eds) Proceedings of VIII Symp. on cerrados: Biodiv. sustain. prod. food fibres in cerrados, Proc. 1st int. symp. trop. savannas, Brasilia, D. F., Brazil. 24–29 March 1996

Brader G (2014) Metabolic potential of endophytic bacteria. Curr Opin Biotechnol 27:30–37
Brewin B, Woodley P, Drummond M (1999) The basis of ammonium release in nifL mutants of *Azotobacter vinelandii*. J Bacteriol 181:7356–7362
Çakmakçi R, Donmez F, Aydın A, Sahin F (2006) Growth promotion of plants by plant growth promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biol Biochem 38:1482–1487
Carvalho TLGE, Balsemão-Pires RM, Saraiva PCG, Ferreira ASH (2014) Nitrogen signalling in plant interactions with associative and endophytic diazotrophic bacteria. J Exp Bot 65(19):5631–5642
Castorph H, Kleiner D (1984) Some properties of a *Klebsiella pneumoniae* ammonium transport negative mutant (Amt^-). Arch Microbiol 139:245–247
Chauhan H, Bagyaraj DJ, Sharma A (2012) Plant growth-promoting bacterial endophytes from sugarcane and their potential in promoting growth of the host under field conditions. Exp Agric 49(1):43–52
Chen B (2011) A bacterial endophyte from banana:its isolation, identification, activity to Fusarium Wilt and PGPR effect to banana seedlings. Microbiol/Weishengwuxue Tongbao 38(2):199–205
Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71(9):4951–4959
Danso SKA (1985) Methods for estimating biological nitrogen fixation. In: Ssali H, Keya SO (eds) Biological nitrogen fixation in Africa. Nairobi MIRCENS, Nairobi, pp 213–244
Danso SKA (1995) Assessment of biological nitrogen fixation. Fertilizer Res 42:33–41
Das S, De TK (2018) Microbial assay of N_2 fixation rate, a simple alternate for acetylene reduction assay. Methods X 5:909–914
Day DA, Poole PS, Tyerman SD, Rosendahl L (2001) Ammonia and amino acid transport across symbiotic membranes in nitrogen-fixing legume nodules. Cell Mol Life Sci 58:61–71
Etesami H, Emami S, Alikhani HA (2017) Potassium solubilizing bacteria (KSB): mechanisms, promotion of plant growth, and future prospects, A review. J Soil Sci Plant Nutr 17(4)
Forchetti G, Masciarell O, Alemano S, Abdala G (2007) Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. Appl Microbiol Biotechnol 76(5):1145–1152
Hafeez FY, Yasmin S, Ariani D, Zafar Y, Malik KA (2006) Plant growth promoting bacteria as biofertilizer. Agron Sustain Dev 26:143–150
Hardoim PR, van Overbeek LS, Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471
Holliday P (1989) A dictionary of plant pathology. Cambridge University Press, Cambridge
Hossain GMA, Solaiman ARM, Karim AJMS, Rahman GKMM, Mia MAB (2020) Influence of Diazotrophic bacteria on growth and biomass production of sugarcane *in vitro*. Int J Curr Microbiol Appl Sci 9(3):3077–3088
Hungria M (2011) Inoculação com *Azospirillum brasilense*: inovação em rendimento a baixo custo. Embrapa Soja Documentos 325
Hungria M, Nogueira MA, Araujo RS (2013) Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. Bioll Fertil Soils 49(7):791–801
Indiragandhi, P., Anandham,R. Madhaiyan, M. & Sa, T.M. (2008). Characterization of plant growth-promoting traits ofbacteria isolated from larval guts of diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae). Curr Microbiol*,* 56, 327–333
Islam MT, Hossain MM (2013) Biological control of *Peronosporomycete phytopathogens* by antagonistic bacteria. In: Maheshwari DK (ed) Bacteria in agrobiology: plant disease management. Springer, Berlin, pp 167–218
Ji SH, Gururani MA, Chun SC (2014) Isolation and characterization of plant growth pro- moting endophytic diazotrophic bacteria from Korean rice cultivars. Microbiol Res 169(1):83–98

Joo, G.J., Kang, S.M., Hamayun, M., Kim,S.K., Na,C.I., Shin, D.H. & Lee, I.J. (2009). *Burkhoderia* sp. KCTC 11096BPas a newly isolated gibberellins producing bacterium. J Microbiol, 47, 167–171

Kang, S.M., Joo, G.J., Hamayun,M. Na,C.I.,Shin, D.H., Kim,Y.K., Hong,J.K. & Lee,I.J. (2009). Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. Biotechnol Lett, 31, 277–281

Kleiner D (1982) Amonium (methylamonium) transport by Klebsiella pneumonia. Biochim Biophys Acta 688:702–708

Knoth JL (2014) Biological nitrogen fixation and biomass accumulation within poplar clones as a result of inoculations with diazotrophic endophyte consortia. New Phytol 201(2):599–609

Kraepiel A (2009) Multiple roles of siderophores in free–living nitrogen–fixing bacteria. Biometals 22(4):573–581

Leite HA, Silva AB, Gomes FP, et al. (2013) *Bacillus subtilis* and *Enterobacter cloacae* endophytes from healthy *Theobroma cacao* L. trees can systemically colonize seedlings and promote growth. Appl Microbiol Biotechnol, 97,2639–2651

Long H, Schmidt D, Baldwin I (2008) Native bacterial endophytes promote host growth in a species–specific manner; phytohormone manipulations do not result in common growth responses. PLoS One 3(7):e2702

Mareque C, Taulé C, Beracochea M et al (2015) Isolation, characterization and plant growth promotion effects of putative bacterial endophytes associated with sweet sorghum (*Sorghum bicolor* (L) Moench). Ann Microbiol 65:1057–1067

Marschner, H. (1995). *Mineral nutrition of high plants* (2nd Edn), London aca-demic press, London, 889

Matos ADM, Izabela CP, Nietsche S, Xavier AA, Gomes WS, Neto JDS, Pereira MCT (2017) Phosphate solubilization by endophytic bacteria isolated from banana trees. An Acad Bras Cienc 89(4):2945–2954

McAuliffe C, Chamblee DS, Uribe-Arango H, Woodhouse WW (1958) Influence of inorganic nitrogen on nitrogen fixation by legumes ilS revealed by N-15. Agron J 50:334–347

Meena VS, Maurya BR, Verma JP, Meena RS (2014) Potassium solubilizing microorganisms for sustainable agriculture. Springer

Mehta P, Walia A, Kakkar N, Shirkot CK (2014) Tricalcium phosphate solubilisation by new endophyte Bacillus methylotrophicus CKAM isolated from apple root endosphere and its plant growth-promoting activities. Acta Physiol Plant 36:2033–2045

Mia MAB (2015) Nutrition of crop. Plants. Nova Science Publisher, New York

Mia MAB, Shamsuddin ZH (2010) Nitrogen fixation and transportation by rhizobacteria: A scenario of rice and bananas. Int J Bot 6(3):235–242

Mia MAB, Shamsuddin ZH (2013) Biofertilizer for banana production. Lambert Academic Publisher, Germany

Mia MAB, Shamsuddin ZH, Marziah M (2007) Associative nitrogen fixation by *Azospirillum* and *Bacillus* spp. in bananas. Infomusa 16(1&2):11–15

Mia MAB, Shamsuddin ZH, Wahab Z, Maziah M (2009) The effect of rhizobacterial inoculation on growth and nutrient accumulation of tissue-cultured banana plantlets under low N-fertilizer regime. Afr J Biotechnol 8(21):5855–5866

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2010a) Rhizobacteria as bioenhancer for growth and yield of banana (*Musa* spp. cv. "Berangan"). Sci Hortic 126(2):80–87

Mia MAB, Shamsuddin ZH, Marziah M (2010b) Rhizobacterial inoculation on growth and nitrogen incorporation in tissue-cultured *Musa* plantlets under nitrogen-free hydroponics condition. Austr J Crop Sci 4(2):85–90

Mia MAB, Hossain MM, Shamsuddin ZH, Islam MT (2013) Plant-associated bacteria in nitrogen nutrition in crops, with special reference to rice and banana. In: Maheshwari DK (ed) Bacteria in agrobiology: crop productivity. Springer, Berlin/Heidelberg

Mia MAB, Naher UA, Panhwar QA, Islam MT (2016) Growth promotion of non-legumes by the inoculation of *bacillus* species. In: Islam et al (eds) Bacillus and agrobiotechnology. Springer

Miller SH (2010) Biochemical and genomic comparison of inorganic phosphate solubilization in pseudomonas species. Environ Microbiol Rep 2(3):403–411

Mohite B (2013) Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. J Soil Sci Plant Nutr 13(3):638–649

Nisa H (2015) Fungal endophytes as prolific source of phytochemicals and other bioactive natural products:A review. Microb Pathog 82:50–59

Panhwar QA, Radziah O, Sariah M, MohdRazi I (2009) Solubilization of phosphate forms by phosphate solubilizing bacteria isolated from aerobic rice. Int J Agric Biol 11:667–673

Péret B, BertDe R, Casimiro I, Benková E, Swarup R· Laplaze L, Beeckman T, Bennett MJ (2009) *Arabidopsis* lateral root development: an emerging story. Trends Plant Sci, 14 (7):399–408

Prakash J, Arora NK (2019) Phosphate-solubilizing Bacillus sp. enhances growth, phosphorus uptake and oil yield of *Mentha arvensis* L. 3 Biotech 9:126

Radhakrishnan R, Hashem A, AbdAllah EFA (2017) *Bacillus*: a biological tool for crop improvement through bio-molecular changes in adverse environments. Front Physiol, 8:667

Rajkumar M (2010) Potential of siderophore–producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28(3):142–149

Ribeiro VP, Marriel IE, Evódio I, de Sousa SM, Lana Gomes U, Mattos BB, de Oliveira CA, Gomes EA (2018) Endophytic *Bacillus* strains enhance pearl millet growth and nutrient uptake under low-P. Braz J Microbiol 49:SuI

Roger PA, Ladha JK (1992) Biological N_2 fixation in wetland rice fields: estimation and contribution to nitrogen balance. Plant Soil 141:41–55

Rubio LM, Ludden PW (2008) Biosynthesis of the iron-molybdenum cofactor nitrogenase. Annu Rev Microbiol 62:93–111

Saad MS, Shabuddin ASA, Yunus AG, Shamsuddin ZH (1999) Effects of *Azospirillum* inoculation on sweet potato grown on sandy tin-tailing soil. Commun Soil Sci Plant Anal 30(11&12):1583–1592

Saeid A, Prochownik E, Dobrowolska-Iwanek J (2018) Phosphorus solubilization by *Bacillus* species. Molecules 23(11):2897

Saha M, Sarkar S, Sarkar B, Kumar B, Bhattacharjee S, Tribedi P (2016) Microbial siderophores and their potential applications: a review. Environ Sci Pollut Res 23:3984–3999

Schulz B, Boyle C (2002) Siegfried Draeger, et al. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res 106(9):996–1004

Schulz B, Boyle C (2006) What are endophytes? In: Schulz BJE, Boyle CJC, Sieber TN (eds) Microbial root endophytes, Soil biology, 9. Springer, Berlin/Heidelberg

Seefedt LC, Hoffman BM, Dean DR (2009) Mechanism of Mo-dependent nitrogenase. Annu Rev Biochem 78:701–722

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus 2(587):1–14

Shen FT, Yen JH, Liao CS, Chen WC, Chao YT (2019) Screening of rice endophytic biofertilizers with fungicide tolerance and plant growth-promoting characteristics. Sustainability 11:1133

Shi Y, Lou K, Li C (2010) Growth and photosynthetic efficiency promotion of sugar beet (*Beta vulgaris* L.) by endophytic bacteria. Photosynth Res 105:5–13

Shrestha RK, Ladha JK (1996) Genotypic variation in promotion of rice dinitrogen fixation as determined by nitrogen-15 dilution. Soil Sci Soc Am J 60:1815–1821

Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67(2):257–268

Suzane AS, Adelica AX, Márcia RC, Acleide MSC (2013) Endophytic bacterial diversity in banana 'Prata Anã' (Musa spp.) roots. Genet Mol Biol 36(2):252–264

Szilagyi-Zecchin VJ, Ikeda AC, Hungria M, Adamoski D, Kava-Cordeiro V, Glienke C, Galli-Terasawa LV (2014) Identification and characterization of endophytic bacteria from corn (Zea mays L) roots with biotechnological potential in agriculture. AMB Express 4:26

Upadhyay SK, Singh JS, Saxena AK, Singh DP (2012) Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. Plant Biol 4(14):605–611

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586
Wahyudi AT, Astuti RP, Widyawati A, Meryandini A, Nawangsih AA (2011) Characterization of *Bacillus sp.* strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting Rhizobacteria. J Microbiol Antimicrob 3:34–40
Wahyudi AT, Astuti RP, Widyawati A, Meryandini A, Nawangsih AA (2020) Characterization of bacillus sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting Rhizobacteria. J Microbiol Antimicrob 3(2):34–40
Waidmann S, Sarkel E, Kleine-Vehn J (2020) Same same, but different: growth responses of primary and lateral roots. J Exp Bot 23 71(8):2397–2411
Wang Y, Li H, Zhao W, He X, Chen J, Geng X, Xiao M (2010) Induction of toluene degradation and growth promotion in corn and wheat by horizontal gene transfer within endophytic bacteria. Soil Biol Biochem 42(7):1051–1057
Wang Z, Yu ZX, Solanki MK, Yang L-T, Xing Y-X, Dong D-F, Li Y-R (2020) Diversity of sugarcane root-associated endophytic *Bacillus* and their activities in enhancing plant growth. J Appl Microbiol 128, 3:814–827
Xia Y, De Bolt S, Dreyer J, Scott D, Williams MA (2015) Characterization of culturable bacterial endophytes and their capacity to promote plant growth from plants grown using organic or conventional practices. Front Plant Sci 6:490
Xie G, Su B, Cui Z (1998) Isolation and identification of N_2-fixing strains of *bacillus* in rice rhizosphere of the Yangtze River valley. Wei Sheng Wu XueBao (Article in Chinese) 38(6):480–483
Zhao L, Xu Y, Lai XH, Shan C, Deng Z, Ji Y (2015) Screening and characterization of endophytic *Bacillus* and *Paenibacillus* strains from medicinal plant Lonicera japonica for use as potential plant growth promoters. Braz J Microbiol 46(4):977–989