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Rhizosphere Ecology of Plant Beneficial Microorganisms

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Appendix GF
### Publication Summary (numbers)

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- **Cooperation**, *briefly explain whether synergistic, complementary or supportive.*

The research cooperation was synergistic and involved collaborative research conducted by a visiting Israeli Postdoctoral researcher in Crowley's laboratory, as well as several exchange visits by the PIs during which time new ideas and directions were discussed.

Appendix GF
Rhizosphere Ecology of Plant-Beneficial Microorganisms

Submitted by

Prof. David Crowley, Prof. Yitzhak Hadar, and Prof. Yona Chen

Abstract. Microorganisms associated with plant roots contribute to plant nutrition and health through the production of growth factors, siderophores, degradation of toxic substances, and suppression of plant disease. Siderophores, which serve as metal chelators for uptake of essential trace elements for plants and microorganisms, play a key role in plant-microbial ecology in iron-limiting soils that represent 1/3 of the world's land surface area. The objectives of this research were 1) to determine the relationships between carbon and iron availability with respect to microbial growth and physiological status; 2) ascertain the importance of soil organic matter as a source of bioavailable iron; and 3) model the biochemical ecology of plant and microbial siderophores with respect to chelation kinetics and ligand exchange. Experiments focused on model systems involving strategy I plants that use organic acids, humic iron complexes, and microbial siderophores to acquire iron, and on strategy II plants that produce phytosiderophores for iron acquisition.

In studies with the microbial siderophore rhizoferrin, it was shown Fe-rhizoferrin complex is an efficient iron carrier that can be used by both strategy I and strategy II plants. The mechanisms of iron uptake by dicotyledonous plants were further shown to involve reduction and transport of ferric iron at the root surface; whereas strategy I plants acquired iron by ligand exchange with phytosiderophores. Discovery of this latter mechanism was in contradiction to thermodynamic equilibrium models, but was substantiated using a kinetic approach to monitor metal exchange between siderophores and other metal chelating substances. The results of this research were further extended to siderophore and phytosiderophore involvement in plant uptake of cadmium.

Plant iron stress and concomitant iron chelate production by plant roots was directly influenced by the presence of soil organic matter. Critical levels needed to supply iron to plants and presumably to rhizosphere microorganisms were estimated to be 50 mg/L of soluble organic matter in the soil solution. Changes in microbial physiology and microbial community structure as related to carbon and iron supply were examined using molecular techniques employing an iron stress reporter system. Phytosiderophore production by plants decreased the iron stress of a plant beneficial rhizosphere pseudomonad, and also provided a carbon source for selective enrichment of certain bacterial species in different root zones. Changes in community structure were examined using PCR-DGGE of 16S rDNA from rhizosphere microorganisms. Results showed that the plant iron nutritional status is a significant factor in determining the bacterial species composition in the rhizosphere, and revealed complex interactions between plants and microorganisms with respect to metal chelation and rhizosphere ecology. These findings have significance for the development of new iron fertilizers, better understanding of mechanisms of metal acquisition in plants, and future studies aimed at altering the rhizosphere community to promote beneficial plant microbial interactions.
Achievements:

This research was aimed at improving fundamental knowledge of the role of plant and microbial siderophores in rhizosphere microbial ecology and in the trace metal nutrition of plants and microorganisms. All of the short term goals and objectives of the original proposal were met and several additional studies were conducted. Dynamic processes involving ligand exchange and cross feeding were shown to be involved in plant and microbial iron uptake from siderophores. Related research confirmed the role of soil organic matter as an iron source for plants and rhizosphere microorganisms, and experiments were conducted to determine the role of siderophores and phytosiderophores in uptake of the heavy metal cadmium by graminaceous plants. Lastly, experiments were conducted to examine the influence of plant iron nutritional status on the species composition and community structure of the rhizosphere microflora.

Better understanding of the mechanisms of iron uptake from siderophores will aid in the development and application of microbial siderophores as alternative iron fertilizers, or possibly even the use of microbial inoculants to enhance plant iron nutrition. Previously, equilibrium based models suggested that microbial siderophores would not function effectively for delivery of iron to plants because of their high stability constants, and that phytosiderophores may be preferentially complexed with other ions in such a way that they would not be effective for enhancing iron uptake by graminaceous plants. Moreover, it was previously thought that microbial siderophores would strip iron from phytosiderophores and thereby cause competition between plants and microorganisms for iron. In contrast to these expectations, experiments conducted here showed that the plant production of siderophores actually is the controlling factor in determining availability of iron to both plants and microorganisms. Using an iron stress induced ice-nucleation reporter system contained in a rhizosphere pseudomonad, we showed that microbial siderophore production by pseudomonads is suppressed by plant production of phytosiderophore. Moreover, phytosiderophore was shown to be capable of removing iron by ligand exchange from the microbial siderophore rhizoferrin,
and it was shown that this mechanism may also function for low level uptake of iron from highly stable siderophores such as ferrioxamine B.

Demonstration of the role of soil organic matter as an iron source for plants and microorganisms was also an important finding in that soil organic matter amendments derived from biosolids, compost, and urban yard waste are becoming increasingly utilized as soil amendments in agriculture. Using a thermodynamic model, organic matter amendments would be predicted to have low stability with iron as compared to other ions and metals. Based on our findings, there is now substantial evidence for recommending the use of organic matter supplements to enhance plant and microbial iron nutrition. This has also opened up new questions as to the role of organic matter amendments in shaping the species composition and structure of rhizosphere microbial communities through the effects of these substances on carbon and iron availability to microorganisms.

The implications of our research findings are further relevant to the current interest in heavy metal acquisition by plants. Among one of the primary metals of concern is cadmium, which can form a complex with phytosiderophores and microbial siderophores, and thus could potentially be taken up by plants and microorganisms. On one hand there is interest in the role of metal chelators in uptake of metals by plants that are being considered for use in phytoremediation of heavy metal contaminated soils. From a human health point of view, there has been concern about uptake and food chain transfer of cadmium in grains. Lastly, cadmium is a potent toxic metal for microorganisms and causes a loss of functional diversity. In studies on the role of rhizoferrin and phytosiderophore on cadmium uptake by barley and wheat, we showed that plants accumulated cadmium in the roots when grown in high cadmium solutions, but that phytosiderophore production and mobilization of cadmium for uptake by plants was inconsequential. In the U.S., high cadmium grains have been prohibited for export to Europe, and there is much interest in the mechanisms that are responsible for cadmium uptake by gramineous plants. Demonstration of the lack of a role for phytosiderophores in cadmium uptake may help to better direct
plant breeding efforts away from this consideration and towards a more meaningful solution to this problem.

Lastly, one of the great challenges of the coming decade in agricultural biotechnology is the design and optimization of plant microbial systems to enhance nutrient acquisition in nutrient limiting soils, and the colonization of roots by plant beneficial microorganisms that promote plant growth and disease resistance. A major obstacle has been the lack of methods to understand the species composition of the rhizosphere microbial community and how the rhizosphere bacteria are influenced by the plant nutritional status and other factors. Research conducted here used molecular biology techniques involving PCR-DGGE of 16S rDNA to fingerprint microbial communities at different root locations and in response to plant iron deficiency. Results of these experiments clearly demonstrated the importance of plant iron nutritional status in shaping the rhizosphere community and in determining the complexity of the community that colonizes the new root tips. This experimental approach will ultimately lead to improved ability to determine the relationship between microbial community function and species composition, and eventually to a better ability to manipulate the rhizosphere to enhance plant nutrition and root health.
Project Background

The plant rhizosphere is one of the most important components of terrestrial ecosystems with respect to microbial processes that affect primary productivity, plant nutrition, disease, nutrient cycling, and detoxification of xenobiotic pollutants. The future success of agricultural biotechnology, as well as our ability to manage and predict the behavior of natural systems depends on understanding the bases of competitive fitness in soil microorganisms that carry out these processes, and how various environmental factors influence microbial communities that colonize plant roots. The objectives of this research were to examine plant microbial interactions that influence the survival and activity of introduced bacteria, with a particular focus on the role of iron in plant and microbial trace metal nutrition.

One of the most important factors governing microbial competition in soil has been speculated to be the differential production and utilization of biosynthetic iron chelators, termed siderophores (Crowley and Gries, 1994). In addition to mediating iron competition, these compounds also have been shown to function as potential iron sources for plants in calcareous soils (Wang et al., 1994, Jurkevitch et al., 1992, Shenker et al., 1993), and may also facilitate plant uptake of heavy metals other than iron. In preliminary research conducted before this project was funded, identified a siderophore produced by Rhizopus arrhizus, which is as effective as the commercial synthetic chelate Fe-EDDHA for remedy of iron chlorosis in tomato. However, both siderophore production and the ability to colonize the rhizosphere also depend on the amount of carbon that is available for microbial growth. This carbon is provided mainly in the form of root exudates that selectively enrich for different bacteria, depending on their ability to use these various compounds. Root exudates themselves may also influence plant and microbial iron nutrition, through the secretion of phytosiderophores by plants and by release of organic acids such as citrate from dicotyledonous plants. Further, the amount and type of carbon substrates that are available for microbial growth is highly variable for different root zones. For example, phytosiderophores produced by grasses are released primarily behind the root tips.
Iron deficiency in plants is the most common trace metal deficiency in nature and is one of the most difficult to correct. This problem occurs on over 1/3 of the world's land surface area and is particularly prevalent in arid, high pH, calcareous soils typical of the western United States and almost all of the agricultural land in Israel. Under these conditions, iron deficiency is also predicted to occur for root colonizing microorganisms. Both plants and microorganisms that are adapted to these soils have iron stress responses that involve the production and utilization of siderophores and phytosiderophores, respectively. Plant strategies include Strategy I that is used by dicotyledonous plants, involves the induction of an iron chelate reductase and rhizosphere acidification; whereas, Strategy II monocotyledonous plants produce phytosiderophores (Römheld, 1987). The influence of these stress responses on microorganisms and their role in iron uptake from microbial siderophores is still debated, but was one of the primary emphases of this research.

The specific objectives of this research were to examine factors that influence the utilization of a prototypical microbial siderophore, rhizoferrin, by plants. We also sought to examine the mechanisms by which this siderophore is used, and to model its efficacy in mobilizing iron and other metals from soil. In the soil environment, much of the pool of microbially produced siderophore is bound to clay; therefore, we also sought to examine the influence of bound siderophores on plant iron nutrition. In collaborative studies conducted in the US at UC Riverside, experiments were conducted to examine the physiological status of rhizosphere microorganisms and how carbon limiting conditions that occur in most of the rhizosphere might influence microbial siderophore production in the vicinity of roots. While plant microbial interactions in the iron nutrition of plants and microorganisms are highly complex, new molecular tools and analytical techniques provided us with the ability to dissect these processes and to examine the importance of iron in shaping the species composition and community structure of the rhizosphere. Lastly, experiments were conducted to examine the role of siderophores and phytosiderophores in uptake of lead and cadmium by plants.
Methodologies and Materials

Experiments examining plant iron uptake from siderophores and organ matter complexed iron were conducted in hydroponic culture systems with $^{59}$Fe-radiolabeled siderophores and by measurement of plant growth, plant tissue analyses, and measurement of leaf chlorophyll contents. Specific methods are highlighted below in the results sections for the different experiments. Plants examined in the hydroponic studies included barley, wheat, corn, and tomato, and cucumber. Plant tissue analyses for iron, cadmium and lead were determined by ICP-MS, or by liquid scintillation counting of radioactive iron. Leaf chlorophyll contents were determined with a SPAD meter and were calibrated against leaf samples that were extracted and quantified using spectrophotometry.

Ligand exchange experiments were conducted using radiolabeled siderophores, which were established in defined solution mixtures at varying ratios with competing ions that would be present in the soil solutions. Exchange kinetics were determined for samples removed from the mixtures over time, at which time the chelators were separated by thin layer chromatography, and radioactive iron associated with the separated chelates was determined by liquid scintillation counting. Iron reduction rates for reductive release of iron from siderophores was determined spectrophotometrically using the ferrous chelator BPDS, or with ferrozine. Calculations of the stability of the Fe-complexes were determined using GEOCHEM-PC (Parker et al., 1995).

Rhizoferrin was produced from *Rhizopus arrhizus* and was purified according using ion chromatography as described in published protocols. Phytosiderophore was collected as root exudates from corn and barley and its concentration determined by HPLC or by the Cu-chrome-azurol assay (Shenker et al., 1995). DFOB and synthetic chelates were purchased from Sigma Chemical Company.

Sorption isotherms of chelates to clay minerals were determined for ferrated and deferrated chelates of ferrioxamine B, rhizoferrin, and phytosiderophore for the clay minerals bentonite, montmorillonite, and kaolinite.
Sorption isotherms were determined over a range of pH under different ionic conditions. Plant availability of sorbed chelates was determined in hydroponic experiments using the various chelators as iron sources for plants.

Physiological status of rhizosphere bacteria at different locations in the rhizosphere was determined using a bioluminescent strain of \textit{P. fluorescens} containing a luciferase reporter system coupled to a ribosomal promoter (Brennerova and Crowley, 1994). Other experiments examining the iron stress status of \textit{P. fluorescens} on barley roots using an ice-nucleation reporter system in which expression of the ice nucleation genes is coupled to an iron-stress inducible promoter for siderophore synthesis (Loper and Henkels, 1997; Marschner and Crowley, 1997). This latter reporter system was calibrated for bacteria grown in pure culture with different levels of iron supplied as inorganic ferric hydroxide, iron citrate, or phytosiderophore. Plants were grown in root box microcosms with a removable plate that allowed access to the roots. The soil was inoculated with $10^6$ cfu/g of the marked bacteria, packed into the microcosms, and then used to grow plants from seedlings that were transplanted into the microcosms and grown for 2 to 3 weeks. Bacteria associated with different locations of the rhizosphere were sampled by removing the cover, and pressing nitrocellulose filter paper strips on to the roots, which were then transferred to sterile saline in 1.5 ml Eppendorf tubes to dislodge the bacteria. The bacteria were enumerated on selective agar media, and subsamples of the saline suspension were analyzed immediately for their luciferase activity, or for their iron-stress activated ice-nucleation activity.

The influence of plant iron nutritional status on microbial communities associated with different root zones was examined for barley plants under iron stress and iron sufficient conditions in soil. Barley plants were germinated and transferred into microcosms containing an iron-limiting, sandy-loam soil. To alter the plant iron status, the plant shoots were sprayed with iron citrate or with distilled water on a daily basis. After 12 weeks, bacterial DNA associated with the root tips, sites of lateral root emergence, and older mature roots was extracted from excised root samples and adhering soil using a Fastprep beadbeater (BIO 101 Visita, CA). Total DNA from the resulting homogenate was
isolated and purified. Three locations of the same root area were taken from each plant, and a total of 72 rhizosphere samples were analyzed. To selectively amplify bacterial DNA, the primers, PRBA338f and PRUN518r, located at the V3 region of 16SrRNA genes from bacterioplankton were used (Ovreas et al. 1997). The PCR products were separated by DGGE using 8% (wt/vol) acrylamide gels containing a linear chemical gradient ranging from 20 to 70% denaturant (7 M urea and 40% [vol/vol] formamide).

Results

1. Organic matter iron complexes as iron sources for plants. The phenomenon of increased plant growth due to organic matter application was attributed mainly to increased absorption of trace elements, either by increased solubility or by colloids formation. The magnitude of this phenomenon is dependent on pH and the concentration and type of the organic matter. It is assumed that in soils containing 100-300 mg L\(^{-1}\) organic matter in the soil solution, effect of added organic matter on plant growth through improvement of microelements solubility is negligible. An improved solubility of microelements, which induces a positive plant response, was attained when organic matter (humic or as an additive containing humic substance) was added to soils containing less then 50 mg L\(^{-1}\) of organic matter in the soil solution (Chen et al., 1998).

2. Role of ligand exchange in uptake of iron from Graminaceous plants. Iron uptake mechanisms from microbial siderophores were examined for barley and corn in studies with radiolabeled 59Fe-rhizoferrin, ferrioxamine B, and the phytosiderophore deoxymugineic acid (DMA). A control included the synthetic chelate FeEDTA. Plants were grown in nutrient solutions at pH 6 and Fe uptake from the chelates was examined on a diurnal basis during the periods of peak production of phytosiderophore (PS) in the morning, and during the evening when there is no phytosiderophore release. Uptake and translocation of iron paralleled the diurnal release of PS release in barley, but not in corn. The results demonstrated that Fe uptake from rhizoferrin involves an indirect mechanism in which phytosiderophores strip iron from the microbial siderophore and transport it into the plant. This hypothesis was further verified by in vitro ligand exchange experiments.
3. Compare the utilization of different siderophores by dicotyledonous and monocotyledonous plants to determine their relative efficacy in providing iron to plants under iron-limiting conditions. Iron uptake from a ferric complex of rhizoferrin was compared to that from EDDHA, EDTA, and ferrioxamine B (FOB) in studies with tomato, barley, and corn. Iron uptake was monitored using radioactive iron 59Fe-siderophores and chelates. Rhizoferrin was shown to be much more effective in supplying iron to plant than was FOB. This phenomenon was shown to be related to the relatively low stability constant of rhizoferrin and its ability to undergo ligand exchange with phytosiderophores, or to release iron via the iron stress inducible chelate reductase in tomato and other dicotylendous plants.

4. Characterize the kinetics of iron complexation and metal exchange by rhizoferrin. In contrast to results observed above, calculations based on stability constants and equilibrium models suggested that rhizoferrin would not readily supply Fe to plants. To address this question, the stability of Fe3+ complexes for this and several other model chelators was examined in nutrient solutions. Ferric complexes of siderophores were stable for at least 31 days, whereas EDTA and the phytosiderophore mugineic acid lost 50% of their iron in less than 2 days. Ligand exchange experiments Fe extraction experiments on the other hand showed that MA was more effective than rhizoferrin for solubilizing iron. Moreover, MA was shown to be remove iron from rhizoferrin by ligand exchange. These data strongly support the need for a kinetic based approach to predict chelate behavior in soil and solution.

5. Influence of phytosiderophores and microbial siderophores on cadmium uptake by plants. Previous work by our group had shown that siderophores may complex metals other than iron and thereby influence uptake of heavy metals (Chen et al., 1994). In new experiments, Cd extraction from solid phase of Cd-phosphate at pH 7.3 was examined in the presence and absence of iron hydroxide and with Ca and Mg as additional competing ions for phytosiderophores (PS), the synthetic chelate HEDTA, and the fungal siderophore rhizoferrin. While rhizoferrin did not extract Cd, PS and the synthetic chelate HEDTA did extract Cd even in the presence of Fe. However uptake of Cd by wheat and barley plants
was low as compared to rates observed for Fe complexes, and was not significantly influenced by the iron stress status of the plant, but instead was controlled by the metal ion activity of Cd in solution. These results suggested that even though cadmium may be mobilized by PS, there is no significant uptake and translocation of the CdPS complex by the plant roots.

6. **Iron stress status of rhizosphere bacteria in the rhizosphere of barley under iron deficient and iron sufficient growth conditions in soil.** The iron stress status of P. fluorescens was determined using a plasmid reporter system consisting of an iron-stress regulated ice nucleation gene construct that can be used to assay iron stress (Loper and Lindow, 1994). With this assay, relative iron stress can be quantified with a droplet freezing assay. Results of experiments in liquid medium showed that iron stress was regulated both by the carbon and iron supply. Under conditions of rapid growth, siderophore production was induced uniformly and irrespective of iron concentration. However, at stationary phase, the amount of siderophore that accumulated in solution was directly correlated with iron concentrations in the medium. Using this assay, we calibrated siderophore accumulation versus ice nucleation on a per cell basis to estimate siderophore accumulation in the rhizosphere. Results showed that bacteria sampled from the rhizosphere of rice and barley were relatively nonstressed as compared to cells grown in liquid medium. The bacteria were less stressed in the rhizosphere of barley plants, which produces high quantities of siderophores than those associated with rice. Foliar iron treatments which alleviated iron stress in the plant tissues resulted in an increase in iron stress for the bacteria associated with the roots. These results strongly suggest that pseudomonads rely on phytosiderophores for iron, and only produce siderophores to augment that which is not provided by the plant iron stress response.

7. **Bacterial community structure and species composition in the rhizosphere of iron stressed and iron-sufficient barley plants.** Changes in plant iron stress status caused striking changes in the community DNA profiles at the root tip zones of the barley plants, but had little or no effect on communities associated with the older root zones and sites of lateral root emergence. In general, alleviation of iron stress caused an increase in the number of species and
increased evenness in the relative dominance of different bands representing different species in the rhizosphere. An interpretation of this result is that iron stressed plants produced phytosiderophore as a major root exudate component, which caused predominance of a few species that were best able to exploit this single carbon source. When iron citrate was supplied to the foliage, phytosiderophore production was reduced and the relative complexity of the exudate increased, which allowed greater numbers of different microbial species to proliferate.

Discussion

This collaborative research project investigated many aspects of plant and microbial iron nutrition that together provide a new integrated view of the function of plant and microbial siderophores in metal uptake, adaptation to iron stress, and rhizosphere ecology. Plants and microorganisms are commonly subjected to iron deficiency in high pH, arid soils that are well aerated and typically calcareous. These soils are common to many agricultural areas in the midwest and western United States and in almost all of Israel. Currently, production of many crops on these soils is limited due to problems with iron deficiency, and fertilization with synthetic iron chelators is an expensive and impractical treatment for all but very high value crops. Even with high value horticultural crops, these treatments are short lived and must be repeated on a frequent basis. For these reasons, there is considerable interest in how iron-efficient plants have adapted to calcareous soils, and the role of microorganisms in plant trace metal nutrition. Another facet of this research problem is the interest in manipulating the rhizosphere microflora to enhance plant fitness to these soils, and to improve their yield and disease resistance.

Siderophores produced by plants and microorganisms play a key role in the adaptation of these organisms to iron stress conditions. Soil organic amendments also can contribute to iron and trace metal nutrition by providing a reservoir of exchangeable metals that can be easily mobilized by biosynthetic chelates or transported to the cell surface as metal complexes. Results of our
research show that organic matter additions are effective for increasing iron availability to plants, and show that certain microbial siderophores such as that produced by Rhizopus sp. are highly effective and comparable to the best synthetic chelates. Our studies on the mechanisms of plant uptake from this chelator show that this involves a ligand exchange process with monocots and a reductive mechanism with dicotyledonous plants. This mechanism was not predicted based on equilibrium models, but was demonstrated empirically and strongly emphasizes the need for using a kinetically based approach to studies on plant metal uptake. Moreover, in soils, much of the siderophore that is secreted by microorganisms is readily sorbed to clay and organic matter, which may influence their efficacy. Results of our research show that microbial siderophores continued to provide iron to plants even when sorbed to clay, which again emphasizes the kinetic processes of sorption and desorption as controlling factors in metal availability as opposed to equilibrium based models.

Previously much research has focused on the role of siderophores produced by root colonizing pseudomonads in plant nutrition, competitive fitness of plants, plant growth promoting pseudomonads, and in disease suppression. In pure culture, very large quantities of siderophore can be produced under high carbon conditions, but relatively low levels are found in soils. Research conducted here has helped to solve this paradox, and shows that the plant stress response is the overriding control on production of pyoverdin by these bacteria. Changes in microbial community structure and species composition in different root zones were shown to occur in relation to plant iron nutritional status. This latter finding and the methods that were developed to explore plant microbial interactions in metal uptake have opened the door to new research on the plant rhizosphere that will continue to employ high resolution techniques for examining microbial communities. Eventually this may lead to diagnostic analysis of the rhizosphere and ways to manipulate plant microbial interactions to increase plant fitness and agricultural yields.

Scientific Implications
This research was aimed at improving fundamental knowledge of the role of plant and microbial siderophores in rhizosphere microbial ecology and in the trace metal nutrition of plants and microorganisms. Specific achievements included advances in our knowledge of the dynamic processes involved in metal uptake from siderophores; determination of the role of soil organic matter as an iron source for plants and rhizosphere microorganisms; determination of the factors that influence siderophore production by plant beneficial pseudomonads; characterization of the interaction of siderophores and phytosiderophores with respect to ligand exchange. In addition, powerful reporter gene techniques were used to assess the influence of plant iron nutritional status on the physiological status of bacteria in the rhizosphere, estimates of the levels of siderophore production by fluorescent pseudomonads, and the effects of plant iron stress on species composition and community structure of the rhizosphere microflora.

Better understanding of the mechanisms of iron uptake from siderophores will aid in the development and application of microbial siderophores as alternative iron fertilizers, or possibly even the use of microbial inoculants to enhance plant iron nutrition. Previously, equilibrium based models suggested that microbial siderophores would not function effectively for delivery of iron to plants because of their high stability constants, and that phytosiderophores may be preferentially complexed with other ions in such a way that they would not be effective for enhancing iron uptake by graminaceous plants. In experiments conducted here, we showed that a kinetic based approach was the only valid method for predicting the efficacy of metal chelators, as compared to equilibrium based models that have predominated in the literature over the past two decades. Previous models suggested phytosiderophores would not function in the presence of siderophores, whereas in fact it was shown that these compounds are capable of removing iron by ligand exchange from the microbial siderophore rhizoferrin, and that this mechanism may also function for uptake of iron from even more stable siderophore complexes such as desferrioxamine B. The use of the kinetic based approach thus provides a powerful method for evaluating metal chelators as iron sources for plants.
Demonstration of the role of soil organic matter as an iron source for plants and microorganisms was also an important finding in that soil organic matter amendments derived from biosolids, compost, and urban yardwaste are becoming increasingly utilized as soil amendments in agriculture. Again, using a thermodynamic model, organic matter amendments would be predicted to have low stability with iron as compared to other ions and metals. Based on our findings, there is now substantiated evidence for the validity of using organic matter supplements to enhance plant and microbial iron nutrition. This has also opened up new questions as to the role of organic matter amendments in shaping the species composition and structure of rhizosphere microbial communities that will be examined in future research.

The implications of our research are further relevant to the current interest in heavy metal acquisition by plants. Among one of the primary metals of concern is cadmium, which can form a complex with phytosiderophores and microbial siderophores, and thus could potentially be taken up by plants and microorganisms. In contrast to expectations, however, our research showed that both cadmium and lead are not readily taken up or translocated when supplied to plants as phytosiderophore complexes.


One of the great challenges of the coming decade in agricultural biotechnology is the design and optimization of plant microbial systems to enhance nutrient acquisition in nutrient limiting soils, and the colonization of roots by plant beneficial microorganisms that promote plant growth and disease resistance. A major obstacle has been the lack of methods to understand the species composition of the rhizosphere microbial community and how the rhizosphere bacteria are influenced by the plant nutritional status and other factors. Experiments conducted here demonstrated the value of new molecular biology techniques for genetic fingerprinting of microbial communities on plant roots. Experiments were conducted in which the plant iron nutritional status was
deliberately altered by foliar application of iron, after which changes in microbial community structure were assessed using for bacteria associated with different root locations. Results of these experiments clearly demonstrated the importance of plant iron nutritional status on shaping the rhizosphere community and in determining the complexity of the community that colonizes the new root tips. This experimental approach will ultimately lead to improved ability to determine the relationship between microbial community function and species composition, and eventually, the manipulation of the rhizosphere to enhance plant nutrition and root health.

Lastly, from a human health point of view, there has been concern about uptake and food chain transfer of cadmium in grains. Cadmium is a potent toxic metal for microorganisms and causes a loss of functional diversity for degradation of unusual but potentially phytotoxic substances that would otherwise accumulate in soils. In studies on the role of rhizoferrin and phytosiderophore on cadmium uptake by barley and wheat, we showed that plants accumulated cadmium in the roots when grown in high cadmium solutions, but that phytosiderophore production and mobilization of cadmium for uptake by plants was inconsequential. In the U.S., high cadmium grains have been prohibited for export to Europe, and there is much interest in the mechanisms that are responsible for cadmium uptake by gramineous plants. Demonstration of the lack of a role for phytosiderophores in cadmium uptake may help to better direct plant breeding efforts away from this consideration and towards a more meaningful solution to this problem.

References


List of all publications resulting from the project.


