Chapter 5

Making Soil More Accessible to Plants: The Case of Plant Growth Promoting Rhizobacteria

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http://dx.doi.org/10.5772/64826

Abstract

Plant Growth Promoting Rhizobacteria (PGPR) are beneficial soil bacteria that can live either symbiotically with plants at rhizosphere or as endophytes living on or inside of the host plants. There are two main mechanisms via PGPR contribute to the plant growth. Direct mechanism consists of phytohormone production (i.e. auxins (IAA), cytokinins and gibberellins), biological nitrogen fixation, solubilizing inorganic phosphates, mineralizing organic phosphate and producing organic matter such as amino acids. As indirect mechanisms, PGPR aid plants in combat against the pathogen microorganisms by means of stimulating the disease-resistance mechanism of plants, promote favorable symbiosis, decontaminate the soil of xenobiotics. PGPR can also help plants to cope against abiotic stress by lowering ethylene levels, or against pathogenic microorganism by means of secreting antibacterial/antifungal substances. Exact mechanisms of PGPR characteristics which stimulate the plant growth or product formation are still under investigation, yet in agriculture, PGPR are used as environmental friendly biofertilizers, biocontrol agents or biostimulants. These beneficial bacteria are usually introduced to the plants either in powder or liquid form or the seeds are covered with the inoculants before sowing. Plants are subject to many different environmental elements. Abiotic factors such as drought or water stress have been one of the main plant growth limiting factors. Agricultural PGPR application is an alternative solution against loss due to the environmental stresses, since breeding a plant with stress resistance trait is a very long and tricky process due to the fact that such traits are controlled by multiple genes. PGPR phytohormone and enzyme (i.e. ACC deaminase) production can decrease the stress levels of plants while enhancing the root structures.

Keywords: plant growth–promoting rhizobacteria, enzymes, plant nutrient use efficiency, nitrogen fixation, phosphorus solubilizing, plant stress

1. Introduction

Soil is a composed of minerals, organic matters, water, and microorganisms and it covers the surface of the earth. Soil not only provides an attachment surface for plants but also the necessary materials for their growth. It also acts as host to many types of bacteria. The number of bacterial species living in the soil varies according to the environmental conditions such as temperature of the soil, amount of salt, chemicals, and moisture in the soil, and plants growing nearby in the soil [1]. Bacteria are usually found abundant around the rhizosphere. The term "rhizosphere" was first coined by Lorenz Hiltner in 1904 to define the layer of soil around the plant root that is populated by microorganisms. The relationship between the plant and the soil bacteria can be beneficial, harmful, or neutral according to the environmental conditions surrounding the plant [2]. For example, bacterial species that has the trait to increase phosphate solubility of the plant can only be beneficial when the plant is growing on a phosphate-poor soil. When the phosphate is given to the plant as fertilizer, the bacterium species becomes neutral from the plant point of view.

The human population has been increasing rapidly and the industrialization grows accordingly. This contributes to the fact that not only the current food sources would not be enough even every condition would stay the same but also industrialization has very negative effects on the environment, such as decrease in the available land for agriculture available land, global warming, and air and water pollution. New strategic solutions should be addressed to improve agricultural yields and sustainability so that the food requirements for the human population will be met with the lowest environmental impacts. A likely solution can be the use of "plant growth promoting rhizobacteria (PGPR)", soil bacteria which colonize at the rhizosphere of the plants stimulating the plant growth. The term PGPR was first coined by Kloepper and Schroth in 1978. The type of PGPR is directly related to the products exudate by the plant root such as sugars, organic acids, and proteins. Understanding how the plant chooses which type of PGPR would form the microbial community in the rhizosphere would give us insight when choosing PGPR inoculants for increased plant crop yields and is a major scientific issue [3].

2. Mechanisms of PGPR

There are numerous types of bacteria which have been observed to possess at least one PGPR trait such as *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, and *Bacillus*. These are commercial inoculant species used as biofertilizers which enhance the crop yield, bioprotectors which defend the plant against pathogens and biostimulators which produce phytohormones beneficial to the plant [4]. The

PGPRs are offering a cheaper and more environmental friendly option compared to using chemical pesticides, herbicides, and fertilizers [5]. They affect the plants in couple of different mechanism, yet none of them is well understood to this day.

2.1. Direct mechanisms

2.1.1. Plant growth substances

Phytohormones, another name for plant growth substances, are plant hormones or messengers that influence the plant's response to its environment. These organic compounds are produced in one part of plant in a very low amount and carried into the other locations of the plant [4]. The physical responses gained by these hormones are ripening or growth of roots and leaves.

There are five main types of phytohormones: auxins, gibberellins, ethylene, cytokinins, and abscisic acid. PGPR usually produces cytokinins, gibberellins, and IAA as phytohormones.

Cytokinins are compounds whose structure is similar to adenine. As the name suggests this hormone induces cytokinesis (cell division) in plants thus involves in growth, root initiation, increase in root surface area [6, 7]. This hormone can be synthesized by plant, some PGPRs, and yeast strains [8]. Some phytopathogens are also reported to synthesize cytokinins, but the amount of the produced hormone regulates whether it promotes or induces plant growth. Various bacterial strains of *Azotobacter* spp., *Rhizobium* spp., *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Paenibacillus polymyxa* are recorded to produce cytokinins [9].

Gibberellin, another phytohormone is also synthesized by some cytokinin-producing PGPR. Gibberellin has a role in flowering, germination, dormancy, sex expression, and plant growth. The gibberellin and cytokinin mechanisms for bacterial production and regulations are now fully understood. Thus, the known effects of these hormones come from the plant physiological knowledge [10].

IAA (indole-3-acetic acid) is the most significant and most studied auxin produced by plants [9] and PGPR which has role in cellular responses such as cell division, organogenesis, gene expression, pigment formation, seed germination, root development, photosynthesis, and tropic responses (such as to gravity and light) [9, 11]. IAA also has role in stress resistance of plants [12]. Like cytokinin, the amount of IAA can be both inhibitory and stimulatory. The amount of IAA that required for the plant growth promotion is influenced by the plant species and the bacterial species [4]. Since IAA is responsible for root formation and lengthening, one of the effects of IAA on plants is increasing the amount of nutrients by the root and the amount of exudation from the root [13]. The increase in exudations promotes the increase in biomass of PGPR and the nodule formation in the rhizosphere [14].

Phytohormone ethylene is responsible for ripening of fruit, promoting root growth, activation other phytohormones, inhibiting formation of *Rhizobia* spp. nodule formations. It is also synthesized when the plant is faced with biotic and abiotic stresses [15].

2.1.2. Biological nitrogen fixation

Nitrogen is an essential element for life as it is present in the structures of important biochemicals such as proteins and nucleotides. Although the air is rich with N_2 (g), plants, and many other complex organisms cannot use nitrogen in this form. Biological nitrogen fixation (BNF) process by N-fixing bacteria produces the ammonia which can be used by plants as a nitrogen source. Plants biomass and product yields are limited by the amount of nitrogen available, thus applications of N-containing fertilizers is heavily used in agriculture. The downside of using chemical fertilizers is they are expensive and have negative impact on environment. Using PGPR and providing needed nitrogen by the BNF can be an alternative way to increase agricultural yield [16, 17].

Biological nitrogen fixation fortunately not limited to the PGPR that forms symbiotic nodules with legumes, but there are nonsymbiotic free living nitrogen fixing bacteria as well. *Azospirillum*, *Azoarcus*, *Azotobacter*, *Bacillus polymyxa*, *Burkholderia*, *Gluconoacetobacter*, or *Herbaspirillum* are such bacterial species reported to have PGPR properties [18].

2.1.3. Phosphate solubilizing bacteria (PSB)

Nitrogen is not the only crucial element for life which can limit the plant growth. For example, phosphorus is also essential for the plants. Soil holds large amounts of phosphate, yet it is found in insoluble form. Some PGPR are reported to solubilize the phosphate in the soil through acidification, chelation, or enzymatically [19] *Gluconacetobacter diazotrophicus* is a PGPR native to sugarcane that has the property to solubilize phosphate via acidification [20].

2.2. Indirect mechanisms

2.2.1. Biocontrol via antibiotics and lytic enzymes

It has been known that, microorganisms compete against each other for nutrients, colonization sites in their natural environments. Many PGPR species evolved mechanism to reduce competition such as releasing of antibiotics, lytic enzymes, or weak organic acids to the environment. This characteristic of PGPR makes it a valuable tool against plant pathogens [18]. Yet, increase in the usage of antibiotic producing bacteria might result in development of resistant pathogens.

The enzymes that PGPR secrete to eliminate pathogens such as *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Phytophthora* spp., *Rhizoctonia solani*, and *Pythium ultimum* are chitinases, cellulates, proteases, and lipases which can destroy the cell walls of the pathogens [9].

2.2.2. Induced systemic resistance (ISR)

Induced systemic and systemic acquired resistances are response mechanisms that plants evolved against pathogens. Unlike systemic acquired resistance (SAR) which is triggered by infection by a pathogen, in ISR, the trigger is a PGPR which will make the plant resistance to

http://dx.doi.org/10.5772/64826

phytopathogens. ISR starts at the root and spreads to the shoots [21]. This phenomenon was first observed in 1991 by van Peer et al. They infected *Arabidopsis thaliana* plant root with nonpathogenic *Pseudomonas* spp. and found out the rest of the plant also gained resistance to pathogenic bacteria. Since this discovery, ISR has been studied in many plants such as bean, tobacco, and tomato [18]. Plants with ISR response react to pathogenic bacteria faster and stronger. It should be also noted that ISR response is not pathogen-specific and can be used to stimulate plant immune response against more than one pathogen species [9].

2.2.3. Siderophore production

Iron is another essential nutrient for plants. In aerobic conditions, iron is found as Fe^{3+} form which is not soluble for microorganisms and plants. Some microorganisms produce and secrete low mass iron chelators. These chelators are called siderophores and have high affinity for iron. These operate as solubilizing agents for Fe^{3+} in limiting conditions. Fe^{3+} becomes Fe^{2+} for while entering the cell membrane and then unbind from the siderophores inside the cell [22].

Siderophore production is also observed to be a biocontrol mechanism, since with this process, PGPR derives other microorganisms from iron. PGPR also reported to use siderophores to obtain other heavy metals (such as arsenic) from the soil and prevents the heavy metal toxicity in plants [23]. This characterization can be used for bioremediation of the heavy metal toxic soil as well.

2.2.4. Regulation of stress conditions

Ethylene is a phytohormone which is also secreted as response to biotic and abiotic stresses such from salt, drought, or pathogenic bacteria. Although promoting growth and ripening of fruits, in high amounts ethylene have harmful effects on the plant. Many PGPRs synthesis an enzyme called ACC deaminase, which destroys the precursor of ethylene called 1-aminocyclopropane-1-carboxylate (ACC), thus decreasing the ethylene levels and relieving the stress of the plant [24].

Some PGPRs which do not have the ability to produce ACC deaminase, can also promote the growth of plants via secretin of IAA even though other inhibitory factors are found in the environment [9].

3. Examples of PGPR

3.1. Symbiotic PGPR: rhizobacteria

Rhizobacteria are soil bacteria which colonize at the root of legumes forming nodules. They fix the atmospheric nitrogen for the plant benefit in exchange for carbon source. Rhizobacteria are the most known PGPRs. Inoculation with rhizobacteria provides biomass increase in legumes [4, 9].

Rhizobacteria are host-specific bacteria, meaning that they will not form rhizosphere nodules with any type of plants. The most common rhizobacteria are Rhizobium and Bradyrhizobium. They are both Gram-negative, rod-shaped (bacilli) bacteria. Rhizobium forms symbiotic nodule with vetches, peas, lentil, clovers, and beans [4].

3.2. Nonsymbiotic PGPR

Fortunately, nitrogen fixation is not limited to Rhizobacteria. There are many free-living species which can also perform biological nitrogen fixation.

Some important nonsymbiotic nitrogen-fixing bacteria include *Azoarcus* sp., *Gluconacetobacter diazotrophicus*, *Herbaspirillium* sp., *Azotobacter* sp., *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum*, *RhodoPseudomonas*, and *Xanthobacter* [25].

Applications of *Azotobacter* and *Azospirillum* species are reported to increase yield of grass type of crops. Although Azospirillum has been isolated from cereal initially, it has been used to inoculated noncereal crops more frequently. It is stated that Azospirillum bacteria is not a host-specific species but a general root colonizer [4].

The family *Acetobacteriaceae* includes genera, *Acetobacter*, *Gluconobacter*, *Gluconoacetobacter*, and *Acidomonas*. *Gluconacetobacter diazotrophicus* is an acid tolerant, Gram-negative and obligate aerobe bacteria. The bacteria cells can grow on high sucrose content and low acidity. The optimum sugar concentration is 10% and pH is 5.5 for growth although its recorded that they can live at pH 3. It is an endophyte; located at the internal tissues of its host [16].

4. Conclusion

Plant growth promoting rhizobacteria are being intensify researched to increase crop yields, to protect the plants and stimulate the plant growth via phyhormone production. Even though the mechanisms behind PGPR characteristics are not fully discovered, there are many commercialized PGPR strains which are used as agricultural inoculants. These strains are *Agrobacterium radiobacter*, *Azospirillum brasilense*, *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus mucilaginous*, *Bacillus pumilus*, *Bacillus* spp., *Bacillus subtilis*, *Bacillus subtilis* var. *amyloliquefaciens*, *Burkholderia cepacia*, *Paenobacillus macerans*, *Pantoea agglomerans*, *Pseudomonas aureofaciens*, *Pseudomonas chlororaphis*, *Pseudomonas solanacearum*, *Pseudomonas* spp., *Pseudomonas syringae*, *Serratiaentomophilia*, *Streptomyces griseoviridis*, *Streptomyces* spp., *Streptomyces lydicus*, and various *Rhizobia* spp.

The inoculation of agricultural plants with PGPR still makes the minor fraction of crop enhancement methods. To increase the application of PGPRs, the mechanisms that are unknown should be studied, the differences and advantages of using nonsymbiotic PGPR over rhizobacteria species should be determined. The production and storage of the PGPR inoculants should be addressed.

http://dx.doi.org/10.5772/64826

Due to the climate and soil composition, there are inconsistencies between the greenhouse trials' results and the field trials should be minimized. A computational approach can be used to find the interaction of plant and PGPR and simulate physiological responses under certain environmental conditions.

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