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Chapter 2

Plant Health

Munazza Gull

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http://dx.doi.org/10.5772/64869

Abstract

Environment friendly control of plant disease is an emerging need for agriculture in the twenty-first century. Biological control using antimicrobial producing rhizobacteria to suppress plant diseases and promote plant health offers a powerful alternative to the use of synthetic chemicals. Many studies have been conducted to identify the specific traits by which plant growth-promoting rhizobacteria (PGPR) promote plant growth. Most of these studies were limited to examining just one or two of these traits. The plant growth-promoting rhizobacteria produce a wide variety of antimicrobial compounds against pathogens. The addition of antagonistic antimicrobial producing bacterial strains, either individual or as mixture in combination with fungicide, significantly decreased the plant disease stress. A single PGPR strain can produce different kinds of antimicrobial defense compounds to compete pathogens. A biocontrol agent possessing multimechanism systems of defense can antagonize pathogens in a better way. This research chapter highlights the current advancements about plant-PGPR interactions focusing on the principles and defensive mechanisms of PGPR during disease stress conditions and their potential use for the biocontrol of plant diseases. The integrated use of genetic, molecular, and ecological approaches will form the basis for significant future advances in biocontrol research against plant diseases.

Keywords: plant health, plant growth-promoting bacteria, microbial pathogens, antimicrobial compounds, biocontrol

1. Introduction

Pathogenic microorganisms affecting plant health are a major and chronic threat to food production and ecosystem stability worldwide. Agricultural yield and production increased in past few decades due to intensive use of agrochemicals providing more stable and reliable method for crop protection. The increasing use of these fertilizers and pesticides results in

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several negative effects on the environment, i.e., development of pathogen resistance and adverse impacts on nontarget organisms. In addition, the high cost of these fertilizers and pesticides and increasing demand of consumers for chemical-free food have led to a search for alternative natural products. There are many plant diseases for which chemical pesticides and stable protection from pathogens are not available. In this scenario, an alternative way of reducing the use of agrochemicals in agriculture, which also provides an effective disease protection and continuous supply of natural food, is biological control [1].

The ability of microorganisms to respond to stress in their environment is the key to their survival. In general terms, any condition that prevents an organism from growing at its optimal rate may be considered a form of environmental stress. For an organism to survive, it must respond to the environmental conditions imposed upon it, whether it is the absence of a nutrient, extremes in temperature, pH or oxidative state, or the presence of toxic compounds. Bacterial responses to these factors are varied and can include the expression of new proteins, the loss of plasmids, changes in membrane fatty acid content, changes in DNA super coiling and, in some cases, cross-tolerances to yet unencountered forms of environmental stress [2].

The lack of homogeneity and varied make up of soil dictates that organisms living in it must be able to adapt and survive. It was the purpose of this study to examine the interplay of nutrient limitation, specifically iron, and the presence of a wide array of antimicrobial compounds on the ability of the plant growth-promoting rhizobacteria to adapt to its environment and suppress the pathogenic disease. To understand the role of antimicrobial compounds in biocontrol of soil-borne pathogens, an overview of the plant rhizospheric ecology, PGPR, and biocontrol mechanisms is first required.

1.1. Plant rhizosphere

The "rhizosphere" can be defined as the part of soil around plant roots where bacterial growth is stimulated. It is the habitat where several biologically important processes and plant microbe interactions take place. A diverse range of microorganisms are populated in rhizosphere and the bacteria colonizing this habitat are usually named as rhizobacteria.

1.1.1. Plant growth-promoting rhizobacteria (PGPR)

There has been a large body of literature describing potential uses of plant-associated bacteria as agents stimulating plant growth and managing soil and plant health. Plant growth-promoting bacteria (PGPR) are associated with almost all plant species in a range of environments. Plant growth-promoting rhizobacteria (PGPR) colonizing the root surfaces and the closely adhering soil interface are the extensively and widely studied group. These PGPR can also enter into the interior parts of roots and establish populations of endophytic bacteria. Majority of these rhizobacteria transcend the barrier of endodermis, penetrating from the cortex of root to the vascular system, and finally reach in the upper parts of plants like stem, leaves, and tubers [3]. The ability of bacteria to selectively adapt these specific ecological niches depends on the extent of endophytic colonization of host plant organs and tissues. Conse-

quently, without harming the plant, eco-friendly associations between bacteria and host plants become established.

It is generally considered that many endophytic bacteria are the final product of a plant microbe process of colonization occurred in the root zone [4].

1.1.2. Direct plant growth promotion

PGPR can influence plant growth directly. These ways differ species to species and even from one bacterial strain to other strain. Rhizobia as symbiotic plant colonizers contribute to plant growth stimulation by enhancing nitrogen fixation. Free-living rhizobacteria usually do not depend on single plant growth-promoting mechanism. Several PGPR are also able to provide the plant with sufficient iron in iron-limiting soils or other important minerals, e.g., phosphate and zinc [5].

1.1.3. Indirect plant growth promotion

Indirect growth promotion occurs when PGPR promote plant growth by improving growth-restricting conditions. This can happen directly by producing antagonistic substances or indirectly by inducing resistance in host plants to a broad spectrum of pathogens. A bacterium can affect plant growth by one or more of these mechanisms and also use different abilities for growth promotion at various times during the life cycle of the plants. The widely recognized mechanisms of biocontrol mediated by PGPR are competent for an ecological niche or a substrate, production of inhibitory allelochemicals, induction of systemic resistance (ISR), and/or abiotic stresses [6].

1.1.4. Competitive root colonization

Successful application of PGPR has been hampered by inconsistent performance under field conditions. This is usually due to their poor and unstable rhizosphere competence. Effective root colonization with the ability to survive and proliferate along growing plant roots for a definitive time period in the presence of the other indigenous microflora results in effective rhizosphere competence development. Rhizosphere competence is considered as a prerequisite of effective biological control. Understanding root-microbe interactions as affected by genetic and environmental factors in spatial temporal contexts could significantly contribute to improve the efficacy of these biocontrol agents under wide range of field conditions [7]. Successful and stable application of PGPR is most directly affected by competition for root niches and bacterial determinants.

Root exudates determine which microorganism colonizes roots in the rhizosphere. It is now known that plant roots also generate electrical signals and zoospores of oomycetic pathogens take advantage of these signals to guide their movements toward the root surface. Both physical and chemical benefits to plants are provided by exudates, e.g., reduce the friction between root tips and the soil by root mucilages and reduction of root desiccation establish the effective contact between the root tips and the soil and contribute to soil structural stability. Root exudates also attract microorganism. Conversely, rhizobacteria can also elicit root exudation in a specific

manner, e.g., metabolites produced by *Pseudomonas aeruginosa* stimulate root exudates by perennial ryegrass 12-fold [8]. Root exudates can also be used as effective and stable antimicrobial agents, which can provide ecological niche an advantage to organisms that have perfect enzymatic mechanisms to detoxify them. Genetic and environmental conditions control the quantity and composition of chemoattractants and antimicrobials produced by plant roots. This indicates that PGPR competence is highly affected by the ability of rhizobacteria to survive under specific environment and to adapt the changing conditions rapidly [9].

Important bacterial traits identified for effective and stable root colonization are linked to phase variation, a regulatory process for DNA rearrangements controlled by site-specific recombinase enzyme. In some PGPR, efficient root colonization is subject to their ability to secrete an effective site-specific recombinase. This importance has been found when a site-specific recombinase gene from a rhizosphere-competent *P. fluorescens* was transferred into a rhizosphere-incompetent *Pseudomonas* strain and it enhanced its ability of root tips colonization [10].

2. Biocontrol of soil pathogens by antimicrobial producing rhizobacteria

A great diversity of rhizospheric microorganisms has been studied, characterized, and analyzed as biocontrol agents against many soil-borne pathogens over the past decades. Such microorganisms can produce substances that may reduce the damage caused by phytopathogens, e.g., by producing antibiotics, siderophores, and variety of enzymes. These microorganisms can also serve as competitors of pathogens for root colonization sites and nutrients. Biocontrol has not yet become widely popular and applied as alternative source of agrochemicals due to several factors. For example, the efficiency and activity of a biocontrol strain under field condition is usually affected by changing environmental conditions: water contents, pH, temperature, and interactions with other microorganisms. As a result, these biocontrol agents that showed promising plant growth stimulation and disease protection traits in initial laboratory experiments failed to be efficient rhizosphere colonizers under more complex biological field conditions. This highlights the need to address these limitations by extensive study of genetic, biochemical, and physiological factors that contribute to the effective and successful activity of biocontrol agents under wide range of environmental conditions.

2.1. Antibiosis

Antibiotics play a very important role in pant disease suppression by biocontrol agaents. Molecular and genetic tools could be effective in this regard because mutant defective in antibiotic production are easily obtained and studied by *in vitro* assays. With respect to the production of antibiotics, the most widely studied group of rhizosphere bacteria is fluorescent pseudomonads. Phenazine derivatives produce by fluorescent pseudomonads were the first biocontrol antibiotics described. Transposon insertion mutations elucidated their role that results in a defective and insufficient production of phenazine-1-carboxylate. As a result, disease suppressive activity has been reduced in these mutants [11]. The functional genes encoding the metabolic synthesis enzymes had been isolated, identified, and their up- and

down-regulation were studied. The presence of populations of other bacteria can influence phenazine production by *P. aureofaciens*, since mutants lacking the ability to produce, and autoinducer signal required for induction of antibiotic synthesis can use autoinducers produced by other (related) rhizosphere inhabitants. Also, other environmental sensors such as regulatory proteins *Gacy* and *ApdA* can influence the production of secondary metabolites involved in Pseudomonad biocontrol [12]. In addition, sigma factors are important for regulation of antibiotic production in fluorescent pseudomonads; housekeeping factor sigma 70 and the stress-related sigma have critical roles in production of antibiotic metabolites in disease suppression.

Antibiosis as a biocontrol mechanism of PGPR has become increasingly popular, better studied and used over the past decades. A large variety of antibiotics have been identified and formulated such as amphisin, 2,4-diacetylphloroglucinol (DAPG), oomycin A, phenazine, pyrrolnitrin, pyoluteorin, tensin, tropolone, hydrogen cyanide, and cyclic lipopeptides produced by *Pseudomonas* spp., and kanosamine oligomycin A, zwittermicin A, and xantho-baccin produced by *Bacillus*, *Streptomyces*, and *Stenotrophomonas* spp. [13]. Some antibiotics produced by PGPR are finding new pharmaceutical uses and these rhizobacteria opened an untapped and continuous resource for compounds to deal with the alarming arouse of multidrug-resistant pathogenic bacteria in human.

Regulatory cascades of these efficient antibiotics include *GacA*/*GacS* or *GrrA*/*GrrS*, *RpoD*, *RpoS*, and N-acyl homoserine lactone derivatives [14] and positive autoregulation [15]. Antibiotic synthesis is tightly linked and associated to the overall metabolism of the cell. Metabolic regulation of cell is dictated by nutrient availability and other environmental stimuli, such as pH, temperature, water, major and minor minerals, type of carbon source and supply, and other variety of parameters [16]. Genetic stability/instability of bacteria, affecting their ability to produce secondary metabolites, has been influenced by trace elements particularly zinc and carbon source levels. It is interesting to found that many bacterial strains produce pallet of secondary antimicrobial metabolites and the conditions favoring the production of one compound may not favor another metabolite mechanism. This wide variety of biocontrol strains may enable antagonistic bacteria to suppress the pathogens under the widest range of environmental conditions effectively and with stability. For example, in *P. fluorescens* CHA0 biosynthesis of diacetylphloroglucinol (DAPG) is stimulated, and pyoluteorin is repressed if glucose present and used as a carbon source. As glucose is depleted and its concentration decreased, pyoluteorin becomes the more abundant antimicrobial compound produced by this strain. This provides a kind of stability and flexibility as well for the antagonistic bacteria when dealt with a different or a changeable environment. Antibiotic biosynthesis can also be influenced by biotic conditions [17]. For example, bacterial metabolites pyoluteorin and salicylates can increase or decrease DAPG production by *P. fluorescens* CHA0. In addition, plant growth and development also influence antibiotic production because biological activity of DAPG-producing bacteria is not initiated by the root exudates of young plants but is induced by the root exudates of older plants, which gives in a strong selective pressure against other rhizosphere microorganisms sharing same ecological niche. Plant host genotype and their

regulation also play a significant role in the disease-suppressive interaction of plant with a microbial biocontrol agent [18].

2.2. Hydrolytic enzymes production

A variety of microorganisms also shows hyperparasitic mechanism, attacking plant pathogens by excreting cell wall enzymes called hydrolases. *Streptomyces plymuthica* C48 produced chitinase, which inhibited germination of spores and germ tube elongation in *Botrytis cinerea* effectively. The production of extracellular chitinase is considered a strong defensive mechanism for *Serratia marcescens* to act as antagonistic organism against *Sclerotium rolfsii* and for *Paenibacillus* sp. strain 300 and *Streptomyces* sp. strain 385 to suppress *Fusarium oxysporum* f. sp. *cucumerinum*. It has been also studied that extracellular chitinase and laminarinase produced by *Pseudomonas stutzeri* break and lyse mycelia of *F. solani* [19]. Chitinolytic activity found less utilized defensive mechanism in PGPB such as *S. plymutica* IC14 when used against *S. sclerotiorum* and *B. cinerea*, synthesis of proteases and other biocontrol characters are involved [20]. *B. cepacia* produces ß-1,3-glucanase that destroys the cell wall integrity of fungal strains *R. solani*, *S. rolfsii*, and *Pythium ultimum*. Production of lytic enzymes (proteases and chitinases, in particular) is regulated by *GacA*/*GacS* or *GrrA*/*GrrS* regulatory system and colony phase variation [21].

2.3. Detoxification and degradation of virulence factors

Biological control exhibits antagonism by detoxification of pathogen virulence factors also. For example, few biocontrol microorganisms are capable of detoxifying albicidin toxin synthesized by *Xanthomonas albilineans*. The detoxification mechanisms involve production of a protein that binds to the toxin reversibly in both *Klebsiella oxytoca* and *Alcaligenes denitrificans*, as well as an irreversible detoxification of albicidin mediated by an esterase enzyme found in *Pantoea dispersa* [22]. Different strains of *B. cepacia* and *Ralstonia solanacearum* can also lyse phytotoxin fusaric acid produced by different *Fusarium* species. Mostly pathogen toxins exhibit a broad spectrum activity of defense mechanisms and can restrain growth of microbial competitors. They can detoxify antibiotics produced by some biocontrol microorganisms as a self-defense mechanism against biocontrol agents [23].

It has been discovered recently that few PGPB show pathogen quorum sensing ability by degrading autoinducer signals, thereby blocking expression of various virulence genes. Bacterial plant pathogens use autoinducer-mediated quorum sensing to switch on gene cascades for their key virulence factors (e.g., cell-degrading enzymes and phytotoxin production). This approach holds tremendous antagonistic potential for suppression of diseases, even after the onset of infection effectively.

Biocontrol activity of microorgansims by production of allelochemicals has been studied widely with free-living rhizobacteria. Similar antagonistic mechanisms are used by endophytic bacteria as they can also synthesize antagonistic metabolites against plant pathogens. For example, it has been established that antibiotics munumbicins produced by the endophytic

bacteria *Streptomyces* sp. strain NRRL 30562 isolated from *Kennedia nigriscans* can suppress *in vitro* growth of phytopathogenic fungi, *P. ultimum*, and *F. oxysporum*, effectively.

Certain endophytic bacteria isolated from field-grown potato plants can suppress the *in vitro* growth of *Streptomyces scabies* and *Xanthomonas campestris* through production of siderophores, antibiotics, and other antagonistic metabolites [24]. The ability to inhibit pathogenic growth by endophytic bacteria isolated from potato tubers decreases as the bacteria colonize the host plant's interior suggesting that bacterial adaptation to this habitat occurs within their host and may be tissue type and tissue site specific. It has been found that the endophytic bacterial strain *P. fluorescens* FPT 9601 can produce DAPG and deposit DAPG crystals around and in the roots of tomato. This ability of endophytic bacteria to produce antibiotics in plants is very promising and could be used as antagonistic mechanism against pathogens [25].

2.4. Induction of systemic resistance

An advanced level of resistance at sites within that plant distant to those parts where infection had occurred is called systemic resistance. PGPR-triggered ISR provides strength and integrity to plant cell walls and boost host physiological and metabolic responses, leading to an increased production of plant defense chemicals against plant pathogens or abiotic stress factors. This recognition mediates the extracellular to intracellular signals. Then, the metabolite by itself or a signal generated by the plant cell turns on a signal transduction cascade. Consequently, distant plant cells, triggering the activation of defense arsenal of the diseased host plant, recognize the translocated signals. The pathways of signal transduction are activated upon microbial challenge, which results in activation of different sets of effector molecules.

Salicylic acid (SA), jasmonate (JA), and ethylene (ET) are the signaling molecules when accumulating trigger the defense responses and, if used exogenously, are even sufficient to induce resistance and suppress disease [26]. These SA signaling molecules activate genes encoding pathogenesis-related proteins (PRs). These self-defense proteins have antimicrobial potential. ET is involved in the regulation and expression of the defensive genes encoding *Hel* (a hevein-like protein basic chitinase (*ChiB*) and a plant defensin (*Pdf1.2*)) [27]. JA has been found to activate and regulate the genes encoding these three proteins. They possess antifungal activity. Furthermore, JA also activates the gene encoding a vegetative storage protein, *Atvsp*. These proteins accumulate in vacuoles but their potent role in antagonistic mechanism has not yet been confirmed.

Two defense pathways, induced systemic resistance and systemic acquired resistance (SAR), are found induced in *Arabidopsis*. ISR is a bacterial-mediated systemic resistance that causes no damage to plant but SAR is induced by foliar pathogens and results in activation of resistance mechanisms in uninfected parts of plant. It is established that in SAR, a first infection predisposes the plant to resist further attacks of pathogens. SAR mediation relies on the accumulation of SA and requires the regulatory inducer protein NPR1. In addition to SA accumulation, several JA- and ET-dependent resistance defense mechanisms that are independent of SA have also been described [28]. JA and ET act synergistically in inducing cascade

of genes for numerous PR proteins. ET has been found to enhance JA-dependent resistant responses but SA suppresses the JA-dependent defense gene expression. JA has also been reported to interface with SA-dependent defense signaling mechanism. ISR can be induced in plants that are not capable to accumulate SA (*NahG* mutant plants). This shows that SA is not required for ISR induction in *Arabidopsis*. PR proteins do not found accumulated in induced plants. However, the regulator NPR1 protein is required for expression of ISR [29]. ET- or JA-responsive defective genes *etrl*, *ein2*, *ein7*, or *jar1* in *Arabidopsis* mutant plants conferring a decreased sensitivity to ET and JA. They also found defective in their expression of ISR. JA application to wild-type plants induces a defense resistance that is not linked with the accumulation of PRs but is dependent on a functional *npr1* gene. These results showed that response to JA and ET is sequentially required in the ISR signal transduction pathway. ISR-mediated defense mechanisms of PGPR varied widely among species. PsJN-grapevine interaction, a host defense reaction in *Burkholderia phytofirmans*, found associated with phenolic compound accumulation and strengthening of cell walls in the exodermis and in several cortical cell layers during endophytic bacterial colonization [30]. The type of plant response linked to antagonistic bacteria induced after pathogen infection leads to the formation of structural barriers, such as thickened cell wall and papillae due to callose deposition and phenolic compounds accumulation at the site of pathogenic attack.

2.5. Hydrogen cyanide production

Hydrogen cyanide (HCN) is released as a product of secondary metabolism by several microorganisms and affects sensitive organisms by inhibiting the synthesis of ATP mediated by cytochrome oxidase. The percentage of cyanogens found is very low among rhizobacteria [31]. Therefore, depending on the target organisms, HCN-producing microorganisms are regarded as harmful when they impair plant health and beneficial when they suppress unwanted components of a microbial community. It has been reported that an isolate capable of cyanide production could be a better biocontrol agent because cyanide acts as an inducer of plant resistance [32].

2.6. Competition for iron: Siderophores production

Siderophores, from the Greek: "iron carriers," play the role to scavenge iron from environment and to make the mineral, which is always essential, available to microbial cell. Consequently, iron becomes unavailable to microorganisms that are unable to use these siderophores and competition for iron between microorganisms seems probable. Studies of siderophore-producing microorganisms have received much attention because of the clinical application and potential utilization of these chelators in agriculture.

Fungal strains produced both extracellular and intracellular siderophores, as discovered in spores and mycelia of *Neurospora* and *Aspergillus* [33]. Whereas in marine bacteria, lipophilic siderophores have been found that do not readily diffuse into the surrounding medium except that in which vesicles are formed. This shows that environmental distribution of siderophores may vary from strain to strain. However, their general iron transport function is evident and has been analyzed by radioactive labeling experiments in a number of microorganisms.

However, their main function is to get iron from insoluble hydroxides or from iron adsorbed to solid surfaces. Siderophores can also extract iron by Fe (III)/ligand exchange reactions from various other soluble and insoluble iron compounds such as ferric phosphate, ferric citrate, Fe-transferrin, ferritin, or iron bound to sugars, plant flavonoid pigments and glycosides, or even from artificial chelators like EDTA and nitrilotriacetate. Therefore, even if siderophores are not involved directly in solubilization of iron, they work as carriers mediating exchange between extracellular iron storage and membrane-located siderophore transport systems of the cells.

Siderophores detection is mostly achieved in iron-limited media, which means that either a synthetic (minimal) recipe or introduction of a complexing agent will render the iron selectively unavailable. The chrome azurol sulfonate (CAS) assay has become widely used since it is comprehensive, responsive, and more convenient than other microbiological assays, which although sensitive is rigidly specific [34]. Quantitative detection of siderophores can be done by spectrophotometry and by HPLC. The presence of hydroxamate siderophores is usually detected by Csaky's test [35], and catechol siderophores are usually detected by Arnow's test [36].

Siderophores differ substantially in structure, so no uniform procedure is available for its isolation. The siderophore can be isolated as individual compound or as its iron chelate. The iron chelates has the benefit of visual color identification but the iron must be removed before any natural product can be characterized by antimicrobial assays. Complete hydrolysis in the presence of iron could damage oxidizable moieties and direct NMR analysis is ruled out by paramagnetism of the ferric ion. By a combination of NMR and mass spectroscopy, structural characterization is done in the best possible way. These methods are sensitive and capable of providing absolute answers to all arising questions. Less than half of the siderophores could be crystallized. However, by X-ray diffraction technique, the successful configuration of those molecules containing a chiral center-like siderophores could be easily possible.

Among the siderophore-producing microbes, bacteria produce both hydroxamate and catecholate siderophores but fungi produce only hydroxamate-type compounds [37].

In Gram-negative genera such as the *Enterobacteria*, *Pseudomonas*, nitrogen-fixing *azotobacteria*, and the plant-associated *agrobacteria*, catecholate siderophores are usually found. It has been found that lipophilicity, complex stability, high environmental pH, and a weak nitrogen metabolism may lead to the production of catecholates. *Bacillus* and *Streptomyces* Gram-positive bacteria and the ascomycetous fungi produce hydroxamate-type ferrioxamines. The basidiomycetous fungi produce ester- and peptide-containing hydroxamate siderophores mostly which are acid stable and compatible for environmental iron solubilization. Siderophore also favors the development of mycorrhizal symbiosis particularly in all terrestrial plant communities. In almost all tree species in temperate forests, ectomycorrhizal interactions typically form. Only few siderophores have been reported due to the difficulties in cultivating the pure culture of mycorrhizal fungi under iron-limited conditions. It has been reported that three mycorrhizal fungal species, *Hymenoscyphus ericae*, *Oidiodendron griseum*, and *Rhodothamnus chamaecistus*, an ectendomycorrhizal fungus *Wilcoxina*, and an ectomycorrhizal fungus *Cenococcusm geophilum* produce hydroxamate siderophores of the ferrichrome and fusigen class [38].

The production of siderophores has been linked to the disease suppression ability of PGPR either through a direct effect on plant by control of noxious organisms in soil or via some other routes. The involvement of siderophores in plant growth promotion and disease suppression by *Pseudomonas* strains was suggested first time. However, the first real substantiation of this concept was published by Kloepper et al. [39] who isolated the fluorescent siderophore from strain B10 and showed that it mimicked the disease suppression ability of the producing strain.

Furthermore, the inhibitory effects of both the purified siderophore and the producing strain were eliminated under high-iron conditions. Subsequent genetic evidence indicated that the inhibitory properties of certain fluorescent pseudomonads were abolished in siderophore-negative mutants. Specific siderophore-producing rhizobacteria (*Pseudomonas*) rapidly colonize plant roots of several crops, and this colonization can result in significant increase in the yield. Penyalver et al. [40] reported that *Agrobacterium rhizogen*s K84 is used worldwide as biocontrol agent against crown gall disease due to its multimechanisms of defense by the production of antibiotics, agrocin 84 and agrocin 434, and hydroxamate siderophores ALS84 as anti-agrobacterial substance. There is convincing evidence to support a direct role of siderophore-mediated iron competition in the biocontrol ability exhibited by bacterial isolates.The addition of a siderophore-producing *Pseudomonas putida* converted a *Fusarium*-conducive soil into a *Fusarium*-suppressive soil for the growth of three different plants. An isolate of *Pseudomonas cepacia*, positive for siderophore and β-1,3-glucanase production, decreased the incidence of diseases caused by *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Pythium ultimum* [41].

In response to iron-deficiency stress, graminaceous plant species differ widely. Understanding the mechanism of stress responses is significant for increasing crop yields on calcareous soils. It also helps in improving the iron content of grains for human consumption. The response of graminaceous plants to iron deficiency occurs by the exudation of phytosiderophores to increase the availability of iron and by improving the uptake capacity of iron (III)-phytosiderophores. Phytosiderophores are usually hexadentate ligands that coordinate iron (III) with their amino and carboxyl groups. Phytosiderophores chelate sparingly soluble soil iron by forming iron (III)-phytosiderophore complexes that can be subsequently transported across the root plasma membrane via facilitated transport when released to the rhizosphere. In general, plant species releasing high quantities of phytosiderophores, such as barley, rye, and wheat are more resistant to iron deficiency chlorosis than species releasing smaller quantities, such as maize, sorghum, and rice. However, the quantity of phytosiderophores released is not always constant, for example, chlorosis resistance in different maize cultivars has been reported but this is not related to the total amounts of phytosiderophores released, indicating the contribution of other factors regulating iron efficiency process [42].

3. Identification of antagonistic antimicrobial producing rhizobacteria

Identification of bacteria is traditionally performed by isolation of the organisms and study of their phenotypic characteristics, including Gram staining, morphology, culture requirements,

and biochemical reactions. The discovery of PCR and DNA sequencing, comparison techniques of the gene sequences of bacterial species, proved that the 16S rRNA gene is highly conserved within a species and among species of the same genus, and thus can be used for bacterial identification at species level. For bacterial systematic studies at the family, genus, species, and subspecies levels, the 16S rDNA, which codes for the small subunit of ribosomal RNA, is now the most widely and successfully used informational macromolecule. For natural relationships between distantly related species and variable regions that can be used to separate closely related genera, the 16S rDNA conserved sequences can be used by constructing and comparing phylogenetic trees.

Such a 16S rDNA sequence-based identification technique will substantially facilitate the ecological study and the control of microorganisms difficult to culture [43].

Interests in biological control have recently increased due to imminent bans on chemical control, widespread development of fungicide resistance in pathogens, and a general need of sustainable disease control strategies. A wide variety of antagonistic biocontrol agents, such as *Pseudomonas*, *Burkholderia*, and *Trichoderma* spp., have been successfully identified, characterized, and utilized against many plant pathogens [44]. Now the agroindustry must focus on the identification and development of effective biocontrol agents against multiple pathogens as well as to develop the formulations that provide stable shelf-life and efficacy, and persistent user-friendliness.

Biocontrol of plant pathogens is being so popular because it can decrease the disease incidence, reduce the use of chemical fungicides, has no undesirable effects on nontarget organisms and environment, and is safer for the user and community.

4. Conclusions and scope

The plant growth promoting (IAA production, nitrogen fixation, and P-solubilization) and biocontrol traits (production of HCN, siderophores, hydrolytic enzymes, and antibiotics) suggest that these traits are more worthy of screening for plant growth promotion and bioantagonistic potential against plant pathogens. The plant growth-promoting rhizobacteria produce a wide variety of antimicrobial compounds against pathogens. A biocontrol agent possessing multimechanism systems of defense can antagonize root pathogens in a better way. This chapter highlights the need of screening the PGPR capable of producing a wide variety of antimicrobial compounds. Further evaluating/characterizing the biocontrol mechanisms and then testing the efficacy of selected antimicrobial-producing bacteria by lab, green house, and field trials could make them potent and successful biocontrol agents against many plant pathogens. This research chapter will help to minimize the chances of failure of biocontrol activity under field conditions, which is an emerging current problem of agriculture sector, and these tools will allow the isolation of improved antimicrobial bacterial strains and more efficient bioformulation to control pathogens. Molecular methods developed for the study of microorganisms in their environments are key tools for the study of the influence of the microbial community on biocontrol through variety of antimicrobial compounds produced by

rhizobacteria. Further experiments should be initiated to study the optimum formulation and the interaction of these bacteria with the constituent of established PGPR preparations, with a view to incorporating them for field use. Research along these lines will increase the impact of PGPR on the biocontrol of plant diseases in the commercial world.

Acknowledgements

The author acknowledges Dr. Fauzia Yusuf Hafeez, COMSATS University Islamabad, Pakistan, for helping in the collection of the literature (books and journals) about antimicrobial rhizobacteria and antagonistic biocompounds and her expertise advise during the preparation of this chapter.

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