Journal of Food Health and Bioenvironmental Science (May-August 2019), 12(2): 49-56

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Nitrogen-fixing Bacteria and Trends in Agricultural Applications

Junjarus Sermsathanaswadi*

Department of Chemical Technology, Faculty of Science and Technology, Suan Dusit University, Bangkok, 10300 Thailand

Article info

Article history:
Received: 11 March 2019
Revised: 13 August 2019
Accepted: 21 August 2019

Keywords:
Agriculture, Nitrogen-fixing Bacteria, Non-symbiotic, Symbiotic

Abstract

Bacteria that can increase the number of nutrients in the soil are important to plants, especially nitrogen-fixing bacteria that fix atmospheric nitrogen and change into the form that plants can use. In recent years, the use of nitrogen-fixing bacteria in agriculture has received a lot of attention because it offers an economically attractive and environmentally friendly method. Many species of nitrogen-fixing bacteria, symbiotic and non-symbiotic, that promote plant growth are used on a regular basis in order to improve crop yields. In addition to agricultural benefits, there are also potential benefits for environmental applications. Many nitrogen-fixing bacteria which grow and multiply within plant tissues are called endophytes. They illustrate the tight association with the plant tissues without causing damage. Therefore, different types of endophytes which produce plant growth hormone provide benefit for many plants.

Introduction

Nitrogen is one of the most abundant elements in the Earth's atmosphere. Air is composed of 78 percent nitrogen, predominately in the form of nitrogen gas (N_2). Nitrogen is an essential element for all living organisms. Nitrogen exists either in the reduced or oxidized forms in the global nitrogen cycle as shown in Fig. 1. In plants, nitrogen is a major element of amino acids which are the building blocks of proteins such as enzymes, cell membranes, transport proteins, hormones, nucleic acids and ATP (energy currency of the cell). Moreover, nitrogen is also an essential element in chlorophyll, which is the most crucial pigment for photosynthesis. When plants lack nitrogen, the leaves will appear yellow and/or pale green because plants are unable to produce the chlorophyll. Plants will also develop and grow more slowly than we would normally expect. Although the atmosphere contains a large amount of nitrogen, plants cannot use that nitrogen gas. Plants can use nitrogen in the form of nitrogen compounds in the soil, but it is tiny amount (Graham & Vance, 2000). About 90 percent of the nitrogen compounds in the soil are in the form of organic matter or organic nitrogen. When they are transformed into inorganic nitrogen, nitrate ion (NO_3^-) and ammonium ion (NH_4^+), they will be beneficial to the plants. Most plants prefer nitrogen in the form of NO_3^- rather than NH_4^+. Plants can absorb NO_3^- better than NH_4^+, which is toxic for plants (Ruan et al., 2007; Babourina et al. 2007). However, both NH_4^+ and NO_3^- contained in the soil are limited and easily lost by processes such as leaching and other procedures such as

* Corresponding Author
e-mail: junjarus_ser@dusit.ac.th

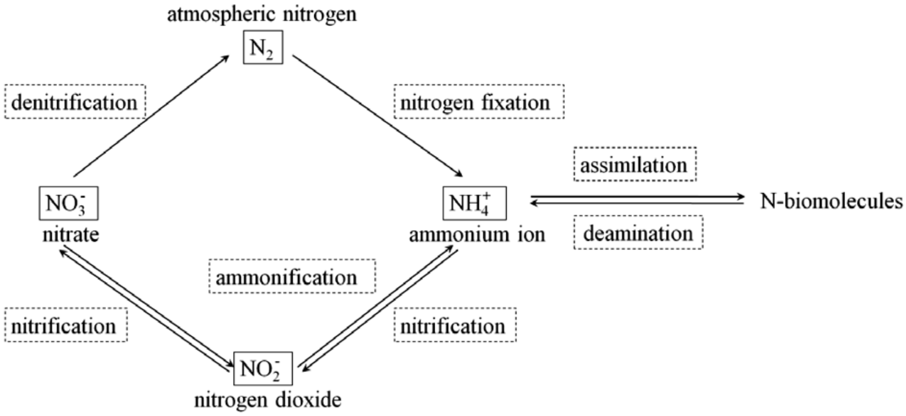

Fig. 1 Steps involved in global nitrogen cycle (modified from Igarashi & Seefeldt, 2003).

biological reduction of NO_3^- (denitrification). Therefore, it can be explained that nitrogen is an element that limits the growth of plants. Even though nitrogen is the most abundant element in the world, plants can only utilize combined nitrogen or reduced forms of nitrogen. Plants acquire these forms of nitrogen from two main sources, first is from the soil through adding fertilizers such as ammonia, manure and/or decomposition of organic substances to the soil. The second source is the conversion of atmospheric nitrogen through nitrogen fixation, such as chemical fixation via the Haber-Bosch process, which reduces N_2 to ammonia using both high temperature and pressure. In other cases, chemical nitrogen fixation can be achieved by oxidation reaction which fixes N_2 by oxidation to nitrate. In nature, lightning strike transforms N_2 into oxide of nitrogen in various forms (finally to NO_3^-). The other nitrogen fixation is symbiotic nitrogen fixation or biological nitrogen fixation by free-living or plant-associated bacteria (Sprent, 2007; Santi et al., 2013). Beijerinck (1901) discovered the biological nitrogen fixation (BNF) for the first time in 1901. It was found that the operation was carried out by a group of prokaryotes including cyanobacteria (*Anabaena* and *Nostoc*) and some bacteria. They have the nitrogenase (EC 1.18.6.1, EC 1.19.6.1), enzymes that catalyze the reduction of N_2 to produce ammonia (NH_3), which is the key step in nitrogen fixation processes. Then, NH_3 will be modified by bacteria to create their organic compounds. The overall reaction of nitrogenase is described by the equation below (Morrison et al., 2017).

$$N_2 + 10H^+ + 8e^- + 16ATP \rightarrow 2NH_4^+ + H_2 + 16ADP + 16Pi$$

Some prokaryotes are able to convert nitrogen in the atmosphere to ammonia which can be used by plants through a biological nitrogen fixation (BNF) which are called diazotrophs (Lam et al., 1996; Franche et al., 2009). These prokaryotes include aquatic organisms, free-living soil bacteria and bacteria that interactions with plants, legumes and other legumes (Postgate, 1982). Nitrogen-fixing bacteria can be divided into three broad categories based on the degree of intimacy and interdependency between them. The first is the free-living bacteria (nonsymbiotic), such as *Azotobacter, Beijerinckia* and *Clostridium.* The second category is endophytic bacteria that can colonize interior the plant tissues and provide benefits to the plant. The third is the endosymbiosis bacteria or mutualistic bacteria (symbiotic), such as *Rhizobium* which are associated with leguminous plants.

Endosymbiosis bacteria

Symbiotic nitrogen fixation is a fixation of nitrogen from the air that relies on microorganisms and plant roots. Many diazotrophs can be found in the rhizosphere, the region of soil around plant roots that influence growth, respiration and nutrient exchange of a plant. The diazotrophs show a tremendous competitive advantage over other bacteria in the rhizosphere, mainly when nitrogen in the soil is limited due to their ability to fix nitrogen from the atmosphere (Döbereiner & Pedrosa, 1987). The habitat of diazotrophs are not only in the rhizosphere, area around the root plant but also in the phyllosphere which are all the above-ground parts of plants (Ruinen, 1956). The well-known mutualistic relationship between bacteria and plant is the association between legume and *Rhizobium* (Fig. 2). The leguminous plants produce the energy from photosynthesis to drive the nitrogen-fixing process and the *Rhizobia* fix the N_2 from the atmosphere, supplying both the bacteria and the plants. Most of the leguminous plants such as soybeans, peanuts, alfalfa, clover and lentils can fix N_2 in association with the *Rhizobia.* The *Rhizobia* have been classified on the species of legume that they nodulate. This type of grouping is called "cross-inoculation". The plants were divided into cross-inoculation groups and *Rhizobium* species would inoculate plants in the same group (Table 1). The interactions between plants and nitrogen-fixing bacteria are the purest form of nitrogen-fixing symbiosis. Plants create the root areas for the symbiotic nitrogen-fixing bacteria which are embedded into the root hairs of plants (generally do not invade the plant tissues). The symbiotic bacteria stimulate the formation of roots nodules when they grow and multiply. In the root nodules, bacteria can turn free nitrogen into ammonia which the plant can absorb and utilize for growth and development. Typically, *Rhizobia* have two sets of genes, *nod* genes for nodulation and *nif* genes for nitrogen fixation (Masson-Boivin et al., 2009). The *Rhizobia* secrete the Nod factors to stimulate the re-orientation of cell wall of the growing root hairs lead to curled root hairs and induce the formation of infection threads. The *Rhizobia* use these tubular structures to enter leguminous plants (Fig. 3). In the actinorhizal plant, a group of angiosperms, is associated with *Frankia* acts similar to legumes and *Rhizobium* (Benson & Silvester, 1993).

Table 1 The cross-inoculation groups

Cross-inoculation Groups	Leguminous plant	*Rhizobium* species
Clover group	*Trifolium* sp. (clovers, trefoil)	*Rhizobium trifolii*
Alfalfa group	*Medicago* sp. (alfafa, burclover) *Melilotus* sp. (melilot, sweet clover, kumoniga)	*Rhizobium meliloti*
Bean group	*Phaseolus* sp. (bean, wild bean)	*Rhizobium phaseoli*
Lupine group	*Ornithopus* sp. (lupines, serradella)	*Rhizobium lupine*
Pea group	pea, sweet pea, lentil, vetch	*Rhizobium leguminosarum*
Soybean group	*Glycine max* (soybean)	*Rhizobium japonicum*
Cowpea group	cowpea, pegionpea, lespedza, groundnut, kudz	*Rhizobium* sp.

Remark: modified from Somasegaran, 1994.

Fig. 2 Nitrogen-fixing bacteria (Rhizobium) nodules on peanut roots.

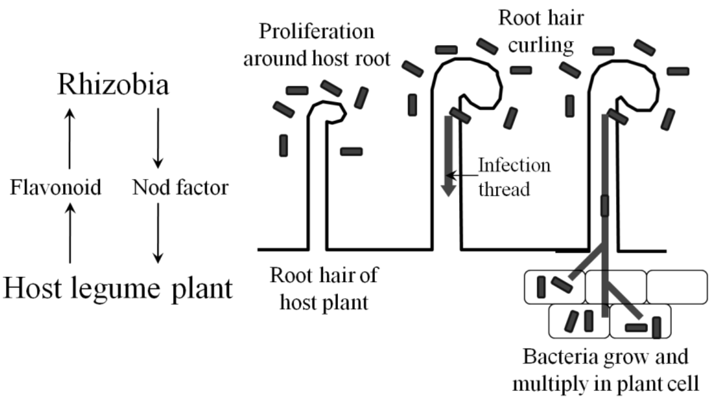

Fig. 3 Scheme of chemical signal exchanges and infection processes involving rhizobia. (modified from Okasaki et al., 2004)

Endophytic bacteria

Although biological nitrogen-fixation using endosymbiosis bacteria is very beneficial for a plant, not all plants have acquired a symbiosis with the *Rhizobia* due to the specificity between *Rhizobia* and host plant (Mylona et al., 1995). The *Rhizobia* require nodules to fix nitrogen from the atmosphere, but in case of some bacteria, endophytic, they not need nodules. It has been reported that the plant-bacteria association can enhance the nitrogen-fixing efficacy of both legume and non-legume plants (Udvarte & Poole, 2013; Santi et al., 2013). In 1961, the diazotrophs in the non-legume plant were first reported by Döbereiner J., Brazilian researcher, and he found the diazotrophs in the rhizosphere of sugarcane (*Saccharum officinarum*) (Döbereiner J., 1961). In subsequent researches, many diazotrophs were isolated from the rhizosphere of sugarcane such as *Azospirillum lipoferum, A. amazonense, Bacillus azotofixans, Enterobacter cloacae, Erwinia herbicola,* and *Paenibacillus polymyxa* (Puri et al., 2017). Many diazotrophic bacteria have evolved to grow, spread and multiply within plant tissues without causing damage or causing plant defense responses such as *Azoarcus, Herbaspirillum* and *Gluconacetobacter.* These bacteria illustrate the tight association with plant tissues and they are classified as endophytes (Pedraza, 2008). They enter to plant tissues through stomata on leaves or through the lateral root (Fig. 4).

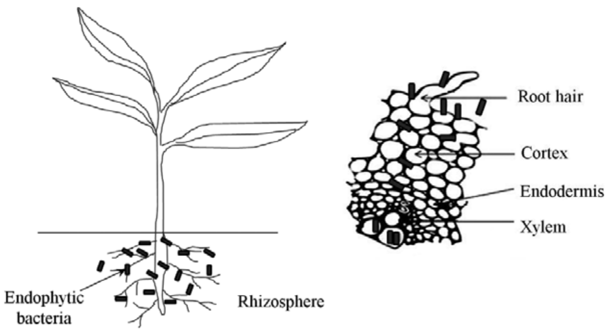

Fig. 4 Plant colonization routes by endophytic bacteria (modified from Audi pudi et al., 2017)

Endophytes are often found as epiphytes, suggesting that endophytes may also colonize surrounding environments of host plants. Many endophytes originate from the rhizosphere and move into the plant cell due to the presence of root exudates and through root colonization. Besides, inside the surface of the stem and leaf can produce exudates that attract microbes as well. Then, bacteria can be found in both areas as well. However, UV light, lack of nutrients and desiccation generally reduce the colonization of the leaf surface.

The most studied association system in the non-legume plant is the association between sugarcane and *Gluconoacetobacter diazotrophicus.* It has been reported that living *G. diazotrophicus* can produce some molecules that activate the sugarcane defense response, protecting the plant against *Xanthomonas albilineans* (pathogenic) attack (Arencibia et al., 2006). This relationship has resulted in increasing of sugarcane production without adding nitrogen fertilizers. There are many experiments which have illustrated that nitrogen-fixing bacteria are beneficial to plants. In 2014, Szilagyi-Zecchin et al. (2014) isolated and identified six endophytic strains from roots of corn growing in the southern Brazilian region of Campo Largo, PR. Out of these six endophytic isolates were found the presence of *nifH* gene and shown nitrogen-fixing activity. Interestingly, they found that two strains, identified as *Bacillus* sp., showed other plant growth–promotion (PGP) characteristics, like production of indole-3-acetic acid (IAA) or auxin plant hormone. It stimulates plant growth, cell division and cell elongation, siderophores and lytic enzymes and antagonism against pathogenic fungi. In 2013, Gupta and coworkers isolated the endophytic diazotrophic strains from pearl millet plants growing in a nutrient-deficient sandy clay loam soil located in Rajasthan. They found that the most dominant *diazotrophic* strain in pearl millet plants was *Pseudomonas aeruginosa* strain PM389. This strain can migrate and live in the stem tissues. *P. aeruginosa* strain PM389 has the ability not only on nitrogen fixation, but also possesses other PGP characteristics such as mineral phosphate solubilization, siderophore production, and antagonistic activity against many pathogenic bacteria and fungi. Moreover, when *P. aeruginosa* strain PM389 was inoculated into wheat, it can promote wheat growth by increasing the, seed germination rate, and root and shoot length (Gupta et al., 2013). The association of endophytic diazotrophic bacteria and their host plants, especially agricultural crops are shown in Table 2.

Because Thailand is the largest rice producer and exporter in the world, then a lot of research focuses on endophytic diazotrophic bacteria and rice. Koomnok et al. (2007) reported that they can be isolated the diazotrophic bacteria which are composed of *Azospirillum,*

Table 2 List of endophytic diazotrophic bacteria recently isolated and associated with agricultural plants.

Endophytic diazotrophic bacteria	Isolated from	Reference
- *Paenibacillus kribbensis* HS-R01, HS-R04; *Bacillus aryabhattai* HS-S05; *Bacillus megaterium* KW7-R08; *Klebsiella pneumonia* KW7-S06, KW7-S22, KW7-S27, KW7-S33; *Bacillus subtilis* CB-R05; *Microbacterium binotii* CB-S18; *Microbacterium trichotecenolyticum* SW521-L21, SW521-L37	Rice (*Oryza sativa* var. *Japonica* c.v. ilpum)	Ji et al. (2014)
- *Enterobacter dissolvens; Brevundimonas aurantiaca; Pantoea agglomerans; Pseudomonas* spp.	Rice (O*ryza sativa* L. cultivar KDML-105)	Prakamhang et al. (2009)
- *Bacillus* sp. BPSAC3, BPSAC6, BPSAC14; *Paenibacillus* sp. BPSAC45; *Bacillus thuringiensis* BPSAC46; *Lysinibacillus sphaericus* BPSAC60; *Pseudomonas* sp. BPSAC75; *Pseudomonas stutzeri* BPSAC43; *Staphylococcus* sp. BPSAC18, BPSAC155	East Indian glory bower (*Clerodendrum cole-brookianum*)	Passari et al. (2016)
- *Bacillus amyloliquefaciens* MBL_B26; *Bacillus subtilis* MBL_B4; *Bacillus firmus* MBL_B5; *Brevibacterium* sp. MBL_B7 ; *Micrococcus* sp. MBL_B10, MBL_B11; *Bacillus pumilus* MBL_B12; *Bacillus subtilis* MBL_B13; *Bacillus* sp. MBL_15, MBL_16, MBL_17, MBL_20, MBL_21; *Micrococcus luteus* MBL_B18; *Micrococcus lylae* MBL_B1; *Kocuria* sp. MBL_19; *Pseudomonas psychrotolerans* MBL_B23, MBL_27; *Pseudomonas monteilii* MBL_B24; *Ralstonia solanacearum* MBL_B6; *Staphylococcus arlettae* MBL_B2, MBL_B3, MBL_B14, MBL_B22; *Staphylococcus hominis* MBL_B8; *Staphylococcus saprophyticus* MBL_B9; *Staphylococcus warneri* MBL_B25;	Jute (*Corchorus olitorius*)	Haidar et al. (2018)
- *Bacillus aryabhattai; Pantoea cypripedii; Bacillus licheniformis; Klebsiella* sp.; *Pantoea dispersa; Klebsiella variicola; Pantoea* sp.; *Agrobacterium larrymoorei; Bacillus* sp., *Bacillus amyloliquefaciens; Lactococcus lactis; Bacillus cereus; Staphylococcus homini*	Maize (*Zea mays* L.)	Marag & Suman (2018)
- *Ancylobacter* sp. UT3R1; *Ochrobactrum* sp. C7HL1; *Novosphingobium sediminicola* C2HL2; *Novosphingobium capsulatum* C34MR1	Sugarcane (*Saccharum officinarum* L.)	Muangthong et al. (2015)
- *Mycobacterium* spp.; *Streptomyces thermolineatus; Micromonospora endolithica; Micromonospora peucetica; Gordonia polyisoprenivorans*	Wheat (*Triticum aestivum*)	Conn & Franco (2004)

Herbaspirillum, Beijerinckia and *Pseudomonas* from cultivated rice (khao dawk mali 105, purple glutinous rice kum doi saket and bue polo) and wild rice (*Oryza granulata, O. rufipogon, O. rufipogon* 18883 and *O. nivara* 18852). In 2016, Raweekul et al. reported that 126 endophytic bacteria isolated from rice (*Oryza sativa*) roots and stems consist of the members of phyla *Firmicutes, Proteobacteria, Bacteroidete* and *Actinobacteria.* From theses phyla, 12 members of isolated in genera *Bacillus, Micrococcus* and *Acinetobactere* showed the increased fresh weight of rice seedlings when compared to the water-treated control group. Theses genera contain *nifH* gene, siderophore production, IAA synthesis and ACC-deaminase activity which represented their potential application as biofertilizers (Raweekul et al., 2016). Many studies showed that endophytic diazotrophic bacteria have the ability to nitrogen-fixation and can act as biofertilizer, especially for highly N-demanding crops such as sugarcane, corn, and rice. Moreover, they present PGP characteristics which can enhance plant growth and antagonistic activity against many pathogenic microorganisms.

Free-living bacteria

There are many free-living microorganisms can fix N_2 from the atmosphere. The free-living nitrogen-fixers live in soil or on soil surfaces such as *Cyanobacteria, Proteobacteria, Archaea* and *Firmicutes.* The free-living nitrogen-fixing bacteria is a group of bacteria that lives independently in soil or other environments. Nitrogen can be fixed from the air without using carbohydrates or energy sources from plants. These microbes rely on energy sources from organic matters in the soil. Therefore, the activity of these microorganisms does not require coexistence with plants (non-symbiotic). The vital enzyme in atmospheric nitrogen fixation is nitrogenase. There are many factors affect the nitrogenase activity, enzymes which convert N_2 to NH_3 or NH_4^+, the composition of free-living nitrogen-fixer communities including nutrients in soil, soil pollution, plant rhizosphere, plant species and temperature (Zhan & Sun, 2012). This nitrogenase enzyme complex is composed of three subunits Nitrogenase 1 which encoded by the *nif* gene and it is dependent on iron and molybdenum. Nitrogenase 2 which encoded by *vnf* gene and it is dependent on vanadium. Nitrogenase 3 which encoded by *anf* gene and it is dependent on iron. In free-living nitrogen-fixing bacteria, the *nif* genes are responsible for encoding highly conserved subunits (Franche et al., 2009). Due to the high conservation of *nif* gene, it has been used to characterize the genetic diversity of diazotrophs using *16S rRNA* gene (Zehr et al., 2003). The significant factor that affects the nitrogenase enzyme complex activity is the high sensitivity to oxygen. The major problem of free-living nitrogen-fixing bacteria is inhibition of nitrogenase by oxygen, especially aerobic species such as cyanobacteria (blue-green algae) and the free-living aerobic bacteria, such as *Azotobacter* and *Beijerinckia*. The aerobic bacteria have different methods to solve this problem. In *Azotobacter* species, the protection of nitrogenase is maintained a deficient concentration of oxygen in their cells by increased in respiration to decrease the oxygen concentration around nitrogenase. Another mechanism for nitrogenase protection is the production of extracellular polysaccharide and maintains water within the layer to limit oxygen diffusion into the cell (Bertsova et al., 2001). In the case of *Beijerinckia derxii,* it contains two lipoid structures consist of poly-β-hydroxybutyrate (PHB). It can produce polysaccharide slime which are exopolysaccharides that can protect the nitrogenase from oxygen, called protective O_2 barrier (Thuler et al., 2003). Most of the biological nitrogen fixation (BNF) is carried out by diazotrophs in symbiosis with legume plant. But in some conditions, free-living nitrogen fixer in soil may fix the massive amounts of nitrogen (up to 60 kg N ha^{-1} $year^{-1}$) (Burgmann et al., 2004). The studies of free-living nitrogen-fixing bacteria are focused on both aerobic and anaerobic bacteria such as *Azotobacter vinelandii, Klebsiella pneumoniae, Clostridium pasteurianum* and *Rhodobacter capsulatus*. The two main steps of the free-living nitrogen-fixing bacteria are ammonia formation and nitrification. The NH_3 is formed by reducing the atmospheric nitrogen. When the bacteria die, NH_3 from bacteria is released from cells into the soil or surrounding ecosystems and can be converted to nitrates by nitrifying bacteria. Nitrates can be absorbed and beneficial to plants.

Application of endophytes in agriculture

Nowadays, modern agriculture has used pesticides excessively which has caused changes in soil microbial populations (Pampulha & Oliveira, 2006). It may have a direct effect on microbial growth and microbial diversity due to the overall changes in ecological structure. Recently, many endophytic bacteria have been studied for application in the agricultural field. They have the ability to produce plant growth-promoting substances to influence the growth of plants directly such as IAA production (Lee et al., 2004), siderophore and ammonia production, increase phosphate solubilization and nitrogen fixation. The endophytic bacteria can control phytopathogens, insects and nematodes through the production of new compounds and antifungal metabolites (Berg et al., 2005; Hallmann et al.,1998; Azevedo et al., 2000). They stimulate the host plant growth by nitrogen fixation, enhance the solubility of minerals and phytohormones production (Audipudi et al., 2007). From the characters of the endophytic bacteria suggesting that they can be used as biofertilizers. Dhevendaran et al. (2013) reported that *Azotobacter chroococcum, Azotobacter beijerinckii* and *Azotobacter vinelandii* produced IAA, a growth-promoting hormone. The increasing pH stimulated the growth and synthesis of IAA lead to higher growth of the seedling of *Ocimum sanctum*. They suggest that the application of *Azotobacter* and *Azospirillum* species as biofertilizers is a testimony to the impact that IAA has on seed growth. From the IAA production of these bacteria dominates the application in agricultural in the next few years.

Pathogenic bacteria including pathogenic *Salmonella* sp., *Escherichia coli, Vibrio cholerae* and *Pseudomonas aeruginosa* were described as endophytic bacteria. They may be associated with the use of manures contaminated with fecal bacteria (Holden et al., 2009). The importance of agricultural practices for preserving the natural diversity of endophytic bacteria is emphasized that plants or crops may become the hosts for human pathogens and the source of foodborne illness (Brandl, 2006). The best methods for using endophytes in agriculture are not yet known. The most obvious way is adding bacteria into the soil or to soak the seeds in the culture solution. However, the addition of bacteria is often unsuccessful on the field level because of many factors in the environment (O'Callaghan, 2016). Many questions have not been answered about the use of endophytic bacteria as food supplements in the agricultural field. However, if properly managed, they can show the ability to pathogenic control and abiotic stress from climate change, osmotic stress, exposure to heavy metals and xenobiotic molecules (Howden et al., 2007; Johnson et al., 2013).

The endophytes show great potential in biotechnology. They produce substances that beneficial to plants and they can decompose contaminated molecules in the soil such as pesticides, makes them a promising tool for bioremediation. Moreover, the inoculation of endophytes in plants is economical because it increases productivity and utilizes low-cost farming techniques that have a little environmental impact. For the development of efficient endophytes in agricultural, the bacteria must be selected formulated concerning the agricultural field and environmental conditions.

Acknowledgment

I am grateful to Dr.Prateep Krasaesindhu, Agricultural Research Officer (Level 9), for the photo of peanut root in Fig. 2.

References

Arencibia, A.D., Vinagre, F., Estevez, Y., Bernal, A., Perez, J., Cavalcanti, J., & Hemerly, A.S. (2006). *Gluconacetobacter diazotrophicus* elicits a sugarcane defense response against a pathogenic bacteria *Xanthomonas albilineans*. *Plant Signaling and Behavior, 1*(5), 265-273.

Audipudi, A.V., Chakicherla, B.V., & Bhore, S.J. (2017). *Biotechnology for Sustainability.* Malaysia: AIMST University.

Azevedo, J.L., Maccheroni, J.J., Pe-reira, O., & Ara, W.L. (2000). Endophytic microorganisms: A review on insect control and recent advances on tropical plants. *Electronic Journal of Biotechnology, 3,* 40-65.

Babourina, O., Voltchanskii, K., McGann, B., Newman, I., & Rengel, Z. (2007). Nitrate supply affects ammonium transport in canola roots. *Journal of Experimental Botany, 58,* 651–658.

Beijerinck, M.W. (1901). Über oligonitrophile mikroben. *Zentralblatt für Bakteriologie, 7,* 561-582.

Benson, D.R., & Silvester, W.B. (1993). Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. *Microbiological Reviews, 57,* 293–319.

Berg, G., Krechel, A., Ditz, M., Si-kora, R.A., Ulrich, A., & Hallmann, J. (2005). Endophytic and ectophytic potato associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. *FEMS Microbiology Ecology, 51,* 215-229.

Bertsova, Y.U., Bogachev, A.V., & Skulachev, V.P. (2001). Noncoupled NADH: ubiquinone oxidoreductase of *Azotobacter vinelandii* is required for diazotrophic growth at high oxygen concentrations. *Journal of Bacteriology, 183,* 6869-6874.

Brandl M.T. (2006). Fitness of human enteric pathogens on plants and implications for food safety. *Annual Review of Phytopathology, 44,* 367–392.

Burgmann, H., Widmer, F., Von Sigler, W., & Zeyer, J. (2004). New molecular screening tools for analysis of free-living diazotrophs in soil. *Applied and Environmental Microbiology, 70,* 240-247.

Conn, V.M., & Franco, C.M. (2004). Analysis of the endophytic actinobacterial population in the roots of wheat (*Triticum aestivum* L.) by terminal restriction fragment length polymorphism and sequencing of 16S rRNA clones. *Applied Environmental Microbiology, 70*(3), 1787-1794.

Dhevendaran, K., Preetha, G., & Hari, B.N.V. (2013). Studies on nitrogen fixing bacteria and their application on the growth of seedling of *Ocimum sanctum*. *Pharmacognosy Journal, 5*(2), 60-65.

Döbereiner J. (1961). Nitrogen fixing bacteria of the genus *Beijerinckia Drex.* in the rhizosphere of sugarcane. *Plant and Soil, 15*(3), 211-216.

Döbereiner, J., & Pedrosa, O.F. (1987). *Nitrogen-fixing bacteria in non-leguminous crop plants*. Madison: Science Tech Publishers.

Franche, C., Lindstrom, K., & Elmerich, C. (2009). Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant and Soil, 321,* 35-59.

Graham, P.H., & Vance, C.P. (2000). Nitrogen fixation in perspective: an overview of research and extension needs. *Field Crops Research, 65,* 93-106.

Gupta, G., Panwar, J., & Jha, P.N. (2013). Natural occurrence of *Pseudomonas aeruginosa,* a dominant cultivable diazotrophic endophytic bacterium colonizing *Pennisetum glaucum* (L.) R. Br. *Applied Soil Ecology, 64,* 252-261.

Haidar, B., Ferdous, M., Fatema, B., Ferdous, A.S., Islam, M.R., & Khan H. (2018). Population diversity of bacterial endophytes from jute (*Corchorus olitorius*) and evaluation of their potential role as bioinoculants. *Microbiological Research, 208,* 43-53.

Hallmann, J., Quadt-Hallmann, A., Rodriguez-Kabana, R., & Kloepper, J.W. (1998). Interactions between Meloidogyne incognita and endophytic bacteria in cotton and cucumber. *Soil Biology and Biochemistry, 30,* 925-937.

Holden, N., Pritchard, L., & Toth, I. (2009). Colonization out with the colon: plants as an alternative environmental reservoir for human pathogenic enterobacteria. *FEMS Microbiology Reviews, 33*(4), 689-703

Howden, S.M., Soussana, J.F., Tubiello, F.N., Chhetri, N., Dunlop, M., & Meinke, H. (2007). Adapting agriculture to climate change. *Proceedings of the National Academy of Sciences of the United States of America, 104,* 19691-19696.

Igarashi, R.Y., & Seefeldt, L.C. (2003). Nitrogen fixation: the mechanism of the Mo-dependent nitrogenase. *Critical Reviews in Biochemistry and Molecular Biology, 38,* 351-384.

Ji, S.H., Gururani, M.A., & Chun, S.C. (2014). Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbiological Research ,169*(1), 83-98.

Johnson, L.J., de Bonth, A.C., Briggs, L.R., Caradus, J.R., Finch, S.C., Fleetwood, D.J., ... & Tapper, B.A. (2013). The exploitation of epichloae endophytes for agricultural benefit. *Fungal Diversity, 60*(1), 171-188.

Koomnok, C., Teaumroong, N., Benjavan Rerkasem, B., & Lumyong S. (2007). Diazotroph endophytic bacteria in cultivated and wild rice in Thailand. *Science Asia, 33,* 429-435.

Lam, H.M., Coschigano, K.T., Oliveira, I.C., Melo-Oliveira, R., & Coruzzi, G.M. (1996). The molecular-genetics of nitrogen assimilation into amino acids in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology, 47,* 569-593.

Lee, S., Flores-Encarnacion, M., Con-treras-Zentella, M., Garcia-Flores, L., Escamilla, J.E., & Kennedy, C. (2004). Indole-3-acetic acid biosynthesis is deficient in *Gluconacetobacter diazotrophicus* strains with mutations in cytochrome C biogenesis genes. *Journal of Bacteriology, 186,* 5384-5391.

Marag, P.S., & Suman, A. (2018). Growth stage and tissue specific colonization of endophytic bacteria having plant growth promoting traits in hybrid and composite maize (*Zea mays* L.). *Microbiology Research, 214,* 101-113.

Masson-Boivin, C., Giraud, E., Perret, X., & Batut, J. (2009). Establishing nitrogen-fixing symbiosis with legumes: how many recipes. *Trends in Microbiology, 17,* 458-466.

Morrison, C.N., Spatzal, T., & Rees, D.C. (2017). Reversible protonated resting state of the nitrogenase active site. *Journal of the American Chemical Society, 139*(31), 10856-10862.

Muangthong, A., Youpensuk, S., & Rerkasem, B. (2015). Isolation and characterisation of endophytic nitrogen fixing bacteria in sugarcane. *Tropical Life Sciences Research, 26*(1), 41-51.

Mylona, P., Pawlowski, K., & Bisseling, T. (1995). Symbiotic nitrogen fixation. *Plant Cell, 7*(7), 869-885.

O'Callaghan, M. (2016). Microbial inoculation of seed for improved crop performance: issues and opportunities. *Applied Microbiology and Biotechnology, 100,* 5729-5746.

Okasaki, S., Nukui, N., Sugawara, M., & Minamisawa, K. (2004). Rhizobial strategies to enhance symbiotic interactions: rhizobiotoxine and 1-aminocyclopropane-1-carboxylate deaminase. *Microbes and Environments, 19,* 99-111

Pampulha, M.E., & Oliveira, A. (2006). Impact of an herbicide combination of bromoxynil and prosulfuron on soil microorganisms. *Current Microbiology, 53,* 238-243.

Passari, A.K., Mishra, V.K., Leo, V.V., Gupta, V.K., & Singh, B.P. (2016). Phytohormone production endowed with antagonistic potential and plant growth promoting abilities of culturable endophytic bacteria isolated from *Clerodendrum colebrookianum* Walp. *Microbiological Research, 193,* 57-73.

Pedraza, R.O. (2008). Recent advances in nitrogen-fixing acetic acid bacteria. *International Journal of Food Microbiology, 125,* 25-35.

Postgate, J.R. (1982). *The fundamentals of nitrogen fixation* (2nd ed.). New York: Cambridge University Press.

Prakamhang, J., Minamisawa, K., Teamtaisong, K., Boonkerd, N., & Teaumroong, N. (2009). The communities of endophytic diazotrophic bacteria in cultivated rice (*Oryza sativa* L.). *Applied Soil Ecology, 42*(2), 141-149.

Puri, A., Padda, K.P., & Chanway, C.P. (2017, December 20). *Nitrogen-fixation by endophytic bacteria in agricultural crops: Recent advances, nitrogen in agriculture - updates, amanullah and shah fahad.* Retrieved from https://www.intechopen.com/books/nitrogen-in-agriculture-updates/nitrogen-fixation-by-endophytic-bacteria-in-agricultural-crops-recent-advances

Raweekul, W., Wuttitummaporn, S., Sodchuen, W., & Kittiwongwattana, C. (2016). Plant growth promotion by endophytic bacteria isolated from rice (*Oryza sativa*). *Thammasat International Journal of Science and Technology, 21*(1), 6-17.

Ruan, J.Y., Gerendas, J.S., Hardter, R. & Sattelmacher, B. (2007). Effect of nitrogen form and root zone pH on growth and nitrogen uptake of tea (*Camellia sinensis*) plants. *Annals of Botany, 99,* 301–310.

Ruinen, J. (1956). Occurrence of *Beijerinckia* species in the phyllosphere. *Nature, 178* (4501), 220-221.

Santi, C., Bogusz, D., & Franche, C. (2013). Biological nitrogen fixation in non-legume plants. *Annals of Botany, 111,* 743-767.

Somasegaran, P. (1994). *Handbook for rhizobia: methods in legume-rhizobium technology.* New York: Springer-Verlag.

Sprent, J.I. (2007). Evolving ideas of legume evolution and diversity: a taxonomic perspective on the occurrence of nodulation. *New Phytologist, 174,* 11-25.

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

Thuler, D.S., Floh, E.L., Handro, W., & Barbosa, H.R. (2003). *Beijerinckia derxii* releases plant growth regulators and amino acids in synthetic media independent of nitrogenase activity. *Journal of Applied Microbiology, 95*(4), 799-806.

Udvarte, M., & Poole, P.S. (2013). Transport and metabolism in legume-rhizobia symbioses. *Annual Review of Plant Biology, 64,* 781-805.

Zehr, J.P., Jenkins, B.D., Short, S.M., & Steward, G.F. (2003). Nitrogenase gene diversity and microbial community structure: a cross-system comparison. *Applied and Environmental Microbiology, 5,* 539-554.

Zhan, J., & Sun, Q. (2012). Diversity of free-living nitrogen-fixing microorganisms in the rhizosphere and non-rhizosphere of pioneer plants growing on wastelands of copper mine tailings. *Microbiological Research, 167*(3), 157-165.