

Plant Growth Promoting Bacteria

The rhizosphere is the narrow zone of soil specifically influenced by the root system. This zone is rich in nutrients when compared with the bulk soil due to the accumulation of a variety of plant exudates, such as amino acids and sugars, providing a rich source of energy and nutrients for bacteria. So a number of bacteria are found around the roots of plants, generally 10 to 100 times higher than that in the bulk soil. The rhizosphere is populated by a diverse range of microorganisms and the bacteria colonizing this habitat are called rhizobacteria.

Plant-associated bacteria can be classified into beneficial, deleterious and neutral groups on the basis of their effects on plant growth. Beneficial free-living soil bacteria are usually referred to as plant growth-promoting rhizobacteria (PGPR). Independent of the mechanisms of vegetal growth promotion, PGPRs colonize the rhizosphere, the rhizo plane (root surface), or the root itself (within radicular tissues). It is well established that only 1 to 2% of bacteria promote plant growth in the rhizosphere. Bacteria of diverse genera have been identified as PGPR, of which *Bacillus* and *Pseudomonas* spp. are predominant.

PGPR affect plant growth in two different ways, indirectly or directly. The direct promotion of plant growth by PGPR entails either providing the plant with a compound that is synthesized by the bacterium, for example phytohormones, or facilitating the uptake of certain nutrients from the environment. **The indirect promotion** of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms. This can happen by producing antagonistic substances or by inducing resistance to pathogens. A particular PGPR may affect plant growth and development by using any one, or more, of these mechanisms.

PGPR, as biocontrol agents, can act through various mechanisms, regardless of their role in direct growth promotion, such as by known production of auxin phytohormone, decrease of plant ethylene levels or nitrogen fixing associated with roots.

PGPR and their interactions with plants are exploited commercially and hold great promise for sustainable agriculture. Applications of these associations have been investigated in maize, wheat, oat, barley, peas, canola, soy, potatoes, tomatoes, lentils and cucumber.

Microbial Antagonism: Bacteria that reduce the incidence or severity of plant diseases are often referred to as **biocontrol agents** whereas those that exhibit antagonistic activity toward a pathogen are defined as antagonists.

The following rhizospheric environment and bacterial antagonistic activities can be highlighted:

- (1) synthesis of hydrolytic enzymes, such as chitinases, glucanases, proteases, and lipases, that can lyse pathogenic fungal cells
- (2) competition for nutrients and suitable colonization of niches at the root surface,
- (3) regulation of plant ethylene levels through the ACC-deaminase enzyme, which can act to modulate the level of ethylene in a plant in response to stress imposed by the infection, and
- (4) production of siderophores and antibiotics.

Siderophores, bacteriocins, and antibiotics production as antagonistic activities

The ability of rhizobacteria to produce siderophores and metabolites contributing to antibiosis has been the focus of many studies dedicated to investigating PGPR. The uptake of ferric ion via siderophore is largely used by pathogenic and non-pathogenic microorganisms from the soil, human body and marine environments. The importance of siderophore is closely related to iron, which is an essential element for different biological processes. On the other hand, bacteria can produce a wide variety of compounds with antimicrobial activity used as defense systems. These include broad-spectrum antibiotics, lactic acid produced by lactobacilli, lytic agents such as lysozymes, numerous types of exotoxins and bacteriocins, which also have a bactericidal mode of action. Siderophores, bacteriocins and antibiotics are three of the most effective and well known mechanisms that an antagonist can employ to minimize or prevent phytopathogenic proliferation.

Siderophores

To satisfy nutritional requirements of iron, microorganisms have evolved highly specific pathways that employ low molecular weight iron chelators termed siderophores. Siderophores are secreted to solubilize iron from their surrounding environments, forming a complex ferric-siderophore that can move by diffusion and be returned to the cell surface. The active transport system through the membrane begins with the

recognition of the ferric-siderophore by specific membrane receptors of Gram-negative and Gram-positive bacteria. Siderophores can chelate ferric ion with high affinity, allowing its solubilisation and extraction from most mineral or organic complexes. In aerobic conditions at physiological pH, the reduced ferrous (Fe) form is unstable and is readily oxidized to the oxidized ferric (Fe) form, which normally occurs as a poorly soluble iron hydroxide basically unavailable to biological systems. Siderophores can be defined as small peptidic molecules containing side chains and functional groups that can provide a high-affinity set of ligands to coordinate ferric ions. Based on their iron-coordinating functional groups, structural features and types of ligands, bacterial siderophores have been classified into four main classes (carboxylate, hydroxamates, phenol catecholates and pyoverdines). Hundreds of siderophores have been identified and reported for cultivable microorganisms, some of which are widely recognized and used by different microorganisms, while others are species-specific. In soil, siderophore production activity plays a central role in determining the ability of different microorganisms to improve plant development. Microbial siderophores enhance iron uptake by plants that are able to recognize the bacterial ferric-siderophore complex and are also important in the iron uptake by plants in the presence of other metals such as nickel and cadmium. However, it is still unclear if bacterial siderophore complexes can significantly contribute to the iron requirements of the plant. Siderophore production confers competitive advantages to PGPR that can colonize roots and exclude other microorganisms from this ecological niche. Under highly competitive conditions, the ability to acquire iron via siderophores may determine the outcome of competition for different carbon sources that are available as a result of root exudation or rhizodeposition. Among most of the bacterial siderophores studied, those produced by pseudomonads are known for their high affinity to the ferric ion. The potent siderophore, pyoverdin, for example, can inhibit the growth of bacteria and fungi that present less potent siderophores in iron-depleted media *in vitro*. A pseudobactin siderophore produced by *P. putida* B10 strain was also able to suppress *Fusarium oxysporum* in soil deficient in iron; this suppression was lost when the soil was replenished with iron, a condition that represses the production of iron chelators by microorganisms. Recent studies have demonstrated the suppression of soil-borne fungal pathogens through the release of iron-chelating siderophores by fluorescent pseudomonads, rendering it unavailable to other organisms.

Antibiotics

Besides siderophore production, the biocontrol abilities of pseudomonas strains essentially depend on aggressive root colonization, induction of systemic resistance in the plant, and production of antifungal antibiotics. The production of one or more antibiotics is the mechanism most commonly associated with the ability of plant growth-promoting bacteria to act as antagonistic agents against phytopathogens. The basis of antibiosis, activity of biocontrol based on secretion of molecules that kill or reduce the growth of the target pathogen, has become better understood over the past two decades. Antibiotics encompass a heterogeneous group of organic, low-molecular-weight compounds that are deleterious to the growth or metabolic activities of other microorganisms. Six classes of antibiotic compounds (for which their modes of action are partly understood) are better related to the biocontrol of root diseases: phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides (all of which are diffusible) and hydrogen cyanide (HCN; which is volatile). More recently, lipopeptide biosurfactants produced by *Pseudomonas* and *Bacillus* species have been implied in biocontrol due to their potential positive effect on competitive interactions with organisms including bacteria, fungi, oomycetes, protozoa, nematodes and plants. Numerous types of antibiotics have been isolated from fungal and bacterial strains and this diversity includes mechanisms of action that inhibit synthesis of pathogen cell walls, influence membrane structures of cells and inhibit the formation of initiation complexes on the small subunit of the ribosome. Pyrrolnitrin, the antibiotic produced by the *P. fluorescens* BL915 strain, is able to prevent the damage of *Rhizoctonia solani* during damping-off of cotton plants. The 2,4-diacetylphloroglucinol (DAPG) produced by pseudomonads, an effective and extensively studied antibiotic, causes membrane damage to *Pythium* spp. and is particularly inhibitory to zoospores of this oomycete. Phenazine, also produced by pseudomonads, possesses redox activity and can suppress pathogens of plants such as *F.oxysporum* and *Gaeumannomyces graminis*. The *P. chlororaphis* PCL1391 strain, isolated from roots of tomato plants, synthesizes phenazine-1-carboxamide, which is able to release soluble iron from insoluble ferric oxides at neutral pH, raising the possibility that phenazines might contribute to iron mobilization in soils. Antibiotics, such as polymyxin, circulin and colistin, produced by the majority of *Bacillus* ssp. are active against Gram-positive and Gram-negative bacteria, as well as many pathogenic fungi. The *B. cereus* UW85 strain, which suppresses

oomycete pathogens and produces the antibiotics zwittermicin A (aminopolyol) and kanosamine (aminoglycoside), contributes to the bio control of alfalfa damping off. Regarding bacteria as biocontrol agents to act as a biological solution, some researchers have highlighted the use of sporulating Gram-positive species such as *Bacillus* and *Paenibacillus* spp., which can confer higher population stability during formulation and storage of inoculant products.

Bacteriocins Other molecules used in microbial defense systems are bacteriocins. Bacteriocins differ from traditional antibiotics in one critical way: they commonly have a relatively narrow killing spectrum and are only toxic to bacteria closely related to the producing strain. Almost all bacteria may make at least one bacteriocin, and many bacteriocins isolated from Gram-negative bacteria appear to have been created by recombination between existing bacteriocins. The colicins, proteins produced by some strains of *Escherichia coli* that are lethal for related strains, are the most representative bacteriocins produced by Gram-negative bacteria. Like colicin, a name derived from *E. coli*, other bacteriocins have been thus defined and named, such as pyocins from *P. pyogenes* strains, cloacins from *Enterobacter cloacae*, marcescins from *Serratia marcescens* and megacins from *B. megaterium*. Interestingly, bacteriocins from *Bacillus* spp. are increasingly becoming more important due to their sometimes broader spectra of inhibition (as compared with most lactic bacterial bacteriocins), which may include Gram-negative bacteria, yeasts or fungi, in addition to Gram-positive species, some of which are known to be pathogenic to humans and/or animals.

Biocontrol Agents - Induced Resistance (ISR and SAR)

Non-pathogenic rhizobacteria have been shown to suppress disease by inducing a resistance mechanism in the plant called "Induced Systemic Resistance" (ISR). Induced resistance is the state of an enhanced defensive ability developed by plants when appropriately stimulated. ISR was formerly described by Van Peer *et al.* (1991) in carnation plants that was systemically protected by the *P. fluorescens* strain WCS417r against *F. oxysporum* f. sp. *dianthi* and in cucumber plants, where rhizobacterial strains protected the leaves against anthracnose caused by *Colletotrichum orbiculare*. The inducing rhizobacteria and the pathogens were inoculated and remained confined and spatially separated on the same plant so that microbial antagonism was excluded and the protective effect was plant-mediated.

Rhizobacteria-mediated ISR resembles pathogen-induced systemic acquired resistance (SAR) in that both types of induced resistance render uninfected plant parts more resistant to plant pathogens, including fungal, bacterial and viral pathogens, as well as nematodes and insects. The same strain induces resistance against several pathogens in the same plant. Specifically, *Pseudomonas* and *Bacillus* spp. are the rhizobacteria most studied that trigger ISR. Vleesschauwer and Höfte (2009) proposed the terminology ISR to depict induced systemic resistance promoted by non-pathogenic rhizobacteria or PGPR, irrespective of the signaling pathway involved in this process, while the term SAR is used to describe salicylic acid-dependent induced resistance triggered by a localized infection.

ISR and SAR act through different signaling pathways. Induction of SAR is through salicylic acid (SA) and ISR requires jasmonic acid (JA) and ethylene (ET) signalling pathways. These accumulating signalling molecules coordinate the defence responses and when applied exogenously, are sufficient to induce resistance. The protection mediated by ISR is significantly less than that obtained by SAR and a degree of dependence on plant genotype is observed in the generation of ISR. However, ISR and SAR together provide a better protection than each of them alone, indicating that they can act additively in inducing resistance to pathogens.

Salicylic acid accumulation occurs both locally and, at lower levels, systemically, in line with the development of SAR. Application of exogenous SA also induces SAR in many plant species. Development of tissue necrosis used to be considered a common and necessary feature for SAR activation, but in many cases, SAR can also be triggered without tissue necrosis as demonstrated in *Arabidopsis thaliana*. In SAR, the first infection predisposes the plant to resist further attacks. SA activates specific sets of defense-related genes called pathogenesis-related proteins (PRs). Generally, ISR is not accompanied by the activation of PR genes. The enhanced defensive capacity characteristic of SAR is always associated with the accumulation of PRs. Treatment of tobacco roots with *P. fluorescens* CHA0 triggers accumulation of SA-inducible PR proteins in the leaves. These PRs serve as hall marks of SAR in several plant species and are thought to contribute to the state of resistance attained. Some of these PRs are 1,3-glucanases and chitinases capable of hydrolyzing fungal cell walls, while other PRs are poorly characterized. SAR associated PRs suggest an important contribution of these proteins to the increased defensive capacity of induced tissues.

The PR-1 gene or protein expression appears to be inducible by SA and it is usually taken as a molecular marker to indicate that SAR has been induced. ISR can be triggered in plants that are unable to accumulate SA. Based on this, one can conclude that PRs are induced concomitantly with SAR, whereas SA and the activation of PR genes is not part of the pathway leading to ISR in *Arabidopsis*. Transduction of the SA signal requires the regulatory (activator) protein NPR1 (or NIM1) that functions in the terminal part of the signaling pathway of SAR. In non-induced plants, NPR1 is present as a multimer and during SAR induction, SA triggers the conversion of NPR1 into a monomeric form. These monomers are translocated to the nucleus, where they interact with members of the TGA/OBF subclass of basic-leucine-zipper (bZIP) transcription factors that are involved in SA-dependent activation of PR genes. A direct interaction between NPR1 and a specific TGA transcription factor is required for the binding of the complex to elements within the promoter of the PR genes. Overexpression of the NPR1 gene leads to enhanced resistance to pathogen attack. The *Arabidopsis* mutant *npr1* does not express PR genes and does not exhibit SAR. Since rhizobacteria mediated ISR is independent of SA and not associated with PRs, it is expected that ISR would still be expressed in this mutant. However, the *npr1* mutant of *Arabidopsis* does not display *P. fluorescens* WCS417r-mediated ISR. Plant growth-promoting rhizobacteria implies that NPR1 regulates defense responses mediated by different signaling pathways that function beyond the expression of PR genes, indicating that SAR and ISR converge at the last part of the signaling pathway. Reports with the reference rhizobacterial strain *P. fluorescens* WCS417r demonstrated that, at least in *Arabidopsis*, WCS417r-mediated ISR functioned independently of SA, depended on NPR1, but required components of the JA and ethylene (ET) response pathways. Infected plants increased their levels of JA and ET as a sign of active defence. These signalling molecules coordinate the activation of a large set of defence responses and when applied exogenously, can induce resistance themselves. The dependency of ISR on JA and ethylene is based on enhanced sensitivity to these hormones rather than on an increase in their production. The *Arabidopsis* JA response mutant *jar1* and the ET response mutant *etr1* were tested in the development of ISR. Both mutants were unable to develop ISR against *P. syringae* pv. tomato upon colonization of the roots by WCS417r bacteria, illustrating the dependency of ISR signalling on these phytohormones. The signal-transduction pathways leading to pathogen-induced SAR and rhizobacteria mediated ISR in *Arabidopsis thaliana* are

summarized in Figure 1. Methyl jasmonate (MeJA) and the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) also promote resistance against *P. syringae* pv. tomato in plants. WCS417r-mediated ISR follows a signaling pathway in which components from the JA and ethylene response pathways are successively engaged to trigger a defence reaction that, like SAR, is regulated by NPR1. One or more bacterial determinant must be recognized by specific plant receptors so that resistance is induced.

A variety of resistance-inducing molecules have been described: lipopolysaccharides and siderophores, including SA, flagella, biosurfactants, N-acyl-homoserine lactones (AHL), N-alkylated benzylamines, antibiotics and exopolysaccharides (EPS). The ability to develop ISR in response to certain rhizobacteria has been demonstrated in several species of plants and appears to depend on the specificity of the interaction between rhizobacteria and plants. Failure to elicit ISR in certain hosts may be due to the absence of production of inducing components in the rhizosphere or an inability of the particular plant species to perceive such compounds. The evidence support that it is necessary specific recognition between the plant and the rhizobacteria for induction of resistance.

Conclusion

The ability of bacterial siderophores and antibiotics to suppress phytopathogens could be of significant agronomic importance. Both mechanisms have essential functions in microbial antagonism but also are able to elicit induced resistance. Resistance-inducing and antagonistic rhizobacteria might be useful in formulating new inoculants, offering an attractive alternative of environmentally friendly biological control of plant disease and improving the cropping systems into which it can be most profitably applied. These new PGPR will require a systematic strategy designed to fully utilize all these beneficial factors, applying combinations of different mechanisms of action allowing crop yields to be maintained or even increased while chemical treatments are reduced.