MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 4, No. 1, pp. 5-13, 1991

Current Review

Siderophores in Microbial Interactions on Plant Surfaces

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Received 10 August 1990. Accepted 21 September 1990.

Iron is the fourth most abundant element in the earth's crust. Iron oxides, comprising minerals such as hematite (Fe_2O_3), magnetite (Fe_3O_4), and limonite [FeO(OH)], are the most abundant of the metallic oxides in soils (Schwertmann and Taylor 1989). Nevertheless, the extreme insolubility of ferric hydroxide ($K_{sp} = 10^{-38}$) (Latimer 1952) limits free iron at pH 7 in an aerobic aqueous environment to an equilibrium concentration of approximately 10^{-18} M (Raymond and Carrano 1979). Minimal concentrations of iron required for normal growth of plants range from 10^{-9} to 10^{-4} M, depending on other nutritional factors (Romheld and Marschner 1981; Schwab and Lindsay 1983; Lindsay and Schwab 1982). Similarly, minimal iron concentrations for the optimal growth of many microbes are approximately 10^{-5} to 10^{-7} M (Lankford 1973). While iron is an essential element to virtually all forms of life, excessive concentrations are toxic, due to the catalytic role of iron in the generation of oxidizing radicals from superoxide and peroxide (Flitter *et al.* 1983). Thus, most organisms have systems for the specific chelation and regulated transport of Fe(III) into the cell. Plants and associated microbes have different though perhaps interacting strategies for obtaining iron from the soil.

Most microorganisms use siderophores and corresponding membrane receptors for iron acquisition (Neilands 1981a). Siderophores are low molecular weight compounds that are produced under iron-limiting conditions, chelate the ferric ion (Fe^{3+}) with a high specific activity, and serve as vehicles for the transport of Fe(III) into a microbial cell (Neilands 1981b). Although siderophores vary greatly in chemical structure, most have either hydroxamate or catechol groups that are involved in iron(III) chelation (Neilands 1981b). Transport of iron into the cell is mediated by specific membrane receptor and transport systems that recognize the iron-siderophore complex (Neilands 1982).

Siderophores produced by fluorescent pseudomonads that inhabit the plant rhizosphere have received much attention over the past decade, largely because of their proposed role in the biological control of soilborne plant pathogens and in disease suppressive soils (Scher 1986; Leong 1986; Loper 1990; Schippers *et al.* 1987). The fluorescent pseudomonads are characterized by the production of yellow-green pigments that fluoresce under ultraviolet light and function as siderophores. The fluorescent siderophores, termed pyoverdines, pyoverdins, or pseudobactins, represent only one class of siderophores produced by the fluorescent pseudomonads (Cox 1980; Demange *et al.* 1987; Teintze *et al.* 1981). Pyoverdines have a common chromophore, which is derived from 2,3-diamino-6,7-dihydroxyquinoline (Demange *et al.* 1987; Teintze *et al.* 1981), linked to a small peptide, which differs among strains by the number and composition of amino acids. The pyoverdines have three bidentate chelating groups that bind iron(III): 1) a catecholate group from the chromophore, 2) a hydroxamate group from a N^{δ}-hydroxyornithnine of the peptide chain, and 3) either an α-hydroxyacid from a β-hydroxyaspartic acid or another hydroxamate group from a second N^{δ}-hydroxyornithine. Thus, pyoverdines are intermediate between the usually strict catechol or hydroxamate siderophores found in a majority of microorganisms.

The mechanisms by which plants avoid iron chlorosis are both more diverse and less investigated than the siderophore-mediated iron uptake systems of microorganisms. Three strategies of iron assimilation have been identified in plants (Bienfait 1989). Strategy I, found in nongraminaceous monocots and all dicots, involves acidification of the rhizosphere, thus increasing the iron solubility by approximately 10^3 per pH unit, the reduction of Fe^{3+} ion and Fe(III) chelates to Fe^{2+} ion, and uptake of Fe(II). Strategy II, observed in graminaceous monocots, involves secretion of iron-chelating agents (phytosiderophores) of the mugineic acid family (Sugiura *et al.* 1981) and uptake of Fe(III) phytosiderophores. Strategy III, the prevalence of which is unknown, is the uptake by plants of microbial Fe(III) siderophores.

The importance of microbial siderophores to plant pathology, as determinants of biocontrol activity, virulence factors or ecological determinants, and factors influencing the iron nutrition of plants, is the subject of many excellent reviews (Leong 1986; Leong and Expert 1990; Neilands and Leong 1986; Schippers *et al.* 1987; Swinburne 1986). The objective of this review is to highlight recent findings in these areas. Our focus, like that of the available literature, is on soil rather than aerial systems.

Detection of siderophores in soil. Knowledge of the nature and concentrations of siderophores present in the rhizosphere and bulk soil is critical to an understanding of the roles of siderophores in plant-microbe interactions. Although diverse groups of siderophores are produced by many soil microbes in culture media (Waid 1975) only

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schizokinen, a citrate-hydroxamate siderophore produced by *Bacillus megaterium* and *Anabaena* sp., has been purified and identified chemically from the soil (Akers 1983a). Concentrations of hydroxamate siderophores, estimated commonly from soil extracts by bioassay with indicator organisms, range from 0 to 300 μg/kg in soils examined in various experiments (Bossier *et al.* 1988) and are greater consistently in rhizosphere than in bulk soils (Akers 1983b; Nelson *et al.* 1988). Unfortunately, the reliability of bioassays is limited by uncertain specificity, difficulties in quantification, and often by a need to extract siderophores, which may be tightly adsorbed to soil components. Nevertheless, the detection of siderophorelike activity in soils by many workers using bioassay approaches (Bossier *et al.* 1988) is strong evidence for the prevalence of microbial siderophores in soil.

No bioassay for the detection or quantification of pyoverdines in soil has been reported. Bakker *et al.* (1988) demonstrated indirectly the *in situ* production of pyoverdines by examining interactions of two *Pseudomonas* strains and their derivatives, which are unable to synthesize pyoverdines (Pvd^-), in the potato rhizosphere. An *in situ* cross-feeding experiment demonstrated that a Pvd^- mutant, which could use the pyoverdine produced by a coinoculated wild-type strain, established a greater population size in the potato rhizosphere than did a Pvd^- mutant that could not use the pyoverdine. Although these data do not provide quantitative estimates of pyoverdine concentrations in the soil, they suggest that pyoverdines are produced in the potato rhizosphere in concentrations adequate to influence bacterial growth.

Immunological techniques and reporter gene systems offer novel approaches for assessing the *in situ* production of pyoverdines. Monoclonal antibodies to ferric pseudobactin, the ferric complex of a pyoverdine produced by *Pseudomonas* sp. strain B10, have been developed (Buyer *et al.* 1990) and will be useful for the detection and quantification of pseudobactin in rhizosphere or bulk soil. A complementary approach that uses a reporter gene system based on ice nucleation activity demonstrated the *in situ* transcriptional activity of a gene involved in pyoverdine biosynthesis in the rhizosphere (Lindow and Loper 1990).

Siderophore-mediated iron competition. The hypothesis that siderophore-mediated iron competition is a mechanism determining microbial interactions is based on the supposition that iron availability sometimes limits microbial growth and development on plant surfaces. Theoretically, in response to the iron-limiting conditions encountered on aerial plant surfaces or in the rhizosphere, microbes produce siderophores *in situ*. Siderophore(s) is produced in culture by virtually every plant-associated microorganism that has been evaluated (Table 1) (Neilands and Leong 1986).

The affinity of a siderophore for iron is represented commonly by a stability constant, or overall formation constant

Table 1. Selected siderophores produced by plants or plant-associated microorganisms

Siderophore	Producing organism(s)	logK (pFe)[a]	References
Pyoverdine	*Pseudomonas fluorescens* *P. putida* *P. syringae*	32	Cody and Gross 1987a, Meyer and Abdallah 1978, Teintze *et al.* 1981
Catechols			
Agrobactin	*Agrobacterium tumefaciens*	···	Ong *et al.* 1979
Chrysobactin	*Erwinia chrysanthemi*	···	Persmark *et al.* 1989
Enterobactin	Enterobacteriacae	52 (35.5)	Harris *et al.* 1979b, O'Brien and Gibson 1970, Pollack and Neilands 1970
Hydroxamates			
Aerobactin	*E. carotovora*	22.5	Crosa *et al.* 1988, Ishimaru and Loper 1988
	Enterobacter cloacae	(23.3)	Ishimaru *et al.* 1989, Harris *et al.* 1979a
Canadaphore	*Helminthosporium carbonum*	···	Letendre and Gibbons 1985
Cepabactin	*P. cepacia*	26.9	Meyer *et al.* 1989
Coprogen	*Alternaria longipipes*	30.2	Diekmann 1970, Jalal *et al.* 1988
	Stemphyllium botryosum	(27.5)	Manulis *et al.* 1987
	Nectria cinnabarina		Matzanke *et al.* 1989
Dimerum acid	*Microdochium dimerum*	···	Diekmann 1970
	Verticillium dahliae		Harrington and Neilands 1982
Ferrichrome	*Ustilago* spp.	29.1	Budde and Leong 1989, Matzanke *et al.* 1989
	Sphacelotheca andopogonis	(25.2)	Neilands 1952
Ferrichrome A	*Ustilago* spp.	32	Budde and Leong 1989, Garibaldi and Neilands 1955, Matzanke *et al.* 1989
Ferrioxamine E	*Streptomyces* spp.	32.5	Berner *et al.* 1988, Matzanke *et al.* 1989
	E. herbicola	(26.6)	Yang and Leong 1982
Ferrirhodin	*Botrytis cinerea*	···	Konetschny-Rapp *et al.* 1988
Fusarinins	*Fusarium roseum*	···	Jalal *et al.* 1986, Sayer and Emery 1968
	Gliocladium virens		
Fusigen	*F. roseum*	···	Diekmann 1967
	Gibberella fujikuroi		
Rhodotorulic acid	*U. montagnei* var. *major*	31.2 (21.9)	Deml and Oberwinkler 1982 Matzanke *et al.* 1989
Other			
Rhizobactin	*Rhizobium meliloti*	17.6	Schwyn and Neilands 1987 Smith *et al.* 1985
Mugineic acid	Graminaceae	18.1	Sugiura *et al.* 1981

[a]pFe values at pH = 7.4 are presented parenthetically (Matzanke *et al.* 1989).

(Table 1). Stability constants for microbial siderophores vary from approximately 10^{23} for citrate-hydroxamates, such as aerobactin (Harris *et al.* 1979a), to 10^{52} for the tricatechol siderophore, enterobactin (Harris *et al.* 1979b). Stability constants, however, do not reflect the protons lost by a ligand upon chelation and are not alone meaningful in judging the relative ability of ligands to compete with one another for Fe(III) at a given pH (Matzanke *et al.* 1989). The chelation of Fe(III) by a siderophore to form a ferric complex, and dissociation of the ferric complex back to Fe(III) and the ligand is described in equation 1.

$$\text{Fe(III)} + \text{H}_n\text{Sid} \rightleftarrows \text{FeSid} + n\text{H}^+ \quad (1)$$

The siderophore gives up n protons when it chelates iron, where n equals three for a trihydroxamate and six for a tricatechol. The equilibrium constant for equation 1 ($K = [\text{FeSid}][\text{H}^+]^n/[\text{Fe(III)}][\text{H}_n\text{Sid}]$) is pH dependent, with the degree of dependence controlled by n and by the ligand protonation constants. Enterobactin loses six protons upon chelation, so that while the stability constant $[\text{FeEnt}]/[\text{Fe}^{3+}][\text{Ent}]$ equals 10^{52}, the ratio $[\text{FeEnt}^{3-}]/[\text{Fe}^{3+}][\text{H}_6\text{Ent}]$ equals 10^{26} at pH = 6.0 (Hider 1984). Predictions of equilibrium concentrations of siderophores and ferric siderophores must rely on equilibrium constants determined at the pH of interest. Siderophores also are compared by calculating pFe ($\text{pFe} = -\log[\text{Fe}^{3+}]$) values at defined concentration and pH. A higher pFe value corresponds to lower free Fe^{3+} and stronger binding of iron by the siderophore. Unfortunately, pFe values are known for few siderophores produced by plants or plant-associated microbes (Table 1).

Two or more siderophores may interact directly through ligand exchange, in which one ferric siderophore loses its Fe(III) to the other (equation 2).

$$\text{FeSid}_1 + \text{Sid}_2 \rightleftarrows \text{Sid}_1 + \text{FeSid}_2 \quad (2)$$

The importance of ligand exchange in competition for iron between two siderophores is determined by the relative equilibrium constants of Sid_1 and Sid_2 for iron (equation 1), the relative concentrations of Sid_1 and Sid_2, and the kinetics of reactions (1) and (2). The kinetics of reaction (1) depends on the speciation and concentration of Fe(III), pH, and concentration of Sid. If reactions (1) and (2) reach equilibrium quickly, iron competition will depend on the relative equilibrium constants and concentrations of Sid_1 and Sid_2. Equilibrium is not always reached quickly, however. For example, the half-life for ligand exchange between two hydroxamate siderophores is 220 hr at pH 7.4 (Tufano and Raymond 1981). Where ligand exchange is slow, iron competition will be controlled by the concentrations of the siderophores and the kinetics of reaction (1).

Siderophore-mediated iron competition between two microbes may be envisioned simplistically as ligand exchange among siderophores used by the competing microbes. A conceptual model based on ligand exchange (Buyer and Sikora 1990) assumes that a utilizable ferric siderophore will be the limiting substrate for microbial growth under iron-limiting conditions. *Escherichia coli* (Braun *et al.* 1987), *Erwinia herbicola* (Berner *et al.* 1988), and *Geotrichum candidum* (Mor *et al.* 1988) are among the organisms known to use, as sources of Fe(III), ferric complexes of siderophores that they do not produce. The ferric complex of a pyoverdine produced by one strain of *Pseudomonas* spp. may or may not be used as an iron-carrier by another strain (Buyer and Leong 1986; Hohnadel and Meyer 1988). To our knowledge, utilization of ferric pyoverdines by organisms other than *Pseudomonas* spp. has not been demonstrated. Thus, the ability of competing microbes to obtain Fe(III) from a ferric pyoverdine will depend generally on ligand exchange and is subject to the factors discussed above. In an iron-limited habitat, significant concentrations of ferric pyoverdines are expected to decrease iron availability to microbes that cannot directly utilize ferric pyoverdines or efficiently obtain Fe(III) from ferric pyoverdines through ligand exchange. Stability constants of siderophores produced by phytopathogens may be several orders of magnitude less than or similar to those of the pyoverdines (Table 1). Given favorable kinetics, ligand exchange will depend on the equilibrium constants at the pH of interest and relative concentrations of siderophores. Siderophore concentrations will be affected by production, utilization, adsorption to components of soil or other substrates, and degradation. Evidence for a determinative role of pyoverdines in interactions of *Pseudomonas* spp. with phytopathogenic bacteria and fungi in culture and on plant surfaces is summarized below.

Effect of pyoverdines on phytopathogens in culture. Fluorescent pseudomonads commonly exhibit iron-regulated antagonism against a variety of phytopathogens in culture (Kloepper *et al.* 1980a; Misaghi *et al.* 1982). Pyoverdine-mediated antagonism depends on the iron concentration of the medium due to the iron-dependent production of pyoverdines by *Pseudomonas* spp. and to the iron-dependent sensitivities of indicator organisms. Pyoverdines produced by *Pseudomonas* spp. in culture may deplete the medium of available iron, thereby inhibiting the growth of an indicator strain, while ferric complexes have no effect. Iron-regulated antagonism cannot be attributed solely to pyoverdine production, however, since *Pseudomonas* spp. are known to produce several antimicrobial compounds, including at least two with activity against phytopathogenic fungi (Azegami *et al.* 1988; Gill and Warren 1988), only under iron-limiting conditions (Weinberg 1986). Therefore, studies evaluating the activities of purified pyoverdines and corresponding ferric complexes are essential for assessing the sensitivities of phytopathogens to pyoverdine-mediated iron competition. Pseudobactin, the pyoverdine produced by *Pseudomonas* sp. strain B10, inhibits growth of the fungal phytopathogens, *Fusarium oxysporum* f. sp. *lini* and *Gaeumannomyces graminis* var. *tritici* (Kloepper *et al.* 1980b), and of the bacterial phytopathogen, *E. carotovora* (Kloepper *et al.* 1980a), on agar plates while ferric pseudobactin does not. The general iron-regulated fungistasis associated with the pyoverdines was established by Misaghi *et al.* (1982), who demonstrated that pyoverdines produced by 156 strains of fluorescent pseudomonads isolated from soil or plants are antagonistic against *G. candidum*. Similarly, a pyoverdine produced by *Pseudomonas tolaasii* decreases mycelial growth of the fungal phytopathogen, *Pythium ultimum*, while the ferric

pyoverdine does not (Meyer *et al.* 1987). These studies suggest that pyoverdines are antagonistic through chelation of iron from the environment of the target pathogen.

Fungal spore germination may be related directly (Simeoni *et al.* 1987) or inversely (Swinburne 1981) to endogenous iron concentrations. Chlamydospores of *F. o.* f. sp. *cucumerinum* require an endogenous iron concentration of at least 0.06 ng of Fe per chlamydospore for optimal germination (Simeoni *et al.* 1987). A partially purified pyoverdine or the synthetic iron chelator, ethylenediaminedi(*o*-hydroxyphenylacetic acid) (EDDHA), inhibits chlamydospore germination and subsequent germ tube elongation (Elad and Baker 1985). The assumption of this study was that EDDHA, which has a stability constant for Fe(III) ($\log_{10}K = 33.9$) (Lindsay 1979) similar to that of the pyoverdines (Table 1), is similar to the pyoverdines in iron-competition with *F. oxysporum*. Optimal suppression of chlamydospore germination by a strain of *P. putida* is observed at an available [Fe^{3+}] of 10^{-22} to 10^{-27} M (Simeoni *et al.* 1987). In contrast, germination of conidia of *Thielaviopsis basicola* (Ahl *et al.* 1986), *Colletotrichum acutatum*, and certain other fungi (Swinburne 1981) is inversely related to endogenous iron concentration. A free ligand may stimulate the germination of such spores while the corresponding ferric pyoverdine has a neutral or toxic effect. Compound S, the pyoverdine produced by the foliar epiphyte, *P. fluorescens* UV3, enhances germination of iron-replete but not iron-deplete conidia of *C. acutatum* (McCracken and Swinburne 1979). Exogenous iron chelators remove iron internal to conidia, thus enhancing germination (Graham and Harper 1983). The ferric pyoverdine of *P. fluorescens* CHA0 inhibits endoconidial germination of the soilborne fungus, *T. basicola*, while the corresponding pyoverdine does not (Ahl *et al.* 1986). The ferric pyoverdine of CHA0 may be directly toxic or may inhibit *T. basicola* indirectly, by making iron more available and thus more toxic. Iron availability clearly plays a role in the growth and development of certain phytopathogens in culture, although no universal pattern describes the role of iron in these processes.

Evidence for a role of pyoverdines in biocontrol of soilborne diseases. The role of pyoverdine-mediated iron competition in biocontrol has been evaluated in studies that 1) varied the iron availability of the soil, 2) added purified siderophores or synthetic iron chelators to soil, or 3) compared the biocontrol activities of parental and derivative strains that are deficient in pyoverdine biosynthesis (Pvd^-). The following is a general presentation of the evidence supporting a role for pyoverdines in biological control by *Pseudomonas* spp. of wilt diseases caused by *F. oxysporum*, of damping-off diseases caused by *P. ultimum*, and in plant growth promotion, which is attributed in part to the biocontrol of minor soilborne pathogens (Kloepper and Schroth 1981; Schippers *et al.* 1987).

Antagonism of a target phytopathogen through pyoverdine-mediated iron competition is related inversely to the level of iron available to the phytopathogen in soil, as in culture. Iron availability in soil has been enhanced by addition of chelators with low affinities for Fe(III) (Kloepper *et al.* 1980a, 1980b; Scher and Baker 1982), $FeSO_4$ (Sneh *et al.* 1984), $FeCl_3$ (Elad and Baker 1985), or H_2SO_4 to lower the soil pH (Elad and Baker 1985), thus increasing the available iron concentration by about 10^3 per pH unit (Lindsay 1979). Strains of *Pseudomonas* spp. promote growth of potato (Kloepper *et al.* 1980a), or suppress wilt diseases of several crops caused by *F. oxysporum* (Kloepper *et al.* 1980b; Scher and Baker 1982; Sneh *et al.* 1984; Elad and Baker 1985) in unamended soils but not in soils amended with one or more of these chemicals. Experiments demonstrating that disease suppression is no longer operative under iron-replete soil conditions provide evidence for the involvement of iron competition in biological control. The potential limitations of this approach include 1) effects of an achieved change in iron availability on the production of iron-regulated metabolites, other than pyoverdines, that may have a role in biological control, 2) effects of soil treatments on the availability of other soil minerals, and 3) difficulties in determining that enhanced iron availability actually is achieved by soil treatments.

Soil amendment with purified pseudobactin mimics the effect of seed inoculation with *Pseudomonas* sp. strain B10 in growth promotion of potato (Kloepper *et al.* 1980a) or suppression of take-all of wheat or fusarium wilt of flax (Kloepper *et al.* 1980b). To our knowledge, purified pyoverdines have not been further evaluated for plant growth promotion or biocontrol, probably because of difficulties in obtaining the large quantities required. Addition of selected strains of *P. putida* or the synthetic iron chelator EDDHA to soil suppresses fusarium wilt symptoms (Scher and Baker 1982), due in part to inhibition of chlamydospore germination in rhizosphere and bulk soil (Elad and Baker 1985; Sneh *et al.* 1984). Experiments demonstrating that pyoverdines or EDDHA mimic the activity of *Pseudomonas* spp. in plant growth promotion or disease suppression, respectively, provide evidence that high-affinity iron chelators have suppressive activity in the soil. This approach provides no evidence, however, for the *in situ* production of pyoverdines in concentrations adequate for disease suppression.

Well-characterized mutants that are deficient in pyoverdine production (Pvd^-) are valuable tools for assessing the *in situ* production of pyoverdines in the rhizosphere and the potential role of pyoverdines in biocontrol. The first study that used the mutagenesis approach demonstrated that Pvd^- mutants, obtained following chemical or ultraviolet mutagenesis, do not promote plant growth as do parental strains of *P. fluorescens* and *P. putida* (Kloepper and Schroth 1981). A limitation of this early study was that the single-site nature of the genetic lesion of each mutant could not be established. More recently, Schippers and colleagues (Bakker *et al.* 1986; Schippers *et al.* 1987) demonstrated that a single Tn*5* insertion inactivates both the pyoverdine production and plant growth promotion phenotypes of *P. putida* WCS358, suggesting a role for siderophores in the biological control of minor pathogens of potato. *Pseudomonas* sp. strain B324 promotes growth of wheat, due largely to protection of plants from *Pythium* spp. Pvd^- derivatives of strain B324 provide no protection from *Pythium* spp. and do not promote growth of wheat (Becker and Cook 1988).

Similarly, *P. fluorescens* 3551 controls *Pythium* damping-off of cotton, while Pvd^- derivatives do not control this disease (Loper 1988). Although the results of these studies are highly suggestive of a role for siderophores in biological control, definitive proof must include an identification of the protein product disrupted by each genetic lesion and an understanding of the role of this protein product in other metabolic pathways of potential importance in biological control.

Potential limitations of the mutagenesis approach for evaluating the role of siderophores in biocontrol include effects on metabolism caused by a mutation altering the iron status of the cell, polar effects of the mutation on the expression of other genes, and effects on the production of other metabolites with biosynthetic steps common to pyoverdine biosyntheses. The biosynthesis by *Pseudomonas* species of iron-regulated metabolites other than pyoverdines may be altered in a mutant with a depleted internal iron pool. For example, a pyoverdine biosynthesis mutant (Pvd^-) of *P. fluorescens* CHA0 suppressed tobacco black root rot disease caused by *T. basicola* only in iron-replete soils (Keel *et al.* 1989). The lack of biocontrol activity of the Pvd^- mutant in iron-deplete soils was attributed to low levels of hydogen cyanide production and a consequent effect on host defense responses, rather than a direct effect of the pyoverdine produced by strain CHA0 on *T. basicola*. The protein products of the many genes involved in pyoverdine biosynthesis (Loper *et al.* 1984; Marugg *et al.* 1988; Moores *et al.* 1984) are unknown. Thus, the potential involvement of these genes in the biosynthesis of other secondary metabolites cannot be excluded.

A combination of the three complementary approaches described above provides convincing evidence for a role of pyoverdines in biological control of certain soilborne plant diseases. Pyoverdines, however, are not universally implicated in the biocontrol effected by fluorescent pseudomonads (Gutterson 1990). For example, pyoverdines contribute only minimally to the biocontrol of take-all of wheat (Weller *et al.* 1988) or black root rot of tobacco (Ahl *et al.* 1986) by *Pseudomonas* spp. The role of pyoverdines in the suppression of plant diseases by fluorescent pseudomonads may vary with the soil environment, target pathogen, plant host, and the *Pseudomonas* strain evaluated.

Role of pyoverdines in biocontrol of aerial pathogens. Pyoverdines are not known to contribute detectably to the biocontrol activity of *Pseudomonas* spp. on aerial plant surfaces. Pyoverdines of certain fluorescent pseudomonads cause iron-regulated antibiosis against ice nucleation active (INA) *P. syringae* in culture, but are of minimal importance in antagonism against *P. syringae* on leaf surfaces or in biocontrol of frost injury (Lindow 1988). Sid^- mutants of four *P. fluorescens* strains protect corn plants treated with *P. syringae* from frost injury at levels comparable to the parental strains. A mutant of *P. aeruginosa* LEC1 that is deficient in pyoverdine production exhibits wild-type levels of inhibition against *Septoria tritici* in culture and on wheat leaves (Flaishman *et al.* 1990).

A series of experiments by Swinburne and colleagues suggest that pyoverdines produced by *Pseudomonas* spp. may stimulate the development of certain phytopathogenic fungi on aerial plant surfaces (Swinburne 1981). For example, inoculation of strawberry stolons with *Pseudomonas* sp. strain UV3 or a pyoverdine (Compound S) purified from this strain increases germination of iron replete conidia of *C. acutatum* and subsequent appressoria formation and maturation (Slade *et al.* 1986). Application of iron chelates to plant surfaces increases conidial germination, formation of mature appressoria, and lesion development on leaves of *Vicia faba* and banana fruits inoculated with *Botrytis cinerea* and *C. musae*, respectively (Brown and Swinburne 1981, 1982; Swinburne and Brown 1983). Thus, in some cases, epiphytic populations of *Pseudomonas* spp. that produce pyoverdines *in situ* may enhance disease development, although results substantiating this supposition have not been reported.

Siderophores and pathogenesis. Siderophores are produced by bacterial and fungal phytopathogens (Table 1), yet their importance as virulence factors or as ecological determinants is virtually unknown. At present, chrysobactin, a catechol produced by *E. chrysanthemi* (Persmark *et al.* 1989), is the only siderophore produced by a plant pathogen that has been identified as a virulence factor in plant disease. Mutants of *E. chrysanthemi* that do not produce chrysobactin cause necrotic lesions on african violet (*Saintpaulia ionantha*) but do not cause a systemic soft rot characteristic of infection by the parental strain (Enard *et al.* 1988). In contrast, comparison of Pvd^- derivative and Pvd^+ parental *P. syringae* strains provided no evidence of a determinative role of pyoverdine production to the growth, survival, or pathogenicity of *P. syringae* on bean (Loper and Lindow 1987) or cherry (Cody and Gross 1987b). *Pseudomonas* spp. are known to produce and use siderophores other than the pyoverdines (Cox 1980), however, that may complement Pvd^- mutants with respect to iron acquisition *in situ*. Agrobactin, a hydroxamate siderophore produced by *Agrobacterium tumefaciens*, is not required for pathogenicity but may be important to the saprophytic growth of this phytopathogenic bacterium (Leong and Neilands 1981). Mutants of *Ustilago maydis* that are deficient in the biosynthesis of ferrichrome, a hydroxamate siderophore, have been constructed and are being evaluated for virulence on corn (Wang *et al.* 1989). Recently, studies have been initiated to evaluate siderophores produced by strains of *E. carotovora* that are targets of pyoverdine-mediated biocontrol by *Pseudomonas* spp. (Bull *et al.* 1989; Ishimaru and Loper 1988). Knowledge of the role of iron acquisition in the virulence and ecology of target phytopathogens will enhance our current understanding of siderophore-mediated iron competition as a mechanism in biological control.

Interactions of plant and microbial iron-aquisition systems. Certain plants use iron supplied by microbial Fe(III) siderophores such as ferrioxamine B (Crowley *et al.* 1988), the ferrichromes (Orlando and Neilands 1982), rhodotorulic acid (Crowley *et al.* 1988; Miller *et al.* 1985), and agrobactin (Becker *et al.* 1985b), although the mechanism of iron uptake is unclear. Strategy I and strategy II plants may transport Fe from microbial Fe(III) siderophores by reduction to Fe(II) and ligand exchange

with phytosiderophores, respectively, or by direct uptake of the Fe(III) siderophores. Since microbial siderophores generally have greater Fe-chelate stability constants (and presumably equilibrium constants) than phytosiderophores such as mugineic acid (Table 1), ligand exchange will require a large excess of phytosiderophore and favorable kinetics. Estimated concentrations of mugineic acid and microbial siderophores in the rhizosphere are 1 mg/kg (Romheld 1989) and about 300 μg/kg (Nelson *et al.* 1988), respectively. Given favorable kinetics, ligand exchange between microbial and phytosiderophores in the rhizosphere appears likely but may not be the sole mechanism of microbial Fe(III) siderophore utilization by plants. Nevertheless, the direct uptake of Fe(III) microbial siderophores by plants is presently a subject of controversy. Experimental systems used for evaluating direct uptake by plants must be buffered carefully and gnotobiotic to maintain conditions of uniform root physiology and predicted Fe-chelate equilibria, ligand exchange rates, and ligand stability. The most convincing evidence for direct uptake of microbial Fe(III) siderophores by plants comes from studies where axenic conditions and consistent pH were maintained (Wang *et al.* 1990; Crowley *et al.* 1990a, 1990b, Abstracts, International Symposium on Iron Transport, Storage, and Metabolism II. June 20–22, Austin, TX).

The utilization of ferric pyoverdines as sources of iron appears to vary among plants. Purified pseudobactin inhibits Fe uptake by pea and maize grown under gnotobiotic conditions (Becker *et al.* 1985a). In contrast, large concentrations of ferric pyoverdines ameliorate lime-induced chlorosis of peanuts in pot experiments (Hadar *et al.* 1986; Jurkevitch *et al.* 1986; Jurkevitch *et al.* 1988). Iron uptake and reduction rates of cotton, peanuts, and sunflower plants are greater when supplied with the more easily reduced FeEDDHA than with a ferric pyoverdine (Bar-Ness *et al.* 1990). These strategy I plants may obtain iron by reduction of Fe(III) in the chelate to Fe(II), which can be used directly, rather than by direct uptake of ferric pyoverdine.

All strategies of iron assimilation by plants influence the form of available Fe in the rhizosphere in ways that may affect the iron status of rhizosphere organisms. Acidification of the rhizosphere by strategy I plants increases the equilibrium concentration of free iron available to plants and possibly to rhizosphere microbes. Reduction of free iron(III) or of microbial Fe(III) siderophores and transport of Fe(II) into strategy I plants may lower the concentrations of iron available for microbial assimilation. Ligand exchange between phytosiderophores and microbial ferric siderophores may have the same effect. These predictions are purely speculative, however, since effects of iron uptake systems of plants on those of associated microflora have not been evaluated.

Conclusions. The ubiquity of iron uptake systems and the prevalence of siderophores in natural habitats provides compelling evidence for the importance of iron to the fitness of plants and associated microbes. Virtually every plant pathogen, symbiont, or saprophyte that has been evaluated produces siderophores. Further, concentrations of pyoverdines produced in the rhizosphere by *Pseudomonas* spp. are adequate to influence microbial interactions (Bakker *et al.* 1988; Leong 1986; Loper 1988; Schippers 1987). This evidence points out an opportunity to antagonize certain soilborne pathogen populations during saprophytic phases of their life cycles through manipulation of the available iron concentration in the rhizosphere.

It also is clear from the literature that iron deprivation is not a universal nor perhaps a prevalent mechanism by which biological control acts on plant surfaces (Gutterson 1990; Lindow 1988; Swinburne 1981). Nevertheless, where siderophores do play a major role in microbial interactions, chemical and physical factors on the plant surface are expected to determine siderophore-mediated iron competition. Perhaps the most important of these is pH, which largely determines iron availability to plants and microbes in the soil (Lindsay 1979). Although pyoverdines determine the biocontrol activity of strains of *Pseudomonas* spp. against certain soilborne pathogens, their consistent production and activity in the varied environments characterizing plant surfaces must be demonstrated before they can be used agriculturally for disease control.

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