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KAHRAMANMARAŞ SÜTÇÜ IMAM UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ANTIFUNGAL ACTIVITY OF RHIZOSPHERIC *PSEUDOMONAS* AND *BACILLUS* SPECIES ISOLATED FROM CEREALS

ABDALLA AZİZ İBRAHİM İBRAHİM

MASTER'S THESIS

DEPARTMENT OF BIOENGINEERING AND SCIENCES

KAHRAMANMARAŞ, TURKEY-2016

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Doç. Dr. E. Banu BÜYÜKÜNAL BAL (Supervisor) ……..

Department of Biology

Kahramanmaraş Sütçü İmam University

Prof. Dr. Sadık DİNÇER (Member) ……

Department of Biology

Çukurova University

Yrd. Doç. Dr. Yaşar ALPTEKİN (Member) …...…….

Department of Plant Protection

Kahramanmaraş Sütçü İmam University

I approve that the above signatures related to the members.

Doç. Dr. Mustafa ŞEKKELİ …….

Director of Graduate School

Kahramanmaraş Sütçü İmam University

DECLARATION

I hereby declare that all information in the thesis has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

ABDALLA AZİZ İBRAHİM İBRAHİM

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TAHILLARDAN İZOLE EDİLEN RİZOSFERİK *PSEUDOMONAS* VE *BACILLUS* TÜRLERİNİN ANTİFUNGAL AKTİVİTELERİ

(YÜKSEK LİSANS TEZİ)

ABDALLA AZİZ İBRAHİM İBRAHİM

ÖZET

Rizosfer toprağın bitki kök sisteminin etkisi altında bulunan toprak kısmını tanımlamaktadır. Bu kısım bitki beslenmesi, sağlığı ve kalitesi için hayati önem taşımaktadır. Bitki-mikroorganizma etkileşimleri hem doğal hem de tarımsal sistemler için karbon salınması, ekosistem işlevi ve besin döngüsünde önem taşımaktadır. Bu nedenle rizosferdeki mikrobiyal komünitenin yapısını ve fonksiyonunu anlamak gereklidir. Bu çalışmada, rizosferik *Pseudomonas and Bacillus* türleri Kahramanmaraş' taki buğday ve arpa tarlalarında yetişen bitkilerden izole edilmiştir. Toplam 38 *Pseudomonas* ve 35 *Bacillus* spp. izolatı in vitro olarak fitopatojenik fungusların (*Fusarium culmorum, Fusarium graminearum, Bipolaris sorokiniana*, *Fusarium* spp. and *Aspergillus* spp.). inhibe etmesi yetenekleri açısından test edilmiştir. Bu testlerde kullanılan, üç fungus türü (*Fusarium culmorum, Fusarium graminearum, Bipolaris sorokiniana*) buğday bitkisinden, *Fusarium* spp. and *Aspergillus* spp. ise domates bitkisinden patojen olarak elde edilmiştir. Sonuç olarak antifungal etkiye sahip izolatlar belirlenmiştir. Buna ilaveten, bazı izolatların (P7, P13, P14, P22, P34, B3, B7, B14, B15, B30, B32) tüm fungal fitopatojenlere karşı güçlü inhibitor etkiye sahip olduğu bulunmuştur. Antifungal aktiviteye sahip izolatlar tarla denemelerinde fungal büyüme inhibitörü olarak yararlı etkileri doğrulandıktan sonra, tahıl yetiştirilmesi esnasında potansiyel biyokontrol ajanları olarak kullanılabilirler.

Anahtar kelimeler: Rizosfer, *Pseudomonas*, *Bacillus*, fungal fitopatojen

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ANTIFUNGAL ACTIVITY OF RHIZOSPHERIC *PSEUDOMONAS* AND *BACILLUS* SPECIES ISOLATED FROM CEREALS

(M.Sc. THESIS)

ABDALLA AZİZ İBRAHİM İBRAHİM

ABSTRACT

Rhizosphere describes the portion of soil where microorganism-mediated processes are under the influence of the root system of plants. It has the central importance for plant nutrition, health and quality. Plant-microorganism interactions in the rhizosphere is important for carbon sequestration, ecosystem functioning and nutrient cycling in both natural ecosystems and agricultural systems, therefore understanding of microbial community structure and function in the rhizosphere is essential. In the present study, rhizospheric *Pseudomonas and Bacillus* species were isolated from wheat and barley growing in the fields of Kahramanmaras. A total of 28 *Pseudomonas* and 35 *Bacillus* spp. isolates were tested in vitro for their ability to inhibit the growth of phytopathogenic fungi (*Fusarium culmorum*, *Fusarium graminearum*, *Bipolaris sorokiniana*, *Fusarium* spp. and *Aspergillus* spp.). Among fungi used in antifungal test, three fungi species (*Fusarium culmorum*, *Fusarium graminearum*, *Bipolaris sorokiniana*) were isolated from wheat, while *Fusarium* spp. and *Aspergillus* spp. were isolated from tomato as pathogen. As a result, isolates having antifungal activities against tested fungi have been determined. Moreover, some isolates (P7, P13, P14, P22, P34, B3, B7, B14, B15, B30, B32) found to have strong inhibitory activities against all fungal phytopathogens. Those isolates with antifungal activity can be potentially used as biocontrol agents during cereal growth after verification of their beneficial effect as fungal growth inhibitor in field tests.

Key words: Rhizosphere, *Pseudomonas*, *Bacillus*, fungal phytopathogens

Kahramanmaraş Sutcu Imam University
Graduate School of Natural and Applied Sciences
Department of Bioengineering and Sciences, June/2016

Supervisor: Doç. Dr. E. Banu BUYUKUNAL BAL

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SYMBOLS AND ABBREVIATIONS

spp. : Species

VOCs : Volatile Organic Compounds

PGP : Plant Growth Promoting Rhizobacteria

IAA : Indole Acetic Acid

HCN : Hydrogen cyanide

AR : Antibiotic Resistance

SAR : Systemic Acquired Resistance

ISR : Induced Systemic Resistance

PDA : Potato Dextrose Agar

1. INTRODUCTION

About hundred years ago, researchers have realized the important roles of microorganisms. Microorganisms have undoubtedly been important components of various ecosystems (Hentschel et al., 2000).

The narrow zone of soil that is influenced by plant roots is defined as rhizosphere. Rhizosphere is a hot spot for numerous organisms and is considered as one of the most complex ecosystems on the Earth (Hinsinger and Marschner, 2006). It is an important ecological environment in soil for plant-microbe interactions. Interactions between microorganisms and plants could be beneficial, neutral or harmful effects to plants (Hynes et al., 2008). In the case of harmful effect, the pathogenic microorganisms can cause various plant diseases that usually weaken or destroy plant tissues and reduce crop yields ranging from 25% to 100%. Root diseases causes around 10-15% yield losses annually in the World. In this regard, a group of organisms defined as plant growth promoting bacteria (PGPR) can be found in close association with plants and can protect plants from diseases and promote plant growth (Germida et al., 1998).

Different plant growth promoting rhizobacteria (PGPR) species belonging to genera of *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Azospirillum*, *Klebsiella*, and *Enterobacter* have been isolated from the rhizosphere of various crops and noted for their synergistic effects on plant growth (Lazarovits and Norwak, 1997). PGPR promote plant growth by direct and indirect mechanisms; however, the exact mechanisms by which PGPR promote plant growth are not fully understood (Kumar et al., 2012). The plant promoting activities can be achieved by different ways: (i) producing or changing the concentration of plant growth regulators like indole acetic acid, gibberellic acid, cytokinins and ethylene (Arshad and Frankenberger, 1993; Glick, 1995), (ii) asymbiotic N_2 fixation (Boddey and Dobereiner, 1995), (iii) antagonism against phytopathogenic microorganisms by production of siderophores (Scher and Baker, 1982), antibiotics (Shanahan et al., 1992) and cyanide (Flaishman et al., 1996), (iv) solubilization of mineral phosphates and other nutrients (De Freitas et al., 1997).

The large majority of plant diseases are mostly controlled by application of chemical pesticides. However, the widespread use of chemical pesticides has been a subject of public concern due to potential harmful effects for the both target and non-target organisms. At first, most of those pesticides can potentially be carcinogenic. Moreover, the pathogens can develop resistance against the pesticides applied. Moreover, chemical fertilizers are known to cause ground contamination, denitrification, leaching and conversion to unavailable forms, which all have negative effects on ecosystems. Therefore, biological control offers an alternative approach to the use of expensive and harmful chemicals, and provides low cost and environmental friendly control measures to reduce the activity of plant pathogens (Spadro and Cullino, 2005; Sindhu et al., 2009; Jiao et al., 2013).

Knowledge of the PGPR diversity and their biocontrol and/or biofertilizing activity are not only essential to understand their ecological roles in the rhizosphere, but also for utilization in sustainable agriculture. Aims of the study present study are;

1- Isolation of *Bacillus* and *Pseudomonas* spp. from wheat and barley rhizosphere grown in Kahramanmaraş, Turkey.
2- Testing antifungal activities of the isolates in vitro against some phytopathogenic fungi that cause diseases in cereal.
3- Selection of most active bacterial isolates against phytopathogenic fungi in vitro to initiate more comprehensive studies for determining their potential as a biocontrol agent.

2. LITERATURE REVIEW

2.1. Rhizosphere

Lorenz Hiltner, the German agronomist and plant physiologist, first introduced rhizosphere term in 1904 for describing the plant-root interface (Hartmann et al., 2008). Hiltner defined the rhizosphere as the area around a plant root that is inhabited by a unique population of microorganisms. Chemicals released from plant roots have impact on the microorganisms. The meaning of rhizosphere has been changed since from that date. Now, it contains three zones with varying distance to root and therefore each zone has been influenced by root at different level (Figure 2.1). One of this zone is called as endorhizosphere and has portions of the cortex and endodermis. This zone contains microbes and cations. The one of the remaining zones defined as rhizoplane and is located between endorhizosphere and ectorhizosphere at the adjacent position to the root. The last zone one is located at the outer and is called as ectorhizosphere. This zone reaches out from the rhizoplane into the bulk soil. Because of the complexity and diversity of plant root systems is inherently complex and diverse for each species. For this reason, the rhizosphere is not an exact region in term of size or shape. Instead, it has a gradient with different chemical, biological and physical properties that change both radially and longitudinally along the root (McNear, 2013).

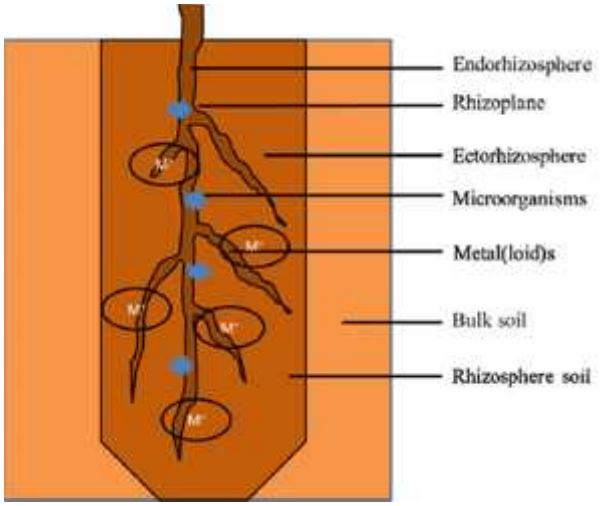


Figure 2.1. A section of soil displaying bulk soil and three zones of rhizosphere (Seshadri et al., 2015)

The properties of rhizosphere alter depending on the plant species. Moreover, the width of rhizosphere has been changed and can be within the range of 2-80 mm from the root surface. For distinguishing rhizosphere and bulk soil zone, concentration of root exudates and degree of microbial activity are used. The rhizosphere and bulk soil separation place is generally called as edaposphere. Although the chemical and biological features help in identifying the rhizosphere region, it is difficult to separate the zones physically from the surface of the root. For this purpose, R/E ratio has been suggested to determine ideal ratio between rhizosphere and bulk soil. A ratio should be between 2 and 20 for describing rhizosphere effect (Badalucco and Kuikman, 2001; Badalucco and Kuikman, 2006).

Many different kinds of organisms like bacteria, fungi, oomycetes, nematodes, protozoa, algae, viruses, archaea, and arthropods live in the rhizosphere. A tight relationships between plant, soil and soil organisms exists in rhizosphere. Organisms in the rhizosphere are responsible from decaying of soil organic matter and cycling of nutrients into available forms for utilization by plants. Therefore, any factor affecting one member of this whole rhizosphere system will affect the others (Wallace, 2001).

The interface between soil and plant root is a dynamic habitat. The growth of microbes are normally limited by a lack of carbon and energy in the surrounding bulk soil, while the continuous release of organic nutrients from the plants facilitates the activity of a large variety of microorganisms in the rhizosphere. Due to release of different organic compounds by different plant species, a different microbiota is formed in the rhizosphere (Olsson and Alstrom, 2000). In this microbiota, pathogenic microorganisms are present in addition to non-pathogenic organisms. Pathogenic microorganisms threat health of plant and affect both food production and ecosystem stability worldwide. For this reason, the producers need to apply on agrochemicals for crop protection and fertilization (Compant et al., 2005).

The rhizosphere is important for plant nutrition, health and quality. In addition, it is important for microorganism-driven carbon sequestration, ecosystem functioning and nutrient cycling in terrestrial ecosystems (Berg and Smalla, 2009). For this reason, the rhizosphere affects community structure, ecosystem processes and patterns of soil

development such as soil type, moisture, pH, and temperature. Several biotic and abiotic factors i.e. climate, season, grazers, other animals, pesticide treatments, soil type, plant health and developmental stage influence the structural and functional diversity of bacterial communities (Lemanceau et al., 1995; Siciliano et al., 2001).

Many factors including plant type, climactic conditions, herbivore insect, nutrient deficiency or toxicity along with chemical, physical and biological properties of the surrounding soil affect the composition and amount of the root released compounds (exudates). The root released products to the surrounding soil have been classified based on their chemical composition, mode of release or function, they are classically defined to include collapsed root cap and border cells, mucilage, and exudates (Rovaria, 1969; Rasche et al., 2006; Hai et al., 2009).

Root exudates contain both secretions (including mucilage) and diffuses. Both differ in the way of release. Secreatins are actively released from the root, however diffuses are passively released due to osmotic differences between soil solution and the cell, or lysates. The released organic compounds by plants can be further split into two groups; as high and low molecular weight (HMW and LMW, respectively) compounds. The complex HMW compounds (e.g. mucilage, cellulose) are not easily used by microorganisms and make up the majority of C released from the root. On the other hand, the LMW compounds are more diverse with known functions. The LMW compounds are divided into organic acids, amino acids, proteins, sugar, phenolics and other secondary metabolites. As a common feature, they are generally more easily used by microorganisms. A little information has been gathered on LMW influencing rhizosphere processes. Most of the information indicates root exudates functions as acquisition of nutrient (e.g. Fe and P), agents of invasiveness (i.e. allelopathy), chemical signals to attract symbiotic partners (e.g. rhizobia and legumes) or the promotion of beneficial microbial colonization on root surfaces (e.g. *Bacillus subtilis*, *Pseudomonas florescence*) (Park et al., 2003; Kloepper et al., 2004).

Most studies to date on the rhizosphere have focused on the number and diversity of bacterial taxa instead of other rhizosphere inhabitants. Numbers on reported in rhizosphere studies range from <100 to more than 55 000 OTUs depending on the

techniques used. For instance, a meta-analysis of 19 clone libraries obtained from the rhizosphere belonging to 14 plant species displayed more than 1200 distinguishable bacterial taxa from 35 different taxonomic orders. Among all taxa, the Proteobacteria phylum members were represented with higher number (Hawkes et al., 2007). A total of 5619 OTUs with dominating in Acidobacteria and Proteobacteria were detected in bacterial community of the rhizosphere of oak based on 454 pyrosequencing, Moreover, the bacterial diversity has been found higher in the bulk soil than in the oak rhizosphere (Uroz et al., 2010).

2.2. Rhizobacteria

The rhizosphere bacteria with beneficial effect to plants can either be symbiotic or free-living. The symbiotic relationship generally involves the formation of specialized structure or nodules on host plant roots, whereas free-living bacteria in the soil are often found near, on or within plant tissues (Frommel et al., 1991).

The symbiotic bacteria develop in root nodules of leguminous plants, such as peas, beans, soybean, peanuts, clover and chickpea etc. Rhizobia-legume interactions are highly specific. In symbiotic relationship, the symbiotic bacteria produce nutrition for the host plant, while the host provides anaerobic conditions and nutrients for the bacteria (Benson, 2001). The symbiotic bacteria in root nodules are represented in Figure 2.2. The symbiotic bacteria have major contributions to soil enrichment. The principal genera of the symbiotic bacteria include *Rhizobium* and *Bradyrizobium* spp. (Long, 2001; Mateos et al., 2001).

Figure 2.2. An example of symbiotic bacteria on plant (Samuel, 2006)

Beneficial free living soil bacteria are generally referred to as plant growth-promoting rhizobacteria (PGPR) (Penmetsa and Cook, 1997) (Figure 2.2). Although numerous free living soil bacteria are present, not all bacterial strains of a particular species are considered to be PGPR. PGPR should have distinct metabolic capabilities and interactions with plants. *Azotobacter, Klebsiella* and *Clostridium* are some samples of PGPR (Kloepper et al., 1989).

2.2.1. Plant growth promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) are composed from heterogeneous group of bacteria that can be found in the rhizosphere either at root surface and in association with root. They can improve the plant growth directly and/or indirectly (Janardan Yadav et al., 2010) (Figure 2.3). Rhizosphere possesses a huge and active microbial population which is capable of exerting beneficial, neutral and detrimental effects on the plant. The plant-microbe interactions in the rhizosphere are responsible for increasing plant health and soil fertility. These mechanisms can be active simultaneously or independently at the different stage of plant growth. PGPR stimulated direct mechanisms could be in several different ways such as fixation of atmospheric Nitrogen (N_2), solubilization of minerals such as phosphorus, production of siderophore, and synthesis of plant growth hormone, for example, indol-3-acetic acid (IAA). The indirect mechanisms involve the biological control of plant pathogen and harmful microbes,

through the production of antibiotics. Lytic enzymes, hydrogen cyanide, catalase, siderophores or competition for nutrient can significantly affect plant health and growth as evidenced by increase seeding emergence and high yield (Verma et al., 2001).

In addition to these traits, PGPR strains must be able to survive and colonize in the rhizospheric soil (Cattelan et al., 1999). In most cases, the interaction between associative PGPR and plants is unstable. For this reason, the positive results obtained in vitro cannot always be reproduced under field conditions (Zhender et al., 1999).

The variability in the performance of PGPR may be due to various environmental factors include climate, soil characteristics or the composition or activity of the indigenous microbial flora of the soil.

Significant increase in crop yield has been reported by applying PGPR microbial inoculants (Raaijmakers et al., 2009; Glick, 2014). Biofertilizers such as microbial inoculants support plant growth and increase the nutrient status of the host plant. They have been accepted as an alternative source of chemical fertilizer (Glick, 2014; Santoyo et al., 2012). Biofertilizer differs from organic fertilizers, which contains organic compounds that increase soil fertility either directly or indirectly as a result of their decay. Not all plant-growth promoting bacteria are considered a biofertilizer. Instead, they are called as biopesticides, if they control plant growth by control of deleterious organisms.

2.2.1.1. *Bacillus* species

Bacillus species are considered within members of PGPR. These bacteria are Gram-positive, endospore-forming, chemoheterotrophic, rod-shaped, usually motile with peritrichous flagella. They are also aerobic or facultative anaerobic with positive catalase activity (Waites et al., 2008).

Bacillus species are commonly found in soil and has characterized with a wide range of physiological abilities. *Bacillus* spp. spores can resist extreme conditions. (Kuta, 2008). The *Bacillus* species can produce a broad variety of metabolites with antimicrobial activity, therefore, it is more important for pharmaceutical industry in controlling numerous diseases in humans, animals and plants. Therefore, they are valuable for treatment of diseases as biological control agent (McKeen et al., 1986; Leifert et al., 1995).

Plants possess a various active defense mechanisms. Those mechanisms can be actively expressed in response to biotic stresses (pathogens and parasites). The timing of this defense response is important and determines the fate of coping and dying to such biotic challenge. If defense mechanisms are started by a stimulus prior to infection by a plant pathogen, disease can be reduced. Induced resistance is defined as a state of improved defensive capacity developed by a plant when appropriately stimulated. Two types of induced resistance, namely systemic acquired resistance (SAR) and induced systemic resistance (ISR), are present in plants. In both, plant defenses, prior infection or treatment arrange resistance against subsequent challenge by a pathogen or parasite (Choudhary et al., 2007).

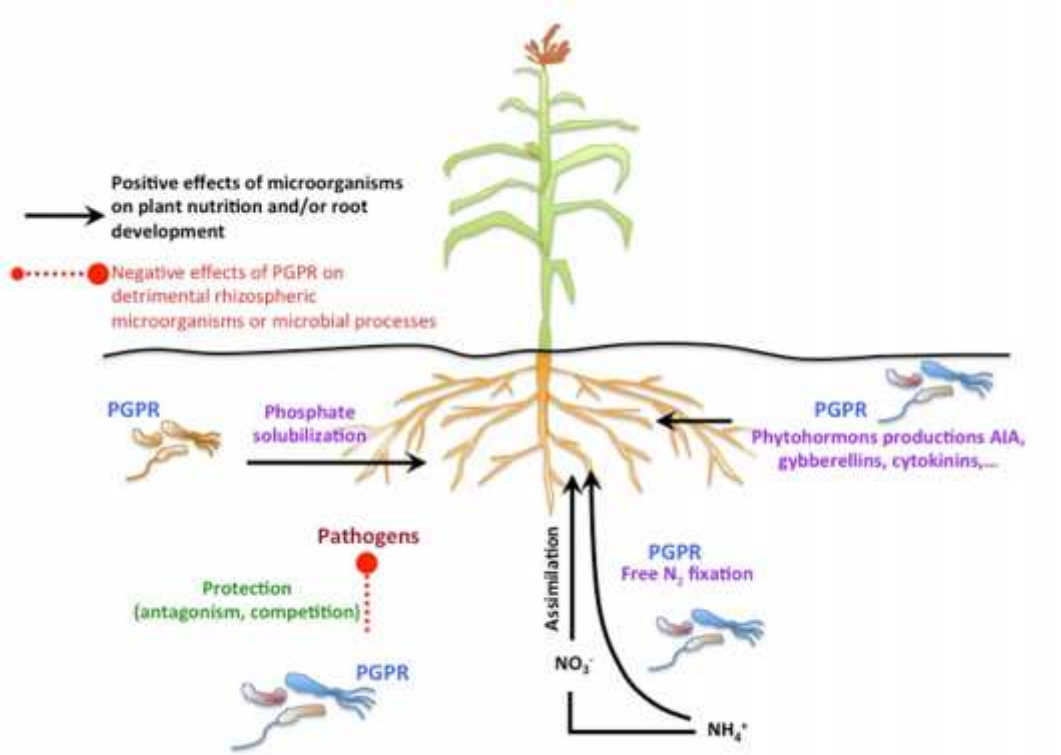


Figure 2.3. A schematic illustration for possible roles of PGPR for plants (Peña and Loyola, 2014)

Certain bacteria can play important roles in induced systemic resistance (ISR) resistance and in controlling diseases in plants which caused by phytopathogens such as virus, fungi, nematodes and pathogen bacteria (Boehm et al., 1993; Boehm et al., 1997; Bargabus et al., 2004). Researchers found that strains of some plant growth-promoting

rhizobacteria (PGPR) suppress diseases by antagonism between the bacteria and soil-borne pathogens as well as by inducing a systemic resistance in plant against both root and foliar pathogens (Choudhary et al., 2007). These bacteria consist from *Pseudomonas* spp. and *Bacillus* spp. (*B. amyloliquifaciens*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides*, and *B. sphaericus*) (Krause et al., 2003; Kloepper et al., 2004).

2.2.1.2. *Pseudomonas* species

Pseudomonas species are taxonomically placed within Proteobacteria subclass of gamma subdivision. They have been found in the order of *Pseudomonadales* and family of *Pseudomonadaceae*. They are Gram-negative, aerobic, motile (having at least one polar flagellum), rod-shaped about 1-5 µm long and 0.5-1.0 µm wide. Optimal growth temperatures are within the range of 25-30 °C. They are often oxidase and arginine dihydrolase.

The genus *Pseudomonas* is well known for its metabolic variability and genetic plasticity. The genus *Pseudomonas* can utilize a wide range of organic and inorganic compounds and live under diverse environmental conditions such as soil and water. They can also be important pathogen for plant, animal and humans (Schroth et al., 1992). Strains of *Pseudomonas* species are often resistant to antibiotics, disinfectants, detergents, heavy metals, and organic solvents. Some strains have ability to produce metabolites that stimulate plant growth or inhibit plant pests (Moore et al., 2006).

The synthesis of yellow-green, fluorescent, water-soluble pigments under certain growth conditions is characteristic for some *Pseudomonas* spp. (Stanier et al., 1966). Many different environmental factors suh as organic carbon and energy source affect the synthesis of these pigments (Sullivan, 1905). Furthermore, the degree of aeration of the culture medium, pH and light affect the pigment synthesis (Elliot, 1958; Lenhoff, 1963).

The sequence information of the gene encoding 16S rRNA has been widely used for phylogenetic studies of *Pseudomonas* species similar to other bacterial species. For *Pseudomonas* species, two distinct intrageneric divisions designated as "*P. aeruginosa intrageneric cluster*" (I) and "*P. fluorescens* intrageneric cluster" (II) were detected. Most species are fall within those intrageneric group.

P. fluorescens and *P. putida* are widely found in soils, in water and especially in the plant rhizosphere. Various studies on these bacteria resulted in that these bacteria improved plant health, plant growth and yield. They can also prevent some soil borne disease (Weller, 2007; Mavrodi, et al., 2011; Singh et al., 2012; Singh et al 2013).

Pseudomonas species comprised from fluorescent *Pseudomonas* spp. and non-fluorescent species. The genus of *Pseudomonas* contains (i) cytochrome c oxidase-positive organisms either phytopathogenic fluorescent *Pseudomonas* such *as P. cichorii*, *P. marginalis* and *P. tolaasii* (Young et al., 1992) or nonphytopathogenic, non-necrogenic strains such as *P. fluorescens*, *P. putida*, *P. chlororaphis*, *P. aureofaciens* and the *P. aeruginosa* type species (ii) necrogenic phytopathogenic fluorescent *Pseudomonas* spp. lacking cytochrome c oxidase: *P. syringae* and *P. viridiflava*; (iii) non-fluorescent *Pseudomonas* spp. suh as *P. stutzeri*, *P. mendocina*, *P. alcaligenes* and *P. pseudoalcaligenes*.

Pseudomonas has currently 156 species, although 48 of these species have been reclassified and are now considered to be basonyms or synonyms of species placed in other genera. Two species, *P. aureofaciens* and *P. perfectomarina*, are recognized as later heterotrophic synonyms of *P. chlororaphis* and *P. stutzeri* (genomovar 2), respectively (Moore et al., 2006).

2.2.2. Importance of PGPR

The importance of rhizosphere microbial population for maintenance of root heath, nutrient uptake and tolerance of environmental stress is well noticed. Starting from the first studies on PGPR around 1950, hundreds of PGPR strains have been screened and evaluated in the laboratory, greenhouse and field studies across the world. Today PGPR are commonly used as inoculant in developing countries on millions of hectares of land (Martinez-Viveros et al., 2010). Over the years, PGPR have gained worldwide importance and acceptance for agricultural benefits. Recently, production of volatile organic compounds (VOCs) with gaseous nature by PGPR has also shown to be responsible for growth-promoting activities of plants such as *Arabidopsis thaliana* and *Medicago truncatula* (Farag et al., 2006; Orozco-Mosqueda et al., 2013).

According to the mode of action, PGPR have been divided into two groups. The first group indirectly provides advantage to the plant growth (biocontrol PGPR) and the second group that directly affects plant growth (Nelson, 2004), seed emergence or improve crop yields (Glick et al., 1999). Most of the efforts have been made in the past two decades to elucidate both the direct and indirect mechanisms by PGPR for enhancing plant growth. Phytohormone production and enhancing plant nutrition by PGPR were the two prominent mechanisms to contribute to the plant growth. Enhancement of plant nutrition by PGPR is mainly achieved through increased phosphorous uptake by solubilization of inorganic phosphates and iron uptake by production of iron chelating siderophores (Ryu et al., 2004). PGPR also indirectly provide the plant growth by suppression of deleterious microorganisms that inhibit plant growth or root pathogens through several ways such as antibiosis, parasitism and competition for nutrients within the surroundings of plant roots and/or activation of host defense responses.

2.2.3. Traits of PGPR

2.2.3.1. Nitrogen cycle and nitrogen fixation

Plants take nitrogen from the soil by absorption through their roots in various forms i.e. amino acids, nitrate ions, nitrite ions, or ammonium ions. The nitrogen cycle is the process which nitrogen is converted to its various chemical forms. This conversion can be carried out through both biological and physical processes. The nitrogen cycle contains important processes, namely fixation, ammonification, nitrification, and denitrification (Figure 2.4). Nitrogen fixation process is performed by rhizobacteria. Moreover, fixation ensures the conversion of gaseous nitrogen (N_2) to ammonia (NH_3) which is the usable form for plants as nutrient to support and enhance plant growth. In the nitrification process, conversion of ammonium to nitrite and nitrate has been achieved by soil-living bacteria and other nitrifying bacteria. Plants can obtain nitrate or ammonium from the soil by absorbing their root hairs (Hill et al., 2011, 2012). If nitrate is absorbed, it is first gone through reduction to nitrite ions and then ammonium ions before incorporation into amino acids, nucleic acids, and chlorophyll. This process called as assimilation and ensures building process of amino acids. Besides, there is a more complex cycling of amino acids between *Rhizobia* bacteroids and plants depending on the formation of an interdependent

relationship. In this cycling, the plant provides amino acids to the bacteroids without requirement of ammonia assimilation and the bacteroides pass amino acids (with the newly fixed nitrogen) back to the plant (Tegeder and Rentsch, 2010; Willey, 2011). Ammonification (also called as mineralization) is part of the decaying process. When a plant or animal dies or discards waste, decomposers like fungi and bacteria transform the nitrogen back into ammonium (NH_4^+). Then, ammonium can re-enter the nitrogen cycle (Erskine et al., 1998).

Denitrification is the reduction of nitrates (NO_3^-) back into the molecular nitrogen (N_2) for completion of the nitrogen cycle. Nitrogen gas is inert and unavailable to plants. Various types of heterotrophic (some *Pseudomonas* spp.) and autotrophic (*Thiobacillus denitrificans*) bacteria can perform denitrification process (Kubota et al., 1999; Smil, 2000; Zhou et al., 2001). Denitrification is commonly used to remove nitrogen from sewage and municipal wastewater. Denitrification can be either aerobic or anaerobic. Denitrifiers are often facultative anaerobes. In aerobic denitrification, simultaneous use of both oxygen (O_2) and nitrate (NO_3^-) as oxidizing agents takes place. Compared to anaerobic denitrification, aerobic process creates higher amount of harmful byproduct (nitrous oxide). During anaerobic denitrification, the nitrate is used as an electron acceptor by bacteria instead of oxygen during respiration. Due to smaller reduction potentials of NO_3^- than O_2, less energy is released per oxidized molecule.

Nitrogen (N) is one of the principal plant nutrients, however; it can be a limiting factor in the agricultural ecosystems due to high losses by emission or leaching. For this reason, bacteria that make atmospheric nitrogen available for plants are important. There are two typical ways of biological nitrogen fixation: symbiotic and non-symbiotic.

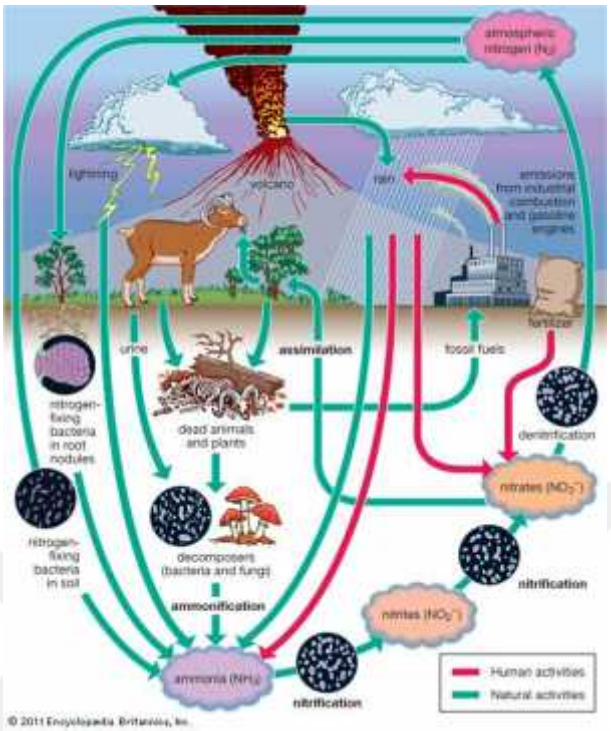


Figure 2.4. Illustration of the processes in Nitrogen cycle (Stanley, 2001)

The first is themost important mechanism for the fixation of most atmospheric nitrogen, but it is limited to plant species such as legumes and actinorhizal plants. Most of the bacteria found in symbiotic association with legume plant are considered as PGPR except in the case of their association with the non-legume plant.

On the other hand, non-symbiotic biological nitrogen fixation is carried out by *Azotobacter*, *Azospirillum*, Cyanobacteria such as *Nosto*c, *Anabaena*, *Oscillatoria* etc. can stimulate non-legume plant growth. There are studies showing that nitrogen fixing by free-living bacteria, as well as *Rhizobium* strains can stimulate the growth of non-legumes such as barley, wheat and rice (Lam et al., 2009).

2.2.3.2. Phosphate solubilization

Phosphorus (P) is an important plant macronutrient which makes about 0.2% of a plant dry weight. It found in the structure of key molecules such as nucleic acids, phospholipids, ATP. As a result, the plants cannot grow without a reliable supply of this nutrient. P is also involved in controlling key enzyme reactions and in the regulation of metabolic pathways (Theodorou and Plaxton, 1993).

Amount of phosphorus found in the soil is generally fairly high (often between 400 and 1,200 mg kg^{-1} of soil), however, most of this phosphorus is not available plant growth since it stays insoluble. The insoluble phosphorus is present as either inorganic form such as apatite or as one of several organic forms including inositol phosphate (soil phytate), phosphomonesters, and phosphotriesters (Khan et al., 2007). Thus, solubilisation and mineralization of phosphorus by phosphate-solubilizing bacteria is an important trait in PGPB as well as in plant growth promoting fungi such as mychorrizae (Rodriquez and Fraga, 1999; Richardson, 2001).Commonly, the action of low molecular weight organic acids such as gluconic and citric acid, which both of them are synthesized by various soil bacteria serve the solubilization of inorganic phosphorus. On the other hand, the mineralization of organic phosphorus takes place through the synthesis of different phosphatases which catalyze the hydrolysis of phosphoric esters (Rodriquez and Fraga, 1999). Importantly, the same bacterial strain can have coexistence of phosphate solubilization and mineralization (Tao et al., 2008).

2.2.3.3. Zinc solubilization

Zinc is a micronutrient required by living organisms. In soil, it goes through a complex dynamic equilibrium of solubilization and precipitation that is greatly influenced by the soil pH and microflora. Zinc deficiency is particularly common in high-pH soils. Zinc deficiency is an important limitation for crop production in many parts of the world. The cropland of about half of Turkey and India, a third of China, and most of Western Australia have been composed from zinc-deficient soil. This problem could only be balanced by the application of expensive zinc fertilizers either as foliar or soil applications (Li et al., 2005; Liu et al., 2007). Alternatively, numerous microorganisms, especially those associated with roots, have the ability to increase plant growth and productivity by increasing the supply of mineral nutrients (i.e. Zn) with low mobility in the soil. Among these microorganisms, a group of bacteria referred to as plant growth promoting rhizobacteria (PGPR) are involved in nutrient cycling and therefore deserve particular attention for agriculture purposes (Muhammad Tariq et al., 2007). Microbes solubilize the metal forms by various ways such as protons, chelated ligands, and oxido reductive systems present on the cell surface and membranes. Thus, microbial strains with ability of

solubilizing minerals can be utilized for conservation of existing resources and for avoiding environmental pollution hazards caused by heavy metals.

2.2.3.4. Phytohormones

Phytohormones (plant growth regulators) are the group of the chemical compounds influencing the plant growth. Five major groups of phytohormones are recognized. These are; Auxins, Gibberillins, Ethylene, Cytokinins, Ethylene and Abscisic acid. As one of the important phytohormone, indole acetic acid (IAA) influences the plant growth, organogenesis, tropic responses, cell division and cell differentiation. Diverse bacterial species have the ability to produce the auxin (Indole acetic acid). Such bacteria include *Rhizobium*, *Microbacterium*, *Sphingomonas*, *Mycobacterium*, Azospirillum, *Burkholderia* spp. (Ramprasad et al., 2014).

2.2.3.4.1. Indole acetic acid (IAA)

Indole-3-acetic acid (IAA) is the main auxin in the plants and controls many important physiological processes such as cell enlargement, cell division, tissue differentiation and responses to light and gravity (Dazzo and Yanni, 2006). In addition, IAA generally stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes related to vegetative growth; initiates root formation; mediates responses to light, gravity and florescence; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stress conditions (Ahemad and Kibret, 2014) (Figure 2.5). Bacterial IAA producers have the potential to interfere with any of these processes by the input of IAA into the plant's auxin pool. The significance of IAA for the plant function is usually related to the amount of IAA that is produced. For

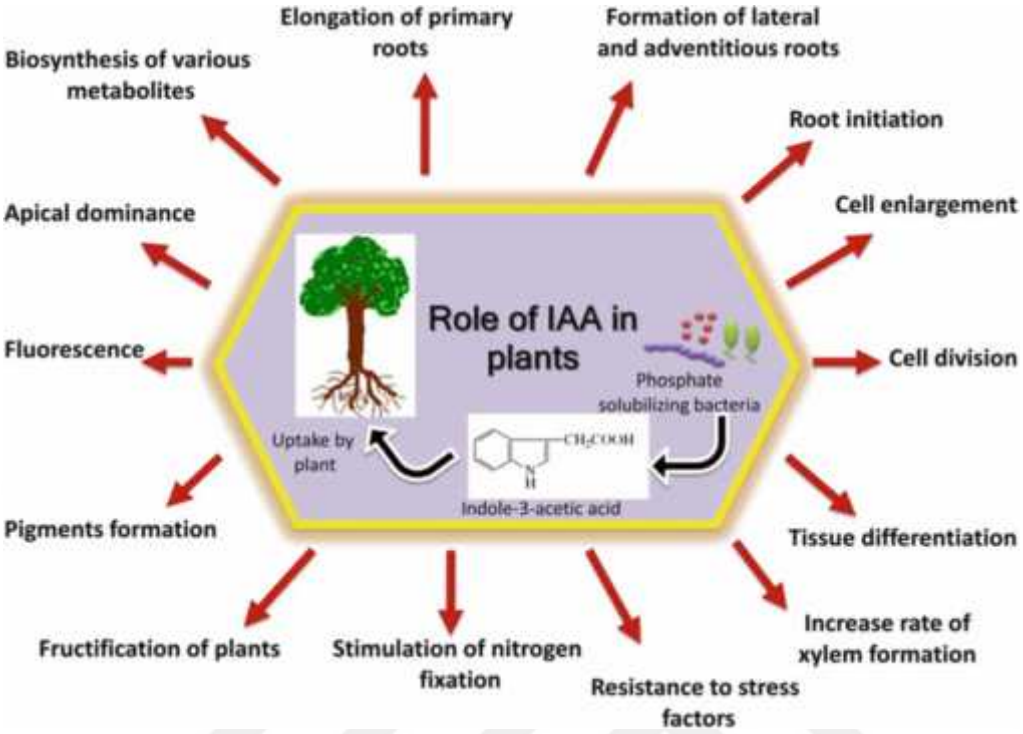


Figure 2.5. Roles of IAA in plants

instance; a root is one of the plant's organs with highest sensitivity to fluctuations in IAA and increase in the amount of exogenous IAA causes elongation of the primary root and the formation of lateral and adventitious roots (Finnie and Van staden, 1985).

Biosynthesis of IAA does not only occur in higher plant. Organisms such as bacteria, fungi and algae are able to make physiologically active IAA that may have clear effects on plant growth and development. Many bacteria isolated from the rhizosphere have the capacity to synthesize IAA in vitro in the presence or absence of tryptophan (Strobel et al., 2004). Among PGPR species, *Azospirillum* is one of the best studied IAA producers. Other IAA-producing bacteria belonging to *Aeromonas, Azotobacter, Bacillus, Burkholderia, Psedomonas* and *Rhizobium* genera have been isolated from different rhizosphere soil. Inoculation with IAA producing PGPR has been used to stimulate seed germination, accelerate root growth, modify the architecture of the root system and increase the root biomass. In addition to stimulating root growth, IAA-producing bacteria can also be used to stimulate tuber growth (Nenwani et al.; 2010; Oves et al., 2013).

PGPR can also indirectly stimulate of the plant auxin pathway to promote plant growth. For example, several PGPR strains like *Azospirillum brasilense* can produce NO by the activity of a nitrite reductase during root colonization. NO is involved in the auxin signaling pathway controlling lateral root formation (Creus et al., 2005). DAPG is a well-known antimicrobial compound produced by biocontrol fluorescent pseudomonads (Couillerot et al., 2009). DAPG can interfere with an auxin-dependent signaling pathway (Brazelton et al., 2008; Radhakrishnan et al., 2013).

2.2.3.4.2. Ethylene

Ethylene in low levels has been observed to promote growth, but it may inhibit root elongation at moderate to high levels. Plants typically respond to the presence of phytopathogens by synthesizing stress ethylene that stimulates the effects of the stress on the plant (Abeles et al., 1992). Thus, one way to decrease the damage in plants caused by various phytopathogens is to lower the plant's ethylene response (Glick and Bashan, 1997). The simplest way to accomplish this is to treat plants (generally the roots or seeds) with ACC deaminase containing PGPB (Glick et al., 1998). Normally, in plants, 1-aminocyclopropane-1-carboxylate (ACC) and 5'-deoxy-5'methylthioadenosine (MTA) is converted to ACC by ACC synthase (Glick et al., 2007). ACC deaminase cleaves and separates the plant ethylene precursor ACC and thus lowers the level of ethylene in a developing or stressed plant (Glick, 2005). To date, this strategy has been utilized in greenhouse and growth chamber experiments to reduce the damage in several plants (cucumber, potato, tomato, carrot, and soybean) (Hao et al., 2007; Husen et al., 2011).

2.2.3.5. Ammonia production

Ammonia production is one of the essential phase of the nitrogen cycle and involves degradation of nitrogenous biopolymers for subsequent release of ammonia. This process is initiated by excretion of an extracellular proteolytic enzyme that is commonly produced by some soil microorganism such as *Bacillus* spp. This enzyme sequentially hydrolyzes the proteins of the plant and animal origins into their constituent acids (Rogers et al., 1996; Clay et al., 2009). Amino acids are afterwards enzymatically deaminated with the release of the ammonia. Therefore, ammonia producing rhizobacteria is important in the regulation of nitrogen cycle (Millet et al., 2010; An and Mou, 2011; Bednarek, 2012).

2.2.3.6. Hydrogen cyanide (HCN)

HCN is a volatile, secondary metabolite that suppresses the development of microorganisms and that also affects negatively the growth and development of the plant. HCN is a powerful inhibitor of many metal containing enzymes, especially copper containing cytochrome c oxidase. HCN is formed from glycine through the action of HCN synthese enzymes, which is associated with the plasma membrane of certain rhizobacteria. To date, many different rhizobacteria have shown to be capable of producing HCN, including species of *Bacillus, Pseudomonas, Rhizobium, Alcaligenes* and *Aeromonas.* HCN production is a common trait within the group of *Pseudomonas* present in the rhizosphere. Some studies showed that about 50% of *Pseudomonas* isolate from wheat and potato rhizosphere are able to produce HCN *in vitro.* Various studies attributed to disease protective effect of HCN for "root-knot" and "black rot" diseases caused by the nematode in Tomato and Tobacco root (Oracz et al., 2007, 2008).

2.2.3.7. Siderophore production

Iron is the fourth most abundant element on the earth's crust, however iron is not readily assimilated by either bacteria or plants because it predominantly found as ferric ion or Fe^{+3} in aerobic soils. This form has low solubility at near-neutral pH so that the amount of iron available for assimilation by living organisms is extremely limited (Müller et al., 1984; Ma, 2005; Sandy and Butler, 2009). Both microorganisms and plants require a high level of iron for the variety of biochemical reactions in the cell, however obtaining sufficient iron can be problematic in the rhizosphere due to competition for iron among organisms (Guerinot and Ying, 1994). A low-molecular weight ($\backsim$400-1500 Da) compounds called siderophores with an exceptionally high affinity for Fe^{+3} (Ka ranging from 10^{23} to 10^{52}) is synthetised by bacteria. Besides siderophores, bacteria can synthesis membrane receptors which are able to bind the Fe-siderophore complex. Both siderophores and receptors facilitate iron uptake by microorganisms to survive limited iron amount (Hider and Kong, 2010). Siderophores are also called as iron (Fe) chelating agents. By the help of siderophore synthesis, most bacteria living in the soil can solubilize and transport iron from iron precipitates (hydroxide polymers, heme proteins, ferritin), then use it for their own energy and growth needs (Goetz *et al.*, 2002; Bellenger et al.,2008).

As evidenced from experimental studies, biocontrol PGPB siderophores can suppress of fungal pathogen-caused plant disease. For example, some studies have included the use of mutants that were defective in siderophore production and found that these strains were less effective than the wildtype strains at protecting plants against fungal pathogens (Buysens et al., 1996). On the other hand, in another study, siderophore overproducing mutants have been shown to be more effective at protecting plants against fungal pathogens (Vandenbergh and Gonzalez, 1984).

2.2.3.8. Exopolysaccharide production

Salinity is the serious problem of agriculture in arid and semi-arid regions of the world. Salt-tolerant plant growth-promoting rhizobacteria (PGPR) can play an important role in reducing soil salinity stress during plant growth. Bacterial exopolysaccharide (EPS) that produced by PGPR as mucoid substance can also help to decrease salinity stress by reducing the content of Na^+ available for plant uptake (Paul and Nair, 2008; Upadhyay et al., 2011). Exopolysaccharides also help microorganisms to survive in other disadvantageous environmental conditions such as heavy metal presence (Upadhyay et al, 2011).

2.2.3.9. Antifungals produced by rhizobacteria

Rhizospheric bacteria play an important role as major biofertilizer. In addition, they have important roles for biocontrol of plant pathogens including fungi. Therefore the biological control of fungal diseases by microbial agent comes into view for a perfect option and endeavours to detach and describe endogenous biocontrol specialists are progressing in numerous test labs around the world (Selin et al., 2010; Yanes et al., 2012; Lagzian et al., 2013; Kakar et al., 2014).

In general, many fungal phytopathogens are most destructive when the soil temperature is low during cold and temperate climates. In those environments, cold tolerant (psychrotrophic) biocontrol plant growth-promoting bacteria (PGPB) are likely to be more effective in the field than mesophilic biocontrol strains. Moreover, in countries with the cool soil temperatures i.e. Canada, Sweden, Finland, Russia, the function of PGPB must be more important. Under cold conditions, the biocontrol PGPB effectively

outcompete fungal pathogens for available iron. (Santoyo et al., 2012; Martinez- Absalon et al., 2014).

2.2.3.10. Antimicrobial metabolites produced by bacteria

Antimicrobial materiales produced by various bacteria can inhibit growth of certain bacteria. The antibiotics formed by certain *Bacillus* species include Gramicidin, Tyrocidine, Bacitracin, Mycobacillin, Bacilysin and Subtilin (Egorov, 1985; Mannanov and Sattarova, 2001). Several antimicrobial materials produced by bacteria such as Gramicidine, Polymyxins, Bacitracins, are used in medicine, while others such as Subtilin or Nisins are used for food canning. Bacitracins are used in agriculture to support animal feeds (Egorov, 1985; Ridgway et al., 2001).

Root-colonising pseudomonads produce a diversity of extracellular metabolites with antimicrobial activity that have an important role in disease suppression. These substances include 2,4-diacetylphloroglucinol (2,4-DAPG), pyoluteorin, phenazines, pyrrolnitrin, cyclic lipopeptides and hydrogen cyanide (HCN) (Weller, 2007).

2.2.3.11. Quorum sensing

Quorum sensing (QS) ensures cell-to-cell communication among bacteria and is regulated by the expression of specific sets of genes in response to changes in cell density (Figure 2.6). The perception of population density has mediated by specific molecules called auto inducers (AIs) or auto inducing peptides (AIPs). Autoinducers like N-acylhomoserine lactones (AHLs) are widely conserved signal molecules in quorum sensing systems in Gram negative bacteria including the ones causing diseases in the plant. Bacterial growth directs a proportional increment in the AI extracellular concentration. Once a cell density reaches to be a certain level, the bacterial population recognizes the AI and reacts to it through the expression of the particular gene (Fuqua et al., 1994; Dong et al., 2000; Miller and Bassler, 2001).

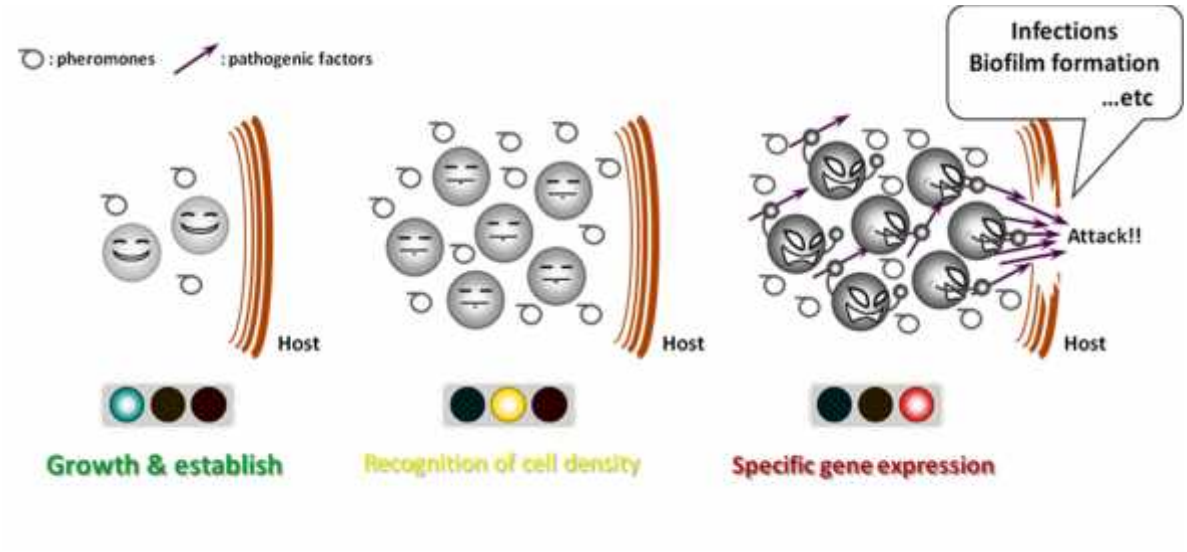


Figure 2.6. Quorum sensing in bacteria

QS control are useless at the point when directed by an individual cell but become more effective when performed collectively (Novick et al., 1995; Seed et al., 1995). When the information goes out, more and more bacteria are collected at the site of the attack. This site can be a wound, for example. When they reached to a sufficient level at certain point, they start acting like multicellular organisms. Then, they can form biofilms and thick structures allowing resistance against both anti-infectional agents and the immune defense system of the host. At the same time, they become more aggressive and increase their mobility. All of these changes are triggered when the communication molecules affect regulation various genes by turning either on or off (Novick and Geisinger, 2008; Ng and Bassler, 2009).

Most of the bacterial plant pathogens depend autoinducer-mediated quorum-sensing to turn on gene cascades related to key virulence factors (e.g. cell-degrading enzymes and phytotoxins) (von Bodman et al., 2003). Recently, it has been demonstrated that certain PGPB suppress pathogen quorum-sensing capacity by degrading autoinducer signals, thereby blocking expression of numerous virulence genes (Dong et al., 2000; Molina et al., 2003; Newton and Fray, 2004). This approach holds tremendous potential for alleviating disease, even after the initiation of infection.

2.3. Wheat

Wheat (*Triticum* spp.) is a cereal grain that is cultivated worldwide. In 2013, world production of wheat was reported as 713 million tons. Wheat is the third most-produced cereal after maize (1,016 million tons) and rice (745 million tons). Globally, wheat is used in human food as significant source of vegetable protein. Compared to other major cereals (maize or rice), the protein content of wheat is higher. It also contains vitamins, minerals, antioxidants, enzymes, and phytonutrients.

Einkorn wheat is a general term for indication of either the wild species of wheat, *Triticum boeoticum*, or the domesticated form, *Triticum monococcum*. Einkorn wheat was also one of the first plants to be domesticated and cultivated. Evidence from two archaeological sites in southern Turkey (Weiss and Zohary, 2011) pointed out the earliest date for domestication of Einkorn was 10,600 to 9,900 years before present (8,650 BC to 7,950 BC) (Weiss and Zohary, 2011).

Wheat genetics is more complicated than most of the other domesticated species (Hancock, 2004). Some wheat species are diploid having two sets of chromosomes, but many are polyploids possessing four (tetraploid) or six (hexaploid) sets of chromosomes. Einkorn wheat (*T. monococcum*) is diploid (AA, two complements of seven chromosomes, 2n=14). Most tetraploid wheats (e.g. emmer and durum wheat) are derivatives of wild emmer, *T. dicoccoides*. Wild emmer resulted from hybridization between two diploid wild grasses, *T. urartu* and a wild goatgrass such as *Aegilops searsii* or *Ae. speltoides*. The results of studies showed that the hybridization during formation of wild emmer (AABB) occurred in the wild long before domestication (Hancock, 2004) and was driven by natural selection. However, evolution of hexaploid wheats occurred in farmers' fields. It is believed that either domesticated emmer or durum wheat hybridized with yet another wild diploid grass (*Aegilops tauschii*) to make the hexaploid wheats, spelt wheat and bread wheat (Hancock, 2004). Hexaploid wheats have three sets of paired chromosomes, therefore three times as many as diploid wheat.

It has been estimated that about 65% of wheat grain is used directly as human's consumption. The most of the remaining (21%) is used as forage for livestock, while 8% is used as seed, and 6% is for other use (Kathlak, 2000).

2.3.1. Wheat classification

Kingdom: Plant

Division: Spermatophyte

Sub-division: Angiosperm

Class: Monocotyledon

Order: Poales

Family: Poaceae

Triticum durum (Queensberry, 1967; Kathlak, 2000*).*

2.3.2. Growth period of wheat

The developing time of spring wheat ranges from 100 to 130 days while winter wheat needs around 180 to 250 days to develop. Day length period and temperature necessities are key features for different varieties. Winter wheat varieties are sown at spring in Europe (Al-Younis et al., 1987). A few frameworks (Feekes, Zadoks, and Haun scales) have been created to describe the growth stages of this crop. The ten major growth stages include : germination, seedling, tillering, stem elongation or jointing, booting, heading, flowering or anthesis, milk, dough (Anderson et al., 1995).

A soft winter or white wheat variety is suitable for soft wheat milling and for production of cakes, cookies, and cracker. The quality of durum wheat is defined by its suitability for semolina and macaroni production. Hard red winter and spring wheats are suitable for hard wheat milling and bread production (Queensberry, 1967; Kathlak, 2000).

2.4. Barley

Barley (*Hordeum vulgare*) is an annual cereal grain which is mainly consumed by animals and is less utilized by human. Barley has also been used as animal fodder. A large part of this crop is used for malting which is a key ingredient in beer and whisky production. A non-alcoholic drink such as barley water and Munich are also made from unhulled barley. Barley is also used in soups and stews. A small amount is used in health

food and coffee substitutes. Barley is used as a medicine for many diseases (Kathlak, 2000).

Similar to wheat, this grass is cultivated in the entire world (Taiz and Zeiger, 1998). Barley is a extensively adaptable crop. It is currently popular in mild areas where it is grown as a summer crop and tropical areas where it is sown as a winter crop. Its' germination time is between one to three days. Barley grows under cool conditions, but is not resistant to too cold conditions. Barley has a short growing season. It is also relatively drought tolerant. Depending on its tolerance to water stress, they are classified as tolerant, semi-tolerant, and non-tolerant. Barley is also more tolerant to salty and gravel soil than wheat.

Barley was one of the first domesticated grains in the Fertile Crescent, which an area of relatively abundant water in Western Asia, and near the Nile River of northeast Africa (Maroof and Sbo, 2006). The grain appeared in the same time as einkorn and emmer wheat.

2.4.1. Barley classification

Kingdom: Plant

Division: Spermatophyte

Sub-division: Angiosperm

Class: Monocotyledon

Order: Poales

Family: Poaceae

Hordeum vulgare L.

Hordeum spontaneumL. (Queensberry, 1967; Kathlak, 2000).

Wheat and barley have many similarities in growth stage progress and management need until harvesting. Day length period and temperature requirements are key factors in variety selection (Kuktaite, 2004). Several systems (Feekes, Zadoks, and Haun scales)

have been developed to provide a numerical appointment for growth and developmental stages to harvest.

2.5. General Characteristics of Fungi

Fungi are eukaryotic, filamentous and spore delivering living things that can be either saprophyte or parasite on animal and plant. Fungi are multi-cellular organisms that don't contain chlorophyll; however they have a cell wall. Fungi are widespread in the environment and roughly 200.000 species have been recognized (Jensen et al, 1997; Hunt, 2007). All fungi growths are heterotrophic life forms that they require organic compound for nourishment. When they grow on dead plant and animals in soil, fresh water or salty water; they are known as saprophytes. Saprophytes decompose complex plant and animal remains by separating them into the basic synthetic substance that is come back to the soil, as a result of that increasing it is fertility. In this way, they can be very profitable to human. Saprophytic fungi are additionally important in industrial fermentation (Pelczar et al., 1986). Some fungi are parasitic which live in or on animal host (Talaro and Talaro, 1996). Fungi can reproduce both sexually and asexually. Asexual reproduction (also called somatic or vegetative reproduction) does not include the joining of nuclei of sex cells while the sexual generation is obtained by a combination of the compatible nuclei from two parent cells (Pelczar et al., 1986).

2.6. Antibiotic Resistance (AR)

Antibiotic resistance occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth. The property of antibiotic resistance can be intrinsic and acquired. Intrinsic resistance is related with inherent properties of the bacterium. Certain types of bacteria are inherently or originally resistant to the effect of particular antibiotic, this is also called innate resistance (Ingraham and Ingraham, 1995; Talaro and Talaro, 1996; Hugo and Russell, 1998). Mycoplasma lacks a cell wall, for this reason, not surprisingly, they are resistant to penicillins that exerts its action by interfering with cell wall synthesis. Many gram-negative bacteria are intrinsically resistant to certain antibiotics because the lipid bilayer of their outer membrane rejects entry of the antibiotic (Nester,

2001). Because of the absence of cell walls, they are completely non-susceptible to agents inhibiting peptidoglycan syntheses, such as β-lactam antibiotics and glycopeptides. In addition, mycoplasmas are resistant to Polymyxin, Rifampicin, Sulfonamides, Trimethroprim and Nalidixic acid. The antimicrobials exhibiting the highest inhibitory effect against mycoplasmas are the Tetracycline, macrolides and related antibiotics, including Ketolides. Aminoglycosides possess less inhibitory activity and are not used in vivo against these organisms, which are often located inside the cells (Ridgway et al., 2001).

Acquired resistance is gained by either a mutation in chromosomes of bacteria (mutational resistance) or transferring of resistance determinants via transposons or plasmids (transferable resistance). Transposons are small pieces of DNA sequence and mobile genetic elements have the ability to move from one piece of DNA molecule to another and vice versa. The ability of transposons to move from one DNA molecule to another has led to them being referred to as jumping genes (Hugo and Russell, 1998; Goldstein et al., 2011). Transformation, conjugation, and transformation are main mechanisms in transferring plasmids carrying resistance determinants between bacteria (Talaro and Talaro, 1996; Lyras et al., 1998). The extensive use of antibiotics has also resulted in the evolution of resistant bacteria that are not killed by antibiotics and these bacteria can threat health and life (Dilks et al., 2003).

There are three main mechanisms for antibiotic resistance;

A- The microorganism produces an enzyme that destroys the drug,

B- The target of drug action changes,

C- Adaptations make it difficult for the antibiotic to enter the microbial cells or the cell activity expels it enters (Igraham and Ingraham, 1995; O'Connor et al., 2008; Curry et al., 2009).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Plant materials

Two wheat and one barley root samples with rhizosphere soil were collected from three different fields. Wheat fields are located across the Kahramanmaras Sutcu Imam University Campus. Barley field was located at Seyrantepe Village on Gaziantep-Kahramanmaras road.

3.1.2. Phytopathogenic fungi

Five different phytopathogenic fungi (*Fusarium culmorum*, *Fusarium graminearum*, *Bipolaris sorokiniana*, *Fusarium* spp. and *Aspergillus* spp.) were kindly provided by Assoc.Prof.Dr. Seral Yücel in Adana Biological Combat Research Station.

3.1.3. Instruments

The list of instruments that are used during the present study is shown in Table 3.1.

3.1.4. Tools

The tools used for experiments were included culture tubes, loop, beakers, flasks, graduated cylinders, petri plates, forceps, scissors, aluminium foil, cotton, micropipette tips, parafilm and wooden applicator sticks and falcon tubes.

3.1.5. Culture media

The culture media used for growing of both bacteria and fungi were listed in Table 3.2.

Table 3.1. The list of instruments used in the present study

Instrument	Company	Country
Autoclave	Hirayama	Japan
Electronic balance	Presica	Italy
Incubator	Memmert	Germany
Water bath with shaker	Memmert	Germany
Microbiology safety cabinet	Esco	Singapore
Distilled water	Millipore	France
pH meter	Hanna	U.K
Hot plate with magnetic stirrer	Velp	Italy
Micropipettes	Eppendorf	Germany
Camera attached to gel documentation system (for fluorescent imaging)	Vilber Lourmat	France

Table 3.2. The list of culture media used in the present study

Culture media	Company	Country
Nutrient Agar	Merck	Germany
Pseudomonas Agar F (King Medium B)	Biolife	Italy
Nutrient Broth	Merck	Germany
Potato Dextrose Agar (PDA)	Biolife	Turkey

3.1.5.1. Nutrient agar

Prepared by dissolving 8g of nutrient broth and 15 g of agar in 1liter of distilled water and sterilized by autoclave at 121°C for 15 min.

Composition of Nutrient Agar (g/L)

Peptone from meat	5
Meat extract	3
Agar	15

3.1.5.2. Pseudomonas agar F (King Medium B)

This medium was used for isolation of *Pseudomonas* spp. For preparation of the medium, 38 g was dissolved in 1000 ml of distilled water and then 10 ml of glycerol (Amresco) was added. It was heated to boiling with frequent equitation and sterilize by autoclaving at 121°C for 15 min. After cooling to 45-50°C, mixed well and poured into sterile petri plates.

Composition of Pseudomonas Agar F (King Medium B)	g/L
Tryptone	10
Peptone	10
Magnesium Sulphate ($MgSO_4$)	1.5
Dipotassium Hydrogen Phosphate (K_2HPO_4)	1.5
Agar	15

3.1.5.3. Nutrient broth

Prepared by suspending 8 g nutrient broth in 1liter of distilled water and sterilized by autoclave at 15psi pressure, 121°C for 15 min

Composition of Nutrient Agar	g/L
Peptone from meat	5
Meat extract	3

3.1.5.4. Potato dextrose agar (PDA)

Potato Dextrose Agar (PDA) is a general purpose medium for the cultivation of yeasts and molds. It can be supplemented with acid or antibiotics to inhibit bacterial growth. Many standard procedures use a specified amount of sterile tartaric acid (10%) to lower the pH of this medium to 3.5 +/- 0.1, inhibiting bacterial growth. Chloramphenicol acts as a selective agent to inhibit bacterial overgrowth of competing microorganisms from mixed specimens, while permitting the selective isolation of fungi. Medium was prepared by dissolving 42 g of PDA in 1000 ml of distilled water. Then, it was autoclaved at 121 ^{o}C for 15 min.

Composition of Potato Dextrose Agar (PDA)	g/L
Potato Extract	5
Glucuse	20
Agar	17

3.2. Methods

3.2.1. Sample collection

The root samples with rhizosphere soil were collected from healthy wheat and barley plants by pulling out of roots from soil on March 05, 2015 in Kahramanmaras, Turkey. After removing the shoot from the root by sterilized scissors, samples were transferred to sterile falcon tubes containing sterile saline (0.85% NaCl).

Figure 3.1. Wheat and barley root samples with rhizosphere soil

3.2.2. Isolation of rhizobacteria

Root samples with attached rhizosphere soil were transferred to sterile tubes containing sterile saline after separating from shoot aseptically and transported to the laboratory. By using sterilized forceps and scissor, a smaller sample was excised from the root sample. To isolate bacteria, such as *Bacillus and Pseudomonas* spp. from root samples with rhizosphere soils were weighted and serial dilutions were prepared (Figure 3.2). Subsequently, 100 µl from each dilution was spread onto Nutrient Agar or Pseudomonas Agar F plates for isolation of *Bacillus* and *Pseudomonas* spp., respectively. For isolation of *Bacillus* spp., diluted samples were heat treated at 80^{o}C for 10 min for killing vegetative forms. The plates were then incubated at 28 ^{o}C for 3 days. Flourescent *Pseudomonas* colonies were selected on UV light.

Representative colonies with typical *Bacillus* and *Pseudomonas* spp. appearance were picked up and restreaked on Nutrient Agar or Pseudomonas Agar F plates for purification. Then, they were transferred to Nutrient Broth and grown for antifungal test (Figure 3.3).

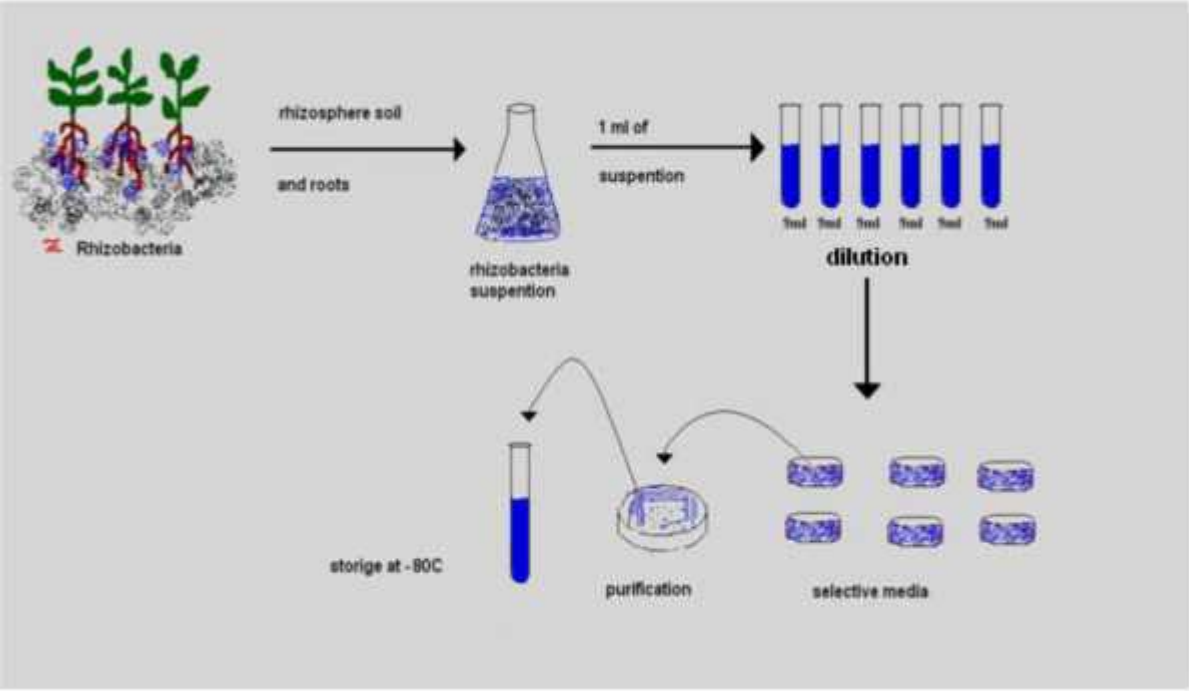


Figure 3.2. Schematic representation of rhizobacteria isolation procedure

3.2.3. Biochemical reactions

Some biochemical tests were performed for presumable identification of Rhizosphere bacteria.

Figure 3.3. Performing antifungal test

3.2.3.1. Oxidase test

Filter paper was placed in a empty petri plate and saturated with oxidase reagent (1% dimethyl-pphenylene diamine- dihydrochloride). A colony on the plate was then transferred to the filter paper with a loop. Development of a violet to purple color in 10 seconds was scored as positive reaction. (Mahon and Manuselis, 2000).

3.2.3.2. Catalase test

A bacterial colony was transferred from the plate to a glass microscope slide containing a drop of water. Then, the catalase test was performed by adding a drop of 3% hydrogen peroxide (H_2O_2). The release of oxygen bubble indicated the presence of catalase activity (Mahon and Manuselis, 2000).

3.2.4. Detection of fungal inhibitor activities of rhizospheric bacteria

In vitro inhibition of phytopathogenic fungi by rhizobacteria was determined by using the spot test method (Sindhu et al., 1999). Briefly, fluorescent *Pseudomonas* and *Bacillus* spp. isolates (n=59) were grown in nutrient broth at 28^{o}C. Then, spores belonging to five phytopathogenic fungi (*Fusarium culmorum*, *Fusarium graminearum*, *Bipolaris sorokiniana*, *Fusarium* spp. and *Aspergillus* spp.) were spread onto PDA plates after dilution in sterile distilled water (Figures 3.4. and 3.5). Following to fungi inoculation, grown rhizobacteria were spotted onto plates. Subsequently, plates were incubated at 25^{o}C for 2-3 days. The zones of inhibition around bacteria were measured.

Figure 3.4. *Fusarium graminearum* (left); *Fusarium culmorum* (right)

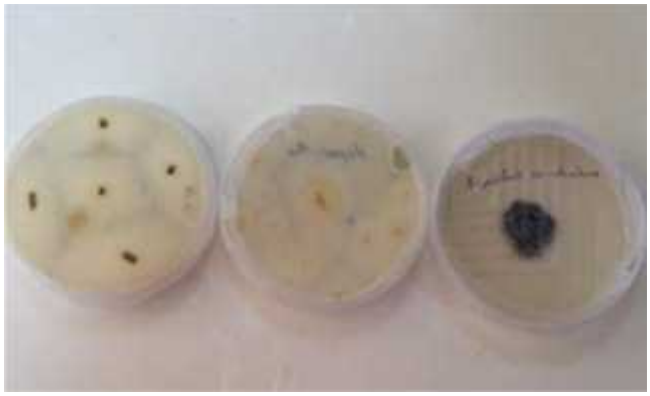


Figure 3.5. *Fusarium* spp. (left); *Aspergillus* spp. (middle); *Bipolaris sorokiniana* (right)

3.2.5. Stock culture preparation from the bacterial isolates

The bacterial isolates grown in nutrient broth (1 ml) were transferred to sterile eppendorf tubes. Subsequently, sterile glycerol was added onto each tube at 10% final concentration and then tubes were mixed by vortex mixer. Furthermore, stock cultures were kept at -80˚C for long-term preservation of bacteria.

4. RESULTS AND DISCUSSION

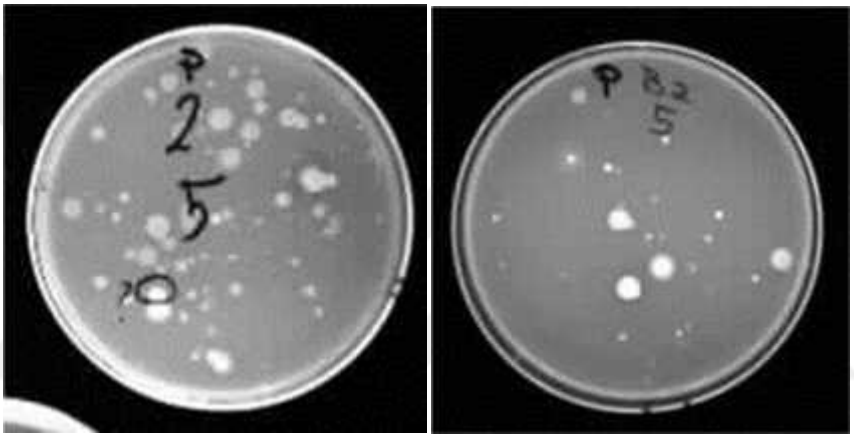
4.1. Isolation of Rhizosphere Bacteria from Wheat and Barley

Numerous plant growth promoting rhizobacteria (PGPR) of the genera *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Azospirillum*, *Klebsiella*, and *Enterobacter* have been isolated from the rhizosphere of various crops. Their synergistic effects on plant growth have been also demonstrated (Kloepper et al., 1992; Egamberdiyeva and Höflich, 2001). Understanding the diversity and beneficial activity of the plant-bacterial association is important to sustain agroecosystems for sustainable crop production (Germida et al., 1998). It is also known that rhizosphere microbial population can be altered by many factors including plant type, so isolation of rhizosphere bacteria specific to a certain crop rhizosphere should be important for selection of strains with biocontrol potentials. Therefore, in the present study, *Bacillus* and *Pseudomonas* strains were isolated from wheat and barley rhizosphere grown in Kahramanmaraş, Turkey. Samples from serial dilutions (10^{-3}, 10^{-4}, 10^{-5}) of root samples with rhizosphere soil were plated onto selective media (King B Agar for *Pseudomonas* and Nutrient Agar following to heat-treatment for *Bacillus*) for preliminary identification of *Pseudomonas* and *Bacillus* strains. Figure 4.1 shows grown *Pseudomonas* colonies on King B Agar after incubation.

Figure 4.1. Appearance of *Pseudomonas* colonies on King B medium

4.1.1. Identification of fluorescent *Pseudomonas* isolates

Colonies grown on King B agar have been examined for fluorescence pigment production under UV light reflection and photos have been taken by using camera attached to gel documentation system (Vilber-Lourmant) (Figures 4.2. and 4.3.). All *Pseudomonas* strains isolated from wheat and barley rhizoshere have been scored based on the fluorescence properties (Table 4.1). As a result; 10 out of 18 wheat rhizosphere isolates and 8 out of 22 isolates barley rhizosphere isolates were found as fluorescent *Pseudomonas* spp.



Figures 4.2. Identification of fluorescent *Pseudomonas* colonies under UV from wheat (left) and from barley (right)

Figure 4.3. Growth of fluorescent and non-fluorescent *Pseudomonas* isolates on King B Agar

Table 4.1. Fluorescent or non-fluorescent presumable *Pseudomonas* isolates

Pseudomonas isolates from wheat	Fluorescein	*Pseudomonas* isolates from barley	Fluorescein
P1	-	P19	-
P2	+	P20	-
P3	-	P21	+
P4	-	P22	+
P5	-	P23	-
P6	-	P24	+
P7	+	P25	-
P8	-	P26	-
P9	+	P27	+
P10	+	P28	-
P11	+	P29	+
P12	+	P30	-
P13	+	P31	+
P14	+	P32	-
P15	+	P33	-
P16	-	P34	-
P17	-	P35	-
P18	+	P36	-
		P37	-
		P38	+
		P39	-
		P40	+

4.2. Morphological Examination of Bacterial Colonies

Morphological characteristics (colony size, surface, margin of colony) of all strains isolated from rhizosphere of wheat and barley were examined and shown in Tables 4.2., 4.3., 4.4., 4.5.

Table 4.2. Morphological characteristics *Pseudomonas* strains isolated from wheat

Cereal type	Isolate number	Colony size	Morphological characteristics of colony
Wheat	P1	+	Smooth surface, entire margin and
	P2	++	Rough surface, wavy margin and white
	P3	++	Smooth surface, entire margin and white
	P4	++	Rough surface, wavy margin and white
	P5	++	Smooth surface, entire margin and
	P6	++	Rough surface, wavy margin and white
	P7	++	Smooth surface, entire margin and
	P8	+	Smooth surface, entire margin and
	P9	++	Rough surface, wavy margin and cream
	P10	++	Smooth surface, entire margin and
	P11	++	Smooth surface, entire margin and cream
	P12	++	Rough surface, wavy margin and cream
	P13	++	Rough surface, wavy margin and cream
	P14	++	Smooth surface, entire margin and cream
	P15	++	Rough surface, wavy margin and white
	P16	++	Smooth surface, entire margin and
	P17	++	Smooth surface, entire margin and
	P18	++	Rough surface, wavy margin and white

Table 4.3. Morphological characteristics *Bacillus* strains isolated from wheat

Cereal type	Isolate number	Colony size	Morphological characteristics of colony
Wheat	B1	++	Smooth surface, entire margin and cream
	B2	+	Rough surface, wavy margin and cream
	B3	++	Rough surface, wavy margin and cream
	B4	++	Smooth surface, entire margin and cream
	B5	+	Smooth surface, entire margin and white
	B6	++	Smooth surface, entire margin and white
	B7	++	Smooth surface, entire margin and cream
	B8	++	Rough surface, wavy margin and white
	B9	++	Rough surface, wavy margin and cream
	B10	++	Smooth surface, entire margin and white
	B11	+	Rough surface, wavy margin and cream
	B12	+	Smooth, surface, entire margin and
	B13	+	Rough surface, wavy margin and cream
	B14	++	Smooth surface, entire margin and cream
	B15	++	Rough surface, wavy margin and white
	B16	++	Rough surface, wavy margin and cream
	B17	++	Smooth surface, entire margin and cream
	B18	+	Rough surface, wavy margin and cream
	B19	++	Rough surface, wavy margin and cream
	B20	++	Rough surface, wavy margin and cream
	B21	+	Rough surface, wavy margin and cream
	B22	+++	Dull surface, filamentous margin

Table 4.4. Morphological characteristics *Pseudomonas* strains isolated from barley

Cereal type	Isolate number	Colony size	Morphological characteristics of colony
Barley	P19	++	Smooth surface, entire margin and cream
	P20	++	Smooth surface, entire margin and cream
	P21	++	Smooth surface, entire margin and cream
	P22	+	Smooth surface, entire margin and cream
	P23	++	Smooth surface, entire margin and cream
	P24	++	Smooth surface, entire margin and cream
	P25	++	Smooth surface, entire margin and cream
	P26	+	Smooth surface, entire margin and cream
	P27	++	Smooth surface, entire margin and yellow
	P28	+	Smooth surface, entire margin and cream
	P29	+	Smooth surface, entire margin and cream
	P30	++	Smooth surface, entire margin and cream
	P31	++	Smooth surface, entire margin and yellow
	P32	++	Smooth surface, entire margin and cream
	P33	+	Smooth surface, entire margin and cream
	P34	++	Rough surface, wavy margin and cream
	P35	+++	Smooth surface, entire margin and yellow
	P36	+	Smooth surface, entire margin and yellow
	P37	+	Smooth surface, entire margin and yellow
	P38	+	Smooth surface, entire margin and yellow
	P39	+	Smooth surface, entire margin and yellow
	P40	++	Smooth surface, entire margin and cream

Table 4.5. Morphological characteristics *Bacillus* strains isolated from barley

Cereal type	Isolate number	Colony size	Morphological characteristics of colony
Barley	B23	+++	Rough surface, wavy margin and white
	B24	+	Rough surface, wavy margin and white
	B25	++	Smooth surface, entire margin and cream
	B26	+	Rough surface, wavy margin and cream
	B27	++	Smooth surface, entire margin and white
	B28	++	Smooth surface, entire margin and white
	B29	+	Rough surface, wavy margin and cream
	B30	++	Rough surface, wavy margin and white
	B31	+	Rough surface, wavy margin and cream
	B32	++	Rough surface, wavy margin and cream
	B33	++	Smooth surface, entire margin and white
	B34	++	Rough surface, wavy margin and cream
	B35	++	Rough surface, wavy margin and cream

As a result, a total of 40 *Pseudomonas* (18 from wheat and 22 from barley rhizosphere) and 35 *Bacillus* (22 from wheat and 13 from barley rhizosphere) strains were isolated and stored at the end of this study.

4.3. Identification of Isolates Based on Some Biochemical Tests

Identification of isolates was performed based on two tests (oxidase and catalase). As known that, most of the *Pseudomonas* spp. were found as oxidase positive as expected as a general feature of *Pseudomonas* genus (Table 4.6). However, some strains showed negative oxidase reaction. This result was also in accordance with the literature, since oxidase negative species of *Pseudomonas* genus have been detected. Almost all *Pseudomonas* species have catalalase positive. For this reason, catalase activity was tested for all *Pseudomonas* isolates. Table 4.7. shows results of catalase test for *Pseudomona*s

isolates. As concordant with the general feature of this genus, all isolates were found as catalase positive.

Table 4.6. Oxidase test results of *Pseudomonas* isolates

Isolate	Oxidase	Isolate	Oxidase
P1		P21	+ weak
P2	+	P22	+
P3		P23	+ weak
P4	+	P24	+
P5	+	P25	-
P6	+	P26	+ weak
P7	+	P27	+
P8	-	P28	-
P9	+	P29	+
P10	+ weak	P30	+ weak
P11	+ weak	P31	+
P12	+	P32	+ weak
P13	+	P33	+ weak
P14	+	P34	+ weak
P15	+ weak	P35	+ weak
P16	-	P36	-
P17	-	P37	-
P18	-	P38	+
P19	+	P39	+
P20	+	P40	+ weak

Table 4.7. Catalase test results of *Pseudomonas* isolates

Isolate	Catalase	Isolate	Catalase
P1	+	P21	+
P2	+	P22	+
P3	+	P23	+
P4	+ weak	P24	+
P5	+	P25	+
P6	+ weak	P26	+
P7	+	P27	+
P8	+	P28	+
P9	+	P29	+
P10	+	P30	+
P11	+	P31	+
P12	+	P32	+
P13	+	P33	+
P14	+	P34	+
P15	+	P35	+
P16	+	P36	+
P17	+	P37	+
P18	+	P38	+
P19	+	P39	+
P20	+	P40	+

4.4. Inhibitory Activity of Bacterial Isolates Against Phytopathogenic Fungi

Beneficial effects of micro-organisms have often included faster seed germination, better seedling emergence, and increased plant growth. However, prior to selection and/or improvement of suitable strains for biocontrol purposes, it is necessary to detect the important traits required for this purpose. The production of fluorescent siderophores (iron-binding compounds) and antibiotic compounds are some of important traits for the inhibition of plant root pathogens. In addition, efficient root colonization is also a prerequisite for successful biocontrol strains (O'Sullivan and O'Gara, 1992). Many strains

of *Pseudomonas fluorescens* show potential for biological control of phytopathogens especially root pathogen.

After recognition the importance of rhizosphere bacteria as biocontrol agent, this technology gained too much interest. Many studies were performed on this subject in worldwide. In one of those study, (Egamberdiyeva, 2008) was were isolated rhizosphere and phyllosphere bacteria (*Pseudomonas*, *Bacillu*s, *Kocuria*, and *Microbacterium*, and *Cellulomonas*) species from wheat and peas and then examined for their plant growth promoting properties. The effects of bacterial inoculants on the growth of peas and wheat were studied in a series of pot experiments using loamy sand soil. The results showed that the colonisation of bacteria was higher in the rhizosphere as compared to the phyllosphere of both plants. It was also found that the response of wheat and peas when inoculated with bacteria was significantly positive over the control. After inoculation with effective bacterial strains, the root and shoot growth, and nodulation of peas were increased. However, the strains stimulated only the roots of wheat. Sixteen bacterial isolates inhibited the growth of *R. solani* and growth inhibition zone varied from 6-15 mm by different rhizobacterial isolates. Two isolates WPS3 and WPS90 caused maximum growth inhibition of the fungi and identified as *Pseudomonas* spp. *Rhizoctonia solani* causes root rot disease in wheat leading to collapsing of the aerial part of the plant (Dua and Sindhu, 2012). In another study, one hundred and thirty bacterial isolates were obtained from the rhizosphere soil of wheat and these rhizobacterial isolates along with 72 reference strains were screened for their antagonistic interactions against *R. solani* under cultural conditions.

Around the world, cereal crops constitute the largest product group with their cultivation and production levels. Cereal farming is carried out on over 75% of cultivated lands in Turkey, and wheat (68.0%) and barley (22.3%) have the largest share among cereal crops. Current data indicate wheat farming lands of Turkey as 8.1×10^6 ha and total production as 21.8×10^6 t with an average yield of 2785 kg ha–1 (http://www.tuik.gov.tr).

In the present study, most of the bacterial isolates were tested in vitro for their ability to inhibit the growth of phytopathogenic fungi (*Fusarium culmorum*, *Fusarium graminearum*, *Bipolaris sorokiniana*, *Fusarium* spp. and *Aspergillus* spp.). Three fungi species *(Fusarium culmorum*, *Fusarium graminearum*, *Bipolaris sorokiniana*) used in this

study are very important for wheat and barley grains as explained below. Another important point about those fungi species is that all have been isolated from infected wheat grown in Turkey. *Fusarium* spp. and *Aspergillus* spp. were isolated from tomato as pathogen.

Gibberella zeae, also known by the name of its anamorph *Fusarium graminearum*, is a plant pathogen which causes fusarium head blight. This disease is very harmful on wheat and barley (Bai and Shaner, 2004). The pathogen causes billions of dollars in economic losses worldwide each year (De Wolf ED et al., 2003). Infection also generates shifts in the amino acid composition of wheat (Beyer and Aumann, 2008), resulting in shriveled kernels and contaminating the remaining grain with mycotoxins. Among them two main types deoxynivalenol and zearalenone are important. Deoxynivalenol inhibits protein biosynthesis, zearalenone is an estrogenic mycotoxin. These toxins cause vomiting, liver damage, and reproductive defects in livestock, and are harmful to humans through contaminated food. *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*) is the causal agent of common root rot, leaf spot disease, seedling blight, head blight, and black point of wheat and barley. The fungus is one of the most serious foliar disease restricts both crops in warmer growing areas and causes significant yield losses. High temperature and high relative humidity assist the outbreak of the disease, in particular in South Asia's intensive "irrigated wheat-rice" production systems (Kumar et al., 2002).

Fusarium culmorum is another fungal plant pathogen and the causal agent of seedling blight, foot rot, ear blight, stalk rot, common root rot and other diseases such as *Fusarium head blight* of cereals (in particular in wheat and barley), grasses, and a wide variety of monocots and dicots. It causes significant yield and quality losses and results in contamination of the grain with mycotoxins (Scherm et al., 2003).

In vitro inhibitory activities by *Pseudomonas* and *Bacillus* isolates were determined in this study (Figures 4.4, 4.5, 4.6, 4.7). The results of those experiments for the tested bacterial isolates were given in Tables 4.8, 4.9, 4.10, 4.11 and 4.12. As seen from data, most *Pseudomonas* and *Bacilllus* isolates had various levels of inhibitory activity against fungal phytopathogens. The degree of inhibition was indicated with +++ for highest, ++ for moderate and + for lowest activity.

A total of 28 *Pseudomonas* and 35 *Bacillus* isolates were tested for inhibitory activities against *Fusarium graminearum*, *Bipolaris sorokiniana* in the present study. Antagonistic activity against *Fusarium graminearum* was detected for 19 out of 28 *Pseudomonas* and 21 out of 35 *Bacillus* isolates. Results indicated that 21 out of 28 *Pseudomonas* isolates and 23 out of 35 *Bacillus* isolates have antagonistic activity against *Bipolaris sorokiniana*. Among 24 *Pseudomonas* and 34 *Bacillus* isolates that were tested for the inhibitory activity, 14 and 16 respectively had inhibitory activity against *Fusarium culmorum*. Therefore, higher number of isolates had inhibitory activity against *Bipolaris sorokiniana* among three wheat specific pathogens.

When the inhibitory activities were evaluated for *Aspergillus* spp. in this study, of 28 *Pseudomonas* and 35 *Bacillus* isolates, 19 and 21 found to be inhibitory. For *Fusarium* spp., 11 of 22 *Pseudomonas* and 19 of 35 *Bacillus* isolates had antagonistic activity.

Moreover, some isolates found to have strong inhibitory activities against all fungal wheat and tomato specific phytopathogens in the present study. Those isolates were identified as P7, P13, P14, P22, P34, B3, B7, B14, B15, B30, B32.

In a similar study performed by Fouzi et al. (2015), three fluorescent pseudomonads from the wheat rhizosphere and one from the endophyte of the halophyte *Atriplex halimus* were isolated and identified as *Pseudomonas putida* AF2, *P. aeruginosa* RB5, *P. fluorescens* RB13 and *P. aeruginosa* EH4. The *Pseudomonas* isolates strongly reduced the mycelial growth of *Fusarium oxysporum* and *Alternaria alternate* with the inhibition rate varying between 25 to 38% and 17 to 27%, respectively.

In the present study, isolates were only tested for their inhibitory activity, however no experiment was performed for determine specific traits of isolates (such as siderophore production, hydrogen cyanide production) associated with their anti-fungal activity. In addition, their utilization possibility as biocontrol agent during barley and wheat growth should be verified with in vivo trials such as fields and greenhouse experiments.

Table 4.8. Inhibitory activites of *Pseudomonas* and *Bacillus* isolates against *Bipolaris sorokiniana*

Cereal Type	*Pseudomonas* isolates	Inhibitory activity against *Bipolaris sorokiniana*	Cereal type	*Bacillus* isolates	Inhibitory activity against *Bipolaris sorokiniana*
Wheat	P2	-	Wheat	B1	+++
	P6	+		B2	+++
	P7	+		B3	+++
	P9	+++		B4	-
	P10	++		B5	+
	P11	-		B6	+
	P12	+		B7	+++
	P13	+		B8	++
	P14	+		B9	-
	P15	+		B10	+
Barley	P19	-		B11	-
	P20	-		B12	++
	P21	+		B13	-
	P22	+		B14	+++
	P23	-		B15	+++
	P24	+		B16	+
	P26	-		B17	+
	P27	++		B18	+++
	P29	+++		B19	+
	P30	+		B20	-
	P31	+		B21	+++
	P32	+		B22	-
	P33	-	Barley	B23	-
	P34	+++		B24	+++
	P35	+		B25	-
	P38	+		B26	+
	P39	+		B27	+
	P40	+++		B28	-
				B29	+
				B30	+
				B31	-
				B32	+++
				B33	+++
				B34	-
				B35	-

The zones of inhibition are indicated with +++ for >15 mm, ++ for 15-10 mm, + <10 mm, - for no inhibition.

Table 4.9. Inhibitory activites of *Pseudomonas* and *Bacillus* isolates against *Fusarium graminearum*

Cereal type	*Pseudomonas* isolates	Inhibitory activity against *Fusarium graminearum*	Cereal type	*Bacillus* isolates	Inhibitory activity against *Fusarium graminearum*
Wheat	P2	-	Wheat	B1	+++
	P6	++		B2	-
	P7	+		B3	+++
	P9	+		B4	+
	P10	-		B5	+
	P11	+		B6	+
	P12	++		B7	+++
	P13	+++		B8	+++
	P14	+		B9	-
	P15	++		B10	+
Barley	P19	-		B11	-
	P20	-		B12	-
	P21	-		B13	-
	P22	+		B14	+++
	P23	-		B15	+++
	P24	+		B16	-
	P26	+		B17	+++
	P27	+		B18	+++
	P29	+		B19	+++
	P30	+++		B20	+++
	P31	++		B21	+++
	P32	+++		B22	-
	P33	-	Barley	B23	+++
	P34	+++		B24	-
	P35	-		B25	-
	P38	++		B26	-
	P39	+		B27	++
	P40	+++		B28	++
				B29	-
				B30	+++
				B31	-
				B32	+++
				B33	+++
				B34	-
				B35	-

Table 4.10. Inhibitory activities of *Pseudomonas* and *Bacillus* isolates against *Fusarium culmorum*

Cereal type	*Pseudomonas* isolates	Inhibitory activity against *Fusarium culmorum*	Cereal type	*Bacillus* isolates	Inhibitory activity against *Fusarium culmorum*
Wheat	P2	++	Wheat	B1	+
	P6	-		B2	+
	P7	+		B3	++
	P9	-		B4	+
	P10	-		B5	-
	P11	+++		B6	+++
	P12	-		B7	++
	P13	+++		B8	-
	P14	+++		B9	+++
	P15	++		B10	-
Barley	P19	nd		B11	+
	P20	nd		B12	-
	P21	-		B13	-
	P22	+++		B14	++
	P23	nd		B15	+++
	P24	+++		B16	+++
	P26	+++		B17	-
	P27	+++		B18	-
	P29	+++		B19	-
	P30	++		B20	-
	P31	-		B21	-
	P32	-		B22	-
	P33	nd	Barley	B23	-
	P34	+++		B24	-
	P35	-		B25	-
	P38	-		B26	-
	P39	+		B27	-
	P40	+		B28	+++
				B29	+++
				B30	+
				B31	nd
				B32	+++
				B33	+
				B34	-
				B35	-

nd: not detected

Table 4.11. Inhibitory activites of *Pseudomonas* and *Bacillus* isolates against *Fusarium spp.*

Cereal type	*Pseudomonas* isolates	Inhibitory activity against *Fusarium spp.*	Cereal type	*Bacillus* isolates	Inhibitory activity against *Fusarium spp.*
Wheat	P2	+++	Wheat	B1	-
	P6	-		B2	+
	P7	+		B3	+
	P9	-		B4	-
	P10	-		B5	-
	P11	+++		B6	+++
	P12	nd		B7	++
	P13	+++		B8	-
	P14	+++		B9	-
	P15	-		B10	-
Barley	P19	-		B11	++
	P20	-		B12	-
	P21	-		B13	-
	P22	++		B14	+
	P23	-		B15	+++
	P24	nd		B16	-
	P26	nd		B17	+++
	P27	nd		B18	++
	P29	nd		B19	-
	P30	-		B20	+++
	P31	-		B21	+++
	P32	+++		B22	-
	P33		Barley	B23	+++
	P34	+++		B24	-
	P35	+++		B25	+++
	P38	+++		B26	-
	P39	-		B27	+
	P40	+++		B28	+++
				B29	+++
				B30	++
				B31	-
				B32	+++
				B33	+++
				B34	-
				B35	-

Table 4.12. Inhibitory activites of *Pseudomonas* and *Bacillus* isolates against *Aspergillus* spp.

Cereal type	*Pseudomonas* isolates	Inhibitory activity against *Aspergillus* spp.	Cereal type	*Bacillus* isolates	Inhibitory activity against *Aspergillus* spp.
Wheat	P2	-	Wheat	B1	+++
	P6	+		B2	+++
	P7	+		B3	+++
	P9	+		B4	+
	P10	-		B5	+
	P11	+		B6	-
	P12	++		B7	++
	P13	+		B8	+
	P14	+		B9	+
	P15	+++		B10	-
Barley	P19	-		B11	+++
	P20	+		B12	-
	P21	+		B13	-
	P22	++		B14	+++
	P23	-		B15	+++
	P24	-		B16	-
	P26	+		B17	+
	P27	+		B18	+
	P29	++		B19	-
	P30	-		B20	-
	P31	+		B21	++
	P32	+		B22	+
	P33	+	Barley	B23	-
	P34	+++		B24	-
	P35	-		B25	-
	P38	+		B26	++
	P39	-		B27	+++
	P40	-		B28	+
				B29	-
				B30	+
				B31	-
				B32	++
				B33	-
				B34	-
				B35	++

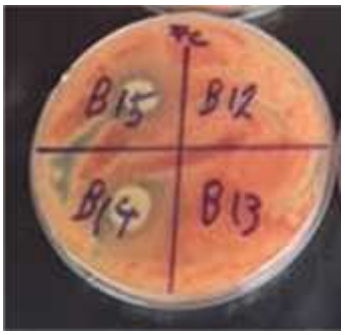


Figure 4.4. Inhibitory activity of *Bacillus* isolates against *Fusarium culmorum*. B14 and B15 have positive activities.

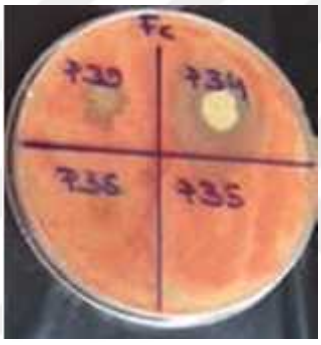


Figure 4.5. Inhibitory activity of *Pseudomonas* isolates against *Fusarium culmorum*

Figure 4.6. Inhibitory activity of some isolates against *Fusarium* spp.

Figure 4.7. Inhibitory activity of some Pseudomonas isolates against *Aspergillus* spp.

In Turkey, fluorescent Pseudomonas (FP) isolates were tested for their inhibitory activity against *Fusarium* pathogen in melon and watermelon and they were found to suppress the disease at 83% in melon (Bora et al.,. 1994). (Akköprü et al. 2005) were performed pot experiments in order to test effectiveness of inoculants containing FP and *Glomus intraradices* Schenck & Smith isolates alone and in combination on plant growth as determined from some morphological parameters (plant height, wet and dry weight) and on prevention of Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* (Sacc) Syd. Et Hans.) (FOL) in tomato. While *G. intraradices* (G.i.) was inoculated (75 spor/10 g soil) during the seed sowing, the FP bacteria suspensions (10^9 cfu/ml) were applied to the roots of tomato seedlings grown in pots. It was detected that combination of FP and G.i was more effective to reduce severity of disease and to provide positive contribution to plant growth parameters.

Effect of rhizosphere bacteria on wheat growth were detected by several studies in Turkey. (Bulut, 2013) evaluated effects of phosphorus-solubilizing (*Bacillus megatherium* var. phosphaticum [13] and nitrogen-fixing (*Stenotrophomonas maltophilia* [82] and *Ralstonia pickettii* [73]) bacteria and chemical fertilizer treatments on wheat yield and quality parameters were compared with control treatment. Significant differences were observed among treatments with regard to entire parameters. The best results were observed in chemical fertilizer treatments, however; single, dual, and triple bacteria combinations yielded significant increases in grain filling period, number of spikes per square meter, number of kernels per spike, 1000-kernel weight, biological yield etc.

5. CONCLUSIONS

Current agricultural practices use unsustainable practices such as chemical fertilizers. Those practices are great concern due to increasing mineral fertilizer costs and negative environmental impacts. For this reason, rhizosphere bacteria with beneficial roles such as biological nitrogen fixation or phosphorus-solubilizing are excellent alternatives for sustainable practices. There is an increased demand to those bacteria in the recent years. Many bacterial preparations have been used in the agriculture.

In the present study, a total of of 40 *Pseudomonas* (18 from wheat and 22 from barley rhizosphere) and 35 *Bacillus* (22 from wheat and 13 from barley rhizosphere) strains were isolated and stored at the end of this study. Most of the bacterial isolates were tested in vitro for their ability to inhibit the growth of phytopathogenic fungi (*Fusarium culmorum*, *Fusarium graminearum*, *Bipolaris sorokiniana*, *Fusarium* spp. and *Aspergillus* spp.). Antagonistic activity against *Fusarium graminearum* was detected for 19 out of 28 *Pseudomonas* and 21 out of 35 *Bacillus* isolates. Results indicated that 21 out of 28 *Pseudomonas* isolates and 23 out of 35 *Bacillus* isolates have antagonistic activity against *Bipolaris sorokiniana*. Among 24 *Pseudomonas* and 34 *Bacillus* isolates that were tested for the inhibitory activity, 14 and 16 respectively had inhibitory activity against *Fusarium culmorum*. Therefore, higher number of isolates had inhibitory activity against *Bipolaris sorokiniana* among three wheat specific pathogens.

When the inhibitory activities were evaluated for *Aspergillus* spp. in this study, of 28 *Pseudomonas* and 35 *Bacillus* isolates, 19 and 21 found to be inhibitory. For *Fusarium* spp., 11 of 22 *Pseudomonas* and 19 of 35 *Bacillus* isolates had antagonistic activity.

In addition, some isolates found to have strong inhibitory activities against all fungal wheat and barley specific phytopathogens in the present study. Those isolates were identified as P7, P13, P14, P22, P34, B3, B7, B14, B15, B30, B32.

Isolates that are obtained in this study were only tested for their inhibitory activity, however no experiment was performed for determine specific traits of isolates (such as siderophore production, hydrogen cyanide production) associated with their anti-fungal activity.

Therefore, further characterization of isolates with antagonictic activity is required in the future. Moreover, their antifungal effect should be tested on field conditions to determine their availability as a biocontrol agent.

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CURRICULUM VITAE

Personal Identity

Name **:** Abdalla Aziz İbrahim İBRAHİM

Nationality **:** Iraqi

Date of birth **:** 20\03\1989 Sulaymaniyah

Place of birth **:** Sulaymaniyah, Iraq

Marital Status **:** Single

Telephone **:** (+964) 750 241 7960

E-mail **:** abdullah.849428@gmail.com

Educational Background

Degree	Place of Education	Date of Graduation
Master Degree	Kahramanmaras Sutcu Imam University Department of Bioengineering and Sciences	2016
Bachelor's Degree	Koya University Faculty of Science Department of Forestry	2013

Language Skills

Kurdish, English, Arabic

Publications

Büyükünal Bal, E.B., İbrahim İbrahim, A.A. Rizosfer Kaynaklı Floresan *Pseudomonas* ve *Bacillus* spp.'nin Tahıllardan İzolasyonu ve Fungal Fitopatojenlere Karşı İnhibitör Etkileri. 4. Ulusal Moleküler Biyoloji ve Biyoteknoloji Kongresi. 21-24 Ağustos 2015, Afyon, 75