Chapter 11
The Role of Siderophores in Plant Growth-Promoting Bacteria

Ana Fernández Scavino and Raúl O. Pedraza

11.1 Introduction

Iron is the fourth most abundant element in the earth’s crust, and living organisms require iron for growth. Although abundant in the environment, iron is not readily available. Under aerobic conditions, free ferrous iron, Fe(II), is oxidized to ferric iron, Fe(III), forming oxy-hydroxide polymers, which are not very easily soluble (Neilands 1995).

Iron is physiologically indispensable since a great number of proteins require iron for their activities, particularly the enzymes involved in redox reactions. Organisms have developed different mechanisms to scavenge iron from the abundant but biologically unusable sources in the environment. Examples of them are (1) reduction of extremely insoluble forms of ferric ion to soluble forms of ferrous iron that can be used easily, (2) use of iron present in hemoglobin by the destruction of erythrocytes and hydrolysis of hemoglobin, (3) direct use of the iron stored in ferritin (complexes that store iron in a form that is soluble, bioavailable, and nontoxic), and (4) enzymatic degradation of compounds that bind ironlike transferrin (Vasil and Ochsner 1999). But among the various mechanisms employed, the production of iron-binding compounds called siderophores is the best studied.

The term siderophore stands for “iron carriers” or “iron bearers” in Greek. They are water-soluble, low-molecular-weight, organic ligands with high affinity and specific for iron binding (Kraemer 2004). This constitutes a high-affinity system for the uptake of iron from the external medium, present in many microorganisms. This
system has three components: a siderophore that acts as a high-affinity ferric-ion-specific ligand that is usually released to the extracellular environment by microbes, a membrane receptor for iron-bound siderophore (ferri-siderophore) complex that transports the chelated iron across the microbial membrane, and an enzymatic system that is present within the cell that can release ferric ion bound to the siderophore. The siderophores form soluble complexes with ferric iron which, in natural environments, is extracted from insoluble iron hydroxides, protein-bound iron from cellular debris, or from other iron chelates.

This system of high-affinity acquisition and receptor-dependent transport of ferric ion is associated with growth or germination factors and with virulence factors (Crichton and Charlotteaux-Wauters 1987). Due to which the siderophores production is a common trait of invasive pathogenic microorganisms, synthetic analogs of bacterial siderophores attract increasing interest as potential drugs for the treatment of infections (Bergeron et al. 1999).

Recently, siderophores production proved in different plant growth-promoting bacteria (PGPB) as an important attribute in the plant growth and phytosanitary protection (Compant et al. 2005, Maheshwari 2011). Considering the important role that siderophores production can play in agronomic ecosystems, the iron content as a limiting nutrient for living organisms, the bacterial siderophores production particularly in PGPB, and the biotechnological applications of siderophores in agriculture are presented in this chapter.

11.2 Iron as a Limited Nutrient

Iron is an essential trace nutrient for most known organisms. The abundance of iron in soils is 1–6 % by weight, and its solubility is dependent on pH. In most environments iron deficiency is not caused by low total iron concentrations but by low iron bioavailability (Kraemer 2004). In aerobic environment iron is found as Fe(III), which is insoluble under physiological conditions (Powell et al. 1980; Matzanka et al. 1989).

More than 100 enzymes involved in primary and secondary metabolism possess iron-containing cofactors such as iron–sulfur cluster or heme groups. The reversible Fe(II)/Fe(III) redox pair is best suited to catalyze a broad spectrum of redox reactions and to mediate electron chain transfer (Miettike and Marahiel 2007). These enzymes and cofactors participate in various processes such as respiration, activation of oxygen, degradation of hydrogen peroxide and hydroxyl radicals, amino acid and pyrimidine biosynthesis, the citric acid cycle, DNA synthesis, nitrogen fixation, carbon fixation metabolism, photosynthesis, and oxygen binding (Andrews 1998). In addition, several transcriptional and posttranscriptional regulators interact with iron to sense its intracellular level or the current status of oxidative stress in order to efficiently control the expression of a broad array of genes involved mainly in iron acquisition or in the reactive oxygen species protection (Hantke 2001).

The cellular uptake of iron is restricted to its physiologically most relevant species, ferrous, i.e., Fe(II), and ferric, i.e., Fe(III). Ferrous form is more soluble in aqueous solutions at neutral pH and then sufficiently available for living cells if remains in the reductive status. Generally, Fe(II) form can be taken up by ubiquitous divalent metal transporters, although specific ferrous uptake systems are known in bacteria and yeasts (Miettike and Marahiel 2007).

Though iron is required by a majority of microorganisms, there are some exceptions like the lactic acid bacteria, as they do not contain heme enzymes and the iron-containing ribonucleotide reductase (Neilands 1995). On the other hand, iron can be toxic for certain organisms. High intracellular concentration of ferrous iron may produce hydroxyl radicals (Crichton and Charlotteaux-Wauters 1987). This problem is alleviated with enzymes such as superoxide dismutase, catalase, and peroxidase that can degrade reactive oxygen species. Iron toxicity is also alleviated by the presence of antioxidants such as glutathione and endonucleases that repair damages caused to DNA during redox stress (Andrews 1998). It is also well known that the iron imports toxicity towards rice plants in lowland environments. After inundation, reduction of iron oxides and hydroxides results in the accumulation of large amounts of ferrous iron that disrupt or overexpress metabolic processes that result in damage of the rice plant (Becker and Asch 2005).

11.2.1 Iron Bioavailability

The iron pools in soils and aquatic environments contain iron complexes (ferric complexes with other ligands different from siderophores), iron-bound enzymes from detritus plant and microbial cells, iron bound to humic and fulvic substances, and iron-bearing minerals. A major iron pool in terrestrial and aquatic systems is constituted by iron oxides (Kraemer 2004). Some pathogens can mobilize ferric iron directly from iron-containing eukaryotic host proteins, like transferrin, lactoferrin, and ferritin, or from heme using a heme oxygenase (Winkelmann 2007). The siderophores production is a particularly efficient and specialized iron-acquisition system that confers competitive advantage to many organisms in biotic and abiotic ecosystems. Most of the information in biological iron acquisition is focused on aerobic systems since reducing conditions lead to a strong increase of iron solubility and is unlikely to encounter iron-limiting conditions in reduced systems (Kraemer 2004). The iron availability is limited by the solubility, and the slow dissolution kinetics of iron-bearing mineral phases particularly occurs in neutral or alkaline environments. The solubility of iron oxides in aerobic systems depends on the properties of the solids, on the particle size, and on the pH, ionic strength, and concentration of organic ligands in solution (Kraemer 2004). At neutral pH and oxic conditions, Fe(II) quickly oxidizes to Fe(III) (Stumm and Morgan 1995). In the absence of a strong organic ligand, Fe(III) precipitates rapidly as a hydrous ferric oxide, and citrate is too weak to bind iron and prevent Fe(III) precipitation in the culture medium (Konigsberger et al. 2000).
In the soil environment, at around neutral pH, the free Fe(III) concentration in equilibrium with ferric oxide hydrates is around $10^{-11}$ M (Budziszewicz 2010). But living microorganisms require higher concentrations ($10^{-6}$ M), and when cells detect concentrations below this threshold, the siderophore production begins (Miethke and Marahiel 2007). Siderophores have a pronounced effect on the solubility of iron oxides over a wide range of pH due to the extraordinary thermodynamic stability of soluble siderophore-iron complexes. Very small concentrations of free siderophores in solution have a large effect on the saturation state of iron oxides. This siderophore-induced disequilibrium can drive dissolution mechanisms such as proton-promoted or ligand-promoted iron oxide dissolution. The adsorption of siderophores to oxide surfaces also induces a direct siderophore-promoted surface-controlled dissolution mechanism (Kraemer 2004). In addition, iron can also be mobilized by exudation of non-siderophore ligands that are ubiquitous in soil. Organic acids such as lactate, succinate, fumarate, malate, acetate, and amino acids exuded by roots of iron-stressed plants can also contribute to the Fe(III) solubilization and influence microbial iron acquisition (Fan et al. 1997).

### 11.2.2 Siderophores from Different Organisms

Various plants belong to family Poaceae (graminaceous grasses); fungi and several bacterial genera are known to sequester iron using siderophores (Nielands 1957; Takagi 1976; Winkelmann 1992).

A specialized mechanism for iron uptake is observed in Poaceae plants which, via roots, release iron-chelating nonproteinogenic amino acids called phytosiderophores. According to Römhild and Marschner (1986), there are two strategies for the acquisition of iron by plants under iron deficiency. Strategy I (in most non-Poaceae species) is characterized by an inducible plasma membrane-bound reductase and an enhancement of H$^+$ release. Strategy II (in grasses) is characterized by an enhanced release of phytosiderophores and by a highly specific uptake system for Fe(III) phytosiderophores. This strategy seems to have several ecological advantages over strategy I, such as solubilization of sparingly soluble inorganic Fe(III) compounds in the rhizosphere and less inhibition by high pH. Thus, mugineic acid is produced by barley, distichonic acid by barley, avenic acid A by oats, deoxymugineic acid by wheat, hydroxymugineic acid by rye, and nicotianamide by tobacco. Some plant like barley is able to take up ferriferrisiderophores 100–1,000 times faster than other ferri-chelators (Castignetti and Smarrelli 1986). It has been observed that the lower affinities of phytosiderophores by iron, compared to microbial siderophores, are partly compensated by high exudation rates by Poaceae plant roots resulting in local ligand concentrations in the millimolar range in the rhizosphere, whereas the bacterial hydroxamate siderophore concentration is four orders lower (Römhild 1991).

### 11.2.3 Siderophores in Soil

In most environmental systems, siderophores mainly exist in complexed form (Kraemer 2004). Their concentrations in soil depend on the soil horizons, but the rhizosphere shows higher concentrations than bulk soil (Bossier et al. 1988). Powell et al. (1980) have estimated hydroxamate siderophore concentrations in soil solutions between $10^{-7}$ and $10^{-3}$ M. Römhild (1991) has estimated that phytosiderophore concentrations can reach local concentrations of up to $10^{-3}$ M since plants are able to exude phytosiderophore at high rates into the rhizosphere. The concentration of microbial siderophores depends on the environmental conditions. Ferrioxamine B-type siderophores, produced by most actinomycetes (Nielands and Leong 1986), were the most abundant siderophore producer in a tiller-amended soil system, whereas the ferrichrome type produced in smaller quantities by several fungi (Crowley et al. 1987). On the other hand, Holmström et al. (2004) identified the main siderophores in coniferous forest soils intensively colonized by ectomycorrhizal hyphae as ferrichrome and ferricrocin, with the former detected in nanomolar concentrations in humic layers overlying granitic rock and soils (Holmström et al. 2004). Ferricrocin is a widespread siderophore in forest soils that seems to be resistant to the proteases excreted by plants and
Gram-positive bacteria (Winkelmann 2007). It is now considered that organic non-
siderophore ligands, as several amino acids and organic acids like citrate, can be
exudated by plants and influence the iron availability. These ligands are ubiquitous
in soil and might have a synergistic or inhibitory effect on the siderophores
dissolution rates (Kraemer 2004).

Furthermore, Kraemer (2004) proposed that the compressive understanding of
the role of siderophores in increasing iron oxide solubility and promoting dissolution
in soils requires the consideration of the rates of various processes that
occurred simultaneously. Thus, the siderophore exudation rates, the uptake, and
the degradation rates, as well as the loss of siderophores by adsorption on other
mineral surfaces, the partitioning of iron into humic substances, and the complexa-
tion of metal other than iron (which stability may be significant, specially for
similar ions as Al(III) or for Cu(II) that is often present in much higher concentrations),
should be considered. In addition, iron oxides in natural terrestrial environments are often coated with humic and fulvic acids, exo-polysaccharides, or biogenic low-molecular-weight organic acids, and the inhibitory, competitive, or
synergistic effects of such substances on siderophore-controlled iron acquisition
need to be investigated.

- **11.3 Microbial Siderophores**

Microbial siderophores show great variability in their chemical structures. This
may be due to genetic factor or biochemical

**11.3.1 Chemical Structures**

Siderophores are iron-chelating secondary metabolites with masses below 2,000 Da
(Budzikiewicz 2010). Almost 500 siderophores with known structure have been
reported (Boukhalfa and Crumbliss 2002), and several hundred active iron-chelator
compounds have been characterized and purified (Hider and Kong 2010). Most, but
not all, of siderophores are hexadentate ligands forming 1:1 complexes with Fe(III)
(Kraemer 2004), and their capability to form stable complexes with Fe(II) is rather
low (Miethke and Marahiel 2007).

The major Fe(III) ligand types are catecholates, hydroxamates, and alpha-
hydroxycarboxylates and often combined in the same molecule of siderophore
(Budzikiewicz 2010). Carboxylate siderophores are produced by microorganisms
that live in acidic environments, e.g., fungi, but these could not compete with
stronger siderophores such as catecholates at physiological pH (Oertel and
Raymond 2003), since catecholates have higher affinity for Fe(III). These ligands
are supported in different chemical structures such as peptides, di- and tri-
aminoalkanes, and siderophores based on citric acid along with miscellaneous
siderophores. The peptide chain carrying the ligand sites usually contains cyclic
structures at the extremes that prevent their degradation by proteolytic enzymes.
The peptidic siderophores are produced by fluorescent *Pseudomonas* (pyoverdines), as well as by species of the genera *Azotobacter*, *Mycobacterium*,
*Rhodococcus*, and by many enterobacteria and by most of fungi. This also includes lipopeptide siderophores produced by species of the genera *Burkholderia*,
*Nocardia*, and *Mycobacterium*. The siderophores based on di- and tri-aminoalkane
skeletons are produced by few *rhizobia*, *Paracoccus*, *Burkholderia*,
*Agrabacterium*, and several *Actinomycetes*. Siderophores based on citric acid are
produced by bacteria from the genera *Bacillus*, *Acinetobacter*, *Arthrobacter*,
*Ochrobactrum*, *Rhizobium*, *Synechococcus*, *Vibrio*, *Ralstonia*, *Staphylococcus*,
and *Marinobacter* (Budzikiewicz 2010). Thus, the stability of Fe(III) siderophore
complexes varies in a range about 30 orders of magnitude depending on the
siderophore structure and on the ligand type. Also, the pH of the environment
strongly influences the chelation efficiency (Miethke and Marahiel 2007). Although
Gram-negative and Gram-positive bacteria have differences in their cell structure,
they share some genes in common for both specific siderophores transport and iron-
binding proteins (Clarke et al. 2000).

Many bacteria produce more than one type of siderophore or have more than one
iron uptake system to take up multiple siderophores (Neilands 1981). Recently,
other compounds able to bind iron with comparable affinity to the known bacterial
siderophores have been reported. The degradation product of an acylhomoserine
lactone (signal molecule in the Quorum Sensing system) produced by *Pseudomonas
eruginosa* possibly is an unrecognized mechanism for iron solubilization
(Kaufmann et al. 2005). Recently detail description on types and chemistry of
siderophores is reviewed by Desai and Archana (2011).

**11.3.2 Biochemical and Genetic Determinants Involved in Bacterial Siderophores Production**

Siderophores production as a response to iron limitation is widespread among
aerobic microorganisms (Neilands et al. 1987). It has been reported that among
302 different fluorescent *Pseudomonas* strains isolated from soils, 297 produced
detectable siderophores under iron deficiency (Cocozza and Ercolani 1997).

Although this iron-acquisition system is induced under iron-limiting conditions,
other environmental factors such as pH, the presence of other trace elements, and
the availability of carbon, nitrogen, and phosphorous sources also influence the
siderophores production (Duffy and Defago 1999). This system involves several
steps: intracellular biosynthesis of siderophores, exudation of siderophores in the
extracellular space, iron mobilization by competitive complexation or dissolution of
iron-bearing minerals, and recognition and uptake of ferric siderophore
complexes by highly efficient transport systems or liberation of iron from the siderophore complex and uptake of iron (Boukhalia and Crumblish 2002).

The system requires tightly regulated enzymes and transport systems that allow concerted siderophore biosynthesis, secretion, siderophore-delivered iron uptake, and iron release. In bacteria, gene regulation of siderophore utilization and iron homeostasis is mediated mainly at the transcriptional level by the ferric uptake regulator Fur (in Gram-negative and low mol % GC Gram-positive bacteria) or by the diphtheria toxin regulator DtxR (in Gram-positive high GC contents as streptomycetes and corynebacteria) (Hantke 2001). The synthesis of catecholates mostly depends on the nonribosomal peptide synthetases, whereas hydroxamate and carboxylate siderophores are assembled by diverse enzymes such as monooxygenases, decarboxylases, and aminotransferases (Miethke and Marahiel 2007).

In bacteria, the main route for the uptake of Fe complexed in siderophores is the import of the complex into the cytosol through specific transporters. Moreover, the organisms that can use exogenous siderophores (synthesized by other organisms) showed frequently a greater battery of Fe-siderophore importers than siderophore exporters (Miethke and Marahiel 2007). The iron release from the Fe(III) siderophore complex into the cytosol comprises either the reduction to Fe(II) by relatively unspecific ferric siderophore reductases or the hydrolysis of the complex by specific enzymes that liberate Fe(III) which is further reduced or complexed by other cellular iron components (Miethke and Marahiel 2007).

Moreover, the role of siderophore might not be limited to the iron chelation. The nitrogen-fixing bacterium *Azotobacter vinelandii* produces at least five different siderophores, where concentration increases sharply at low iron concentration in diazotrophic cultures although their production is not suppressed at high iron concentration (Bellenger et al. 2008). Kraepiel et al. (2009) suggested that *A. vinelandii* may produce siderophores to acquire molybdenum (Mo) and vanadium (V), two important metals required for nitrogen fixation, when these metals are limiting in diazotrophic cultures.

### 11.3.3 Siderophores Influence the Interaction Among Organisms

Siderophores production can modify the interaction among organisms in the environment leading to mechanisms of cooperation or competence. The capability of sensing iron in the environment is an advantage by the siderophore-producing organism and may help other microorganisms that do not have this capability or that are not so competitive. Many microorganisms are able to utilize the Fe(III) complexes of siderophores which they have not synthesized. The persistence in soils of ferrichromes, the most common fungal siderophores, benefits other microorganisms that have the receptors for the uptake of these siderophores as in case of several enterobacteria like *Pantoea, Enterobacter, Erwinia*, and *Yersinia* (Winkelmann 2007). Also, the uptake of bacterial siderophores by fungi, like *Saccharomyces* and *Aspergillus*, has been observed (Haas 2003, Heymann et al. 2000; Lesuisse et al. 1998), and the enterobactin, the predominant siderophore produced by enterobacteria, can also be utilized by *Saccharomyces*, a non-siderophore-producing microorganism (Winkelmann 2007).

In addition, it has been proposed that partial degradation of fungi siderophores or iron exchange between bacterial siderophores and phytosiderophores is involved in the iron nutrition of *Pacaeae* plants (Yehuda et al. 1996; Winkelmann 2007). An indirect effect of cooperation has been postulated by Kraepiel et al. (2009) between non-nodulating plants and free-living diazotrophs inhabiting their rhizosphere. Besides iron, several metals are complexed and accumulated in plant leaves that when decomposed in topsoil constitute a source of essential minerals for the nitrogen fixation, from which the plants benefit. The diazotrophic bacteria extract these essential minerals through the excreted siderophores.

Competence among microorganisms is well illustrated by several examples and can benefit or be negative for the siderophore-producing microorganism. In general bacterial siderophores, though differing in their abilities to sequester iron, deprive pathogenic fungi of this essential element since the fungal siderophores have lower affinity for Fe(III). This constitutes one of the main mechanisms of biocontrol of plant pathogenic fungi (Loper and Hanks 1999). Their ability to use a large number of heterologous siderophores has been confirmed by the presence of many homologues of iron-siderophore receptor genes in their genomes (Cornelis and Matthijis 2002; Kaufmann et al. 2005). Conversely, siderophore producers can be invaded by nonproducing cheats from the same or different species that have the siderophore receptors. Siderophore production is metabolically expensive to individual producers but benefits all cells in the vicinity able to capture iron-siderophore complexes produced by other cells of the same species (Harrison et al. 2008). On the other hand, certain microorganisms synthesize structurally distinct siderophores apparently as a strategy to overcome the competition of cheats. *Streptomyces* species produce two different siderophores with two independent uptake systems; whereas ferrioxamines can be taken by several organisms, the ferric coelicichelin complex can be selectively absorbed into *Streptomyces coelicolor* cells through an independent uptake system (Challis and Hopwood 2003). Additionally, the capability of microorganisms to degrade siderophores in soil can modify the interaction established through siderophores production. It has been reported that bacteria of the genus *Azospirillum* in pure cultures are able to degrade ferrioxamines when present as iron-free compounds (Winkelmann et al. 1999).

A singular case may be the endophytic bacteria that colonize internal tissues of the plants and their relationship with the siderophores production. In Uruguay it has been shown that at the end of the cropping cycle, the leaves of three different rice varieties were colonized by high amounts of siderophore-producing bacteria (Fig. 11.1), with *Pantoea* and *Psedomonas* as the predominant genera. Furthermore, the proportion of siderophore-producing bacteria to heterotrophic bacteria
Fig. 11.1 Enamtement endophytic heterotrophic bacteria (HB) and endophytic heterotrophic siderophore-producing bacteria (HSPB) in leaves of three rice varieties cultivated in Uruguay at the end of the crop season. EP, El Paso 144; IT, INIA Tacurú; IO, INIA Olimar. The values represent the mean of triplicate plots in a field experiment.

augmented in leaves when the plant grew, and they increased in roots compared to rhizospheric soil after the flooding, when the environment becomes anoxic (Loaces et al. 2011). They remained strongly associated to the plant tissues although in vitro inhibition towards pathogenic fungi or PGPB was not observed. Apparently, siderophore-producing bacteria were selected into the plant tissues, though the benefit for the plant results is still unclear. Their role capturing Fe(III) generated by the oxidation of Fe(II) in oxic micro-niches into the plant or in the rhizosphere, increasing the iron availability locally, or reducing the Fe(II) toxicity towards the plant by accumulation of the sequestered metal into the bacterial cells should not be dismissed (Loaces et al. 2011).

Finally, the role of siderophore-producing bacteria as bacterial growth promoters should be also considered. The (until now) uncultured bacteria may be stimulated and become culturable in the presence of siderophore-producing bacteria. Recently D’Onofrio et al. (2010) have shown that previously uncultured isolates from marine sediment biofilm, grown on a Petri dish in the presence of cultured organisms from the same environment. This helper strain produces a grow factor identified as new acyl-desferrioxamine siderophore.

11.4 Siderophores Production in Plant Growth-Promoting Bacteria

Siderophores have been implicated for both direct and indirect enhancement of plant growth by rhizospheric microorganisms. The ecological significance of microbial siderophores in soil and plant surfaces has attracted the attention of workers.

11.4.1 Plant Growth-Promoting Bacteria: Mechanisms of Action

PGPB are a heterogeneous group of bacteria, such as the genera Azotobacter, Azospirillum, Azorarcus, Herbaspirillum, Pseudomonas, and Rhizobium, among others, that can be found in the rhizosphere at root surfaces and in association with inner root tissues and other habitats (Ahmad et al. 2008). The enhancement of plant growth using PGPB is well documented (Reed and Glick 2004; Bashan and de Bashan 2010), and these organisms have also been used to reduce plant stress associated with phytoremediation strategies for metal-contaminated soils (Reed and Glick 2005).

PGPB enhance plant growth through different mechanisms, such as (1) enhancing asymbiotic nitrogen fixation (Khan 2005) or indirectly affecting symbiotic N2 fixation, nodulation, or nodule occupancy (Fuhrmann and Wollum 1989); (2) reducing ethylene production, allowing plants to develop longer roots, and better establishment during early stages of growth, due to the synthesis of 1-aminoacyclopropane-1-carboxylate (ACC) deaminase which modulates the level of ethylene by hydrolyzing ACC, a precursor of ethylene, in ammonia and α-ketobutyrate (Glick et al. 1998); (3) production of hormones such as auxins, cytokinins, and gibberellins (Glick 1995; Ahmad et al. 2008); (4) raising the solubilization of nutrients with resulting increase in the supply of bioavailable phosphorous and other trace elements for plant nutrition (Glick 1995); and (5) synthesis of antibiotic and other pathogen-depressing substances such as siderophores, volatiles, and chelating agents that protect plants from that antagonize phytopathogens. (Karnve and Lelie 2000; Tortora et al. 2011, 2012). These microorganisms can also increase plant tolerance to environmental stresses such as flooding (Griccho and Glick 2001), salt stress (Mayak et al. 2004a), and water deficiency (Mayak et al. 2004b). PGPB are not only significant from an agricultural point of view, as they can also play an important role in soil remediation strategies, not only by enhancing growth and successful establishment of plants in contaminated soils but also by increasing the availability of contaminants, as reported for heavy metals, namely, Zn and Ni, in Thlaspi caerulescens (Whiting et al. 2001) and in Alyssum murale and Thlaspi goesingense (Abou-Shanab et al. 2003; Idris et al. 2004). Recently Kumar et al. (2010) observed reduction of chemical fertilizer by using combination of root-nodulating Sinorhizobium fredii KCC5 and rhizospheric Pseudomonas fluorescens LPK2.

11.4.2 Siderophores as a Competitive Advantage for Plant Growth

Given that iron is an essential nutrient, plants have evolved strategies for its acquisition, which, in dicotyledonous plants such as cowpea (Vigna unguiculata),
is based on strategy I. Unlike strategy II found in grass monocotyledonous plants, strategy I does not involve the release of phytosiderophores. Rather, it is characterized by an enhanced Fe(III) reductase activity, release of reductants such as phenolics, and acidification of the rhizosphere (Römhild and Marschner 1986). Furthermore, in strategy I plants, microbial siderophores have been reported to promote plant growth under Fe deficiency (Crowley et al. 1991).

In a work about enhanced plant growth by siderophores produced by PGPB, specific strains of the Pseudomonas fluorescens-puvida group have been used as seed inoculants on crop plants to promote growth and increase yields (Kloepper et al. 1980). Several workers observed that these bacteria rapidly colonized plant roots of potato, sugar beet, radish, and other crop plants, which caused statistically significant yield increases in field tests (Maheshwari 2011). These results prompted them to investigate the mechanism by which plant growth was enhanced. Most of these workers have concluded that these bacteria exerted their plant growth-promoting activity by depriving native microflora of iron as they were able to produce extracellular siderophores which efficiently complexed environmental iron, making it less available to certain native microflora (Kloepper et al. 1980).

Sharma and Johri (2003) reported about maize seeds inoculated with siderophore-producing pseudomonads with the aim to develop a system suitable for better iron uptake under iron-deficient conditions. They found that inoculation of maize seeds with fluorescent Pseudomonas spp. strains GRP3A and PRS showed significant increase in germination percentage and plant growth. Maximum shoot and root length and dry weight were observed with 10 μM Fe(III) along with bacterial inoculants, suggesting that application of siderophore-producing plant growth-promoting bacterial strains positively influences the crop productivity in calcareous soil system. Pandey et al. (2005) found Pseudomonas aeruginosa GRC1 having prolific production ability of hydroxamate siderophore in iron-deficient conditions. The siderophore of GRC1 was purified and characterized. The purified siderophore appeared to be of pyoverdine type with typical amino acid composition. In field trials, P. aeruginosa GRC1 enhanced the growth of Brassica campestris var Pusa Gold (Indian mustard).

Although extensive research has been directed to correct chlorosis (iron deficiency) by the application of available iron compounds to the soil and by selective plant breeding to produce Fe-chlorosis-resistant cultivars, during the last years, the possible implication of siderophores production by PGPB has been considered as a potential way to improve plant growth, nodulation, and N2 fixation in iron-deficient conditions. The beneficial effect of using siderophore-producing strains of Bradyrhizobium sp. and Rhizobium melloti was reported by O’Hara et al. (1988) and Gill et al. (1991), respectively. In addition, siderophore-producing ability might favor the persistence of rhizobia in iron-deficient soils (Leenheer et al. 1995).

### 11.4.3 Importance of Siderophores in Plant Protection Against Diseases

Nowadays, control of plant diseases is performed by the intensive use of chemical products that may cause environmental pollution, pathogen resistance, increase in production costs, and serious risks to the environment and human health. An alternative of crop protection against pathogens is the biological control exerted by some PGPB. Several factors can affect the efficacy of siderophores as control agents against plant pathogens, the most important among them being type of microorganism, target phytopathogen, and medium composition (Glick and Bashan 1997). Because of their catabolic versatility, their excellent root-colonizing abilities, and their capacity to produce a wide range of antifungal metabolites, the soil-borne fluorescent pseudomonads have received particular attention as efficient biological control agents (Nautiyal et al. 2003). They produce several siderophores such as pyoverdine, pyochelin, azotobactin, salicylic acid, and pseudomonine (Dave and Dube 2000; Mercado-Blanco et al. 2001; Labuschagne et al. 2010). All these siderophores contribute to disease suppression through the competition for iron.

However, siderophores production in the genus Azospirillum, an important member of PGPB, is a biocontrol mechanism that has been scarcely studied. Saxena et al. (1986) and Shah et al. (1992) reported the production of salicylic acid (SA) among siderophores produced by Azospirillum lipoferum under iron-starved conditions. Salicylic acid (SA) besides being a compound with siderophore activity (Visca et al. 1993) is a precursor in the biosynthesis of microbial catechol-type siderophores, such as yersiniabactin, pyoverdine, and pyochelin (Cox et al. 1981; Jones et al. 2007; Serino et al. 1995). Moreover, it was demonstrated to play a crucial role as an endogenous regulator of localized and systemic acquired resistance (SAR) against pathogen infection in many plants (Delaney et al. 1994). Therefore, SA-producing strains may increase defense mechanisms in plants. However, bacterial SA participation on plant-induced systemic resistance (ISR) is still controversial (Siddiqui and Shaukat 2005; Cornelis and Matthys 2007). It was hypothesized that bacterial SA excreted to the medium was recognized by plant roots inducing signals for systemic resistance (Maurhofer et al. 1998), although in some interactions, it has been proposed that SA may not be the primary signal for ISR induction (Press et al. 1997), but other siderophores could be implicated (Siddiqui and Shaukat 2004).

Recently, it was reported that A. brasilense siderophores contain antifungal activity against Colletotrichum acutatum, the causal agent of anthracnose disease in strawberry crop (Tortora et al. 2011). They demonstrated that under iron-limiting conditions, different strains of A. brasilense produce siderophores, exhibiting different yields and rates of production according to their origin. The bacteria strains have also been isolated from rhizosphere or inner tissues of strawberry roots and stolons (Fig. 11.2).
total soluble phenolic compounds and callose depositions and the transient accumulation of SA. The latter brings about the upregulation of defense-related genes, such as those encoding pathogenesis-related proteins like PR1, chitinases, and glucanase. Therefore, the activation of a systemic defense response, together with the plant growth-promoting effect exerted by _A. brasilense_ REC3 (Pedraza et al. 2010), could, in part, explain the increase of strawberry plants’ tolerance to anthracnose disease caused by _C. acutatum_ M11.

### 11.4.4 Biotechnological Application in Agriculture

In agriculture, the increasing introduction of new biotechnological products has allowed the achievement of higher yields in almost every present-day commercial crop, leading at the same time to a higher quality and minimizing ecological damage. In this context, agro-biotechnology may be used to develop environmentally safe and economically sound alternatives to chemical fertilizers and pesticides. New products are currently being developed through the stimulation of plant self-defense by the application of PGPB for biological control disease and as plant growth promoters (biofertilizers), applied as inoculants. In Table 11.1 are shown examples of PGPB siderophore producers, some of them already used as inoculants.

Much research has been dedicated to the development of _Pseudomonas_ inoculants and other biological products constituted by active metabolites such as antibiotics and siderophores as biocontrol agents (Mark et al. 2006). _Pseudomonas_ spp. have been efficiently used for biocontrol in the past decade, and at present time, there are several commercial products already in the market. For example, there is a biological product constituted by antimicrobial metabolites such as siderophore pyoverdine and SA produced by _P. aeruginosa_ PSS, very effective against _Peroonospora tabacina_ in tobacco culture, _Alternaria solani_ in tomato, and _Pseudoperonospora cubensis_ in cucumber (Díaz de Villegas 2007).

Microbe-assisted phytoremediation provides plants with natural metal-solubilizing chelators which do not represent a potential source of environmental pollution. At the same time as with microbial chelators, plant growth promotion can be enhanced through bacterially produced phytohormones (e.g., auxins). Recently, Dimkpa et al. (2006) studied the simultaneous production of siderophores and auxins by _Streptomyces_ aiming for future application in plant growth and phytoremediation in a metal-contaminated soil. Standard auxin and siderophore detection assays indicated that different _Streptomyces_ strains can produce these metabolites simultaneously. However, AP, Cu²⁺, Cu²⁺, Fe³⁺, and Ni²⁺ or a combination of Fe³⁺ and Cd²⁺ and Fe³⁺ and Ni²⁺ affected auxin production negatively, as revealed by spectrophotometry and gas chromatography–mass spectrometry. This effect was more dramatic in a siderophore-deficient mutant. In contrast, except for Fe, all the metals stimulated siderophores production. Mass spectrometry showed that siderophore and auxin-containing supernatants from a representative
Table 11.1 Examples of some siderophore producers within the plant growth-promoting bacteria (PGPB) and their main features

<table>
<thead>
<tr>
<th>PGPB</th>
<th>Main features</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azotobacter vinelandii</td>
<td>Produces at least five different siderophore types</td>
<td>Bellenger et al. (2008)</td>
</tr>
<tr>
<td>Azotobacter vinelandii</td>
<td>May produce siderophores to acquire Mn and V for nitrogen fixation when these metals are limiting in diazotrophic cultures</td>
<td>Krepeil et al. (2009)</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>Used as seed inoculants on crop plants to promote growth and increase yields</td>
<td>Kloepper et al. (1980)</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>Improve nodulation and N2 fixation in iron-deficient conditions</td>
<td>O'Hara et al. (1988),</td>
</tr>
<tr>
<td>Bradyrhizobium sp.</td>
<td>Produces salicylic acid among other siderophores under iron-starved conditions</td>
<td>Satena et al. (1986),</td>
</tr>
<tr>
<td>Azospirillum lipoflavorum</td>
<td>Produces siderophores with antifungal activity against Colletotrichum acutatum, the causal agent of anthracnose disease in strawberry crop</td>
<td>Shah et al. (1992),</td>
</tr>
<tr>
<td>Azospirillum brasilense</td>
<td>Produces siderophores in tobacco culture, Alternaria solani in tomato, and Pseudomonas aeruginosa</td>
<td>Tocora et al. (2011)</td>
</tr>
<tr>
<td>Gluconactobacter diazotrophicus</td>
<td>Hydroxamate-type siderophores</td>
<td>Logeshwaran et al. (2009)</td>
</tr>
</tbody>
</table>

Streptomyces species contain three different hydroxamate siderophores, revealing the individual binding responses to Cd2+ and Ni2+ and, thus, showing their auxin-stimulating effects. They concluded that siderophores promote auxin synthesis in the presence of Al3+, Cu2+, Cr3+, and Ni2+ by chelating these metals. Chelation makes the metals less able to inhibit the synthesis of auxins and potentially increases the plant growth-promoting effects of auxins, which in turn enhances the phytoremediation potential of plants.

11.5 Concluding Remarks

Agrochemicals, including fertilizers and pesticides, are extensively used in agricultural production to control pests, diseases, and weeds, minimizing the yield losses and maintaining high product quality. The increasing cost and the negative impact of agrochemicals and their degradation products in the environment are major ecological and health problems. Therefore, the use of PGPB as biofertilizers or biocontrol agents, most of which are siderophores producers, is quite promising to support an eco-friendly and sustainable agriculture.

Literature available revealed that siderophores production is not directly linked to the plant growth promotion neither to plant protection; siderophores are involved on iron availability in soil and in the interaction between plant and microorganisms in this habitat. The importance of siderophores is known since more than 30 years, and many siderophore-producing bacteria that benefit the crops, promote their growth, or protect them against pathogens have been reported. However, it is still not entirely known if this mechanism effectively operates in the interaction and whether it is the only one. Assuming that siderophore-producing microorganisms can obtain certain competitive advantages in the soil, where Fe(III) is not easily available, they are not the only attribute obtaining that benefit as siderophore–iron complexes may persist, be destroyed, or utilized by other organisms. Nevertheless, the role that siderophores can play as signal molecules or regulators in the microbe-plant interaction is evident and opens great perspectives for biotechnological applications in agriculture. 

Acknowledgments

The laboratory work of ROP was partially supported by CIUNT and ANPCIYT grants (PICT 2007 N°472). The research of FPS was supported by CIC (Comisión Sectorial de Investigación Científica, Universidad de la República), PEDECIBA (Programa de Desarrollo de Ciencias Básicas), and ANI (Agencia Nacional de Investigación e Innovación, Uruguay). The authors acknowledge the support of CYTED through the DIMAGRI network project.

References


The Role of Siderophores in Plant Growth-Promoting Bacteria

11
Gill PR, Barton LL, Scoble MD, Neilands JB (1994) A high affinity iron transport system of Rhizobium meliloti may be required for efficient nitrogen fixation in plants. Plant Soil 130:211–216
Gschicho VP, Glick BR (2001) Flooding tolerance of transgenic tomato plants expressing the bacterial enzyme ACC deaminase controlled by the 3SS, rolD or PRB-1b promoter. Plant Physiol Biochem 39:19–25


