Biochemistry and Molecular Biology
2021; 6(4): 92-98
http://www.sciencepublishinggroup.com/j/bmb
doi: 10.11648/j.bmb.20210604.12
ISSN: 2575-5064 (Print); ISSN: 2575-5048 (Online)

Nitrogen Fixation Using Symbiotic and Non-Symbiotic Microbes: A Review Article

Mamo Bekele[1, 2, *], Getachew Yilma[3]

[1]Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia
[2]Holeta National Agricultural Biotechnology Research Center, Holeta, Ethiopia
[3]Fogera National Rice Research and Training Center, Fogera, Ethiopia

Email address:
mamob27@gmail.com (M. Bekele), getachewmk@gmail.com (G. Yilma)
[*]Corresponding author

To cite this article:
Mamo Bekele, Getachew Yilma. Nitrogen Fixation Using Symbiotic and Non-Symbiotic Microbes: A Review Article. *Biochemistry and Molecular Biology*. Vol. 6, No. 4, 2021, pp. 92-98. doi: 10.11648/j.bmb.20210604.12

Received: August 12, 2021; **Accepted**: November 26, 2021; **Published**: December 7, 2021

Abstract: Nitrogen (N) is one of the key drivers of global agricultural production and needs 150-200 million tons each year by plants in agricultural systems to produce the world's food, animal feed and industrial products. Hence, to minimize this problem industrially producing nitrogen fertilizer is necessary. However, this industrially produced nitrogen fertilizer affect the world ecosystem through different mechanisms and effective exploitation and utilization of biologically fixed nitrogen in agricultural systems is necessary. This naturally fixed nitrogen minimizes the rate of cost of crop production, urea volatilization and is the sustainable soil fertility maintenance. Despite the importance of biological nitrogen fixation is sustainable and environmentally friend approach, some researches were done on legume crops through exploring variety specific rhizobia species for legume crops and shortage of information on free living nitrogen fixer of bacteria species for cereal crops which will be the future concerned research. This paper review discusses biological nitrogen fixation mechanism symbiotically and non-symbiotically either through free living bacteria or associative with host plant. It also focused on types of bacteria in which fix atmospheric nitrogen in cereal crops and factors affecting biological nitrogen fixation in lesser amount.

Keywords: Bacteroid, Legume, Nifgene, Nodgene Nodule, Nitrogenase, Rhizobia

1. Introduction

Nitrogen is the supreme abundant macronutrient and a principal component of chlorophyll, having the most important pigment needed for photosynthesis, and plays a critical contribution for plant growth and crop production [70]. It has a vital role in the building blocks of life in all living things when bonded with carbon, hydrogen and oxygen. Though the amount of nitrogen in the atmosphere occupy more percentage (80%) [19], its availability is extremely insignificant and do not uptake by plants. Hence, plant use only in the form of ammonia and nitrate [26]. Even the small amount of accessible nitrogen in the soil solution is logically losses by erosion, volatilization, leaching etc leads to limits crop production. To substitute such constraints, continuous application of industrially synthesized chemical nitrogen fertilizer is the common practices across the globes. However, applications of nitrogen fertilizer to soil have negative impact on underground water and environment. It also lessens to the atmosphere among others as nitrogenous gases, notably in form of N_2O, which is an important greenhouse gas lead to global warming [4].

Lightning and the roles of microorganism (biological nitrogen fixation) also the natural mechanism in which unavailable nitrogen nutrient is fixed and changed to available form to replace the lost available nitrogen and nitrogen fertilizer as well [60]. Therefore, the goal of this paper review is consider and recap mechanism of re-establishing naturally depleted nutrient, biological nitrogen fixation and over all process going in. Additionally, it overlooks at factors affecting biological nitrogen fixation and some major microbes associated with cereal crops.

2. Nitrogen Mineralization-immobilization

Nitrogen mineralization is the transformation of organic nitrogen such as crop residue, leaf, weed and etc., to plant available form, inorganic forms of nitrogen usually ammonium (NH_4^+) through microbial degradation of nitrogen rich organic matter. [15]. This process have three steps aminization, ammonification and nitrification. In aminization step, there are hydrolytic decomposition of protein and release of amines and amino acides by heterotrophic microbes such as bacteria like bacillus, pseudomonas in the absence of oxygen whereas during ammonification, their transformation of organic nitrogenous compounds such us amino acids, amides, ammonium compounds nitrates etc into ammonia. This process occurs as a result of hydrolytic and oxidative enzymatic reactions under aerobic condition by heterotrophic microbes. Finally the nitrification process, there are conversion of ammonia to nitrites by the help of Nitrosomonas bacteria and then to nitrate by nitrobacterer which is available for plant uptake. It is an aerobic processs by autotrophic bacteria.

As whole most nitrogen mineralization is done by aerobic microorganisms such as (bacteria, fungi, actinomycetes) and macroorganisms such as earthworms, termites, slugs and snails generally called decomposers. Those decomposers use extracellular enzymes and these enzymes initiate degradation of plant polymers. Microorganisms use proteases, lysozynmes, and nucleases to degrade nitrogen containing molecules.

Nitrogen mineralization can be estimated based on organic matter percentage and adversely affecded by carbon to nitrogen ratio, temperature, aeration, moisture and soil organic matter content. Immobilization is the conversion of inorganic form of nitrogen to organic form of nitrogen and when the organic matter in the soil is high and carbon to nitrogen ration also high as well. The nitrogen immobilization is assumed to be proportional to the net build-up of organic matter in different sites determined by the rates of production and decomposition. The long-term immobilization of nitrogen in organic matter deposited on the soil depends on the soil conditions and litter quality.

3. Nitrogen Fixation

Nitrogen fixation is the transformation of non-reactive N_2 into nitrite, ammonia and nitrates which is suitable and support for plant growth. This nitrogen is use as part of chlorophyll molecules and as part of amino acid to build protein which participate in metabolism and energy storage. Plenty of amino acid is produced by nitrogen fixing bacteria like Azosprillum, azotobacter, rhizobium and mesorhizobium and etc which have close association with plant roots. [25] Hence those nitrogen fixing bacteria (biological nitrogen fixation) add much amount of nitrogen during they die the accumulated biomass releases to soil and the decomposer split plant nitrogenous compounds and release ammonium and ammonia to the soil and increase soil fertility. During this process, symbiotics and asymbiotics are the two methods of biological nitrogen fixation [59]. Free-living soil bacteria are non-symbiotic nitrogen fixations and only associated with root zone of cereal crops and gramineciaous family also in termite habitats. Those nitrogen fixing microorganism could endosymbiont, free living ones or exosymbiont. And symbiotic nitrogen fixing bacteria. Those bacteria survive dwell on/ in or not endophytes versus exophytes. Comparatively, endosybiont are more putative microbes than exosybiont due to there are no competition with other microbes in case of endosybiant on resources and ready to access carbon and water in the rhizosphere and rhizoplane. Therefore, they are most likely very effective in biofertilizer production.

The amount of nitrogen fixed by microorganism in different ecosystem per year is not the same. According to the report of [13], the potential global biological nitrogen fixation (symbiotic and asymbiotic) in natural ecosystems is between 100 and 290 million tones nitrogen per year. In soils under agricultural production, estimates of biologically fixed nitrogen range nearly 33 million tone of nitrogen per year [60]. Other research finding also stated that the amount of nitrogen fixed ranged between 50-70 million tones nitrogen per year [27].

4. Symbiotic Nitrogen Fixation

Symbiotic nitrogen fixing bacteria are represented by a phylogenetically unlike class of alpha- and beta-proteobacteria usually collectively termed rhizobia that have achieved the function of fixing atmospheric nitrogen (N_2) in symbiosis, which inhabit the root nodules of most leguminous crops such as groundnut, soybean and cowpea [42, 53]. Most of the symbiotic species are represented in the alpha-proteobacteria order *Rhizobiales*, which, amongst many others, contain the agriculturally important nitrogen fixing genera of *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Azorhizobium* [47]. Legumes are unique in that they have the ability to form a symbiotic relationship with soil bacteria collectively called "rhizobia". Non-legume tree parasponia also forms nitrogen-fixing nodules with rhizobia, and actinomycete soil bacteria called 'Frankia' form nitrogen-fixing nodules on non-legume plants like Casuarina, Datisca and Alnus) [28].

Process of symbiotic nitrogen fixation, consisting of three parts; (a) the formation of nodules which give the right environment for housing the nitrogen-fixing bacteria; (b), the regulation of symbiotic tissue (*i.e.*, nodule numbers) by internal and external factors, and (c), the actual conversion of atmospheric nitrogen into ammonia by the invading bacteria using the nitrogenase enzyme complex and its associated biochemical machinery [10].

All plant release a significant amount of organic carbon into the soil in the form of cell lysates intact border cells, mucilage's and root exudates [18]. Nutrient exchange between plant and microbes is the driving force of symbiosis between a plant and nitrogen fixing microorganism and the exchange of nutrients between the two partners. Both organisms change their metabolic routines in order to accommodate to each other's need, a process that is monitored and regulated by both partners [48]. The fully compatible symbiosis proceeds from recognition, penetration, and stimulation of host cell division and differentiation of the endosymbiont.

4.1. Nodule Formation and Nitrogen Fixation

Rhizobia is a symbiotic nitrogen fixer, it is present inside the root nodules of leguminous plants. The growth of rhizobia over other microbes is encouraged by the crop root secretes of sybiont such as biotin, amino acids and thiamines. They are called growth stimulating substances. The reactions between polysaccharide (callose) existing on the surface of rhizobial cell and the lectin secreted by the root hairs helps in the recognition of the correct host plant by the specific *Rhizobium* [6].

In biological nitrogen fixation, nodule formation is mandatory through involving multiple interactions between rhizobia and plant host roots. This occurs through the invasion of the rhizobia around the rhizosphere and high number of rhizobia spread around the legume roots and there are huge amount of production of mucopolysaccharides by bacteria to react with component of root hairs for the stimulation the root cell to produce polygalcturonase and infect the rot hairs [35]. Then their existence of the penetration of bacteria to the root through releasing of nod factor and the root become curled [21]. This process called infection thread and this process have the continuation of the wall of the infection threads with the cell wall of the root hairs [45]. After the bacteria inter the root cortex, there become rod bacteroids and initiation of nodule formation and bacteria divided into different mass of cell called nodule [7]. However, the amount of nodule number and the nitrogen fixation among different bacteria is different according to the research finding in Tunisian arid soil [41]. The fixed nitrogen distributed to other plant part through vascular connection with the host for the exchange of nutrients. Flavonoids are another compounds released by root hairs which activate the node gene in the bacteria for the formation of nodule [6, 61].

Nitrogen is very sensitive element no nitrogen fixation occurs in the presence of oxygene and need anaerobic environment. The free-living aerobic microorganisms develop mechanisms to shield nitrogenase from oxygen such as high rates of metabolism, physical barriers etc. In nodules, oxygen is scavenged by leg-haemoglobin. Nodules contain O_2 tied heme protein and as a result nodules appear red or rosy in colour. The bacteroids are the sites of nitrogen fixation. Bacteroids may be distended, rough, star shaped, Y-shaped, branched etc [9]. The protohaem is prosthetic group which is synthesized by the bacteroids, while the making of the protein part includes the plant cell. It supplies O_2 to the respiring symbiotic bacterial cells. It increases the transportation of oxygen at little partial pressure and also offers the defense to nitrogenase against oxygen and motivates ATP production necessary for N_2-fixation. Finally, when the nodule dies, bacteria are released into soil and this also important decomposition of organic matter added to soil from different sources [43].

4.2. Genes Involved in Nodule Formation and Nitrogen Fixation

Gene involved in nodule formation is called *Nod* gene (nodulation gene) whereas nitrogen fixing gene is *nif gene.* Genes involved in nitrogen fixation are nif regulon and whose structures and organization are different among various microorganisms [36]. Nodulation gene i.e., *nod* gene and nitrogen fixing gene i.e., *nif* gene are located in the plasmid which is also called mega plasmid (Sym plasmid) (pR me4 ib) [63, 64] which is adjacent to nod gene. But in some of the free living bacteria, these are localized on the chromosome. The expression of nif gene is repressed by ammonia and other nitrogenous compounds [69]. The *nod* gene is consisted of 4 genes (a) *nodA, (b) nodB, (c) nodC* and (d) *nodD*. *NodA* code for protein of 196 amino acid residues, *nodB* of 217 amino acid residues, *nodC* of 426 amino acid residues and *nodD* of 311 amino acid residue. The *nod* gene is controlled by *nodD* gene. *nodD* proteins act as transcriptional activator of inducible *nod* gene [38].

4.3. Factors Affecting Nodule Formation, Nitrogen Fixation and Regulation Mechanisms

The amount of nitrogen in the soil, the rhizobia strain infecting the legume, stage of legume plant growth, native rhizobia in the soil and the length of growing season affect nodule formation and nitrogen fixation [46, 56]. Nitrogenase (convert dinitrogen to ammonia) is irreversibly inactivated by oxygen; there are specific cells (heterocyst) in photosynthesizing microorganism to protect them. The heterocysts are thick-walled cell inclusions that are impermeable to oxygen; they deliver the anaerobic (oxygen-free) environment essential for the operation of the nitrogen-fixing enzymes [37].

The nitrogenase enzyme is mainly found in *Bacteria* and *Archaea* and predominantly in chemotrophs, phototrophs and heterotrophs [8] in soils, termite guts, lakes, rivers, estuaries, algal mats and sediments and oligotrophic oceans. Nitrogen fertilizers inhibit nodule formation and nitrogen fixation. Availability of molybdenum (Mo) in the soil is essential because the enzyme, nitrogenase, present in root nodules contains molybdenum element [72].

Nitrogen fixation is regulated at the transcriptional level (*ntr* gene) and post translational level (*draT* and *draG*) in response to environmental oxygen and ammonia levels [73]. The *ntrA* is a regulatory gene which allows RNA polymerase to bind at the *nif* promoters and for the initiation of transcription. *NtcA* controls nitrogen fixation in cyanobacteria which is almost different from *ntr* system [71]. Optimum temperature for bacteria is necessary. Interaction of temperature, light and moisture can influence the nitrogen fixation. Soil physiochemical properties such as organic matter; available

Nitrogen and salt are factors affecting nitrogen fixation [5].

5. Non-symbiotic Nitrogen Fixation

The most active representatives of the non-symbiotic nitrogen-fixing bacteria are Azotobacter and Clostridium *(Bac. amylobacter)* groups [22]. From the roots of a native grass (*Saccharum spontaneum* L.) several nitrogen-fixing bacteria were isolated which capable of enhancing shoot growth, root density and yield of rice, corn and sugar cane. The bacteria were found to possess at least 57% of the characteristics of the genus *Azospirillum* [17]. They are associative N_2 fixing bacteria found to be capable of producing growth regulators like gibberellins and cytokinins, which were thought to contribute to the stimulated plant growth [12]. Inoculation of crops with Azospirillum or other diazotrophs often resulted in enhanced plant growth or nitrogen content under environmental conditions [54, 2], improve nutrient assimilation, alter root size [50] and function [20].

They are also called free-living diazotrophs. Both aerobic and anaerobic bacteria are free-living diazotrophs where water, oxygen and nutrients are required in optimum amount, so that, the microorganism can grow. Cyanobacteria grow mainly in the crop fields. The site of nitrogen fixation in the cyanobacteria is the heterocyst because the enzyme (nitrogenase) required for nitrogen fixation acts under anaerobic condition [24]. Some cells in these microorganisms become specialized i.e. have oxygen level reduced. Typically, they fix nitrogen in dark and photosynthesize in light.

6. Types of Free Living N_2 Fixer and Associative Nitrogen Fixation

6.1. Azotobacter

Although free-living, N_2-fixing microorganisms are widely distributed in soils, the quantity of N_2 they fix seldom approaches that of the symbiotic systems [34] whereas, one anticipates a fixation of 100-200 kg of N fixed annually per hectare from a highly effective symbiotic association, one may find only 1-5 kg of N fixed per hectare by the free living microorganisms. It is note that, they are naturally unable for vigorous fixation, but abundant substrates to support their growth and fixation are commonly lacking in the soil.

Azotobacter species (Azotobacter vinelandii and Azotobacter chroococcum) are free-living heterotrophic diazotrophs that depend on an adequate supply of reduced carbon compounds such as sugars for their energy source [32]. In the laboratory, it grows vigorously and fixes N_2 actively when supplied a substrate such as a sugar or an organic acid. Their activity in rice culture can be increased by straw application [30], presumably as a result of microbial breakdown of cellulose into cellobiose and glucose. *Clostridia* are obligatory anaerobic heterotrophs only capable of fixing N_2 in the complete absence of oxygen [31].

6.2. Azospirillum

Azospirillum species are aerobic heterotrophs that grow extensively in the rhizosphere of gramineous plants and fix nitrogen under micro aerobic conditions [62, 31]. The Azospirillum–plant association leads to enhanced development and yield of different host plants [20]. This increase in yield is attributed mainly to an improvement in root development by an increase in water and mineral uptake, and to a lesser extent biological nitrogen fixation [51].

Azospirillum brasilense shows both chemotaxis and chemokinesis in response to temporal gradient of different chemo effectors, thereby increasing the chance of root–bacterial interactions. Phytohormones synthesized by *Azospirillum* influence the host root respiration rate, metabolism and root proliferation and hence improve mineral and water uptake in inoculated plants [51].

Azospirillum brasilense have a greater role in agricultural production of cereal crops such as wheat, maize, sorghum and etc through helping in nutrient uptake NO_3^-, K^+ and H_2 PO_4 which is used in plant growth [3, 57]. They are also boost grain yield of wheat up to 30% (Okon) and increase up to 0.91 mg/plant of nitrogen content of cotton through stimulating plant root growth by inoculating with *Azospirillum brasilense* [2]. Soil applications with Azospirillum can expressively rise cane yield in both plant and ratoon crops in the field [58].

Finally, *Azospirillum brasilense* isolated from wheat roots [1], *Azospirillum lipoferum* from the roots of wheat and maize, [67], *Azospirillum amazonense* from roots and rhizosphere soil of *Gramineae*, [40] and *Azospirillum irakense* from roots and rhizosphere of rice, [33] are the commonly associative nitrogen fixing bacteria in cereal crops.

6.3. Gluconacetobacter Diazotrophicus (Gd)

Gluconacetobacter diazotrophicus is an associative bacteria first discovered within sugarcane plants in Alagoas, Brazil, [11]. According to this research findings, the bacteria was isolated from sugarcane root and stem and have the capacity to grow in acidic level less than three hydrogen ion concentration and an obligate aerobic rod, motile by 1 to 3 lateral flagella. The enzyme called nitrogenase which produced by this bacteria is also inhibited by ammonium partially leads to continue fixing nitrogen inside the plant even when nitrate (and ammonium)-containing fertilizers are applied. It has the ability to excrete almost half of the fixed nitrogen as ammonium, which becomes potentially available to plants; this is the distinctive characteristic of this bacterium [14].

Gluconacetobacter diazotrophicus association with arbuscula mychorriza fungi were evaluated on the basis of root colonization, fresh and drymatter yield, N, P, soluble sugars and photosynthetic pigments in leaves of Sorghum bicolor leads significant increment of fresh weight and dry weight of the *sorghum bicolors* [44]. These bacteria could be wide range of host and isolated from agricultural crops apart from sugarcane such as sweet potato [55], coffee [29], pineapple [66], tea, mango, banana [49], corn [68], carrot, raddish, beet root [39]. Further the plant growth promoting characters such as significant increase the activity of nitrogenase enzyme, production of phytohormone indole acetic acid (IAA) production in the absence of tryptophan, phosphorus and have significant zinc solubilization were assessed [39].

7. Creation of Artificial Symbiosis/Association

There are different biotechnological approach to develop cereal products and other non-legume crops that can fix nitrogen. There are two approaches in these strategies. The first approach is introduction of nitrogen fixation to plants directly through introduction of nitrogenase encoding bacterial *nif* genes into plants [52]. The second approach is engineering of non-legume plants to nodulates and establishes symbiotic nitrogen fixation. However, both approaches have its own challenges; complexity of nitrogenase biosynthesis and sensitivity of nitrogenase to oxygen present. The appropriate solution for these challenges is setting genes/ gene products required for functional nitrogenase biosynthesis and plastids and mitochondria offers potential sub-cellular low oxygen environment to express active nitrogenase in plants [65, 16].

8. Conclusion

Nitrogen is the mainly relevant in our today's life and need special attention to improve our livelihoods. From time to time the amount of available of it in the soil which feed the crops we eat is almost depleted by different factors. This enforces as to replenish the lost nitrogen by diverse mechanism we are capable to do. Enhancement of biological nitrogen fixation through isolating and selecting best elite strain capable of nitrogen fixation is the possible methods for legumes crops as a supplemental. In addition, exploring cereal crops strians with the capability to fix their atmospheric nitrogen and evaluation of endosybiont bacteria is more useful system in compared to rhizospheric bacteria. Therefore, such organisms are more likely to be successful as inoculants and need to isolate from the host. Since legumes don´t need external N supply to grow, the idea of trying to transfer their nitrogen fixing capacity to important non-fixing cereal crops and wood plants, such as wheat or maize, is one of the best solutions for future. Therefore, the researchers from different area should have given great attention towards microbial roles of nitrogen fixation with great significant of non-legume crops with different disciplines and encouraging biological nitrogen fixation. Hence, improved understanding could clue to more workable exploitation of the biodiversity of nitrogen-fixing organisms both in legumes and cereals crops to fix atmospheric nitrogen symbiotically and as-symbiotically. Isolating and evaluating those microbes for the alternative usage of biofertilizer is key future direction in ecosystem management.

References

[1] Baldani, V. L. D., Alvarez, M. D. B., Baldani, J. I., & Döbereiner, J. (1986). Establishment of inoculatedAzospirillum spp. in the rhizosphere and in roots of field grown wheat and sorghum. *Plant and Soil*, *90* (1-3), 35-46.

[2] Bashan, Y., & Levanony, H. (1990). Current status of Azospirillum inoculation technology: Azospirillum as a challenge for agriculture. *Canadian Journal of Microbiology*, *36* (9), 591-608.

[3] Bashan, Y. (1998). Azospirillum plant growth-promoting strains are nonpathogenic on tomato, pepper, cotton, and wheat. *Canadian Journal of Microbiology*, *44* (2), 168-174.

[4] Bashir, M. T., Ali, S. A. L. M. I. A. T. O. N., Ghauri, M. O. I. N. U. D. D. I. N., Adris, A. Z. N. I., & Harun, R. A. Z. I. F. (2013). Impact of excessive nitrogen fertilizers on the environment and associated mitigation strategies. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, *15* (2), 213-221.

[5] Belnap, J. (2001). Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. In *Biological soil crusts: structure, function, and management* (pp. 241-261). Springer, Berlin, Heidelberg.

[6] Bonaldi, K., Gherbi, H., Franche, C., Bastien, G., Fardoux, J., Barker, D.,... & Cartieaux, F. (2010). The Nod factor–independent symbiotic signaling pathway: development of Agrobacterium rhizogenes–mediated transformation for the Legume Aeschynomene indica. *Molecular plant-microbe interactions*, *23* (12), 1537-1544.

[7] Buhian, W. P., & Bensmihen, S. (2018). Mini-review: nod factor regulation of phytohormone signaling and homeostasis during rhizobia-legume symbiosis. *Frontiers in plant science*, *9*, 1247.

[8] Bürgmann, 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* (1), 240-247.

[9] Callaham, D. A., & Torrey, J. G. (1981). The structural basis for infection of root hairs of Trifolium repens by Rhizobium. *Canadian Journal of Botany*, *59* (9), 1647-1664.

[10] Carnahan, J. E., Mortenson, L. E., Mower, H. F., & Castle, J. E. (1960). Nitrogen fixation in cell-free extracts of Clostridium pasteurianum. *Biochimica et biophysica acta*, *44*, 520-535.

[11] Cavalcante, V. A., & Dobereiner, J. (1988). A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane. *Plant and soil*, *108* (1), 23-31.

[12] Chavada Nikul and Girish K. Goswai, (2020). Biofertilizer: sustainable tools for modern agriculture practise. World Journal of Pharmaceutical Research. 9 (1) 1957-1967.

[13] Cleveland, C. C., Townsend, A. R., Schimel, D. S., Fisher, H., Howarth, R. W., Hedin, L. O.,... & Wasson, M. F. (1999). Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. *Global biogeochemical cycles*, *13* (2), 623-645.

[14] Cojho, E. H., Reis, V. M., Schenberg, A. C. G., & Döbereiner, J. (1993). Interactions of Acetobacter diazotrophicus with an amylolytic yeast in nitrogen-free batch culture. *FEMS Microbiology Letters*, *106* (3), 341-346.

[15] Crohn, D. (2004, December). Nitrogen mineralization and its importance in organic waste recycling. In *Proceedings, National Alfalfa Symposium* (pp. 13-5).

[16] Curatti, L., & Rubio, L. M. (2014). Challenges to develop nitrogen-fixing cereals by direct nif-gene transfer. *Plant Science*, *225*, 130-137.

[17] Dommergues, Y., Balandreau, J., & Rinaudo, G. (1973). Non-symbiotic nitrogen fixation in the rhizospheres of rice, maize and different tropical grasses. *Soil Biology and Biochemistry*, *5* (1), 83-89.

[18] Dennis, P. G., Miller, A. J., & Hirsch, P. R. (2010). Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities?. *FEMS microbiology ecology*, *72* (3), 313-327.

[19] Egorov, V. I. (2007). The nitrogen regime and biological fixation of nitrogen in moss communities (the Khibiny Mountains). *Eurasian soil science*, *40* (4), 463-467.

[20] Fallik, E., Sarig, S., & Okon, Y. (1994). Morphology and physiology of plant roots associated with Azospirillum. *Azospirillum/plant associations*, 77-85.

[21] Fisher, R. F., & Long, S. R. (1992). Rhizobium–plant signal exchange. *Nature*, *357* (6380), 655-660.

[22] Franche, C., Lindström, K., & Elmerich, C. (2009). Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant and soil*, *321* (1-2), 35-59.

[23] Geisseler, D., Horwath, W. R., Joergensen, R. G., & Ludwig, B. (2010). Pathways of nitrogen utilization by soil microorganisms–a review. *Soil Biology and Biochemistry*, *42* (12), 2058-2067.

[24] Golden, J. W., Robinson, S. J., & Haselkorn, R. (1985). Rearrangement of nitrogen fixation genes during heterocyst differentiation in the cyanobacterium Anabaena. *Nature*, *314* (6010), 419-423.

[25] González-López, J., Rodelas, B., Pozo, C., Salmerón-López, V., Martínez-Toledo, M. V., & Salmerón, V. (2005). Liberation of amino acids by heterotrophic nitrogen fixing bacteria. *Amino acids*, *28* (4), 363-367.

[26] Hachiya, T., & Sakakibara, H. (2017). Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *Journal of Experimental Botany*, *68* (10), 2501-2512.

[27] Herridge, D. F., Peoples, M. B., & Boddey, R. M. (2008). Global inputs of biological nitrogen fixation in agricultural systems. *Plant and soil*, *311* (1-2), 1-18.

[28] Jensen, E. S., Peoples, M. B., Boddey, R. M., Gresshoff, P. M., Hauggaard-Nielsen, H., Alves, B. J., & Morrison, M. J. (2012). Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. *Agronomy for sustainable development*, *32* (2), 329-364.

[29] Jimenez-Salgado, T., Fuentes-Ramirez, L. E., Tapia-Hernandez, A., Mascarua-Esparza, M. A., Martinez-Romero, E., & Caballero-Mellado, J. (1997). Coffea arabica L., a new host plant for Acetobacter diazotrophicus, and isolation of other nitrogen-fixing acetobacteria. *Applied and Environmental Microbiology*, *63* (9), 3676-3683.

[30] Kanungo, P. K., Panda, D., Adhya, T. K., Ramakrishnan, B., & Rao, V. R. (1997). Nitrogenase Activity and Nitrogen-Fixing Bacteria Associated with Rhizosphere of Rice Cultivars with Varying N Absorption Efficiency. *Journal of the Science of Food and Agriculture*, *73* (4), 485-488.

[31] Kennedy, I. R., Choudhury, A. T. M. A., & Kecskés, M. L. (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited?. *Soil Biology and Biochemistry*, *36* (8), 1229-1244.

[32] Kennedy, I. R., & Tchan, Y. T. (1992). Biological nitrogen fixation in non-leguminous field crops: Recent advances. *Plant and Soil*, *141* (1-2), 93-118.

[33] Khammas, K. M., Ageron, E., Grimont, P. A. D., & Kaiser, P. (1989). Azospirillum irakense sp. nov., a nitrogen-fixing bacterium associated with rice roots and. *Research in microbiology*, *140* (8), 679-693.

[34] Knowles, R. (1983). Free-living dinitrogen-fixing bacteria. *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*, *9*, 1071-1092.

[35] Kumarı, S., & Sınha, R. P. (2011). Symbiotic and asymbiotic N_2 fixation. *Advances in Life Sciences*, 133-148.

[36] Laranjo, M., Alexandre, A., & Oliveira, S. (2014). Legume growth-promoting rhizobia: an overview on the Mesorhizobium genus. *Microbiological research*, *169* (1), 2-17.

[37] L'taief, B., Sifi, B., Zaman-Allah, M., Drevon, J. J., & Lachaâl, M. (2007). Effect of salinity on root-nodule conductance to the oxygen diffusion in the Cicer arietinum–Mesorhizobium ciceri symbiosis. *Journal of plant physiology*, *164* (8), 1028-1036.

[38] Long, S. R. (2001). Genes and signals in the Rhizobium-legume symbiosis. *Plant physiology*, *125* (1), 69-72.

[39] Madhaiyan, M., Saravanan, V. S., Jovi, D. B. S. S., Lee, H., Thenmozhi, R., Hari, K., & Sa, T. (2004). Occurrence of Gluconacetobacter diazotrophicus in tropical and subtropical plants of Western Ghats, India. *Microbiological Research*, *159* (3), 233-243.

[40] Magalhaes, F. M., Baldani, J. I., Souto, S. M., Kuykendall, J. R., & Dobereiner, J. (1983). New acid-tolerant Azospirillum species. *Anais-Academia Brasileira de Ciencias*.

[41] Mahdhi, M., De Lajudie, P., & Mars, M. (2008). Phylogenetic and symbiotic characterization of rhizobial bacteria nodulating Argyrolobium uniflorum in Tunisian arid soils. *Canadian journal of microbiology*, *54* (3), 209-217.

[42] Martínez-Romero, E. (2009). Coevolution in Rhizobium-legume symbiosis?. *DNA and cell biology*, *28* (8), 361-370.

[43] Meena, R. S., Das, A., Yadav, G. S., & Lal, R. (Eds.). (2018). *Legumes for Soil Health and Sustainable Management*. Springer.

[44] Meenakshisundaram, M., & Santhaguru, K. (2010). Isolation and nitrogen fixing efficiency of a novel endophytic diazotroph Gluconacetobacter diazotrophicus associated with Saccharum officinarum from Southern districts of Tamilnadu. *International Journal of Biological and Medical Research*, *1*, 298-300.

[45] McAdam, E. L., Reid, J. B., & Foo, E. (2018). Gibberellins promote nodule organogenesis but inhibit the infection stages of nodulation. *Journal of experimental botany*, *69* (8), 2117-2130.

[46] Mohammadi, K., Sohrabi, Y., Heidari, G., Khalesro, S., & Majidi, M. (2012). Effective factors on biological nitrogen fixation. *African Journal of Agricultural Research*, *7* (12), 1782-1788.

[47] Mousavi, S. A., Willems, A., Nesme, X., de Lajudie, P., & Lindström, K. (2015). Revised phylogeny of Rhizobiaceae: proposal of the delineation of Pararhizobium gen. nov., and 13 new species combinations. *Systematic and applied microbiology*, *38* (2), 84-90.

[48] Mus, F., Crook, M. B., Garcia, K., Costas, A. G., Geddes, B. A., Kouri, E. D.,... & Udvardi, M. K. (2016). Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Applied and environmental microbiology*, *82* (13), 3698-3710.

[49] Muthukumarasamy, R., Revathi, G., Seshadri, S., & Lakshminarasimhan, C. (2002). Gluconacetobacter diazotrophicus (syn. Acetobacter diazotrophicus), a promising diazotrophic endophyte in tropics. *Current Science*, 137-145.

[50] Okon, Y., & Kapulnik, Y. (1986). Development and function ofAzospirillum-inoculated roots. *Plant and soil*, *90* (1-3), 3-16.

[51] Okon, Y., & Itzigsohn, R. (1995). The development of Azospirillum as a commercial inoculant for improving crop yields. *Biotechnology advances*, *13* (3), 415-424.

[52] Oldroyd, G. E., & Dixon, R. (2014). Biotechnological solutions to the nitrogen problem. *Current opinion in biotechnology*, *26*, 19-24.

[53] Oldroyd, G. E., Murray, J. D., Poole, P. S., & Downie, J. A. (2011). The rules of engagement in the legume-rhizobial symbiosis. *Annual review of genetics*, *45*, 119-144.

[54] Patriquin, D. G., Döbereiner, J., & Jain, D. K. (1983). Sites and processes of association between diazotrophs and grasses. *Canadian Journal of Microbiology*, *29* (8), 900-915.

[55] Paula, M. D., Reis, V. M., & Döbereiner, J. (1991). Interactions of Glomus clarum with Acetobacter diazotrophicus in infection of sweet potato (Ipomoea batatas), sugarcane (Saccharum spp.), and sweet sorghum (Sorghum vulgare). *Biology and Fertility of Soils*, *11* (2), 111-115.

[56] Quesada, A., Leganés, F., & Fernández-Valiente, E. (1997). Environmental factors controlling N 2 fixation in Mediterranean rice fields. *Microbial ecology*, *34* (1), 39-48.

[57] Saubidet, M. I., Fatta, N., & Barneix, A. J. (2002). The effect of inoculation with Azospirillum brasilense on growth and nitrogen utilization by wheat plants. *Plant and soil*, *245* (2), 215-222.

[58] Shankariah, C., & Hunsigi, G. (2001, September). Field responses of sugarcane to associative N_2 fixers and P solubilisers. In *International society of sugar cane technologists congress* (Vol. 24, pp. 40-45).

[59] Simon, T. (2003). Utilization of the biological nitrogen fixation for soil evaluation. *Plant, Soil and Environment-UZPI (Czech Republic)*.

[60] Smil, V. (1999). Nitrogen in crop production: An account of global flows. *Global biogeochemical cycles*, *13* (2), 647-662.

[61] Sprent, J. I., & Sprent, P. (1990). Nitrogen fixing organisms: Pure and applied aspects. *Nitrogen fixing organisms: pure and applied aspects.*

[62] Rogel, M. A., Ormeno-Orrillo, E., & Romero, E. M. (2011). Symbiovars in rhizobia reflect bacterial adaptation to legumes. *Systematic and Applied Microbiology*, *34* (2), 96-104.

[63] Roper, M. M., & Ladha, J. K. (1995). Biological N 2 fixation by heterotrophic and phototrophic bacteria in association with straw. In *Management of Biological Nitrogen Fixation for the Development of More Productive and Sustainable Agricultural Systems* (pp. 211-224). Springer, Dordrecht.

[64] Rosenberg, C., Boistard, P., Dénarié, J., & Casse-Delbart, F. (1981). Genes controlling early and late functions in symbiosis are located on a megaplasmid in Rhizobium meliloti. *Molecular and General Genetics MGG*, *184* (2), 326-333.

[65] Rubio, L. M., & Ludden, P. W. (2008). Biosynthesis of the iron-molybdenum cofactor of nitrogenase. *Annual review of microbiology*, *62*.

[66] Tapia-Hernández, A., Bustillos-Cristales, M. R., Jiménez-Salgado, T., Caballero-Mellado, J., & Fuentes-Ramirez, L. E. (2000). Natural endophytic occurrence of Acetobacter diazotrophicus in pineapple plants. *Microbial Ecology*, *39* (1), 49-55.

[67] Tarrand, J. J., Krieg, N. R., & Döbereiner, J. (1978). A taxonomic study of the Spirillum lipoferum group, with descriptions of a new genus, Azospirillum gen. nov. and two species, Azospirillum lipoferum (Beijerinck) comb. nov. and Azospirillum brasilense sp. nov. *Canadian journal of microbiology*, *24* (8), 967-980.

[68] Tian, G., Pauls, P., Dong, Z., Reid, L. M., & Tian, L. (2009). Colonization of the nitrogen-fixing bacterium Gluconacetobacter diazotrophicus in a large number of Canadian corn plants. *Canadian Journal of Plant Science*, *89* (6), 1009-1016.

[69] Vintila, S., & El-Shehawy, R. (2007). Ammonium ions inhibit nitrogen fixation but do not affect heterocyst frequency in the bloom-forming cyanobacterium Nodularia spumigena strain AV1. *Microbiology*, *153* (11), 3704-3712.

[70] Wagner, S. C. (2011). Biological Nitrogen Fixation. Nature Education Knowledge. 3, 15.

[71] Vega-Palas, M. A., Flores, E., & Herrero, A. (1992). NtcA, a global nitrogen regulator from the cyanobacterium Synechococcus that belongs to the Crp family of bacterial regulators. *Molecular microbiology*, *6* (13), 1853-1859.

[72] Vlassak, K. M., Vanderleyden, J., & Graham, P. H. (1997). Factors influencing nodule occupancy by inoculant rhizobia. *Critical Reviews in Plant Sciences*, *16* (2), 163-229.

[73] Yan, Y., Ping, S., Peng, J., Han, Y., Li, L., Yang, J., & Li, D. (2010). Global transcriptional analysis of nitrogen fixation and ammonium repression in root-associated Pseudomonas stutzeri A1501. *BMC genomics*, *11* (1), 11.