UNIT-II

BIOFERTILIZERS

biofertilizer (also **bio-fertilizer**) is a substance which contains living micro-organisms which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant.[1] Biofertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. The microorganisms in biofertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of biofertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Biofertilizers can be expected to reduce the use of synthetic fertilizers and pesticides, but they are not yet able to replace their use. Since they play several roles, a preferred scientific term for such beneficial bacteria is "plant-growth promoting rhizobacteria" (PGPR).

Biofertilizers today

Biofertilizers provide "eco-friendly" organic agro-input. Biofertilizers such as *Rhizobium*, *Azotobacter*, *Azospirilium* and blue green algae (BGA) have been in use a long time. *Rhizobium* inoculant is used for leguminous crops. *Azotobacter* can be used with crops like wheat, maize, mustard, cotton, potato and other vegetable crops. *Azospirillum* inoculations are recommended mainly for sorghum, millets, maize, sugarcane and wheat. Blue green algae belonging to a general cyanobacteria genus, *Nostoc* or *Anabaena* or *Tolypothrix* or *Aulosira*, fix atmospheric nitrogen and are used as inoculations for paddy crop grown both under upland and low-land conditions. *Anabaena* in association with water fern *Azolla* contributes nitrogen up to 60 kg/ha/season and also enriches soils with organic matter.[2][3]seaweeds are rich in various types of mineral elements (potassium, phosphorus, trace elements etc) hence they are extensively used as manure by people of coastal districts. Seaweed - manure also helps in breaking down clays. Fucus is used by Irish people as manure on a large scale. In tropical countries bottom mud of dried up ponds which contain abundant blue green algae is regularly used as manure in fields. The mixture of seaweeds and blue green algae may serve as ideal fertilizer.

Phosphate-solubilizing bacteria

Other types of bacteria, so-called phosphate-solubilizing bacteria, such as *Pantoeaagglomerans* strain P5 or *Pseudomonas putida* strain P13,[4] are able to solubilize the insoluble phosphate from organic and inorganic phosphate sources.[5] In fact, due to immobilization of phosphate by mineral ions such as Fe, Al and Ca or organic acids, the rate of available phosphate (P_i) in soil is well below plant needs. In addition, chemical P_i fertilizers are also immobilized in the soil, immediately, so that less than 20 percent of added fertilizer is absorbed by plants. Therefore, reduction in P_i resources, on one hand, and environmental pollutions resulting from both production and applications of chemical P_i fertilizer, on the other hand, have already demanded the use of phosphate-solubilizing bacteria or phosphate biofertilizers.

Benefits

1. Biofertilizers are means of fixing the nutrient availability in the soil. Generally Nitrogen deficiencies.
2. Since a bio-fertilizer is technically living, it can symbiotically associate with plant roots. Involved microorganisms could readily and safely convert complex organic material into simple compounds, so that they are easily taken up by the plants. Microorganism function is in long duration, causing improvement of the soil fertility. It maintains the natural habitat of the soil. It increases crop yield by 20-30%, replaces chemical nitrogen and phosphorus by 30%, and stimulates plant growth. It can also provide protection against drought and some soil-borne diseases.
3. It has also been shown that to produce a larger quantity of crops, biofertilizers with the ability of nitrogen fixation and phosphorus solubilizing would lead to the greatest possible effect.[6]
4. They advance shoot and root growth of many crops versus control groups.[7] This can be important when implementing new seed growth.
5. Biofertilizers also promote healthy soil, leading to greater farming sustainability.

Groups of biofertilizers

1. *Azolla-Anabena* symbiosis: Azolla is a small, eukaryotic, aquatic fern having global distribution. Prokaryotic blue green algae Anabena azolla resides in its leaves as a symbiont. Azolla is an alternative nitrogen source. This association has gained wide interest because of its potential use as an alternative to chemical fertilizers.[*citation needed*]

2. *Rhizobium*: Symbiotic nitrogen fixation by *Rhizobium* with legumes contribute substantially to total nitrogen fixation. *Rhizobium* inoculation is a well-known agronomic practice to ensure adequate nitrogen.[8][9]
3. *Streptomyces grisoflavus*[10]
4. Unigrow (UniGrow): a commercial bio fertilizer that is currently in use. It is made with a by-product of palm oil production and it contains a microbial element[11] It has been shown to have promising results in studies.[12]

Rhizobia are diazotrophic bacteria that fix nitrogen after becoming established inside the root nodules of legumes (Fabaceae). To express genes for nitrogen fixation, rhizobia require a plant host; they cannot independently fix nitrogen.[1] In general, they are gram negative, motile, non-sporulating rods.

Rhizobia are a "group of soil bacteria that infect the roots of legumes to form root nodules".[2] Rhizobia are found in the soil and after infection, produce nodules in the legume where they fix nitrogen gas (N_2) from the atmosphere turning it into a more readily useful form of nitrogen. From here, the nitrogen is exported from the nodules and used for growth in the legume. Once the legume dies, the nodule breaks down and releases the rhizobia back into the soil where they can live individually or reinfect a new legume host.

Importance in agriculture

Rhizobia nodules on *Vigna unguiculata*

Although much of the nitrogen is removed when protein-rich grain or hay is harvested, significant amounts can remain in the soil for future crops. This is especially important when nitrogen fertilizer is not used, as in organic rotation schemes or some less-industrialized countries.[7] Nitrogen is the most commonly deficient nutrient in many soils around the world and it is the most commonly supplied plant nutrient. Supply of nitrogen through fertilizers has severe environmental concerns.

Specific strains of rhizobia are required to make functional nodules on the roots able to fix the N_2.[8] Having this specific rhizobia present is beneficial to the legume, as the N_2 fixation can increase crop yield.[9] Inoculation with rhizobia tends to increase yield.[10]

Legume inoculation has been an agriculture practice for many years and has continuously improved over time.[9] 12–20 million hectares of soybeans are inoculated annually. The technology to produce these inoculants are microbial fermenters. An ideal inoculant includes some of the following aspects; maximum efficacy, ease of use, compatibility, high rhizobial concentration, long shelf-life, usefulness under varying field conditions, and survivability.[9][11][12]

These inoculants may foster success in legume cultivation.[13] As a result of the nodulation process, after the harvest of the crop there are higher levels of soil nitrate, which can then be used by the next crop.

Symbiotic relationship

Rhizobia are unique in that they are the only nitrogen-fixing bacteria living in a symbiotic relationship with legumes. Common crop and forage legumes are peas, beans, clover, and soy.

Nature of the mutualism

The legume–rhizobium symbiosis is a classic example of mutualism—rhizobia supply ammonia or amino acids to the plant and in return receive organic acids (principally as the dicarboxylic acids malate and succinate) as a carbon and energy source. However, because several unrelated strains infect each individual plant, a classic tragedy of the commons scenario presents itself. Cheater strains may hoard plant resources such as polyhydroxybutyrate for the benefit of their own reproduction without fixing an appreciable amount of nitrogen.[14] Given the costs involved in nodulation and the opportunity for rhizobia to cheat, it may be surprising that this symbiosis should exist at all.

Infection and signal exchange

The formation of the symbiotic relationship involves a signal exchange between both partners that leads to mutual recognition and development of symbiotic structures. The most well understood mechanism for the establishment of this symbiosis is through intracellular infection. Rhizobia are free living in the soil until they are able to sense flavonoids, derivatives of 2-phenyl-1.4-benzopyrone, which are secreted by the roots of their host plant triggering the accumulation of a large population of cells and eventually attachment to root hairs.[15][16] These flavonoids then promote the DNA binding activity of NodD which belongs to the LysR family of transcriptional regulators and triggers the secretion of nod factors after the bacteria have entered the root hair.[16] Nod factors trigger a series of complex developmental changes inside the root hair, beginning with root hair curling and followed by the formation of the infection thread, a cellulose lined tube that the bacteria use to travel down through the root hair into the root cells.[17] The bacteria then infect several other adjacent root cells. This is followed by continuous cell proliferation resulting in the formation of the root nodule.[15] A second mechanism, used especially by rhizobia which infect aquatic hosts, is called crack entry. In this case, no root hair deformation is observed. Instead the bacteria penetrate between cells, through cracks produced by lateral root emergence.[18]

Inside the nodule, the bacteria differentiate morphologically into bacteroids and fix atmospheric nitrogen into ammonium, using the enzyme nitrogenase. Ammonium is then converted into amino acids like glutamine and asparagine before it is exported to the plant.[15] In return, the plant supplies the bacteria with carbohydrates in the form of organic acids.[15] The plant also provides the bacteroid oxygen for cellular respiration, tightly bound by leghaemoglobins, plant proteins similar to human hemoglobins. This process keeps the nodule oxygen poor in order to prevent the inhibition of nitrogenase activity.[15]

Recently, a *Bradyrhizobium* strain was discovered to form nodules in *Aeschynomene* without producing nod factors, suggesting the existence of alternative communication signals other than nod factors, possibly involving the secretion of the plant hormone cytokinin.[15][19]

It has been observed that root nodules can be formed spontaneously in *Medicago* without the presence of rhizobia.[20] This implies that the development of the nodule is controlled entirely by the plant and simply triggered by the secretion of nod factors.

Evolutionary hypotheses

The sanctions hypothesis

There are two main hypotheses for the mechanism that maintains legume-rhizobium symbiosis (though both may occur in nature). The **sanctions hypothesis** theorizes that legumes cannot recognize the more parasitic or less nitrogen fixing rhizobia, and must counter the parasitism by post-infection legume sanctions. In response to underperforming rhizobia, legume hosts can respond by imposing sanctions of varying severity to their nodules.[21] These sanctions include, but are not limited to reduction of nodule growth, early nodule death, decreased carbon supply to nodules, or reduced oxygen supply to nodules that fix less nitrogen. Within a nodule, some of the bacteria differentiate into nitrogen fixing bacteroids, which have been found to be unable to reproduce.[22] Therefore, with the development of a symbiotic relationship, if the host sanctions hypothesis is correct, the host sanctions must act toward whole nodules rather than individual bacteria because individual targeting sanctions would prevent any reproducing rhizobia from proliferating over time. This ability to reinforce a mutual relationship with host sanctions pushes the relationship toward a mutualism rather than a parasitism and is likely a contributing factor to why the symbiosis exists.

However, other studies have found no evidence of plant sanctions.[23]

The partner choice hypothesis

The **partner choice hypothesis** proposes that the plant uses prenodulation signals from the rhizobia to decide whether to allow nodulation, and chooses only noncheating rhizobia. There is evidence for sanctions in soybean plants, which reduce rhizobium reproduction (perhaps by limiting oxygen supply) in nodules that fix less nitrogen.[24] Likewise, wild lupine plants allocate fewer resources to nodules containing less-beneficial rhizobia, limiting rhizobial reproduction inside.[25] This is consistent with the definition of sanctions, although called "partner choice" by the authors. Some studies support the partner choice hypothesis.[26] While both mechanisms no doubt contribute significantly to maintaining rhizobial cooperation, they do not in themselves fully explain the persistence of the mutualism. The partner choice hypothesis is not exclusive from the host sanctions hypothesis, as it is apparent that both of them are prevalent in the symbiotic relationship.[27]

Evolutionary history

The symbiosis between nitrogen fixing rhizobia and the legume family has emerged and evolved over the past 66 million years.[28][29] Although evolution tends to swing toward one species taking advantage of another in the form of noncooperation in the selfish-gene model, management of such symbiosis allows for the continuation of cooperation.[30] When the relative fitness of both species is increased, natural selection will favor the symbiosis.

To understand the evolutionary history of this symbiosis, it is helpful to compare the rhizobia-legume symbiosis to a more ancient symbiotic relationship, such as that between endomycorrhizae fungi and land plants, which dates back to almost 460 million years ago.[31]

Endomycorrhizal symbiosis can provide many insights into rhizobia symbiosis because recent genetic studies have suggested that rhizobia co-opted the signaling pathways from the more ancient endomycorrhizal symbiosis.[32] Bacteria secrete Nod factors and endomycorrhizae secrete Myc-LCOs. Upon recognition of the Nod factor/Myc-LCO, the plant proceeds to induce a variety of intracellular responses to prepare for the symbiosis.[33]

It is likely that rhizobia co-opted the features already in place for endomycorrhizal symbiosis, because there are many shared or similar genes involved in the two processes. For example, the plant recognition gene, SYMRK (symbiosis receptor-like kinase) is involved in the perception of both the rhizobial Nod factors as well as the endomycorrhizal Myc-LCOs.[34] The shared similar processes would have greatly facilitated the evolution of rhizobial symbiosis, because not all the symbiotic mechanisms would have needed to develop. Instead the rhizobia simply needed to evolve mechanisms to take advantage of the symbiotic signaling processes already in place from endomycorrhizal symbiosis.

NIF GENES

The ***nif* genes** are genes encoding enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen available to living organisms. The primary enzyme encoded by the *nif* genes is the nitrogenase complex which is in charge of converting atmospheric nitrogen (N_2) to other nitrogen forms such as ammonia which the organism can use for various purposes. Besides the nitrogenase enzyme, the *nif* genes also encode a number of regulatory proteins involved in nitrogen fixation. The *nif* genes are found in both free-living nitrogen-fixing bacteria and in symbiotic bacteria associated with various plants. The expression of the *nif* genes is induced as a response to low concentrations of fixed nitrogen and oxygen concentrations (the low oxygen concentrations are actively maintained in the root environment of host plants). The first Rhizobium genes for nitrogen fixation (nif) and for nodulation (nod) were cloned in the early 1980s by Gary Ruvkun and Sharon R. Long in Frederick M. Ausubel's laboratory.[1]

Regulation

In most bacteria, regulation of *nif* genes transcription is done by the nitrogen sensitive NifA protein. When there isn't enough fixed nitrogen available for the organism's use, NtrC triggers NifA expression, and NifA activates the rest of the *nif* genes. If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein is activated: NifL. NifL inhibits NifA activity resulting in the inhibition of nitrogenase formation. NifL is regulated by the products of *glnD* and *glnK*. The *nif* genes can be found on bacterial chromosomes, but in symbiotic bacteria they are often found on plasmids or symbiosis islands with other genes related to nitrogen fixation (such as the *nod* genes).

Examples in nature

The expression and regulation of *nif* genes, while sharing common features in all or most of the nitrogen-fixing organisms in nature, have distinct characters and qualities that differ from one diazotroph to another. Examples of *nif* gene structure and regulation in different diazotrophs include:

Klebsiella pneumoniae—a free-living anaerobic nitrogen-fixing bacterium. It contains a total of 20 *nif* genes located on the chromosome in a 24-Kb region. *nifH*, *nifD*, and *nifK* encode the nitrogenase subunits, while *nifE*, *nifN*, *nifU*, *nifS*, *nifV*, *nifW*, *nifX*, *nifB*, and *nifQ* encode proteins involved the assembly and incorporation of iron and molybdenum atoms into the nitrogenase subunits. *nifF* and *nifJ* encode proteins related to electron transfer taking place in the reduction process and *nifA* and *nifL* are regulatory proteins in charge of regulating the expression of the other *nif* genes.[2][3]

Rhodospirillum rubrum—a free-living anaerobic photosynthetic bacterium which, in addition to the transcriptional controls described above, regulates expression of the *nif* genes also in a metabolic way through a reversible ADP-ribosylation of a specific arginine residue in the nitrogenase complex. The ribosylation takes place when reduced nitrogen is present and it causes a barrier in the electron transfer flow and thereby inactivates nitrogenase activity. The enzymes catalyzing the ribosylation are called DraG and DraT.[3][4]

Rhodobactercapsulatus—a free-living anaerobic phototroph containing a transcriptional *nif* gene regulatory system. *R. capsulatus* regulates *nif* gene expression through *nifA* in the same manner described before, but it uses a different *nifA* activator which initiates the NtrC. NtrC activates a different expression of *nifA* and the other *nif* genes.[3][4]

Rhizobium spp.—Gram-negative, symbiotic nitrogen fixing bacteria that usually form a symbiotic relationship with legume species. In some rhizobia, the *nif* genes are located on plasmids called 'sym plasmids' (sym = symbiosis) which contain genes related to nitrogen fixation and metabolism, while the chromosomes contain most of the housekeeping genes of the bacteria. Regulation of the *nif* genes is at the transcriptional level and is dependent on colonization of the plant host.[3][4]

Biofertilizers contain microorganisms that are able to activate a biological process which stimulates the development of plants and ensures healthy growth. These microorganisms do not function only as a fertilizer. They transform the inaccessible forms of soil elements into ones accessible to plants. Although they are called fertilizers, they do not contain all nutrients that may be added directly into the soil to increase soil fertility. On the contrary, microorganisms slowly and reliably improve the soil stability and phytosanitation. The difference between biofertilizes and composts lies in the amount of microorganisms contained in them. Biofertilizes can comprise only a specific strain of microorganism, which is intended for a specific activity in the soil. These microorganisms are classified into three main groups: nitrogen-fixing, phosphate-transforming and cellulose-degrading microorganisms. They help to fix atmospheric nitrogen and to convert the phosphorus into a form usable to plants.

Microorganisms also help plants to produce hormones, vitamins and amino acids that are of substantial importance for building resistance to pathogens. Almost all crops need different types of biofertilizers depending on their needs. The various types of biofertilizers which help plants grow at different phases of growth can be grouped into four categories:

- N-fixing biofertilizers: These include the bacteria *Rhizobium*, *Azotobacter*, *Azospirillum, Clostridium* and *Acetobacter* among others; blue-green algae (BGA), or cyanobacteria, and the fern *Azolla* (which works in symbiosis with BGA).
- P-solubilizing/mobilizing biofertilizers: These include phosphate-solubilizing bacteria (PSB) and phosphate-solubilizing microorganisms (PSMs) like *Bacillus, Pseudomonas* and *Aspergillus. Mycorrhizae* are nutrient-mobilizing fungi, also known as vesicular-arbuscular mycorrhizae, or VA-mycorrhizae or VAM.
- Plant-growth-promoting rhizobacteria (PGPR): Mainly represented by species of *Pseudomonas*. These bacteria do not provide plant nutrients but they enhance plant growth and performance.
- Composting accelerators: cellulolytic (*Trichoderma*) and lignolytic (*Humicola*) fungal species and different Gram-positive and Gram-negative bacteria.

2.1. *Nitrogen-fixing biofertilizers*

Nitrogen is the most limiting nutritional factor for plant growth. Suitable nitrogen application to growing plants has a favourable enhancing effect on growth, yield and quality. Since nitrogen is the main element in the composition of amino acids, which are required for the synthesis of proteins and other related compounds, it plays a role in almost all plant metabolic processes. Nitrogen is also an integral part of the chlorophyll molecule responsible for plant photosynthesis. Symptoms of nitrogen deficiency generally appear on the bottom leaves first; the lower leaves on the tips turn brown, usually disintegrate, and fall off. However, the excessive use of nitrogen fertilizers increases the total costs of crop production, pollutes the agro-ecosystem and enhances the deterioration of soil fertility. Therefore, it became essential for researchers to develop and adopt a strategy of supplementing or substituting inorganic nitrogen with organic sources, especially ones of microbial origin. Nitrogen-fixing biofertilizers were the ones majorly utilized in the industry in 2012, accounting for over 78% of the global demand. These biofertilizers are mainly used for crop yield improvement and involve several potential benefits in environmental application, in addition to their agricultural usefulness. Furthermore, increasing consumption of leguminous and non-leguminous plant products is also expected to augment the demand for nitrogen fixing biofertilizers over the forecast period.

Nitrogen biofertilizers help agriculturists to determine the nitrogen level in the soil. The type of crops also determines the level of nitrogen. Some crops need more nitrogen for their growth, while others need fewer amounts. The type of soil is an important factor which determines which type of biofertilizers is needed for a crop.

Though the atmospheres contain 79% N_2, eukaryotes cannot utilize it directly. Atmospheric N_2 must be first reduced to nitrogen compounds that can be assimilated by plants (either NH_{4+} or NO_{3-}). This process is called biological nitrogen fixation (BNF) and is exclusively carried out by prokaryotes (bacteria and cyanobacteria) (Fig.1).

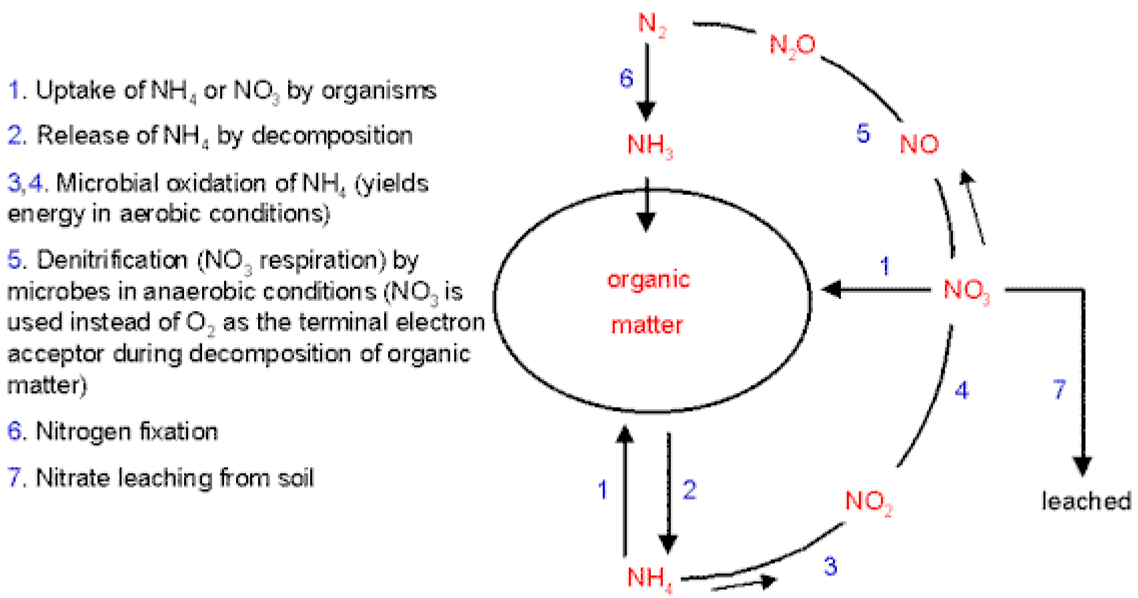

Fig. 1 Nitrogen cycle in nature

The diagram above shows an overview of the nitrogen cycle in soil or aquatic environments. At any time, a large proportion of the total fixed nitrogen will be locked up in the biomass or in the dead remains of organisms (shown collectively as "organic matter"). So, the only nitrogen available to support new growth will be that supplied by nitrogen fixation from the atmosphere (pathway 6 in the diagram) or by the release of ammonium or simple organic nitrogen compounds through the decomposition of organic matter (pathway 2).

Biological nitrogen fixation was discovered by the Dutch microbiologist MartinusBeijerinck. It accounts for 60% of the total nitrogen fixation. The microorganisms that fix nitrogen are called diazotrophs.

In this way, they increase the soil nitrogen level and, respectively, the soil fertility. Biological nitrogen fixation is catalyzed by a microbial multimeric enzyme complex, nitrogenase. The nitrogenase complex exists in all diazotrophs. It consists of two conserved proteins: an iron (Fe)-containing dinitrogenase reductase (Fe protein) encoded by the *nifH* gene and a molybdenum iron (Mo Fe) dinitrogenase (or Mo Fe protein), which is encoded by the *nifDK* genes (Matthew et al., 2008). The reactions occur while N_2 is bound to the nitrogenase enzyme complex. The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N_2, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin), HN=NH is reduced to $H_2N\text{-}NH_2$, and this in turn is reduced to $2NH_3$. Depending on the type of microorganism, reduced ferredoxin, which supplies electrons for this process, is generated by photosynthesis, respiration or fermentation. There is a remarkable degree of functional conservation between the nitrogenase proteins of all nitrogen-fixing bacteria. The Fe protein and the Mo-Fe protein have been isolated from many of these bacteria, and nitrogen fixation can be shown to occur in cell-free systems in the laboratory when the Fe protein of one species is mixed with the Mo-Fe protein of another bacterium, even if the species are very distantly related. The nitrogenase is irreversibly inhibited by molecular oxygen and reactive oxygen species, because the oxygen reacts with the iron component of the proteins. Although this is not a problem for anaerobic bacteria, it could be a major problem for the aerobic species such as cyanobacteria (which generate oxygen during photosynthesis) and the free-living aerobic bacteria of soils, such as *Azotobacter* and *Beijerinckia*. These microorganisms have various defense mechanisms to overcome the problem. For example, *Azotobacter* species have the highest known rate of respiratory metabolism of any organism, so they might protect the enzyme by maintaining a very low level of oxygen in their cells. These species also produce extracellular polysaccharide, which retains water and in this way limits the diffusion rate of oxygen to the cells.

Plant growth-promoting bacteria (PGPB) have been used as biofertilizers worldwide, due to their ability to promote plant growth and therefore crop yields and soil fertility and hence, the potential to contribute to more sustainable agriculture and forestry.

Generally, PGPB facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development, in the form of biocontrol agents. They suppress the activity of pathogens by producing numerous metabolites like siderophores, hydrolytic enzymes, and antibiotics. PGPB live freely in soil, colonize plant roots aggressively and establish symbiotic association with plants. The existence of PGPB with the plant roots is generally classified by two environments; rhizosphere and endosphere. The rhizosphere is the soil volume under the direct influence of roots, while the endosphere is the internal root tissue. The strains inhabiting the rhizosphere and endosphere are called rhizobacteria and endophytes, respectively.

Only N-fixing microorganisms bring additional supplies of a nutrient (N) into the soil/plant system. All other biofertilizers simply solubilize or mobilize the nutrients that are already present in soils. Microorganisms that have the capacity to fix atmospheric N_2 can be used as efficient biofertilizers. Their application in soil improves the soil biota and reduces the need of chemical fertilizers. Among all PGPB, the diazotrophic (N_2-fixing) bacteria, which are involved in the transformation or fixation of N_2 from the unavailable gaseous form in the atmosphere, are divided into:

- Free-living heterotrophic or autotrophic bacteria;
- Bacteria in associative symbiotic relationships;
- Bacteria in symbiotic relationships with plants.

2.1.1. Free-living nitrogen fixers

The free-living, or non-symbiotic, nitrogen-fixing bacteria live outside plant cells and are associated with the rhizosphere, the part of soil under the influence of plant roots and their exudates. They are of four types:

- Free-living non-photosynthetic aerobic nitrogen-fixing bacteria such as *Azotobacter*, *Beijerinckia* and *Derxia*;
- Free-living non-photosynthetic anaerobic nitrogen-fixing bacteria such as *Clostridium*;
- Free-living photosynthetic nitrogen-fixing bacteria such as *Chromatium*, *Rhodopseudomonas*, *Rhodospirillum*, cyanobacteria;
- Free-living chemosynthetic nitrogen-fixing bacteria such as *Desulfovibrio.*
- *Free-living non-photosynthetic nitrogen-fixing bacteria*:

Although many genera and species of N_2-fixing bacteria are isolated from the rhizosphere of various cereals, mainly members of the *Azotobacter* and *Azospirillum* genera have been widely tested to increase the yield of cereals and legumes under field conditions. *Azotobacter* is an obligate aerobe, although it can grow under limited O_2 concentration. Its six species are: *Azotobacter armeniacus*, *A. beijerinckii*, *A. chroococcum*, *A. nigricans*, *A. paspali* and *A*. *vinelandi*. These species play an important role in nitrogen fixation in rice crops and are used as a biofertilizer for wheat, barley, oat, rice, sunflower, maize, line, beetroot, tobacco, tea, coffee and coconuts. *Azotobacter* species are different in terms of morphological and physiological characteristics. Some of them have higher nitrogen-fixing ability than others. Inoculation of soil with *Azotobacter* species lead to increase in crop yields due to the increase in the concentration not only of nitrogen, but also of other substances, such as vitamins, gibberellins, naphthalene and acetic acid, which improve plant growth. *Azotobacter* also synthesizes growth-promoting substances, produces group B vitamins such as nicotinic acid and pantothenic acid, biotin and heteroauxins, gibberellins and cytokinin-like substances, and improves the seed germination in several crops. Both carrier-based and liquid-based *Azotobacter* biofertilizers are available.

- *Free-living photosynthetic nitrogen-fixing bacteria*

Free-living nitrogen-fixing photosynthetic cyanobacteria (blue-green algae) belong to 15 genera, which are found freely in the soil where they fix free N_2 into nitrogenous and ammonium compounds. Mostly they are heterocysts, e.gNostoc, Anabaena, Aulosira, Cylindrospernum, Calothrix, Totypothrix and Stigonema. Cyanobacteria are photosynthetic and hence add organic matter and extra nitrogen into the soil. Amongst these, Aulosira is the most active nitrogen fixer in the rice fields of India. Nitrogen fixation occurs in special thick walled cells called heterocysts, or heterocytes (H), which occur at intervals along the cyanobacterial filaments. This separation of cellular functions is necessary because cyanobacteria have oxygen-evolving photosynthesis but the nitrogen-fixing enzyme, nitrogenase, is unstable in the presence of oxygen. This problem is overcome because the heterocysts contain only part of the photosynthetic apparatus, photosystem I, which can be used to generate energy (as ATP). But the heterocysts do not contain photosystem II, which is used to split water into hydrogen (for combination with CO_2 to produce organic products) and oxygen. There are fewer non-heterocystous nitrogen-fixing blue-green algae, e.g. Oscillatoria, Phormidium and Gleocapsa.

2.1.2. Associative symbiotic nitrogen fixers

This group comprises bacteria from the family Spirillaceae with two main genera, *Azospirillum* and *Herbaspirillum*. Bacteria of the genus *Azospirillum* are widespread in the soils of tropical, subtropical and temperate regions where they live in symbiotic mutualism around the root of various wild and agricultural plants, which is also known as a risosphere association. They are a good example of the so-called associative nitrogen fixers. *Azospirillum* belong to the facultative endophytic diazotrophs groups, which colonize the surface and the interior of non-legume plants. They are able to fix a considerable quantity of nitrogen in the range of 20– 40 kg N/ha in the rhizosphere in non-leguminous plants such as cereals, millets, oilseeds, cotton, rice, sugar cane etc. Nitrogen fixers such as *Azospirillum* benefits plant by improving shoot and root development and increasing the rate of water and mineral uptake by roots (Gonzales et al., 2005). The yield increases can be substantial, up to 30 percent, but generally range from 5 to 30 percent. These yield increases by *Azospirillum* are possibly a result of the production of growth-promoting substances rather than N_2 fixation (Okon, 1985). The main problem that limits the use of *Azospirillum* on a large scale is the great uncertainty and unpredictability of the results. Regardless of these uncertainties, *Azospirillum* bears great promise as a growth-promoting N_2-fixing biofertilizer. The species *A. lipoferum*, *A. brasilense* and *A. amazonense* have been commercially used as nitrogen-supplying biofertilizers.

2.1.3. Symbiotic nitrogen fixers

The best known and most exploited symbiotic nitrogen fixers comprise mutualistic (symbiotic) bacteria belonging to the group of Alphaproteobacteria, family Rhizobiaceae, which include the following genera *Rhizobium, Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium* and *Mesorhizobium* and *Allorhizobium* , collectively called rhizobia. Rhizobia participate in mutually useful associations with the roots of leguminous plants where they form noodles and carry out the nitrogen fixation process. Within the nodules, the bacteria convert free nitrogen to ammonia, which the host plant utilizes for its development. To ensure sufficient nodule formation and optimum growth of legumes (e.g. alfalfa, beans, clovers, peas, soybeans), seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species, especially in soils poor or lacking in the required bacterium. *Rhizobium* can fix 15–20 kg N/ha and increase crop yields up to 20% in pulses. It has been estimated that 40–250 kg N/ha/year is fixed by different legume crops by the microbial activities of *Rhizobium*. The N_2-fixing capability of rhizobia varies significantly among host plant species and bacterial strains.

Therefore, for the production of biofertilizers not only the bacterial strain, but also the rhizobia-host compatibility must be taken into account.

The N_2-fixers from the genus *Frankia* also participate in symbiotic relationships with certain dicotyledonous species (actinorhizal plants). *Frankia* are a free-living gram-positive filamentous actinobacteria found in root nodules or soil. Inoculation of actinorhizal plants with *Frankia* significantly improves plant growth, biomass, shoot and root N content, as well as the survival rate after transplanting in fields. However, the success of establishment of an actinorhizal plantation in degraded sites depends upon the choice of effective *Frankia* strains. Species from this genus are capable of infecting and nodulating eight families of actinorhizal plants (mainly woody plants), which are used for wood production, land reclamation, for timber and fuel wood production, in mixed plantations, for windbreaks, as well as for shelterbelts along deserts and coastlines. *Frankia* inoculation can be advantageous in arid environments, disturbed sites, and areas where native actinorhizal plants are absent. The symbiosis between actinorhizal plants and *Frankia* induces the formation of a perennial root organ called nodule, wherein bacteria are hosted and nitrogen is fixed. In the field, actinorhizal nodules can have variable forms and colours. Comparison of actinorhizal and leguminous nodules shows that the morphology, anatomy, origin, and functioning of the nodules are different for these two nitrogen-fixing plants. Two types of nodule formation occur in actinorhizal symbiosis: intercellular and extracellular infection.

Cyanobacteria are ecologically important because they contribute significantly to the global N_2-fixation. Their capability to fix molecular nitrogen is essential in rice cultivation and in the remediation of arid soils. Nevertheless, the production and application of cyanobacteria is still fairly poorly developed. However, cyanobacteria should be seriously considered as a biofertilizer supporting sustainable agricultural practices in various environments.

Besides cyanobacteria (blue-green algae), which are an important biological factor in rice cultivation, *Azolla* forms another inexpensive, economical, and ecologically friendly biofertilizer. The important factor in using *Azolla* as biofertilizer for rice crops is its quick decomposition in the soil, efficient availability of its nitrogen to rice plants, requirement of a shallow freshwater habitat, rapid growth, and growth along with rice without competition for light and space. Increase in grain yields of rice from 14% to40 % have been reported with *Azolla* being used as a dual crop. It improves the height of rice plants, the number of tillers, grains and the straw yield. It is supplemented with 8–20 kg phosphate per hectare.

Besides N-fixation, these biofertilizers or biomanures also contribute significant amounts of P, K, S, Zn, Fe, Mb and other micronutrients. Widely cultivated in the Asian regions, *Azolla* is either incorporated into the soil before rice transplanting or is grown as a dual crop along with rice. The Asians have recognized the benefits of growing *Azolla* as a biofertilizer, human food and medicine. It also improves water quality by removal of excess quantities of nitrate and phosphorous and is also used as fodder, feed for fish, ducks and rabbits. *Azolla* is a small floating pteridophyte which has symbiotic associations with cyanobacteria and eubacteria that remain associated throughout its life cycle. It is unique in the sense that it acts as a host to the N-fixing cyanobacteria, after which it is used virtually as a green manure. In this process, it adds not only the biologically fixed N, but also the other nutrients absorbed from the soil and present in its biomass. There are seven species of the Azzolaceae family: *Azolla caroliniana*, *A. filiculoides, A. maxicana, A. microphylla*, *A. pinnata, A. rubra* and *A. nilotica*. In India, *A. pinnata* is commonly observed. The algal symbiont belongs to family Nostocaceae and is generally referred to as *Anabaena azollae*. In the associations between *Azolla* and the cyanobacteria *Anabaena azollae*, the eukaryotic partner *Azolla* houses the prokaryotic endosymbiont *Anabaena azollae* in its leaf cavities and provides carbon sources and, in turn, gets its nitrogen requirements satisfied. The atmospheric nitrogen is harvested by the algal symbiont. The sites of nitrogen fixation are heterocysts. The heterocyst counts increase along the stem from the apex towards the base in the successive leaves. This symbiosis helps in the quick growth and multiplication of the fern and in the creation of a huge amount of biomass on the water surface. It is then harvested, dried and used as biofertilizer to supplement the needs of nitrogen in coffee farms as well.

2.2. *Phosphorus biofertilizers*

Phosphorous (P) is the next essential macroelement after nitrogen. Phosphorus is required in a soluble form for maximizing crop growth and production. It plays a significant role in plant metabolism and is important for the functioning of key enzymes that regulate the metabolic pathways. The phosphate available in soil occurs in three forms: soil solution phosphate, insoluble organic phosphate and insoluble inorganic phosphate. The greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates. This means that soils contain a high amount of total phosphorus, but its availability to plants is very low and it is often a limiting factor for plant growth.

A major characteristic of phosphorus biogeochemistry is that only 1% of the total soil phosphorus (400–4,000 kg P/ha in the top 30 cm) is incorporated into living plant biomass during each growing season (10–30 kg P/ha), reflecting its low availability for plant uptake. Phosphorus deficiency in plants leads to chlorosis, weak stem and slow growth. Therefore, it is considered to be the most important chemical factor that restricts plant growth because of its vital role in the physiological and biochemical functions of plants. The application of chemical phosphorous fertilizers to circumvent the phosphorus deficiency in soil is not a very efficient method due to the high reactivity of phosphate anions through precipitation with cations such as Fe^{3+} and Al^{3+} in acidic soils or Ca^{2+} in calcareous soils. The application of microbial inoculants with phosphate-solubilizing activity will be a promising approach to increase the phosphorus availability in agricultural soil and is an environmentally-friendly alternative to the use of chemical fertilizers. Organic phosphate solubilization is also called mineralization of organic phosphorus, and it occurs in soil at the expense of plant and animal remains, which contain a large amount of organic phosphorus-containing compounds. The decomposition of organic matter in soil is carried out by the action of numerous saprophytes, which release orthophosphate from the carbon structure of molecules. Various bacterial species are able to solubilize inorganic phosphate compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphates. It is important to determine the actual mechanism of phosphorus solubilisation by PSM for optimal utilization of these microorganisms in various field conditions. Microorganisms must assimilate phosphorus via membrane transport, so dissolution of calcium phosphate [$Ca(H_2PO_4)_2$] to dihydrogen phosphate anion (H_2PO_{4-}) is considered essential to the global phosphorus cycle.

The solubilization of phosphorus in nature is due to the activity of phosphate-solubilizing microorganisms (PSM) which belong to several genera: *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Microccocus*, *Aereobacter*, *Flavobacterium* and *Erwinia.* The symbiotic nitrogenous rhizobia, which fix atmospheric nitrogen into ammonia and export the fixed nitrogen to the host plants, also show phosphate-solubilizing activity. For instance, *Rhizobium leguminosarum* bv. *trifolii*, and *Rhizobium* species nodulating *Crotalaria* species improved plant phosphorus nutrition by mobilizing inorganic and organic phosphorus. Various phosphate-solubilizing bacteria have also been isolated from stressed environments; for example, the halophilic bacteria *Kushneriasinocarni* isolated from the sediment of Daqiao saltern on the eastern coast of China, which may be useful in salt-affected agricultural soils.

Two types of phosphate biofertilizers have been developed based on the application of phosphate-solubilizing bacteria and phosphate-mobilizing microorganisms.

2.2.1. Phosphate-solubilizing biofertilizers

The members of this group are bacterial and fungal species which solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate. The most efficient ones belong to *Bacillus* and *Pseudomonas* among Bacteria and *Aspergillus* and *Penicillium* among Fungi. They could be isolated in higher concentrations from rhizosphere soil rather than non-rhizosphere soil. Their application in biofertilizers aims to increase the yields of legume, cereals, vegetables and fruit crops. The phosphate-solubilizing fungi produce more acids than bacteria and consequently exhibit greater phosphate-solubilizing activity. Among the filamentous fungi that solubilize phosphate, the genera *Aspergillus* and *Penicillium* are the most representative ones, although strains of *Trichoderma* and *Rhizoctonia solani* have also been reported as phosphate solubilizers. A number of theories have been proposed to explain the mechanisms of phosphate solubilization. The most important theories are the acid production theory and the proton and enzyme theory.

- *Acid production theory*

The major mechanism involved in the solubilization of phosphate by phosphate-solubilizing microorganisms is the production of organic acids which either directly dissolve rock phosphate as a result of anion exchange of phosphate by acid anion or chelate Fe, Al, Ca ions to bring the phosphate into solution. Due to the ability of PSM to secrete and release organic acids (citric, oxalic, succinic, tartaric, malic, alpha keto butyric, 2-ketogluconic, gluconic and fumaric acids) in the soil environments, these bacteria lower the pH in their vicinity, which is a prerequisite for solubilization of bound phosphates in soil and consequently dissociate the bound form of phosphates like $Ca_3(PO_4)_2$ in calcareous soil. The microbial organic acids are produced as a result of oxidative respiration or by fermentation of organic carbon sources. Gluconic and fumaric acids have the highest ability to solubilize phosphate from inorganic phosphate compounds. The amount of soluble phosphate released depends on the strength and type of acid. Aliphatic acids are found to be more effective in phosphate solubilization than phenolic acids and citric acids. *Pseudomonas* sp., *Erwinia herbicola*, *Pseudomonas cepacia* and *Burkholderiacepacia* are phosphorus-solubilizing bacteria, which produce a higher amount of gluconic acid. Besides organic acids, inorganic acids such as nitric and sulphuric acids are also produced by the nitrifying *Nitrosomonas* and sulphur-oxidizing *Thiobacillus* bacteria during the oxidation of nitrogenous or inorganic compounds of sulphur which react with calcium phosphate and convert them into soluble forms. The introduction of efficient phosphate solubilizers in the rhizosphere of crops increases the availability of phosphorus and thus increases the crop yield up to 200–500 kg/ha. In this way, microorganisms play a major role in the solubilization and uptake of native and applied phosphorus.

- *Enzyme and proton theory*

Phosphate-solubilizing microorganisms are also known to produce phosphatase enzyme along with acids which cause the solubilization of phosphate in aquatic environment. Esterases are involved in liberating phosphorous from organic compounds. Solubilization without acid production is due to the release of protons accompanying respiration or ammonium assimilation. Besides these mechanisms, some bacterial species synthesize syderophores – iron-chelating compounds which bind the iron present in the root area and, thus, make it unavailable for harmful microorganisms so that crop plants are protected from them. The production of other chelating substances, mineral acids and biologically active substances like indole, acetic acids, gibberellins and cytokinins, is also correlated with phosphate solubilization.

2.2.2 Phosphorus mobilizing biofertilizers: Mycorrhiza

This type of biofertilizers contain mycorrhizal fungi also known as phosphate absorbers. They are a heterogeneous taxonomic group which inhabits the plant root system and establishes a symbiotic association with them. Mycorrhizal fungi live in symbiosis with over 90 % of all vascular plant species, including many important crop species, such as maize, wheat, rice and potato. Mycorrhizal fungi form a bridge between the roots and the soil, gathering nutrients from the soil and giving them to the roots. There are two major types of mycorrhizae: ectomycorrhizal fungi (EM) and endomycorrhizal fungi (AM). Endomycorrhizae are the most common type, and are found in grasses, shrubs, some trees and many other plants. Ectomycorrhizal fungi are usually specific to a certain host species, but most species of endomycorrhizae will form relationships with almost any AM-fungi host plant, and are therefore much easier to specify. The arbuscule-forming mycorrhiza (AMF) are a widespread type of endomycorrhiza associated with crop and horticultural plants, where fungal hyphae of *Glomeromycota* species penetrate root cortical cells and form branched structures called arbuscules. The host plant is benefited by obtaining needed nutrients, especially phosphorus, calcium, copper, zinc etc., which are otherwise inaccessible to it, with the help of the fine absorbing hyphae of the fungus. Phosphorus is a highly immobile element because it is easily absorbed by soil particles and a phosphate-free zone rapidly occurs around plant roots. Some of the external hyphae of mycorrhizal fungi may extend more than 10 cm from the root surface, which allows them to have access to a greater volume of non-depleted soil than the root alone. The small diameter of hyphae (20 to 50 µm) permits access to soil pores that cannot be explored by roots as well. They also produce extracellular alkaline phosphatases which can mobilize phophate from organic sources. Through the excretion of protons, hydroxyls and organic acids, mycorrhizae modify the redox potential around the root and the mycelium, which also enhances the transformation of insoluble phosphate from the soil into a soluble form in the soil solution. Therefore, a root system forming a mycorrhizal network will have a greater effective surface area for absorbing nutrients and exploring a greater volume of soil than nonmycorrhizal roots. AM hyphae also excrete gluey, sugar-based compounds called glomalin, which helps to bind soil particles, and make stable soil aggregates. There is an increasing interest in the use of mycorrhiza to promote sustainable agriculture, considering the widely accepted benefits of the symbioses to nutrition efficiency (for both macronutrients, especially P, and micronutrients), water balance and biotic and abiotic stress protection of plants. Vesicular Arbuscular Mycorrhiza Root Inoculant (VAMRI) is a biofertilizer based on chopped dried corn roots infected with *Glomus* species (*G. mosseae* or *G. fasciculatum*). Besides a microbial inoculant, this product also serves as a biocontrol agent of soil-borne diseases of different crops under various conditions. VAMRI can be applied for pepper, tomato, papaya, onion, corn, peanut, sugarcane, eggplant, banana, fruit crops, watermelon, etc.

2.3. *Potassium (K)-solubilizing biofertilizers*

Potassium (K) is the third essential nutrient necessary for plant growth. Some rhizobacteria are able to solubilize insoluble potassium forms. *Bacillus edaphicus* has been reported to increase potassium uptake in wheat and *Paenibacillusglucanolyticus* has been found to increase the dry weight of black pepper. Sudan grass inoculated with the potassium-solubilizing bacterium *Bacillus mucilaginosus* had higher biomass yields. Moreover, *Bacillus mucilaginosus* in co-inoculation with the phosphate-solubilizing *Bacillus megaterium* promoted the growth of eggplant, pepper and cucumber.

2.4. Biofertilizers for secondary macronutrients: zinc and iron solubilizers

Zinc is of utmost importance. It is found in the earth's crust at a concentration of 0.008%, but there are soils which exhibit zinc deficiency with content far below the critical level of 1.5 ppm of available zinc. The plant deficiencies in absorbing zinc from the soil are overcome by external application of soluble zinc sulphate ($ZnSO_4$). Microorganisms found in the soil can be used as biofertilizers to provide micronutrients like Zn, Fe, Cu, etc. Zinc can be solubilized by *B. subtilis, Thiobacillus thioxidans* and *Saccharomyces* sp*.* These species are responsible for Zn extraction in soils where native zinc is higher or in conjunction with insoluble cheaper zinc compounds like zinc oxide (ZnO), zinc carbonate ($ZnCO_3$) and zinc sulphide (ZnS) instead of costly zinc sulphate. The zinc fixation occurs through two main mechanisms: the first one operates in acidic soils and is based on cation exchange; the second mechanism operates in alkaline soils where fixation takes place by sorption of Zn on $CaCO_3$ and, as a result, a solid-solution of $Zn_xCa_{x-1}CO_3$ is formed.

2.5. *Plant-growth-promoting rhizobacteria (PGPR)*

A group of rhizosphere bacteria (rhizobacteria) that exerts a beneficial effect on plant growth is referred to as plant-growth-promoting rhizobacteria or PGPR. PGPR is a generic acronym that indicates bacteria which, in some often unknown way, can stimulate plant growth. They belong to several genera, e.g. *Agrobacterium*, *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Actinoplanes*, *Azotobacter*, *Bacillus*, *Pseudomonas* sp., *Rhizobium*, *Bradyrhizobium*, *Erwinia*, *Enterobacter*, *Amorphosporangium*, *Cellulomonas*, *Flavobacterium*, *Streptomyces* and *Xanthomonas*. These bacteria vary in their mechanism of plant growth promotion but generally influence growth via phosphate solubilization, nutrient uptake enhancement, plant growth hormone production or production of a variety of antimicrobial compounds that act in different ways. Bertrand et al. (2000) showed that a rhizobacterium belonging to the genus *Achromobacter* could enhance the root hair number and length in oilseed rape (*Brassica napus*). *Achromobacter* increased the NO_3 and K uptake and, consequently, the shoot and root dry weights by 22 to 33 percent and 6 to 21 percent, respectively. One of the plant-growth-promoting mechanisms of rhizobacteria is the antagonism against phytopathogenic microorganisms due to the production of antimicrobial metabolites like siderophores, antibiotics, cyanides, fungal cell-wall-degrading enzymes and gaseous products including ammonia (Idris et al., 2007; Lugtenberg and Kamilova, 2009). The mechanism of antifungal effects lies in the production of a variety of antimicrobial compounds that act in different ways. The antagonistic effects are caused by cytolysis, leakage of potassium ions, disruption of the structural integrity of membranes, inhibition of mycelial growth and protein biosynthesis. Most of the identified *Pseudomonas* biocontrol strains produce antifungal metabolites such phenazines, pyrrolnitrin, pyoluteorin and cyclic lipopeptides like viscosinamide. It was demonstrated that viscosinamide prevents the infection of sugar beet by *Pythium ultimum*. These bacterial strains, besides having an antagonistic effect, also influence the defense system of plants. The siderophore-mediated competition for iron is one of the mechanisms responsible for the antagonistic activity of *Pseudomonas* spp. The secreted iron-chelating compounds bind ferric ions (Fe^{3+}), and are taken up by microbial cells through specific recognition by membrane proteins (Srivastava and Shalini, 2008). The presence of iron-chelating compounds makes the bacteria better competitors for iron, in this way preventing the growth of pathogenic microorganisms. *Pseudomonas* species produce two different types of siderophores: pseudobactin and pyoverdin (Oldal et al., 2002). Siderophores produced by biocontrol bacteria have a higher affinity for iron than those produced by some fungal pathogens, allowing the former microbes to scavenge most of the available iron, preventing the proliferation of fungal pathogens (Hillel, 2005). Some authors have reported that *Pseudomonas fluorescens* belonging to the PGPR class produces siderophores and has a biocontrol effect against *P. ultimum*, *R. batatticola* and *Fusarium oxysporum*. Other *Pseudomonas* species like *Pythium stutzeri* produce extracellular enzymes like chitinase and laminase capable of lysing the mycelia of *Fusarium solani*. *Pseudomonas aeruginosa* produces three types of siderophores under iron-limiting conditions: pyoverdine, pyochelin and its precursor salicylic acid, and induces resistance to plant diseases caused by *Botrytis cinerea* on bean and tomato, *Colletotrichum lindemuthianum* on bean. *F. oxysporum* causes vascular wilt and foot-, root- and bulbrot diseases in a wide variety of economically important crops. *Alternaria* spp., *Sclerotium* spp. cause leaf spots, root rot and stem rot, which also leads to serious yield losses. The antifungal effect of PGPRs is influenced by a lot of environmental and genetic factors. Biotic and abiotic environmental signals may have an important input on the regulation of biocontrol genes in pseudomonads, e.g. on the repression of siderophore biosynthesis. Together with low oxygen concentrations, the available carbon and nitrogen sources that influence the molecular mechanisms are involved in biocontrol activity.