Chapter

Plants and Their Microbes

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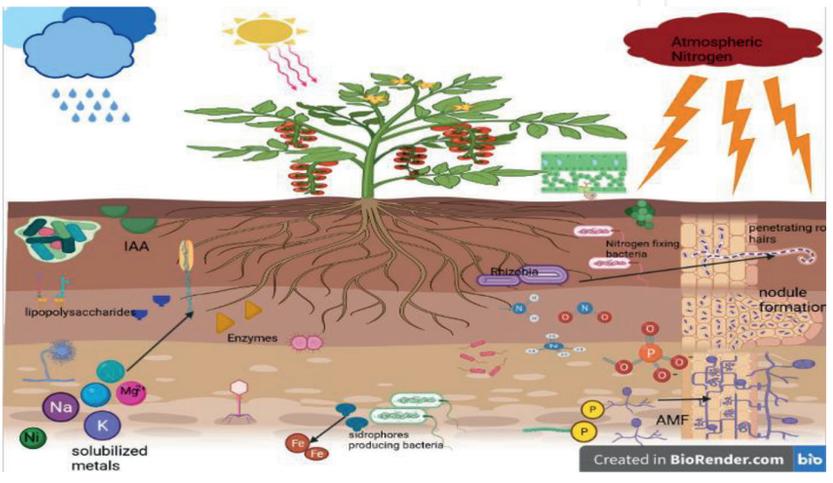
Abstract

The soil harbors billions of Microbes supporting the growth of several plants, resulting in the constant symbiotic or non-symbiotic interactions between the plants and these microorganisms known as plant-microbe interactions. Plants need nutrients (available or available but inaccessible) in the soil to grow while microbes need shelter and nutrients supplied by plants and also promote plant-growth. Microbes are constantly trading with plants either buying or selling nutrients (the nutrients are considered the main currencies for trading and the product being bought in the soil rhizosphere). Plants including legumes inoculated with Rhizobia and microbes in experimental studies show that they are able to solubilizing phosphate and metals, fixing nitrogen fixers, producing IAA, cytokinins, Gibberellins and Ethylene. Microbiomes are therefore essential for plant growth and health as they govern most soil functions affecting plant-growth. For higher crop-yields and increased soil- fertility using ecofriendly manner, researches focused on the new concepts of exotic biomolecules, hormones, enzymes and metabolites which create a suitable environment for the interaction between plant and microbes using molecular and biotechnological approaches are necessary to increase our knowledge of rhizosphere biology and to achieve an integrated management of soil microbial populations that can ultimately enhance the health of plants.

Keywords: plants-microbes, symbiosis, plant-health, soil-fertility, plant growth

1. Introduction



The soil is the habitat for billions of bacteria and the support for the growth of all or most plants. There is therefore bound to be constant interactions between the plants and these microorganism hence the study of plant-microbe interactions (these interactions can be symbiotic or non-symbiotic). Plants are constantly in need of nutrients in the soil (such as zinc, nitrogen, phosphorus, calcium, cobalt, molybdenum, iron, etc.) to grow and these nutrients may be available or sometimes available but inaccessible for the plants. While bacteria are constantly in need of shelter form harsh environmental conditions, and also in need of nutrients that the plants can supply to them. Hence they are constantly in contact with the roots of the plant trying to either buy or sell nutrients with the plant (we may say that nutrients are the main currencies for trading and the product being bought in the soil rhizosphere). The rhizosphere is the narrow region or (zone around the root in which the microbial biomass is impacted by the presence of the root of soil) substrate that is directly influenced by root secretions and associated soil microorganisms known as the root microbiome. The rhizosphere of each plant the roots of each plant is has been shown to be colonized by a specific set of bacteria that are useful to it or benefiting from it and this microbes are able to interact with plant tissues and cells with different degrees of dependence these interactions can be beneficial or harmful especially in the case of pathogenic microbes that attack the plants making them fall sick. These bacteria are referred to as Plant Growth Promoting Rhizobacteria (PGPR). In essence plants and their microbiomes need each other but can they survive without each other in the soil? Most likely not.

2. Common microbiomes associated with plant the good and the bad

Microbiomes commonly associated with plants are largely dependent on the environmental conditions of the soil such as the pH, the temperature, the moisture content, the soil texture, availability of nutrients etc. For example, in mountainous regions (such as Indian Himalayan Region and Tropical Andes), the climate is mainly characterized by low soil and atmospheric temperatures especially during the winter months, and the soils are generally acidic and severely lack nutrients. On the other hand in the desert regions, the climate is mainly characterized by high temperature, low moisture content, poor soil structure, high salinity and is mostly unfavorable for microgranisms. These two regions face several major challenges in agriculture while in temperate regions the weather and environmental conditions are more favorable to microorganisms although it does not come without its own challenges. Several of the PGPR found in the soil belong to four phyla namely Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes respectively. And the most common genera found in the soil includes *Pseudomonas* spp., *Bacillus* spp., *Stenotrophomonas* spp., *Methylobacterium* spp., *Arthrobacter* spp., *Pantoea* spp., *Achromobacter* spp., *Acinetobacter* spp., *Exiguobacterium* spp., *Staphylococcus* spp., *Enterobacter* spp., *Providencia* spp., *Klebsiella* spp., *Leclercia* spp., *Brevundimonas* spp., *Flavobacterium* spp., *Kocuria* spp., *Kluyvera* spp., *Rhizobia* spp., *Serratia* spp., and *Planococcus* spp.

2.1 Phosphate solubilizers

Plants are able to access phosphorus in two forms which are the monobasic and dibasic. It can be found in the soil in the inorganic forms, such as apatite, or as organic

forms such as inositol phosphate, phosphomonoesters, and phosphotriesters, but it is mostly insoluble. Phosphate solubilizing bacteria are able to solubilize the insoluble forms of phosphorus thereby making it available for plants to use. They inculde bacteria such as *Achromobacter* spp., *Agrobacterium* spp., *Bacillus* spp., *Enterobacter* spp., *Erwinia* spp., *Flavobacterium* spp., *Gluconacetobacter* spp., *Mycobacterium* spp., *Pseudomonas* spp., *Rhizobium* spp., *Serratia* spp.

2.2 Nitrogen fixers

They are able to fix nitrogen which is generally unavailable to plants although it is an essentially important element for developing and producing of fruits and seeds in plants. Leguminous plants in particular are able to form symbiotic associations with *Rhizobia* sp., which include the genera Bradyrhizobium, Sinorhizobium, Mesorhizobium, Rhizobium, Azorhizobium, Neorhizobium, and Pararhizobium. They enter into the plant tissue of the roots by exchanging chemical signals with the plant forming nodules where they are able to fixing atmospheric nitrogen and converting it into ammonia a form of nitrogen that plants can use. Some other bacteria (non-nitrogen-fixing bacteria), for example Pseudomonas, can stimulate symbiosis between legumes and their associated rhizobia while helping to increase levels of nitrogen fixation, ultimately helping to improve the nutrition and growth of the plants. For example, when the activity level of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase is high in Pseudomonas it essentially helps to enhance the process of nodulation in rhizobia, an indication that interactions among bacteria are also beneficial to plants.

2.3 Pathogenic microrganisms

These are bacteria or fungi that produce substances, enzymes or metabolites that affect the plant they also feed on the plant or its fruits causing disease, infection and yield losses they impair the normal state of the plant and interrupt the normal functioning in the plant. Such microbes include fungi, viruses, bacterium.

2.4 Metal solubilizers bioremediators

PGPR are able to stimulate solubilization of metals, such as zinc, potassium, Iron and manganase are generally essential for the fertility of the soil and improvement of crops. Iron is naturally highly abundant, but for it to be available to plant, there is a need for the formation of Fe-siderophore complexes. Bacteria, can secrete siderophores as iron-chelating compounds that are able to reduce iron (Fe^{3+} to Fe^{2+}) intra and intercellularly which are then made available to plants and endophytes. Siderophores are therefore highly have a relevant as they are able to perform the function of making Fe available when it is scarce in the rhizosphere.

2.5 IAA producer

Most PGPR can produce auxins that have very strong effects on the growth of plant root and structure and they are therefore involved in the plant-microbe interactions examples include IAA (Indole-3-acetic acid) produce cytokinins and gibberellins. They are able to give rise to transcription changes in hormones, defense-related and cell wall related genes, resulting in longer roots, root biomass increase and stomata size and density decrease and activate auxin response genes that enhance plant growth.

2.6 Cytokinins and gibberellins producers

PGPR are able to produce cytokinins and gibberellins, which are able to enhance plant shoot growth. Gibberellins interact with auxins to alter the structure of the root favorably while cytokinins act by enhancing root exudate production in plants this helps to encourage the presence of PGPR associated with the plant.

2.7 Ethylene producers

Ethylene gas hormone is produced in response to stress (abiotic and biotic) conditions and is very active in extremely low concentrations (0.05 mL L – 1). It helps plant in tolerating stress and senescence by providing more growth stimulation under drought stress, heat stress, salinity, unfavorable pH and other types of stress. Ethylene plays an important role for improving plant stress tolerance for some PGPR.

2.8 Abscisic acid (ABA) producers

Abscisic acid is a plant hormone that regulates numerous aspects of plant growth, development (seed and bud dormancy, control of organ size, germination, cell development, synthesis of protein etc.) and stress responses (abiotic), modulation of synthesis and inhibition of cytokinins. Their functions are conserved in plants. They are usually produced in small quantities in the root as well as terminal buds.

2.9 ACC (1-aminocyclopropane-1-carboxylic acid producers)

1-aminocyclopropane-1-carboxylic acid is a non- protein amino acid acting as the direct precursor of ethylene (which is a plant hormone that regulates a wide range of vegetative and developmental processes). ACC is the central molecule of ethylene biosynthesis.

2.10 Fungal PGRP

Fungi such as *Trichoderma* spp. (promote the growth of plants), *Beauveria bassiana* (used to control pests), Mychorriza (enhance water utilization and plant growth), have been found to be very useful in plant.

3. Response mechanism of plants to their microbiomes friends or foes

Plants and bacteria respond to the each other by producing hormones known phytohormones which are key requirements for regulating plant growth and development. They do these mostly to facilitate uptake of nutrients are in defense against pathogens or to enhance and stimulate proper growth and development in the plants. They also function as molecular signals in response to environmental factors that otherwise limit plant growth or become lethal when uncontrolled they can have either positive, neutral or negative effects on plants.

PGPR are able to produce acid phosphatases, to mineralize organic phosphorus in the soil and organic acids such as gluconic acid and citric acid to solubilize phosphorus. They also produce organic and inorganic acids such as citrate, oxalate, acetate, sulfuric acid, carbonic acid and nitric acids to stimulate the solubilization of other

elements, such as zinc and potassium, which are essential for soil fertility and crop improvement. Siderophores such as pyochelin, pseudobactin, and pyoverdine are produced by bacteria to reduce iron (Fe^{3+} to Fe^{2+}) intra and intercellularly and are very useful to both the plant by (attacking pathogens by making it unavailable to them) and the PGRP. They increase plant growth through the improvement in iron acquisition, since this element has several important biological functions for the cell. Others like Rhizobia are able to enter the root plant tissue through the exchange of chemical signals and form nodules. Other non-nitrogen-fixing bacteria, such as Pseudomonas are able to enhance nodulation process by producing enzymes like 1-aminocyclopropane-1-carboxylate (ACC) deaminase. They also produce auxins, such as volatile organic compounds (VOC) which stimulate plant growth and are required in several physiological processes such as seed germination, formation of lateral roots, gravidity, and photosynthesis; it affects photosynthesis and the production of metabolites and other relevant compounds involved in the development of the plant.

PGPR also act as antagonist against plant pathogens, they do so by restricting their growth or killing the pathogens promoting plant growth and health. PGPR containing a collection of compounds and enzymes are usually able to restrict or entirely eliminate the activities of pathogens. Bacteria of the genus *Pseudomonas* are able to produce siderophores that chelate Fe available in the rhizosphere, to restrict pathogens. Another mechanism widely used by bacterial endophytes with antifungal activity is the production of enzymes, such as chitinases, cellulases, and β-1, 3-glucanases that degrade or attack the fungal cell wall, chitin (component of the fungal cell walls). They also produce enzymes with antibiotic activity including peptides of ribosomal origin (polyketide synthases), such as subtilin, subtilosin A, TasA, and sublancin and peptides of non-ribosomal origin such as bacillaene, bacilysin, chlorotetain, difficidin, mycobacillin, and some rhizocticins and antibiotics, fluorescent pigment or by cyanide production they are therefore able to act as bio controls against pathogen.

4. Utilizing the useful microbes

New biotechnologies are being developed using the plant defense response, plant beneficial bacteria to control diseases in plants (biopesticides), to control pathogens (biopathogens), as biocontrol agents and for plant growth promotion (biofertilizer) replacing chemical fertilizers and pesticides. They can be in the solid (e.g., powder) or liquid forms and can be either inoculated in the plants in the case of biofertilizers, biopathogens and biocontrol agents or sprayed on the plants biopesticides and biopathogens. They can be applied in the soil or on the aerial parts of the plant depending on the target. If it is intended for attacking soil fungi or oomycetes, it is more suitable to apply them in the soil, near the root while if the potential infection by pathogens is in the aerial part of the plants application in liquid or powder at the foliar parts is more suitable. They can be applied pre-field, on-field or post-field depending on when their action is required.

New biotechnologies are being developed using the plant defense response, plant beneficial bacteria to control diseases in plants (bio-pesticides) [1], to control pathogens (bio-pathogens), as biocontrol agents and for plant growth promotion (bio-fertilizer) replacing chemical fertilizers and pesticides. They can be in the solid (e.g., powder) or liquid forms and can be either inoculated in the plants in the case of biofertilizers, biopathogens and biocontrol agents or sprayed on the plants

biopesticides and biopathogens [2, 3]. They can be applied in the soil or on the aerial parts of the plant depending on the target.

5. Symbiosis in legumes: biological nitrogen fixation

Legumes are crops which are known to fix nitrogen in their roots with the help of nitrogen fixing bacteria known as Rhizobia [4, 5]. Although the roles of rhizobia is well known in legumes we know that several other bacteria are present and associated with legumes in the soil play important roles to facilitate the health and growth of legumes [4]. Plants and bacteria respond to the each other by producing hormones known phytohormones which are key requirements for regulating plant growth and development [6]. They also function as molecular signals in response to environmental factors that otherwise limit plant growth or become lethal when uncontrolled they can have either positive, neutral or negative effects on plants. Plant growth promoting bacteria (PGPR) are able to produce acid phosphatases, to mineralize organic phosphorus in the soil and organic acids such as gluconic acid and citric acid to solubilize phosphorus [7, 8]. They also produce organic and inorganic acids such as citrate, oxalate, acetate, sulfuric acid, carbonic acid and nitric acids to stimulate the solubilization of other elements, such as zinc and potassium, which are essential for soil fertility and crop improvement [9].

6. Other plant growth promoting bacteria and their contribution/roles in legume health

The microbiomes associated with legumes showed abilities to improve $\%N_2$ fixed, biomass of the plants, leaf chlorophyll and seed emergence as shown in the findings of [2, 4]. It is suggested that they are able to do this by able to stimulate solubilization of metals, produce Ethylene gas hormone is produced in response to stress (abiotic and biotic) conditions, and organic acids such as gluconic acid and citric acid to solubilize phosphorus and also produce IAA (Indole-3-acetic acid) produce cytokinins and gibberellins produce acid phosphatases, to mineralize organic phosphorus in the soil and organic acids such as gluconic acid and citric acid to solubilize phosphorus as shown in the findings of [2, 3]. They also produce organic and inorganic acids such as citrate, oxalate, acetate, sulfuric acid, carbonic acid and nitric acids to stimulate the solubilization of other elements, such as zinc and potassium, which are essential for soil fertility and crop improvement as explained in the findings of Andreote and Pereira e Silva [9] and Wille et al. [1].

6.1 Plant biomass

The plant biomass shoot, root, nodule and seed weight for both the wet and dry matter has been found to be enhanced by Plant growth promoting bacteria [10, 11]. The nodule weight and dry weight of plants receiving *Serratia proteamaculans* increased by 33 and 25%, respectively at 15°C RZT, while for plants receiving *Aeromonas hydrophila* P73 they were increased by 72 and 31% at 17 ± 5°C RZT. Rhizobia has also been shown to significantly increase the yield of legumes and influence the plant biomass positively, ultimately enhancing the plants growth and also the nodule biomass of legumes [10, 12–14].

6.2 Nutrient uptake

The uptake of nutrients such as nitrogen, phosphorus, macronutrient and micronutrients has been shown to be significantly increased [15] in plants by the presence of plant growth promoting bacteria. The bacteria make them available by solubilizing them thereby releasing them into the rhizosphere of the plant and making them assessable for the roots of the plants to absorb for the plant to use for various biochemical processes [10, 16]. In legumes the percentage nitrogen fixed in the plant is also significantly increased [10, 12, 16]. *Herbaspirillum sp*. is another gram-negative which can be found associated with most agricultural crops like rice (*Oryza sativa L*.) [17, 18], maize (*Zea mays L*.) [19], sorghum (*Sorghum bicolor L*.) [20], and sugarcane [17]. Studies using electron and fluorescence microscopy were able to revealed that the *Herbaspirillum sp*. strain B501 is able to colonize the intercellular space in the leaves of wild rice (Oryza officinalis), and fix N_2 in vivo [21]. It also is able to express nif genes in the shoot of wild rice [22]. *Herbaspirillum sp* can also colonize the intercellular space in the root and stem tissue of sugarcane plant, they are therefore non-specific to host plants [23]. When plants of barley (*Hordeum vulgare L*.) and Miscanthus are inoculations with *Herbaspirillum frisingense* strains colonization occurs showing that it is a true plant endophyte [24].

6.3 Flowering, height and maturation (Podding and yield)

Flowering and maturation (Podding and yield) have been shown to be enhanced by the activities of Plant growth promoting bacteria. In cow pea plants inoculated with PGPRs (*Rhizobial* spp., *Agrobacterium* spp.*, Pseudomonas sp*. *Paenibacillus sp*., *Azotobacte*r), the first signs of flowering commenced by the 6th week while plants to which PGPRs were added commenced flowering ahead of plants with N^+ (fertilizer application) and in non-sterile soils, an indication of faster maturity than the control plants. By the end of the 7th week, flowering had occurred in all plants expect for the plants in non-sterile soils (showed no signs of flowering), and by the 8th week no signs of flowering could still be seen [25]. In 82% of the plants to which PGPRs were applied, podding rate and size was enhanced with visible cowpea pods before harvested at the 8th week, while neither controls i.e. N^+ and plants in non-sterile soil showed signs of pod formation. Flowering and podding were also enhanced on co-inoculation of *Rhizobial* spp. alongside a consortium of *Agrobacterium* spp., *Pseudomonas* sp. *Paenibacillus* sp. and a consortium of *Azotobacte*r. Co-innoculation of *Bradhyrizobium* with PGPRs increased seed yield in soybean plants [26], this may vary from plant to plant and strain type as rhizobial inoculation were shown to induce late flowering and pod formation in soybean [27].

Much study has been done on PGPRs related to genus Azospirillum largely because of their efficiency to promote the growth of plants with of different agronomical interest. The work of Garcia de Salamone et al. [28], shows that *Azospirillum sp*. is a major contributor to plant fitness through BNF. The genus Burkholderia also includes bacteria species that are able to fix N_2. *Burkholderia vietnamiensis*, a human pathogenic species, was efficient in colonizing rice roots and fixing N_2 [29]. Other bacteria genera asides Burkholderia, such as *Pantoea*, *Bacillus* and *Klebsiella*, have the potentials for BNF and endophytic colonization in different maize genotypes [30]. Another extensively studied endophyte is *Gluconacetobacter diazotrophicus* [31–34]. Plant height was also significantly enhanced on inoculation with PGPRs, where the plants with PGPR were taller than their counterpart containing only rhizobia, with only 22–33% of the

plants, being shorter than the N^+ (nitrogen fertilizer) plants. Also increased availability of nutrient with inoculation of PGPRs in the rhizosphere enhanced to plant height.

Co-innoculation of Bradhyrhizobium with PGPRs increased soybean plant vigor [35, 36]. Plant height, flowering and podding were enhanced by co-inoculation of *Rhizobial* spp. with a consortium of *Agrobacterium* spp., *Pseudomonas* sp. *Paenibacillus* sp. while a consortium of *Azotobacte*r spp. showed no ability to improve plant height [25]. The plant growth-promoting rhizobacteria (or PGPR) generally belong to heterogeneously beneficial groups of microorganisms residing in the plant rhizosphere, either on associated or on the surface of the root surface with the capacity to enhance plant-growth and provide protection for the plants from disease and other abiotic stresses [37–39].

6.4 Phosphorus uptake

Phosphorus (P) is required for several molecular and biochemical plant processes, especially in acquisition, storage and utilization of energy. When phosphorous supply is deficient, it results in severe limitations in the nitrogen fixing and symbiotic interacting processes. It's requirement maybe more for the roots or shoots of the host plant. There are definite differences in Rhizobial and plant requirements for P [40]. Usually, the slow- growers are more tolerant to low P than the fast-growing Rhizobia. Nodules are also strong sinks for P and nodulation and N_2 fixation are greatly affected by P availability [40]. In cases where leguminous plants that depend on symbiotic nitrogen get insufficient supplies of phosphorus, they also suffer nitrogen deficiency. The phosphorus content in a dry weight unit of a plant is usually greater in nodules than in the roots and shoots, especially when there are low supplies of phosphorus. It has been discovered that Nitrogen fixing plants require more P than plants getting nitrogen directly from fertilizer, this is likely because it is needed for nodule development and signal transductions, and for P-lipids in the very numerous bacterioids [40].

6.5 Nitrogen fixation

Biological nitrogen fixation (BNF) is the process by which Rhizobia (which are rod shape, Gram negative soil bacteria) that relate symbiotically with legumes, play the crucial role of replenishing the soi1 nitrogen by converting atmospheric nitrogen to ammonia [41]. They have the potential to fix up to 300 kg/ha of nitrogen. The biological fixation of atmospheric nitrogen by *Rhizobium*/legumes association has been estimated to be 50% of all nitrogen fixed on the earth [42, 43].

The common *nodABC* genes is found in all *Azorhizobium*, *Rhizobium*, and *Bradyrhizobium* isolates which have been studied so far are structurally conserved and functionally interchangeable between *Rhizobia* spp. [44, 45]. They encode enzymes involved in the synthesis of the lipo-oligosaccharide core. And their inactivation of abolishes the ability to elicit any symbiotic reaction in the plant [44, 46]. *nodZ*A and *nodZ*B are two genes that have been shown to have specificity for soybean associated rhizobia and most *Rhizobia* spp. that have ability to nodulate soybean [47, 48] as they play a major role in the nodulation of soybean.

Both *nodA* and *nodB* were found to be present in all the strains confirming that they are rhizobia strains as these two nod genes are found in all rhizobia and are functionally similar in *Rhizobia* spp. and was similar to the findings of Toolarood et al. [49] and Reeve et al. [50] where both genes have been found to be conserved in *Rhizobia* spp. *nodZ*A and *nodZ*B may also be found in a Bambara symbiotic

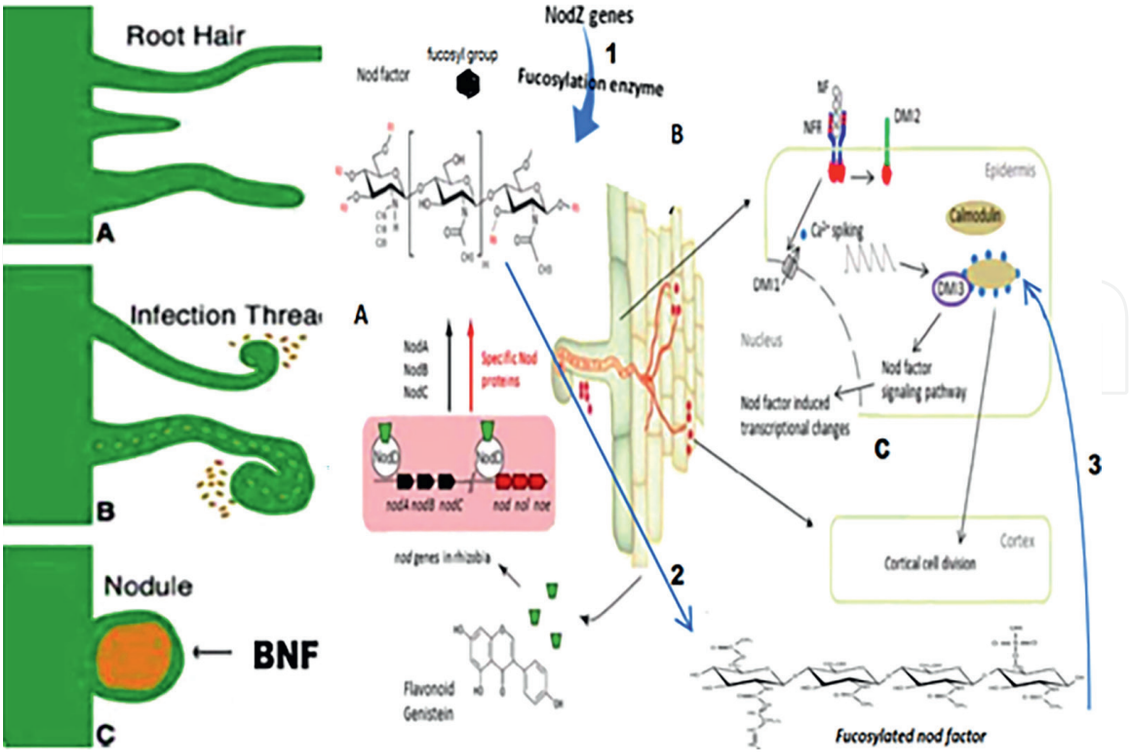


Figure 1.
(A) Legume secrete flavonoid eg genistein into the rhizosphere as signal and are perceived by rhizobial nodD regulatory proteins which binds to appropriate flavonoid, which are transcription factors that activate transcriptones of various nod genes that induce synthesis of rhizobial nod factors (NF). (B) Nod factors NFs are perceived by the Nod factor receptors (NFR) at the legume root. (C) DM12 causes root hair curling. DM11 encodes an ion channel that is localized to the nuclear membrane. DM13 encodes a calcium-calmodulin-dependent protein kinase which is required for the induction of transcriptional changes done to Nod factors binding as well as cortical cell division coined from Oldroyd and Downie [51] and Masson-Bovin et al. [52]. 1—The product of the nodZ genes are responsible for the addition of a fucosyl group to Nod factors; 2—Fucosylation of nod factors occurs; and 3—Fucosylation results in sharp increase of cytosolic ca^{2+}.

strains [15], implying that these strains may have the ability to form nodules in soybean plants as these two genes are shown to be specifically found in soybean symbiotic rhizobia because their products is the fucosyl group which a common product for the symbiont of soybean and most *Rhizobia* spp. that have ability to nodulate soybean as confirmed by [47, 48] (**Figure 1**).

The importance of bacteria endophytes with N_2-fixing ability have been an interest of study in non-leguminous plants like sugarcane (*Saccharum officinarum L.*), [53] and several other plants. Other studies suggest that bradyrhizobia is able to colonize the root nodules in legume plants and also the roots of tubers such as sweet potatoes (*Ipomoea batatas L.*), expressing nifH, and also acting as diazotrophic endophytes [54]. In conditions of N deficiency, the *G. diazotrophicus* strain Pal5 isolated from sugarcane is able to increase the N content when compared with plants inoculated with a Nif – mutant or uninoculated plants [55].

6.6 ACC (1-aminocyclopropane-1-carboxylic acid producers)

1-aminocyclopropane-1-carboxylic acid (ACC) is also a fairly simple molecule and is the biochemical precursor of ethylene. It's amino acid has a triangularly shape with several more features in addition to being a precursor for the lead-role player ethylene. For instance, ACC could be conjugated into three types of derivatives, although

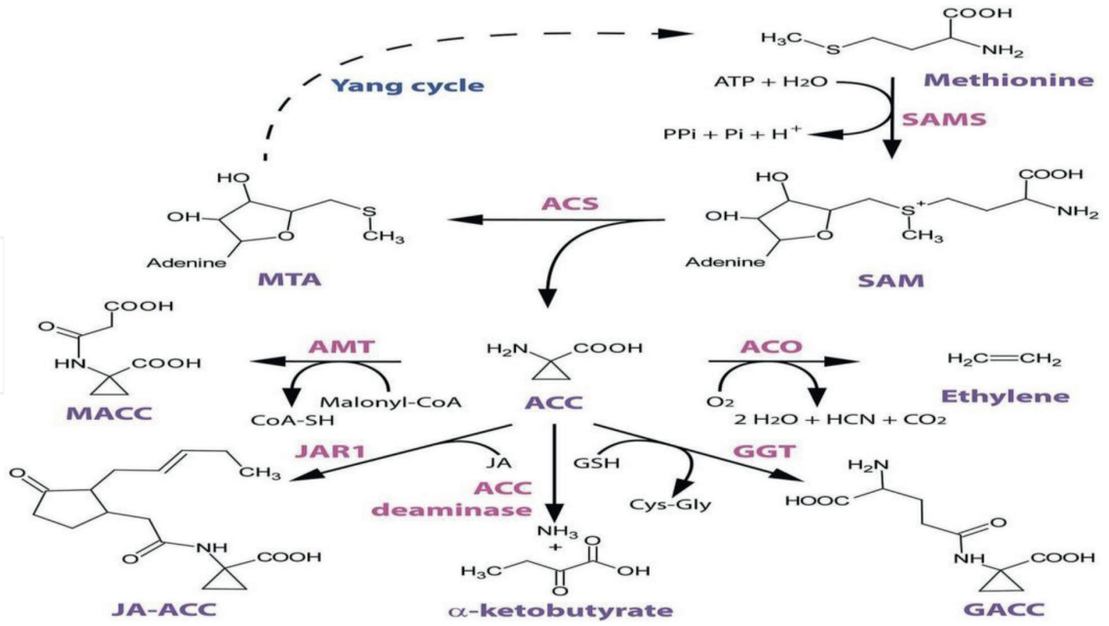


Figure 2.
Structural scheme of ethylene biosynthesis and 1-aminocyclopropane-1-carboxylic acid (ACC) conjugation/metabolism.

their role biological is still uncertain. ACC favors plant growth and lowers susceptibility to stress when they are metabolized by bacteria using the enzyme ACC-deaminase. ACC is also subjected to a sophisticated transport mechanism to ensure local and long-distance ethylene responses. ACC has been reported to function as a signal itself, independently from ethylene. Plants possess several mechanisms to control their pool of ACC, for example by converting it to ethylene or to conjugates like MACC, GACC, or JA-ACC. ACC can also be metabolized by deamination (**Figure 2**). The ACC deaminase enzyme was discovered in bacteria first with PGPRs having the ability to process plant-borne ACC and convert it to ammonia and α-ketobutyrate by metabolizing it with the ACC deaminase enzyme [56]. ACC deaminase enzyme has been retrieved in bacteria such as *Pseudomonas sp*. strain ACP [56], *Pseudomonas putida* GR12-2 [57] Pseudomonas chloroaphis 6G5 [58] and *Pseudomonas putida* UW4 [59].

The ACC deaminase enzyme produced by bacterial is PLP-dependent having low affinity for ACC (Km value is 1.5–15 mM have been reported [60];). Nonetheless, expressions of the ACC deaminase gene (acdS) can be induced under low relative concentrations of ACC (100 nM), much-like other amino acids including DL-alanine, L-alanine, and DL-valine [57] (**Figure 2**). The expression of acdS is also regulated by the nitrogen fixation (nif) promotors of most Rhizobia strains, with links ACC deaminase with the process of nodule formation [61, 62].

The amino acid methionine is converted to S-adenosyl-L-methionine (SAM) by SAM-synthetase (SAMS) with the requirement of ATP. The general precursor SAM is then converted to ACC by ACC-synthase (ACS). This reaction also involves the cleavage of 5′-methylthioadenosine (MTA), which is recycled back to methionine by the Yang cycle (dotted line indicates multiple enzymatic steps). ACC can be converted to ethylene by ACC-oxidase (ACO) in the presence of oxygen. ACC can also be converted to its major conjugate 1-malonyl-ACC (MACC) by the yet uncharacterized ACC-N-malonyl transferase (AMT) with the requirement of malonyl-Coenzyme-A. A second derivate of ACC is γ-glutamyl-ACC (GACC) which is formed by γ-glutamyl-transpeptidase (GGT) with the requirement of glutathione (GSH). Another novel

derivate of ACC is jasmonyl-ACC (JA-ACC), which is formed by jasmonic acid resistance 1 (JAR1). ACC can also be metabolized by the bacterial (and plant) ACC deaminase into ammonium and α-ketobutyrate [63].

6.7 Soil viral infection

Legumes (form symbiotic relationships with nitrogen fixing *Rhizobia* spp) are of major economic importance worldwide but are damaged by viral diseases causing significant economic losses as a result of reduced grain production, seed-quality and costs incurred during disease-control. Most viruses infecting legumes are transferred by insect-vectors, or via seed. Viral infections in plants are currently incurable due to unavailability of antiviral compounds, only the use of efficient measures of control or agents that can suppress viral-activity can be employed to prevent or reduce their effects. Symptoms of infection in Legumes: flowering, leaves, podding, seedling stages, nodule formation, reduced yield and productivity (**Figure 3**).

Recent researches show that virus-carrying-aphids induced salicylic-acid in plants, affecting the nitrogen-fixing-ability of rhizobia in the plants resulting in fewer root nodules and nodule biomass. But fewer essential amino-acids were obtained in the aphids that were collected on *P. sativum* plants inoculated with rhizobia than aphids collected from *P. sativum* plants grown without rhizobia inoculation. Also, in faba-bean plants previously inoculated with rhizobia there was a reduction in disease-occurrence in plants infected with BYMV (bean yellow mosaic virus) when studied using ELISA reads but the volatile emissions from the plant host were suppressed (**Figure 3**).

Rhizobia may be useful as plant-pathogen fighters providing defense against viruses and protect leguminous plants, ultimately reducing susceptibility and damage-effects in plants. In addition, the manipulation of the host by the virus may be dependent on the physiological condition of the host. These mechanisms are not yet well understood and more work needs to be done to discover the interaction between Rhizobia and soil virome in leguminous plants resistance to viral infections.

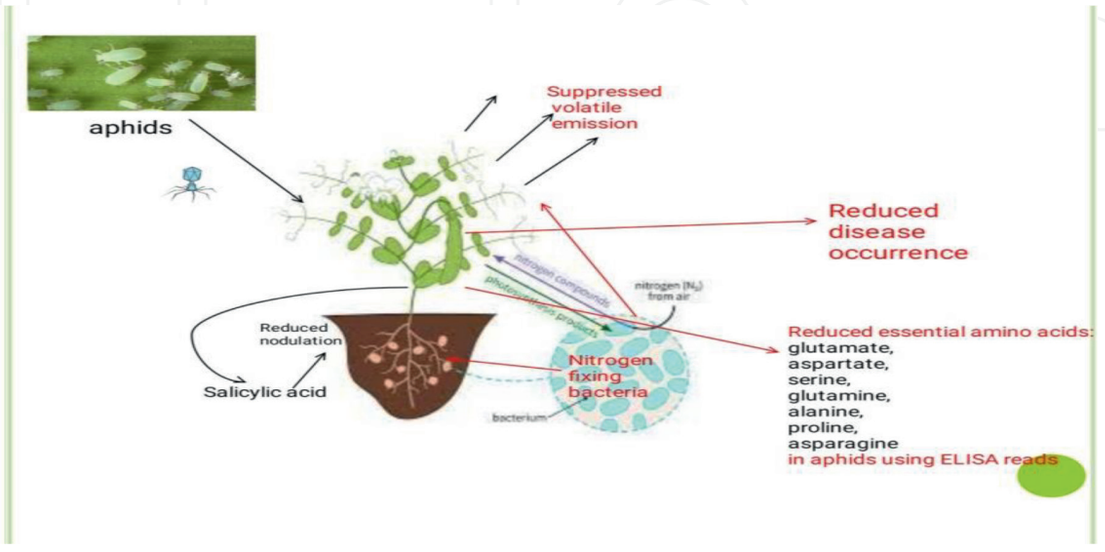


Figure 3.
Soil- plant- virus interaction.

6.8 Other factors

Other factors that are affected by PGPRs are seedling health, fight against diseases, drought tolerance, germination rate. Bacterial inoculation contributes substantially to increasing the agronomic efficiency by reduced production costs and pollution to the environment, reduced or complete elimination of use of chemical fertilizers can be accomplished with inoculant efficiency. To achieve success with the use of bacterial inoculants in improved plant growth and production in plants, many processes which ultimately influence the efficiency of inoculation are involved, these includes the release of plant root exudates, the root colonization by bacteria and soil health. This review gives an overview of how important soil-plant-microbe interactions are for developing efficient bacterial inoculants, and the extensively studied PGPRs that represents a large diversity group of easily accessible beneficial bacteria [37–39].

7. Plant analysis to check the activities of plant growth promoting bacteria in the soil and their effect on the plant

7.1 Determination of chlorophyll

Leaf chlorophyll content is determined determined using a chlorophyll meter SPAD- 502 this can be done weekly starting from the 2nd–3rd week [64]. The measurments are recorded in SPAD units. This is done by placing the leaf in-between the teeth of the meter which has a sensor the measures the chlorophyll based on the color and transpiration rate. Three leaves per plant including the youngest the oldest and the matured leaves [4, 15]. chlorophyll content can also be measured traditionally in the laboratory by extracting the chlorophyll itself from the leaf sample using acetone before calculating the chlorophyll concentration can be measured invitro by using a spectrophotometer at absorption of 663 nm and 645 nm [65, 66]. Its unit are recorded as nmol/cm, mg/L, mSPU (m specific pigment unit), μmol m-2 (micromoles per square meter), mg m-2 (milligrams per square meter).

7.2 Biomass determination harvesting

To determine the biomass of the plant, the plants are harvested between 4 and 8 weeks after planting (WAP) (depending on the plant type), by cutting at the base with a secateurs. The shoots and roots are separated, collected and put inside labeled paper bags before being placed in an oven were they were dried at 68°C for 72 hours or until constant weights are obtained [4].

7.3 Determination of nitrogen

Plant samples (shoot, root) are collected and dried in an oven at 68°C for 2 days or brown dry. The samples are weighed and then grinded to 1 mm using a grinder after which they are packed in small white envelops and transferred to the laboratory and stored under dry conditions. Approximately 0.1 g of the shoot samples is weighed into digest tubes. To digest the samples the following steps are carried out addition of 2.5 mL of acid digest solution to the samples while stirring the solution of the acid digest. Samples are placed in a fume cupboard and 3 mL of H_2O_2 is added to it. The digest tubes are arranged in a digester and the temperature is set to 360°C and heated for 3 hours.

Samples are checked to see if they had turned white (those that were not white can be left for 1 hr. more) and then removed. The digested samples are allowed to cool and 50 mL of distilled water is added. The sample solution is then poured into centrifuge tubes. 10 mL of the digested sample is taken and added to a 50 mL standard volumetric flask the volume was made up to 50 mL. It was allowed to stay for 1 hour and the readings were taken using a spectrophotometer at 650 nm [67].

7.4 Determination of nutrients in plant shoots

The total Nitrogen in plant shoots can be determined using the micro Kjeldahl method [15, 67] while phosphorus is determined by the molybdenum blue method as described by [68]. The concentrations of Ca, K, Na and Mg in plant shoot and root can be determined using the method as described by [15, 69]. Un-inoculated plants or plant with control PGRP inoculation e.g., N^+ treatment can be used as reference plants for comparison. Dried plant samples are digested (as described above) after which they can be used for detections of metals such as sodium and calcium using a flame photometer while the detection of nutrients such as potassium and magnesium are done using an atomic spectrometer from the diluted digested samples.

7.5 Determination of phosphate solubilization

Pakovskaya medium is used for the determination of the ability of the selected strains to solubilize calcium phosphate (Ca_3 $(PO_4)_2$). Pakovskaya's agar [70] was composed (Glucose-10 g, Yeast extract-0.5 g, Ammonium sulphate-0.5 g, Magnesium Sulphate 0.1 g, Calcium Phosphate-5 g, KCl-0.2 g, $MnSO4.7H_2O$-0.0001 g, $FeSO_4$.7H2O-0.0001 g, Agar powder-20 g). The medium is autoclaved at 121°C for 15 min allowed to cool to 45°C, poured into petri dishes and allowed to set. Broth (10 μL) of bacteria with concentrations of $\leq 10^7$cfu/ml are spotted on the plate in triplicates and incubated at 30°C for 7 days. Isolates with visible clear halos around the colonies are considered to be able to solubilize calcium phosphate. The phosphate solubilization index (PSI) can be calculated by dividing the halo diameter formed by the bacteria (colony diameter + diameter of the clear zone around the colony, B) by the colony diameter (b) of the bacteria itself as shown in **Figure 4**. Tests are done using three–four replicates.

7.6 Indole-acetic acid (IAA) production

The production of IAA production can be determined using the method of Gordon and Weber [71] with three replicates. The isolated bacteria strains are cultured in 50 ml Laria Broth (LB) for 2 days in a rotary shaker. The broth is then collected and centrifuged at 10,000 × g for 15 min and the clear supernatant is collected. The supernatant (1 ml) is mixed with 2 ml of Salkovski reagent (1 ml of 0.5 M ferric chloride solution added to 50 ml of 35% perchloric acid (HClO4)), and 10 μl (a drop) of the ortho-phosphoric acid is added. The solution is incubated for 30 min. Pink color change is used to determine positive IAA production. The absorbance of the pink (positive) samples is measured at 530 nm using a spectrophotometer to evaluate the amount of IAA produced. A standard curve is prepared from pure IAA with concentration points ranging from 5 to 50 $\mu g.ml^{-1}$ (or ppm) from the prepared standard concentrations. A standard graph is prepared by plotting the IAA concentrations against their respective optical density (OD530). The linear equation

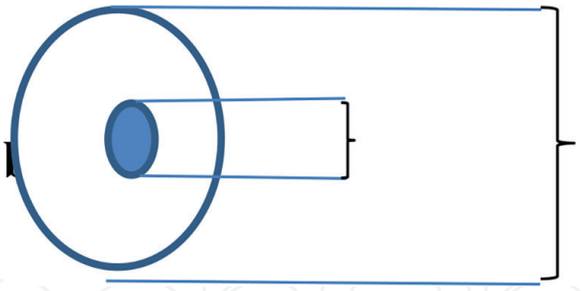


Figure 4.
Phosphorus-solubilizing and parameters for the determination of bacteria PSI (right).

obtained can be used to determine the concentration of IAA in samples from their optic density at 530 nm. IAA sample (μg/ml) = (OD530-(−0.0052)/0.0016). IAA can also be determined using HPLC (high performance liquid chromatography), liquid chromatography, mass spectrometry and GC (gas chromatography). IAA can also be analyzed by gas chromatography-mass spectrometry (GC-MS) using selected-ion monitoring (SIM).

7.7 Nitrogen fixation assay

The amount of nitrogen fixed can be determined using Uriede analysis. Stems and stem segments of the plant is harvested and leaves are collected and placed in properly labeled bags and then dried at 65–80°C in an air oven for 2 days. The dried plant tissue are grounded, passed through a 60-mesh (1.0 mm) screen and then stored in a dry place until extraction. Approximately 0.5 g of the dry grounded material is weighed and transferred into 100 mL beakers or Erlenmeyer flasks to which 25 mL of distilled water is added and boiled for one to 2 minutes. The extract is then filtered while it was hot through a 15 cm Whatman No. 4 filter paper folded into a funnel inserted into a 50 mL volumetric flask and the residue is washed into the filter and rinsed with a little distilled water. The residue is allowed to cool, and the volume made up to 50 mL with distilled water. The extract are then stored in a freezer in small vials until analysis of N solutes.

Into the extract an amount of 0.5 mL of 0.5 N sodium hydroxide (NaOH) is added (forcefully that additional mixing is unnecessary) using a Dispenser and placed in a rack of tubes in a boiling water bath for 10 mins after which 1.0 mL HCl/phenylhydrazine mix is added using a second Dispenser and placed in the boiling water bath for exactly 2 minutes. It is then removed and immediately plunged into an ice bath and left for 15 minutes after which 2.5 mL of cold HCl/$K_3Fe(CN)_6$ is added using a Dispenser, thoroughly mixed and left on the bench for 10 mins after which the optical density is read at 525 nm on a spectrophotometer. A standard curve which covers the 0–0.10 mM allantoin range can be constructed from time to time as part of the standard laboratory's quality control using 2.5 mL of each of the five different concentrations in test tubes using 2 replicates corresponding to 0, 0.025, 0.05, 0.1, and 0.25 μmol of Ureide [72].

7.8 Determination of abscisic acid (ABA) and ethylene production

ABA can be isolated from mature seeds and from plant leaves of at different stages of growth. The seeds or leaves are dried and grinded into powder and 10 g (using

three replicates) are extracted and homogenizied using 40 mL of 80% aqueous methanol for 30 min at 4°C. The mixture is filtered using a conical flask (for each sample) using the filter paper (size No. 1. Whatman). The filtrate is then subjected to vaccum evaporation in a lyophilizer and the dried vacuumed residue is re-dissolved into 10 mL of 0.5 M phosphate buffer (set to pH 8) afterwhich it is stirried for 30 min. The resulting suspension is washed with 20 mL light petroleum sprit and pH re-adjusted to 2.8 (with dil. HCl), and the extraction is done four times using ethyl acetate (4 × 10 mL). The extract is then lyophilized and re-dissolved using 5 mL buffer of 0.5 M phosphate (pH 8). The sample can then be purified by passing it through a sephadex G10 column. To prepare the Sephadex column, Sephdex (1 g) is allowed to swell in 10 mL of distilled water (double) over night before it is packed into a glass column (1 cm diameter and 5 cm length) up to 2 cm length and the column should be equilibrated using a phosphate buffer. The eluted solution can then be lyophilized again and the resulting residue dissolved using 1 mL of acetonitrile and analyzed by HPLC [73]. ABA can also be analyzed by gas chromatography-mass spectrometry (GC-MS) using selected-ion monitoring (SIM). Three main categories of methods commonly used (or having the potential to be used) for ethylene detection in plants: (1) GC detection [74, 75], (2) electrochemical sensing [76] and (3) optical detection [77].

8. Conclusion

Microbiomes are a very essential part of the plant growth and health and they govern most soil functions affecting the growth of plant directly or indirectly. There is a need for higher crop yield production in our current world as well as fertility of soil in an ecofriendly manner and this requires research that is focused on the new concepts of the exotic biomolecules, hormones, enzymes and metabolites which create a suitable environment for the interaction between plant and microbes using molecular and biotechnological approaches to increase our knowledge of rhizosphere biology and to achieve an integrated management of soil microbial populations.

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