INDUCED SYSTEMIC RESISTANCE BY PLANT GROWTH-PROMOTING RHIZOBACTERIA IN CONTROL OF PLANT DISEASES

H. Handan Altinok 1,*, H. Nilüfer Yıldız 2

1 Erciyes University, Faculty of Agriculture, Department of Plant Protection, 38039, Kayseri, Turkey
2 Biological Control Research Institute, 01321, Adana, Turkey

Abstract
Plant Growth Promoting Rhizobacteria (PGPR), colonizing in rhizosphere of plants are able to promote plant growth as well as provide protection against diseases by triggering the defense mechanisms of plants. Bacillus, Pseudomonas and Streptomyces species are licensed as biocontrol agents and/or biological fertilizers and successfully used to control plant pathogens, as a part of integrated disease management. Seed and soil applications of PGPRs are increasing both germination ability and plant resistance to pathogenic microorganisms. Salicylic acid (SA), Jasmonic acid (JA) and ethylene (ET) signaling components are playing an important role on regulation of resistance of plants against various pathogens. SA plays role on pathogen-induced systemic acquired resistance (SAR), while JA and ET take place as key regulators in induced systemic resistance (ISR) promoted by rhizobacteria. Both forms of induced resistance are effective against wide range of pathogens. Several potential defense mechanisms like chitinase, β-1,3 glucanase, pathogenesis-related proteins, phytoalexin accumulation, lignin, callose and hydroxyprolin-rich glycoprotein, protective biopolymer coating are activated in ISR. Siderophores produced by Pseudomonas are able to prevent germination of fungal pathogen spores by binding the iron needed by pathogen. In previous studies, PGPR strains able to fix nitrogen, dissolve phosphate, showing protease activity and produce siderophores and hydrogen cyanide are found to be successful on control of some fungal and bacterial diseases by triggering an increase in synthesis of peroxidase and catalase defense enzymes. This study focused on the roles of PGPRs in ISR.

Keywords: Biological control, Induced systemic resistance, Rhizobacteria.

1. INTRODUCTION
Rhizosphere region contains large amount of soil microorganisms because of stimulation by root activities. These microorganisms include bacteria, fungi, protozoa and algae. Bacteria are the most abundant of them and it is highly probable that they influence the plant physiology to a greater extent, especially considering their competitiveness in root colonization (Antoun and Klopper, 2001; Barriuso et al, 2008). The bacteria inhabiting the rhizosphere and beneficial to plants are termed as Plant Growth Promoting Rhizobacteria (PGPR) (Klopper et al., 1980). Bacterial populations in upper layers of the soil can contain as many as 10^9 cells per gram of soil (Torsvik and Ovreas, 2002). PGPR can affect plant growth by different direct and indirect mechanisms (Glick, 1995). PGPR influence direct growth promotion of plants by fixing atmospheric nitrogen, solubilizing insoluble phosphates, secreting hormones such as indole acetic acid (IAA), gibberellic acid (GA), and kinetins besides ACC (1-Aminocyclopropane-1-carboxylic acid) deaminase production which helps in regulation of ethylene (Glick et al., 1995). Induced systemic resistance
(ISR), antibiosis, competition for nutrients, parasitism, production of metabolites (hydrogen cyanide, siderophores) suppressive to pathogens are some of the mechanisms that indirectly benefit plant growth. Various species of bacteria like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance the plant growth antagonistic effects and their ability to trigger ISR (Kloepper et al., 1989; Joseph and Lawrence, 2007). As PGPR *P. fluorescens*, *P. putida*, *P. aeruginosa* are found to have disease control potentials (Weller et al., 2002). *Bacillus* sp. have reported to be effective on root diseases (Sivasakhti et al., 2014). Their effect was due to being easy colonizers of the soil by producing spores. PGPR may use combinations of different mechanisms of action, leading to a more efficient use for biocontrol strategies to improve cropping systems. Plant growth promoting rhizobacteria are environmentally beneficial for reducing production cost such as fertilizers and best soil and crop management practices to achieve more sustainable agriculture.

2. PGPR: DIRECT MECHANISMS OF ACTION

2.1. PGPR: Biofertilizer

Direct growth promotion of plants by PGPR is fixing atmospheric nitrogen, solubilizing insoluble phosphates, secreting hormones which helps in regulation of ethylene. PGPRs as a biofertilizer promote plant growth by improving the nutrient uptake of the plant. They act as a biofertilizer by increasing the nutrition status of the host plant via root association. Nitrogen is an essential element for plant growth and productivity. It is found in the atmosphere (78%) but plant species are not capable of fixing atmospheric nitrogen into soils for their growth. The atmospheric nitrogen is converted into utilizable forms by biological nitrogen fixation (BNF). Nitrogen fixing microorganisms change nitrogen to ammonia by using a complex enzyme system known as nitrogenase (Gaby and Buckley, 2012). Plant growth promoting rhizobacteria have the ability to fix atmospheric nitrogen. Fixed nitrogen contribute to the nitrogen account of the plant. Bacteria such as *Enterobacter*, *Klebsiella*, *Burkholderia*, and *Stenotrophomonas*, have been gaining attention in the recent years, because of their association with important crops and potential to enhance the plant growth (de Freitas, 2000). N-fixing bacterial strains have a potential on plant growth activity in organic and low-N input agriculture (Canbolat et al., 2006). Hydrogen cyanide (HCN) is produced by many rhizobacteria and plays a role in biological control of pathogens (Defago et al., 1990, Anith et al., 1999; Kremer and Soussi, 2001). Fluorescent pseudomonads located in the rhizosphere region of plants were shown to enhance plant growth and suppress pathogens by HCN production (Shivani et al., 2005; Ramette et al., 2006). HCN synthase activity of fluorescent pseudomonads is found to be encoded by three biosynthetic genes. Phosphorus is present in soil in insoluble form that can not be utilized by plants. Organic substrates in soil can be a source of P for plant growth. Most of phosphate fertilizers are reprecipitated into insoluble mineral complexes and are not efficiently taken up by the plants. Microbial solubilization of inorganic phosphate compounds is of great economic importance in plant nutrition (Gaur, 2002). Some bacterial species have organic and inorganic phosphorus mineralization and solubilization capacity (Khan et al., 2007). Phosphorus solubilizing bacteria, mainly *Bacillus*, *Pseudomonas* and *Enterobacter* are very effective for increasing the plant available P in soil as well as the growth and yield of crops (Tripura et al., 2005). Mineralization of most organic phosphorous compounds is carried out by producing enzymes like phosphatase, phytase, phosphonoacetate hydrolase, D-α-glycerophosphate and C-P lyase (Hayat et al., 2010).

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*Corresponding author, E-mail address: ahandan@gmail.com*
1.2. PGPR: Phytohormon production

Plant-growth promotion by PGPR include bacterial synthesis of the plant hormones of indole-3-acetic acid, cytokinin, and gibberellin and breakdown of plant produced ethylene by bacterial production of 1-aminocyclopropane-1-carboxylate deaminase.

Auxins synthesized by the plant and the microorganisms principally affect plant roots. Soil bacteria in the rhizosphere are mostly (80%) capable of producing auxins. Those released by rhizobacteria mainly affect the root system, increasing its size and weight, branching number and the surface area in contact with soil. All these changes lead to an increase in its ability to probe the soil for nutrient exchange, therefore improving plant nutrition and growth capacity (Barriuso et al., 2008). Diverse bacterial species produce auxins as part of their metabolism including indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) or their precursors.

Indole acetic acid (IAA) is the most common natural auxin found in plants. IAA have positive effect on root growth (Miransari and Smith, 2014). Most of the rhizobacteria can synthesize IAA by colonizing on the seed or root surfaces and enhance the host’s uptake of minerals and nutrients from the soil (Vessey, 2003). Indole acetic acid also affects plant cell division, extension, and differentiation; stimulates seed and tuber germination and also increases the rate of xylem and root development; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions (Spaepen and Vanderleyden, 2011)

Ethylene is a plant growth hormone produced by almost all plants. It is also produced in soil by various biotic and abiotic mechanisms. Ethylene, which is plant growth regulator and stress hormone, also plays a key role in physiological changes in plants at molecular level. Plant growth promoting rhizobacteria (PGPR) mostly contain a vital enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which regulates the ethylene production by metabolizing ACC (an immediate precursor of ethylene biosynthesis in higher plants) into α-ketobutyrate and ammonia (Babaloa, 2010). Inoculation with PGPR containing ACC deaminase activity could be helpful in sustaining plant growth and development under stress conditions by reducing stress-induced ethylene production (Porcel et al., 2003).

Abscisic acid (ABA) is one of the most important phytohormones by playing an important role in many physiological processes in plants. This hormone is crucial for the response to environmental stresses such as desiccation, salt and cold. Some PGPR especially Bacillus species were found to have ABA synthesize ability (Porcel et al., 2003).

Gibberellins are the largest group of plant regulators, including more than 100 different molecules with various degrees of biological activity. They can be translocated from the roots to the aerial parts of the plant. Gibberellin producing bacteria also produce auxins that stimulate the root system, enhancing the nutrient supply to the sink generated in the aerial part of the plants (Atzorn et al., 1988).

Cytokinins are purine derivatives that promote and maintain plant cell division and are also involved in various differentiation processes including shoot formation, primary root growth and callus formation. Plants continuously use cytokinins to maintain the pools of totipotent stem cells in their shoot and root meristems. Auxins and cytokinins interact in the control of many important developmental processes in plants, particularly in apical dominance, and root and shoot development. The balance between auxin and cytokinin is a key regulator of in vitro organogenesis. Some bacterial species are found to stimulate cytokinin production levels (Leibfried et al., 2005).
3. PGPR: INDIRECT MECHANISMS OF ACTION

3.1. BIOLOGICAL CONTROL

The mechanism of action of the PGPR in the promotion of plant growth depends on efficient plant root colonization. In general, their function by preventing plant pathogens “Biocontrol”, facilitating the uptake of certain nutrients from the soil “Biofertilizer” and synthesis of phytohormones “Biostimulants” (Glick et al., 1995; Bhuvaneshwari and Kumar, 2013). In recent years, PGPRs has been used based on their direct essential compound supply capacity to plants or indirectly by inhibiting the phytopathogenic organisms (Glick, 2015). These PGPR mechanisms are presented in Figure 1.

PGPR are the soil bacteria inhabiting function by preventing plant disease conditions “Biocontrol”, for nutrient turn over and sustainable for crop production “Biofertilization” and synthesizing phytohormones “Biostimulants” (Glick et al., 1995; Ahemad and Kibret, 2014). For PGPR species to be an effective biocontrol agent against different phytopathogens, it must utilize some of the following mechanisms; suppress the growth of pathogens, production of siderophores, antibiotics, biocidal volatiles, competition for nutrients and niche, signal interference, hydrogen cyanide and lytic enzymes production and the ability to induce systemic resistance (ISR) (Podile and Kishore, 2006; Lugtenberg and Kamilova, 2009). Rhizobacteria as a component in integrated management systems in which reduced rates of pesticide are used as biocontrol agents.

Non-pathogenic rhizobacteria can suppress disease by various mechanisms of action e. g. antagonism, competition for space and nutrient with pathogens in the rhizosphere, production of some volatile metabolites, production of cell walls degraded molecules and “Induced Systemic Resistance” (ISR) (Van Loon et al., 1998; Araujo et al., 2005; Kai et al., 2009; Zhao et al., 2014). The production of one or more antibiotics is one of the most studied biocontrol strategies displayed by PGPR. Some common examples of different antibiotics include amphisin, oomycin-A, phenazine, pyoluteorin, pyrrolnitrin, tropolone, tensin and the cyclic lipopeptides synthesis (Loper and Gross, 2007). Some recent studies have indicated that biofilm formation of bacterial cells in the rhizosphere is of considerable antifungal and antibacterial activity such as toxins and antibiotics in

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*Corresponding author, E-mail address: ahandan@gmail.com
their periphery, which has an inhibitory effect on phytopathogens in the soil (Kwon and Kim, 2014; Figueiredo et al., 2016).

Rhizospheric bacteria through their fast colonizing ability are able to compete favorably for the available water, nutrient and space which is required to limit the disease incidence and severity. The most competent group of rhizobacteria controls whole metabolic activities. In addition to its advantages through competition with the other properties such as presence of flagellium, chemotaxis, lipopolysaccharide and the usage of secreted root exudates enhances their survival (Lugtenberg and Kamilova, 2009). Siderophore production confers competitive advantages to PGPR and unavailability of iron suppress the phytopathogen. Iron is a vital element needed by all microorganisms for synthesis of ATP, DNA and a number of functions (Saraf et al., 2011).

Pseudomonades are ubiquitous bacteria in agricultural soils and major group of rhizobacteria with potential biological control (Lee et al., 2009). They grow rapidly in vitro, utilize seed and root exudates and fast colonize in the rhizosphere, produce a wide spectrum of bioactive metabolites, adapt to environmental stresses, compete aggressively with the other microorganisms. In addition, pseudomonads are responsible for the natural suppression of soil borne pathogens (Lim et al., 2007). Fluorescent pseudomonads are being used widely as they produce a wide variety of antibiotics, chitinolytic enzymes, growth promoting hormones, siderophores, HCN and catalase, and solubilize phosphorous (Sairam et al., 1998; Kwak et al., 2009). Their weakness as biocontrol agents is difficult to produce resting spores. Bacillus is the most abundant other genus in the rhizosphere. There are a number of metabolites that are released by these strains which increase nutrient availability of the plants (Leegood and Walker, 1982; Gao and Zhang, 2008). The other common rhizobacteria is Azotobacter that generally regarded as a free-living aerobic nitrogen-fixer (Saharan and Nevra, 2011).

3.2. INDUCED SYSTEMIC RESISTANCE (ISR)

The salicylate- and jasmonate-induced pathways are performed by the production of PR proteins which include antifungals (chitinases and glucanases), and oxidative enzymes (peroxidases, polyphenol oxidases and lipoxygenases) respectively. Low-molecular weight compounds such as phytoalexins which is antimicrobial properties can also accumulate (Choudhary and Prakash, 2007). SAR is induced by exposing the plant to virulent, avirulent and nonpathogenic microorganisms (Pieterse and Van Loon, 2001). While SAR includes the accumulation of pathogenesis-related proteins or salicylic acid, ISR relies on pathways regulated by jasmonate and ethylene.

Induced Systemic Resistance (ISR) of plants against phytopathogens is a widespread phenomenon in plant protection. Rhizobacteria-mediated induced systemic resistance (ISR) effectively response to a broad spectrum of plant pathogens. Compared to SAR and non-pathogenic rhizobacteria, inducing ISR trigger a different signal transduction pathway not dependent on the accumulation of the SA and accumulation of pathogenesis-related proteins but dependent on precipitation of jasmonic acid and ethylene (Pieterse et al., 2014). After infection, levels of SA increase locally and systemically in the phloem before ISR occurs. ISR utilizes some plant hormones (salicylic acid, jasmonic acid and ethylene) in signaling and stimulation of the host defense response against variety of phytopathogens (Beneduzi et al., 2012; Pieterse et al., 2014). The colonization of roots by inoculated rhizobacteria is an important step which become constituent molecules of the bacterial cell or synthesized by the bacteria as elicitors of a biochemical signal (Figueiredo et al., 2016).

Induction of proteases, β-1,3-glucanase, and chitinases as catabolic enzymes and small molecules can be secreted by various rhizobacteria and can suppress soilborne pathogens. The cell wall degrading enzymes such as chitinases produced by rhizobacteria cause abnormality of the mycelial
growth (Zhao et al., 2014). Some antibiotics and various toxic compounds to pathogens have been
recovered form Bacillus strains (Esikova et al., 2002). B. subtilis produces lipopeptide antibiotics of
the surfactant group that can inhibit several plant pathogens.

3.3. TOLERANCE TO STRESS
Phytohormones and reactive oxygen species (ROS) are major determinants as stress responses in
plants. In metabolic pathway studies carried out focus on production of phytohormones in the
rhizosphere and stimulation of resistance to biotic and abiotic stress. ROS such as superoxide anion
(O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (HO.) stimulates enzymatic antioxidant defense
systems in plants under stressful conditions as free radicals and non-radical molecules are key
components of the signaling pathways and act as main regulators of cellular responses of plant to
environmental factors (Araujo et al., 2005; Kang et al., 2010). ROS is extremely toxic for the cells
at high concentrations. ROS cause oxidative damage to lipids, proteins and DNA (Gill and Tuteja,
2010). Hydrogen peroxide (H$_2$O$_2$) is the most important ROS that produced by normal aerobic
metabolism in plants.

Rapid accumulation of free proline as an osmoprotectant is a typical response in stressed plants
facilitating water uptake from the soil. Plants produce several antioxidant enzymes that include
superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) glutathione peroxidase
(GPX, EC 1.11.1.9) involved in scavenging free radicals (Simova-Stoilova et al., 2008).

The treatment of PGPR to the rhizosphere can greatly contribute antioxidant enzyme activities (Li
et al. 2008). In tomato and eggplant, the inoculation of B. subtilis caused an increase in the
peroxidase activity in plants (Araujo and Menezes, 2009; Altinok et al., 2013). Antioxidants
beneficial to the health of consumers which were found in certain foods may prevent the damage
caused by free radicals by neutralizing. The main antioxidant compounds in plants are flavonoids
that known with anti-inflammatory, antiallergic, antiviral, and anticarcinogenic properties (Tapas et
al., 2008).

4. CONCLUSIONS
Plant growth promoting rhizobacteria (PGPR) are a group of bacteria found in the rhizosphere, at
root surfaces and in association with roots, which can improve the extent or quality of plant growth
directly and/or indirectly. Bacteria including species of Pseudomonas, Azospirillum, Azotobacter,
Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus, Rhizobium and
Serratia have reported to enhance plant growth. Some PGPR provide plant growth promoting
substances to a plant or facilitate the uptake of certain plant nutrients from the soil which is called
direct promotion. The indirect promotion occurs when PGPR prevent deleterious effects of
phytopathogenic microorganisms. Some of them can produce or change the concentration of plant
growth regulators like indoleacetic acid, gibberellic acid, cytokinins and ethylene. Others were
found to have N$_2$ fixation, antagonism against phytopathogenic microorganisms, solubilization of
phosphate capacity. Plant growth promoting bacterial strains must be rhizospheric competent, able
to survive and colonize in the rhizospheric soil. Unfortunately, the interaction between associative
PGPR and plants can be unstable. Effective strains as in vitro cannot always be the same under field
conditions. The variability in the performance of PGPR may be due to various environmental
factors that may affect their growth and exert their effects on plant. The environmental factors
include climate, weather conditions, soil characteristics or the composition or activity of the
indigenous microbial flora of the soil. Therefore, it is necessary to develop efficient strains in field
conditions. Exploring the soil microbial diversity is important for PGPR to achieve well adaptation

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*Corresponding author, E-mail address: ahandan@gmail.com
for a particular soil environment. The success of PGPR will depend on the ability to manage the rhizosphere to enhance survival and competitiveness of these beneficial microorganisms. Genetic enhancement of PGPR strains to enhance colonization and effectiveness may involve addition of one or more traits associated with plant growth promotion. High output yield and enhanced production of the crop as well as fertility of soil to get in an ecofriendly manner is needed. Future research may focus on rhizosphere in optimizing growth conditions and extend shelf-life of PGPR products which will be cost effective and tolerate adverse environmental conditions better.

6. REFERENCES


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*Corresponding author. E-mail address: ahandan@gmail.com


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*Corresponding author, E-mail address: ahandan@gmail.com*


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*Corresponding author, E-mail address: ahandan@gmail.com*