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**Development of Microbial Siderophores as Iron
Carriers for Higher Plants: Production, Mode of
Action and Effectiveness**

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Development of Microbial Siderophores as Iron Carriers for Higher

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III. Abstract

Many agricultural crops worldwide experience iron deficiencies on calcareous soils, which cover nearly 40% of the earth's land surface. This research addressed the ability of plants to utilize iron via microbial siderophores with emphasis on pseudobactin-type siderophores as compared to other microbial siderophores. The role of the pseudomonad siderophore, pseudobactin, in the iron nutrition of both dicots and monocots was examined in nutrient solution with varying pH and in calcareous soil. Efforts were made to develop a bioassay specific for pseudobactin siderophores, based on the experience and success with other siderophore bioassays. The utilization by several plant species of iron when supplied as pseudobactin was compared with several other hydroxamate siderophores, including ferrioxamine B, and phytosiderophores. The influence of microbial interactions in the rhizosphere on iron availability to the plant was also examined.

A fluorescent siderophore isolated from a strain of Pseudomonas putida grown on peanut roots was shown to be similar to the known class of pseudobactin/pyoverdine siderophores, and was shown to alleviate iron deficiency in peanut plants grown in calcareous soil. In short-term nutrient solution experiments, the uptake of radioactive iron by the dicots of cotton, peanut and sunflower from pseudobactin was relatively low compared to EDDHA, while uptake by the monocot sorghum was much higher than EDDHA. Plants also utilized the siderophore ferrioxamine B (FOB) in iron uptake. Excised root studies with peanut showed high rates of radioactive iron uptake from FOB. Iron uptake rates by dicots from Fe-pseudobactin and FeEDDHA were found to correlate with iron reduction rates and reduction potentials of both chelates.

The utilization of iron from hydroxamate or catecholate microbial siderophores by corn and oat was affected by rhizosphere microbes in short-term experiments. The uptake of radioactive iron by grasses was inhibited under certain rhizosphere microbial densities. In experiments with mycorrhizal roots of pine, the presence of the mycorrhizal fungus also altered the uptake pattern of radioactive iron from FOB.

Anomalies in developing bioassays for individual siderophore classes, e.g., pseudobactin, led to elucidation of specific and unique outer membrane receptors in Escherichia coli for linear ferrioxamines. Comparisons of plant uptake by various dicots and monocots of phytosiderophores, pseudobactins, and hydroxamates have led to models on plant uptake mechanisms and on the ecological interactions involved in iron supply from microbial siderophores in the rhizosphere.

IV. Objectives of Proposed Research

This final report summarizes the collaborative research conducted during a three-year project to investigate the development of microbially-produced chelates as iron carriers for higher plants. The specific aims of this research were to study the influence of siderophores (microbially-produced iron chelates), particularly pyoverdin, on iron stress in plants and to explore the development of new technology using pseudomonads and pyoverdin for alleviating plant iron deficiencies in iron-limiting agricultural soils. As described in the BARD guidelines, this report includes an evaluation of the research achievements with respect to the original proposed objectives, a description of cooperation, and a detailed description of research activities and results.

Summarizing from the original proposal, *Pseudomonas* root-colonizing bacteria produce pyoverdin-(pseudobactin-)type siderophores that have been implicated in regulating iron-availability in calcareous soils. Plants acquire iron under such conditions by one or more iron-stress response mechanisms based on the use of naturally-occurring iron chelates. Iron-efficient plants use a variety of hydroxamate siderophores produced by bacteria and fungi, but the ability of plants to use pyoverdins has only been preliminarily examined. This latter class of siderophores includes a number of compounds with various structures. There are conflicting reports that suggest some pyoverdin-type compounds may inhibit plant iron uptake, whereas other pyoverdin compounds are effective iron sources for plant nutrition.

Since pyoverdins occur naturally in the rhizosphere of plant roots, the first goal of this research was to examine their possible function in plant iron nutrition and to determine if there was a difference in plant utilization of different pyoverdins. Secondly, because pyoverdins have features which may be desirable for commercial applications, e.g., resistance to degradation and ability to control iron availability to plant deleterious root pathogens, a major goal of this research was to explore the potential use of pyoverdins as iron carriers for use in agriculture.

Two hypotheses were proposed in the original proposal:

H0 (the null hypothesis): Plants in the presence of pyoverdin use hydroxamate siderophores produced by other rhizosphere microorganisms.

H1 (the alternate hypothesis): Iron-efficient plants directly utilize iron chelated by pyoverdins.

Proceeding from our original proposal, our research plan focused on plant utilization of pyoverdin produced by specific *Pseudomonas* spp and made extensive comparisons of plant use of pyoverdin with the representative hydroxamate siderophore, ferrioxamine B (FOB).

V. Publications and Abstracts

LIST OF PUBLICATIONS

- Babbitt, M.J., D.E. Crowley and C.P.P. Reid. 1991. Degradation of ferrioxamine B in moist soil and soil-inoculated media. *Soil Biology and Biochemistry* (in revision).
- Bar-Ness, E., Y. Chen, Y. Hadar, H. Marschner and V. Romheld. 1991. Rhizosphere microorganisms involvement in Fe uptake from microbial siderophores by corn and oats in short-term experiments. (submitted)
- Bar-Ness, E., Y. Chen, Y. Hadar, H. Marschner, and V. Romheld. 1991. Siderophores of *Pseudomonas putida* as an iron source for dicot and monocot plants. *Plant and Soil* 130:231-242.
- Bar-Ness, E., Y. Chen, Y. Hadar, H. Marschner and V. Romheld. 1991. Siderophores of *Pseudomonas putida* as an iron source for dicot and monocot plants. pp.271-281. In: Chen, Y. and Y. Hadar (eds). *Iron Nutrition and Interaction in Plants*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Crowley, D. E., V. Romheld, H. Marschner, and P.J. Szaniszlo. 1991. Root-microbial effects on plant iron uptake from siderophores and phytosiderophores. *Plant & Soil* (submitted).
- Crowley, D.E., Y.C. Wang, C.P.P. Reid and P.J. Szaniszlo. 1990. Mechanisms of iron acquisition from siderophores by microorganisms and plants. *Plant and Soil* 130:179-198.
- Jurkevitch, E., Y. Chen and Y. Hadar. 1988. The role of bacterial siderophores in the iron nutrition of plants. *Soil Sci. Soc. Am. J.* 52:1032-1037.
- Jurkevitch, E., Y. Hadar and Y. Chen. 1991. Differential siderophore utilization and iron uptake by soil and rhizosphere bacteria. *Appl. Environ. Microbiol.* (submitted)
- Leyval, C. and C.P.P. Reid. 1991. Utilization of microbial siderophores by mycorrhizal and nonmycorrhizal pine roots. *New Phytologist*. (In Press)
- Nelson, M., C.J. Carrano and P.J. Szaniszlo. Identification of the ferrioxamine B receptor, fox, in *Escherichia coli* K-12. *Biology of Metals* (to be submitted).
- Nelson, M. and P.J. Szaniszlo. TonB-independent ferrioxamine B-mediated iron transport in *Escherichia coli* K-12. *J. Bacteriology* (to be submitted).

ABSTRACTS OF LECTURES PRESENTED IN NATIONAL AND INTERNATIONAL CONFERENCES

- Bar-Ness, E., Chen, Y., Hadar, Y., Marschener, H. and Romheld, V. 1989. Siderophores of *Pseudomonas putida* as an iron source for plants. 5th International Symposium on "Iron Nutrition and Interactions in Plants" June 1989, Jerusalem, Israel.
- Bar-Ness, E., Hadar, Y. and Chen, Y. 1990. The effect of rhizosphere microorganisms on Fe uptake from microbial siderophores by higher plants. Meeting of the Israel Society of Botany, February 28, Tel-Aviv, Israel.
- Crowley, D.E., C.J. Carrano and P.J. Szaniszlo. 1989. Shuttle mediated siderophore-iron transport by oat roots. Amer. Soc. Microbiol. (Abst) 89:295.
- Crowley, D.E., C.P.P. Reid and P.J. Szaniszlo. 1988. Siderophore reduction and iron transport by membrane vesicles from oat roots. Amer. Soc. Microbiol. (Abst) 88:261.
- Crowley, D.E., H.N. Brown and P.J. Szaniszlo. 1990. Iron acquisition from ferrioxamine B by axenic oat and soybean in long-term growth studies. II Intl. Symp. Iron Transport, Storage and Metabolism, Austin, TX (Abst).
- Crowley, D.E., V. Romheld, H. Marschner and P.J. Szaniszlo. 1990. Root-microbial contaminant effects on Fe-uptake from ferrioxamine B and epi-hydroxymugineic acid by corn and oat. II Intl. Symp. Iron Transport, Storage and Metabolism, Austin, TX (Abst).
- Hadar, Y., Y. Chen and E. Jurkevitch. 1987. The role of *Pseudomonas* siderophores in plant microorganisms interactions. Phytoparasitica 15:2.
- Hadar, Y., Chen, Y., Jurkevitch, E. 1987. The role of *Pseudomonas* siderophores in plant-microorganisms interactions. The Annual Meeting of the Israeli Microbiological Society, February 24-25, Rehovot, Israel.
- Jurkevitch, E., Hadar, Y. and Chen, Y. 1990. The effect of various siderophores on soil and rhizosphere populations. International Symposium on Iron Transport, Storage and Metabolism, June 20-22, Austin, Texas.
- Jurkevitch, E., Hadar, Y. and Chen, Y. 1990. The role of Pseudobactin as an iron source for soil and rhizosphere microorganisms. Phytoparasitica 18:91.
- Leyval, C. and C.P.P. Reid. 1990. Influence of an ectomycorrhizal fungus on the utilization of microbial siderophores by slash pine roots. 2nd. PGPR Workshop, Interlaken, 14-19 October 1990 (Abst.).

- Leyval, C and C. P. P. Reid. 1990. Influence of Pseudomonas fluorescens on the utilization of microbial siderophores by mycorrhizal and non mycorrhizal slash pine roots. 2nd. PGPR Workshop, Interlaken, 14-19 October 1990 (Abst.).
- Nelson, M., C.J. Carrano and P.J. Szaniszlo. 1989. Photoinhibition of ferrioxamine B-mediated iron transport in Escherichia coli. Amer. Soc. Microbiol. (Abst.) 89:271.
- Nelson, M., and P.J. Szaniszlo. 1991. Ton B-independent ferrioxamine B-mediated iron transport in Escherichia coli K-12. Amer. Soc. Microbiol. (Abst) 91:233.
- Reid, C.P.P., P.J. Szaniszlo and D.E. Crowley. 1989. Role of microbial siderophores in plant iron nutrition. 5th Intl. Symp. on Iron Nutrition and Interactions in Plants. Kibbutz Ramat-Rachel, Israel. (Abst).
- Szaniszlo, P.J., D.E. Crowley and C.P.P. Reid. 1989. Iron uptake mechanisms from siderophores by plants and microorganisms. 5th Intl. Symp. on Iron Nutrition and Interactions in Plants. Kibbutz Ramat-Rachel, Israel. (Abst).
- Wang, Y.C., D.E. Crowley and P.J. Szaniszlo. 1990. Comparison of iron uptake rates from phyto- and microbial-siderophores by axenic and nonaxenic oat plants. II Intl. Symp. on Iron Transport, Storage and Metabolism. Austin, TX. (Abst).
- Walter, A., V. Romheld, H. Marschner, D.E. Crowley and P.J. Szaniszlo. 1991. Siderophores of Pseudomonas putida YC3 as iron source for cucumber and maize. 6th Intl. Symp. on Iron Nutrition and Interactions in Plants, Logan, UT. (Abst).

VI. Description of Cooperation

During the three years of this research, there was extensive cooperation and collaboration of the three institutions as well as with other research groups with similar interests. Both Drs. Reid and Szaniszlo visited the Hebrew University laboratories in Israel during 1989. Drs Chen, Hadar and Bar-ness, at different times visited the laboratories either at the University of Texas or the University of Florida, or both during the past three years. Dr. Crowley spent the first one and one-half years at the University of Florida, and then continued his work during the last one and one-half years at the University of Texas, ensuring excellent liaison between the two U.S. universities. Dr. Crowley all visited the laboratory of Dr. H. Marschner in Germany, also a collaborator with the Israel group, and conducted collaborative research that is reported here. A reciprocal visit of 6 months by A. Walter from Dr. Marscher's laboratory to the University of Texas also resulted in collaborative work. There was considerable stimulation of ideas and synergism created by the interaction among the three research groups.

VII. Research Achievements and Benefits to Agriculture.

As documented in section VIII below, there was extensive assessment of the role of pseudomonad siderophores (pseudobactin-type) in the iron nutrition of dicotyledonous and monocotyledonous plants in nutrient solutions and in soil in the presence and absence of other microorganisms. Emphasis by the Israel group was placed in examining the utilization of iron by plants in the presence of pseudobactin and some comparison with hydroxamate-type siderophores. The U.S. labs placed emphasis on plant uptake comparisons with hydroxamates and the examination of plant and microbial mechanisms in the utilization of iron from siderophore groups.

The aggregate of the research has greatly extended our knowledge on the role of microbial siderophores in the iron nutrition of plants, especially under calcareous conditions, and has placed this role in perspective with phytosiderophores. Not only has the research shown that microbial siderophores supply iron to plants and can correct chlorosis in Fe-siderophore amended soils, but it has shown the importance of the rhizosphere population of microorganisms in soil and its impact on the utilization of iron by plants. Plants that utilize microbial siderophores may be more iron efficient by virtue of their ability to use a variety of Fe sources. The ability of some microbes, e.g., pseudomonads, to utilize a wide variety of microbial siderophores may confer an ecological advantage that in turn contributes to Fe supply to plants. The involvement of siderophores in the iron nutrition of plants and the rhizosphere organisms, indicates that the development of cultural practices that seek to optimize beneficial plant-microbe interactions must consider the influence of microbially-produced siderophores on the iron nutrition of the plant. Plants that have extensively developed symbiotic mycorrhizal fungi associations are likely to have a unique influence on the rhizoplane and rhizosphere population of free-living microbes and in turn alter iron nutrition of the host plant.

We believe this research has contributed to the foundation upon which agronomists, soil scientists and microbial and plant geneticists will continue to build applied technologies for improving plant iron nutrition in iron-deficient environments.