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INTERACTIONS OF DELETERIOUS AND BENEFICIAL RHIZOSPHERE MICROORGANISMS AND THE EFFECT OF CROPPING PRACTICES

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INTRODUCTION

Evidence is increasing that the saprophytic microflora of the rhizosphere includes both deleterious and beneficial elements that have the potential to influence plant growth and crop yields significantly (13, 16, 25, 27, 57, 72, 77, 78). The deleterious microorganisms affect plant growth negatively, but they do not necessarily parasitize the plant tissue. Their deleterious activities include alterations of the supply of water, ions, and plant growth substances by changing root functions and/or by limiting root growth (1, 57, 67, 77). The mechanisms by which beneficial nonsymbiotic microorganisms are considered to affect plant growth positively include promotion of the availability and uptake of mineral nutrients (6, 7, 27), provision of plant-growth substances (27, 56, 57), and, most of all, suppression of deleterious rhizosphere microorganisms (13, 27, 28, 72, 77). Beneficial rhizobacteria that promote plant growth, supposedly by competing for iron with deleterious rhizosphere microorganisms, have recently become known as plant growth–promoting rhizobacteria (PGPR) (13, 41, 67, 77).

This paper reviews recent knowledge on deleterious and beneficial rhizosphere bacteria, their interactions, and their effect on yield as related to cropping practices.

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DELETERIOUS RHIZOSPHERE MICROORGANISMS

Terminology

Dommergues classified rhizosphere microorganisms as being beneficial (symbiotic), harmful (pathogenic), or having no effect on the plant (neutral) (22). Salt distinguished between major, or true, pathogens that penetrate the stele and disrupt the phloem, causing major disease symptoms, and minor pathogens, either saprophytes or parasites, confined to juvenile tissues such as root hairs, root tips, and cortical cells (65). According to Salt, minor pathogens include facultative parasites and obligate parasites such as *Olpidium* and *Polymyxa*. Within the category of minor pathogens rhizosphere microorganisms that affect plants by their metabolites without parasitizing plant tissue (13, 67, 77), here named *deleterious rhizosphere microorganisms* (DRMO), should be distinguished from those that parasitize plant tissue, named *parasitizing minor pathogens*. The DRMO include deleterious rhizobacteria (DRB) and deleterious rhizofungi (78). The pathogenicity of DRMO is difficult to demonstrate because their effect on plants is usually restricted to retardation of root and/or shoot growth without other distinct symptoms (3, 28).

Identification

Deleterious effects of microorganisms isolated from the rhizosphere have been demonstrated in pot experiments by treating seeds or root systems with bacterial suspensions and studying consequent root or plant growth response (25, 31, 78). Symptoms other than growth retardation, such as root discoloration, wilting, necrotic reactions, distortions of leaves and roots, or stunting of plants (31, 78), observed by this approach are often not obvious under field conditions (17, 23, 69) and may be the consequence of unnaturally high numbers of the introduced DRMO (31, 77). On the other hand, potential DRMO might be overlooked using this approach because environmental factors essential for production or activity of their deleterious metabolites are absent. Reduction in numbers of potential DRMO in the rhizosphere, with simultaneous increase in root or plant growth after introduction of PGPR, is evidence for their growth-reducing ability, but does not constitute proof of pathogenicity. Based on these arguments only, the conclusions that DRMO belong to a variety of genera of different families such as Enterobacteriaceae, Corynebacteriaceae, Pseudomonadaceae, and Bacilliaceae (13, 77, 78) are premature. Research is therefore needed that (*a*) identifies more specifically the mechanisms by which DRMO affect plant growth, and (*b*) determines what factors influence these activities.

Mode of Action

DRMO may affect plant growth by interfering with the delivery of plant growth substances or nutrients to plants by free-living rhizosphere microorganisms. However, increases in solute concentrations of poorly soluble compounds such as phosphates or iron salts by free-living rhizosphere microorganisms are not considered to be of great importance for plant growth because the plant itself can increase the concentrations of these materials by changing the rhizosphere pH or by excreting chelators (9, 33, 79, 80). There is also no consistent and accepted evidence that free-living nitrogen-fixing microorganisms are of much significance for agricultural crop yields (80). Increase in the susceptibility of plants to parasitic pathogens is a possible mode of action of DRMO about which we know very little.

Continued root growth into unexploited soil, number and length of root hairs, and an efficient energy metabolism of root cells are important in enabling plants to obtain water and ions such as phosphorus and potassium that have low diffusion constants (1). Microorganisms producing metabolites that inhibit these processes may have an effect on crop production. Many rhizosphere microorganisms produce auxins, ethylene, cytokinins, and vitamins and other plant growth substances (56). The negative and positive effects of auxins and ethylene on root growth and root morphology and their modes of action are well reviewed (56, 57). Whether microbial sources of plant growth substances have negative or positive effects on plants depends on their total and relative concentrations. Recent studies demonstrated the negative effect of indol acetic acid by rhizosphere-introduced pseudomonads on root elongation of sugar-beet seedlings (54). Many other metabolites of rhizosphere microorganisms, including antibiotics (12, 60), are toxic to plant roots. Most of these metabolites are organic acids (56), e.g. HCN (2). One may question the in vivo effects on roots of microbial substances such as HCN, which is easily inactivated by soil components or assimilated by soil microorganisms (14). However, if DRMO are in direct contact with root cells at various microsites offered by mucigel, epidermis, and cortex, as shown by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (61), or in the intercellular spaces of roots, penetration of their metabolites into the root cells cannot be excluded. Intercellular penetration of rhizosphere microorganisms, often in large numbers, has recently been demonstrated in various crops, including wheat, barley, pea, beet, and citrus (19, 23, 25, 26, 37, 40, 61). In most of these cases *Pseudomonas* spp. are predominant. Fluorescent pseudomonads produce a great variety of secondary metabolites including plant growth substances and antibiotics (49). The microbial metabolites could operate by changing cell wall structure (24), cell permeability, polysaccharide and acid secretion, enzyme release, or by de-

creasing the efficiency of physiological processes (16). Strains of *Pseudomonas* spp. have been suspected since 1961 of being responsible for retarded plant growth and reduced root hair formation (11, 63). No reports of detailed research have been presented on their mode of action and effect on plant growth and yield, nor on possible mechanisms by which their deleterious activity may be counteracted by PGPR, except for the involvement of cyanide production by microorganisms (2, 67).

In our research on the deleterious microbial factor (or factors) responsible for decreasing potato yields with increasing potato cropping frequency (69), we observed that root growth from potato stem cuttings was greatly restricted within the first week after planting in short-rotation (potatoes grown most frequently) soil compared with that in long-rotation (potatoes grown least frequently) soil (see also "Effect of Cropping Practices"). However, disease symptoms such as discolorations of roots, lesions, or impaired root hair formation were not observed (3). The relatively low potato yield where potatoes were grown most frequently was therefore hypothesized to be caused by DRMO that impair root function, possibly by affecting nutrient uptake by the roots (2).

Uptake of nutrients (e.g. P, K, and N) by plant roots is an energy-requiring process (1). Respiratory energy used for nutrient uptake, as shown for maize, is of considerable importance and amounts to 60% of the root respiration under normal conditions (82). The production of ATP, mediated by cytochrome oxidase respiration, can be inhibited by cyanide. This inhibition causes electrons released by oxidation of NADH in mitochondria to follow the alternative cyanide-resistant respiratory pathway to oxygen. Much energy is thereby lost as heat instead of being used for phosphorylation of ADP (47). Uptake of nitrate by *Arabidopsis thaliana* was significantly inhibited by 10-μM KCN (21). Cyanide is a secondary metabolite of several microorganisms. It can be produced directly from glycine and proline, and from cyanogenic glycosides (45), all of which have been demonstrated in root exudates (18, 64). Approximately 4% of the aerobic bacteria, isolated on tryptic soy agar (TSA) from rhizospheres of potato and wheat grown in short- and long-rotation (based on frequency of potatoes) soils, had the potential to produce cyanide and appeared to be *Pseudomonas* spp. At least 40% of the pseudomonads from potato rhizosphere were able to produce cyanide in vitro (2).

In experiments in vitro, the cytochrome respiratory pathway of intact potato roots was very sensitive to cyanide, being more than 40% inhibited by 5-μM KCN (2). The concentrations of P and K in potato plants from short rotations were significantly lower than those in long rotations, although the availability of these elements was the same in both soils or even more available in short-rotation soil (J. Vos & K. Groenwold, personal communication; J.

Lamers, personal communication). These observations support our hypothesis that the low yields of potato in short rotations may at least partly originate from impaired nutrient uptake caused by a depressed root cell energy metabolism imposed by HCN (2).

Cyanide production by isolates of *Pseudomonas* spp. was demonstrated to depend on Fe^{3+} availability (2). Production of HCN by microorganisms might therefore be particularly influenced by competition for iron with PGPR.

Effect of Cropping Practices

The plant growth–promoting and yield-enhancing effects of soil fumigation, pasteurization, pesticide treatment, or introductions of antagonists often cannot be explained by increases in nutrient availability, improved soil structure, detoxification of soils, or suppression of recognized plant-parasitic nematodes or major pathogens. This problem led to earlier statements that minor pathogens or DRMO are involved in plant growth and crop yield reductions (13, 17, 69, 71, 77). Although most field soils are likely to contain populations of minor pathogens or DRMO, the effect of such pathogens or DRMO on plant health and crop yields seems to become more pronounced with certain cropping practices. Our knowledge in this respect is limited. Most attention to the subject has been given only recently, as demonstrated by the following examples.

CROPPING FREQUENCY Detailed crop rotation experiments in the Netherlands, which were started in 1963, demonstrated that yields of wheat, and especially of potato, decreased over the years and stabilized at different levels depending on the frequency of the crop. Since 1975 potatoes grown in the same plots every fourth (1:4) or every third (1:3) year have yielded 10–15% less, in general, than potatoes grown every sixth year (a 1:6 potato-cropping frequency) (Figure 1). Yields had even been lower (30% less) in fields where potatoes had been cropped every second year or every year (35, 69). Although inoculum densities of, or disease incidence caused by, regular soilborne pathogens such as *Verticillium dahliae, Rhizoctonia solani,* and *Streptomyces* spp. have increased with increasing potato-cropping frequency, elimination of these pathogens and of nematodes by pesticide treatment or use of resistant potato cultivars has resulted in little or no yield increases (35). Moreover, retardation of plant growth (dry weight) in the field was already very significant at seven weeks after seeding and before regular pathogens could be isolated from potato plants or their symptoms could be observed (G. J. Bollen, personal communication; A. W. Bakker, unpublished results). These observations strongly suggest that microbial factors other than normally recognized pathogens are a major factor in the observed yield declines. Yet,

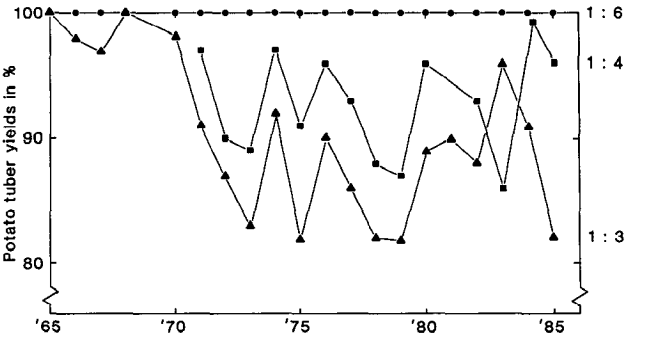

Figure 1 Relative yields of potato tubers (diameter > 35mm) at the optimal nitrogen dressing rate for a potato-cropping frequency of one year in four (1:4) and one year in three (1:3) compared with a one year in six (1:6) (= 100%) at the experimental farm "De Schreef" during 1965–1985.

the involvement of systemic pathogens such as *V. dahliae* that cause latent infections cannot be completely excluded.

The retardation of plant growth could be reproduced within two months in pot experiments in environmental chambers using soil obtained from field plots with different potato-cropping frequencies (28). The plant growth retardation and yield reductions in the field could not be ascribed to differences in soil structure or availability of nutrients (35, 71). A possible role of these factors was excluded in pot experiments in which they were controlled, using plants developed from tubers or rooted stem cuttings (2, 3, 28). Differences in plant growth in pots containing soils of different potato-cropping frequencies were eliminated by soil disinfestation with methyl bromide or soil pasteurization (71). These observations make it very likely that the causal agents are non-spore-forming DRMO. Our hypothesis (2, 67) that cyanide production by rhizosphere pseudomonads is involved is discussed above. Why the deleterious microbial effects on yields increase with increasing frequency of potatoes in the rotation remains to be elucidated. The effect is obviously host specific, as yields of other crops in the rotations, e.g. wheat and sugar beet, are not affected by the frequency of potato cropping (35). Host specificity of deleterious rhizosphere pseudomonads has been demonstrated for wheat and citrus at the cultivar level (25, 26). The DRMO probably survive on root residues that provide a source of inoculum for root colonization of the next potato crop, as stated for other rhizosphere microorganisms (10). They may thus increase in numbers with increasing frequency of potato crops.

Apart from this, an accumulation of factors in soil that stimulate the production of their toxic metabolite (or metabolites) may be involved. In the case of cyanide, such factors could be precursors for cyanide production (e.g. glycine) and/or availability of the ferric iron (2) (see above). Glycine, and to a

lesser extent proline, enhances cyanide production by fluorescent pseudomonads (45; A. W. Bakker, unpublished results), and both are constituents of plant root exudates (36, 64). Wainwright & Pugh (83) demonstrated that glycine can be resistant to microbial degradation. Proline and glycylglycine have a high affinity for certain clay minerals (20), and thus may persist for some time in certain soils.

An increase of iron availability with increasing frequency of a certain crop, e.g. potatoes, would stimulate the production of certain toxic metabolites including HCN, and reduce competition for iron and siderophore production by the DRMO. Whether precursors of cyanide or availability of iron, e.g. as ferric siderophores, increases with increasing frequency of potato crops is currently under investigation.

The possible role of deleterious rhizobacteria in replant diseases and "soil sickness" has been discussed by Suslow (77). Evidence for their involvement in the replant disease of apple has been provided (15, 38). Cultivar-specific inhibition of seedling growth by fluorescent and nonfluorescent strains of *Pseudomonas* spp. has recently been suggested to be a major factor in the replant disease of citrus (26).

A decrease in yield with increasing frequency of the crop in question is not restricted to potato. It has also been noticed for wheat, barley, bean, and maize (69; J. G. Lamers, personal communication) and deserves further attention. Wheat roots in soil with large amounts of crop residues in no-till sowing systems carry higher populations of root growth–inhibiting pseudomonads than those in conventional tillage systems. These pseudomonads were suggested to be responsible for decreased crop yields frequently observed in the US Pacific Northwest and England (23, 25).

In hydroponic systems, the microbial composition and activity of the rhizosphere microflora are less stable than in the microbially complex soil system. Excessive growth of DRMO or excessive production of phytotoxic metabolites can therefore be expected. Evidence has been obtained that as yet unknown DRMO can indeed have considerable negative effects on plant development in hydroponic systems (R. van Peer & B. Schippers, unpublished results).

These examples demonstrate that some populations of the rhizosphere microflora can negatively affect root development and function. Depending on cropping practices, their numbers and/or activity may increase to the extent that they result in considerable losses in crop yield.

BENEFICIAL RHIZOSPHERE MICROORGANISMS

In the broadest sense, beneficial rhizosphere microorganisms include symbionts (*Rhizobium,* certain actinomycetes, and mycorrhizal fungi), and free-living saprophytes that increase the availability of nutrients (9, 33, 79, 80) or

plant growth substances (15, 78) to plants and/or suppress parasitic and nonparasitic pathogens. We focus on those plant growth–promoting rhizobacteria (PGPR) whose plant growth–promotion effects are mainly ascribed to inhibition of minor pathogens, and particularly of DRB.

Plant Growth–Promoting Pseudomonads

The enhanced plant growth resulting from the introduction of specific root-colonizing fluorescent pseudomonads into the rhizosphere is thought to be due to their production of siderophores under iron-limited conditions and to the consequent competition with DRMO for ferric iron in the rhizosphere. Details of this phenomenon have recently been reviewed (13, 34, 50, 70, 77). The siderophores of PGPR have a higher affinity to iron than those of DRMO, which lack the iron-assimilation (or siderophore receptor) system for these PGPR siderophores. Different mechanisms for transfer of iron from the iron chelate into the cell contribute to the specificity of the siderophore-mediated iron-transport systems. Plant growth promotion ascribed to suppression of iron availability for DRMO has been demonstrated in pot experiments for several crops including potato, sugar beet, and radish (42).

The supposition that the main mechanism of plant growth promotion is competition with DRMO for iron is based mainly on the following observations in in vitro and pot experiments: (*a*) Addition of dissolved iron(III) to the environment abolishes in vitro antagonism and also prohibits the plant growth promotion by PGPR strains (41); (*b*) siderophore-negative mutants, obtained by exposure of wild-type PGPR strains to ultraviolet light or mutagenic chemicals (43) or obtained by transposon mutagenesis (58), also lost their plant growth–promoting properties, although they colonized roots as well as wild-type PGPR (3, 43, 67); (*c*) the siderophores produced by PGPR strains B10 and WCS358 mimicked the antagonism in vitro (B10 or WCS358) and also promoted plant growth (B10) (5, 41, 68).

Of the many questions that remain to be answered, the following have recently received attention. (*a*) Do siderophores of PGPR also have a key function in promotion of plant growth and yield in the field? (*b*) Are siderophores of PGPR actually produced in the rhizosphere? (*c*) Can siderophore production be genetically manipulated to improve the efficacy of PGPR?

In our research, growth promotion of potato plants in response to bacterization of tubers or roots in pot and field experiments occurred only in soils cropped most frequently to potatoes and where growth and yields were reduced. Reductions in plant development and tuber production in pot experiments in field soil cropped continuously to potato were counteracted by bacterization of seed tubers with *Pseudomonas* spp. strains WCS358 or WCS374. No growth promotion was obtained in the soil having no history of potato cropping (Table 1) (28), apparently because the DRMO, or more likely

Table 1 Effect of treating seed tubers with the fluorescent *Pseudomonas* spp. strains WCS358, WCS365, and WCS374 on dry weight of potato plants in pots after two months in soil from fields cropped continuously with either potato or wheat

Treatment	Total plant dry weight (%)	
	Potato soil	Wheat soil
Control	100	100
WCS358	128[a]	
WCS365	131[b]	99
WCS374	123[c]	

[a] $p = 0.05$.
[b] $p = 0.005$.
[c] $p = 0.025$.

the factor (or factors) in soil that stimulate the production of their toxic metabolites, had not accumulated in this soil. Root development of potato stem cuttings in soil cropped to potatoes every year (1 : 1) or every third year (1 : 3) was reduced significantly within one week, compared to root development in a soil cropped to potato once every six years (1 : 6). Root bacterization of the potato stem cuttings with *Pseudomonas putida* isolate WCS358 resulted in a significant increase of root development in 1 : 1 soil, whereas siderophore-negative (Sid^-) mutants of WCS358, obtained by transposon Tn5 mutagenesis (84), had no such effect (3). Reduction in seed tuber yield of 13% in field plots cropped every third year to potatoes compared to yields in plots cropped to potatoes every sixth year was prevented by seed tuber bacterization with WCS358, but not with the Tn5 Sid^- mutants (Table 2). Both Tn5 Sid^- and siderophore-producing (Sid^+) Tn5 mutants (which do not differ from the wild type except for kanamycin and streptomycin resistance) colonized the roots equally well until the end of the season (4). This experiment demonstrated for the first time the prerequisite of siderophore production for plant growth promotion and yield increases obtained with selected fluorescent pseudomonads in the field.

Further evidence for the actual production of siderophores in the rhizosphere by PGPR was obtained by studying interactions between transposon Tn5 Sid^- mutants of strains WCS358 and WCS374 and their wild types and with their purified siderophores. Tn5 Sid^- mutants can use the siderophore excreted by the wild type. The numbers (cfu) of Sid^- mutants in potato rhizosphere increased significantly in the presence of the wild type (5, 68). Since induction of siderophore production only occurs with low Fe(III) availability, these results indicate that such a low Fe(III) availability occurs in the rhizosphere.

Table 2 Fresh weight of tubers 86 (seed potatoes) and 130 days after seeding in plots where potatoes had been grown every third (1:3) and every sixth (1:6) year and after treatment of seed tubers with WCS358 wild type or its siderophore-negative Tn*5* transposon mutant (WCS358 Sid$^-$)

Treatment	Tuber fresh weight (kg 100 m^{-2})	
	86 days[b]	130 days[c]
1:3 Rotation		
Control[a]	334 a[d]	535 a
WCS358	372 b	551 a
WCS358 Sid$^-$	339 a	534 a
1:6 Rotation		
Control	376 b	625 b
WCS358	377 b	634 b
WCS358 Sid$^-$	374 b	641 b

[a]Control: 1% carboxymethylcellulose.
[b]MSD = 28.
[c]MSD = 65.
[d]Values with the same letter in a column are not significantly different at $P=0.05$, based on Student's t-test.

The siderophore of *P. putida* strain WCS358 (pseudobactin 358) was isolated and purified, and a tentative structure for it has been proposed (81). Analysis of the molecular genetics of siderophore biosynthesis by *Pseudomonas* spp. (which may have potential as biocontrol agents) is in progress for three isolates: *P. fluorescens-putida* B10 (59), *P. syringae* pv. *syringae* JL2000 (55), and *P. putida* WCS358 (58). These studies create possibilities to manipulate siderophore production by pseudomonads in order to construct improved strains for use as biocontrol agents (50, 84).

The application of the synthetic chelator 8-hydroxyquinoline (8-OHQ) in a low-iron medium has been suggested for the isolation and selection of pseudomonads producing siderophores with a high affinity for Fe(III) (29). However, some pseudomonads selected in this way could use the Fe(III)-8-OHQ as an iron source (P. A. H. M. Bakker, unpublished results).

Root Colonization of PGPR

The success of plant growth promotion by the introduction of PGPR depends largely on their timely establishment and their persistence throughout the growing season at sites where DRMO may become active. This persistence seems to be most difficult with agricultural crops because they usually have a

Table 3 Colonization of potato roots, during the growing season, by kanamycin- and streptomycin-resistant Tn*5* transposon mutants of *P. putida* strain WCS358 in a field plot cropped to potato every third year. The introduced bacteria are expressed as a percentage of the total population of fluorescent pseudomonads

	Tn*5* mutants as a % of the total population of pseudomonads		
	Days after seeding		
Depth from stem base (cm)	26	57	104
0–5	0.95 a[a]	0.20 ab	0.24 ab
20	0.20 ab	0.48 ab	0.06 b
40	1.44 a	0.14 b	0.06 b

[a]Values with the same letter are not significantly different at $p = 0.05$, based on Student's t-test.

long growing season. Moreover, it is difficult to distribute PGPR evenly through agricultural soil. Introductions of PGPR have mainly been effected by applying them to seeds or tubers, from which it is hoped PGPR will colonize the developing root system. PGPR introduced in this way have been detected in the rhizospheres of potato plants throughout the growing season, although their relative numbers gradually decline (Table 3) compared to the total population of fluorescent pseudomonads, which remains at a constant level (4, 30).

Determinations of root colonization by PGPR in the field have been made by plating suspensions of pooled root samples of individual plants on selective media and subsequently counting colonies (4, 30). This method gives no information on whether PGPR are adequately distributed to compete with DRMO. Populations of introduced PGPR strains of *P. putida* or *P. fluorescens*, aerobic bacterial populations, and total populations of fluorescent *Pseudomonas* spp. generally approximate a lognormal distribution (53). The frequency with which a threshold population of an applied beneficial strain is met or exceeded on individual root systems has been suggested to be of predictive value in determining its potential to affect plant growth and yield (53). Such an approach may also be helpful in selecting strains with an optimal root-colonizing ability.

Pseudomonas strains, although growing at similar rates in potato rhizospheres, differ in their capacity to maintain stable populations in such rhizospheres (52). Osmotolerance in vitro was correlated with the abilities of seven strains to establish a stable population in potato rhizospheres (52).

Different processes are involved in the establishment and maintenance of viable bacterial cells on roots. Chemotaxis of fluorescent *Pseudomonas* strains towards asparagine, threonine, and valine at concentrations present in

exudate has been demonstrated, and these compounds induced effects comparable to those of the exudate itself. Chemotaxis was suggested to be the first step of bacterial colonization of seed or roots of soybean (66). Motility of *P. fluorescens* strain WCS374 was essential for efficient colonization of potato roots because nonmotile Tn*5*-induced mutants, defective in the synthesis of functional flagella, were impaired in this respect (L. A. de Weger & P. A. H. M. Bakker, unpublished results). Surface-charge properties of bacteria and plant cells have been suggested to be involved in the next event in the colonization process (39). Interactions of polysaccharides and proteins at the substratum surface probably influence the subsequent bacterial adhesive interactions (62). A better understanding of the role of these different processes and environmental factors in establishing and maintaining stable populations may help in selecting good root colonists.

The Tn*5* transposon Sid$^-$ mutants of *P. putida* strain WCS358 colonized roots as thoroughly as the wild-type strain in both pot and field experiments (3, 4). This finding suggests that the production of siderophores may not be of major importance for root colonization. However, the root colonization by the Sid$^-$ mutants of strain WCS358 probably occurred because this strain can use the Fe(III) siderophore complex of a wide range of rhizosphere pseudomonads, in contrast to the majority of other *Pseudomonas* strains, including WCS374 and B10, which cannot use the siderophores of many other rhizosphere pseudomonads (P. A. H. M. Bakker, unpublished results). The Tn*5* Sid$^-$ mutants of WCS374 colonized roots significantly less than Sid$^-$ mutants of WCS358 (5). The iron-assimilation and -receptor system of pseudomonads thus may be of importance for their root colonization.

INTERACTIONS BETWEEN PGPR AND DRMO

The supposition that plant growth promotion is due to competition for iron between DRMO and PGPR is so far based only on observations that, after introduction of PGPR, various rhizosphere microorganisms causing deleterious effects decrease in numbers, with a simultaneous increase in root and plant growth (78). Although suppression of a great diversity of DRMO, based on competition for iron, may be involved, the wide diversity of their ecological niches and microhabitats does not make this explanation entirely satisfactory. Competition between organisms increases with increasing overlap of their ecological niches (46). The ecological niches of both growth-promoting and cyanide-producing fluorescent pseudomonads are very likely to overlap. Moreover, the dependence of microbial cyanide production on iron availability (2, 32) increases the attractivity of the hypothesis that growth promotion of potato plants in response to bacterization in soil cropped frequently to potato is due to siderophore-mediated suppression of microbial cyanide production

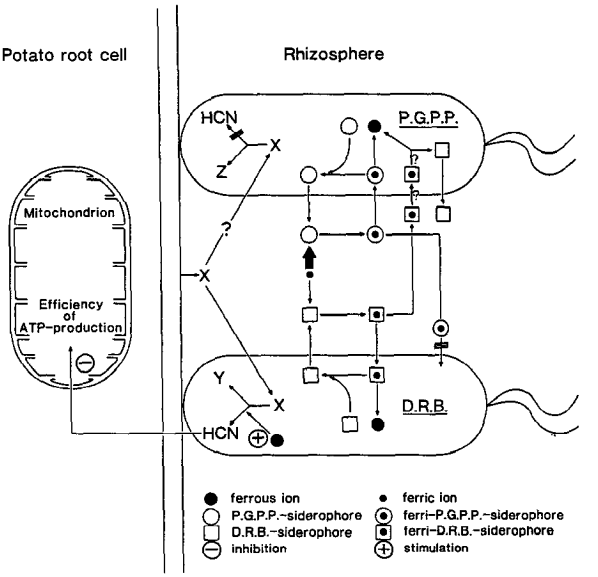

Figure 2 Hypothetical situation in the potato rhizosphere in soil cropped frequently to potato and following bacterization of roots with plant growth–promoting rhizobacteria (PGPR). The production of siderophores by the plant growth–promoting pseudomonads (PGPP) and their ability to use siderophores produced by deleterious rhizobacteria (DRB) interfere with iron acquisition by DRB. The resulting decrease in iron availability for the DRB prohibits their production of HCN from compound X. Y and Z represent metabolic pathways not leading to HCN production.

(Figure 2). The production of cyanide by fluorescent *Pseudomonas* spp. strain WCS A9 was significantly reduced by strain WCS358 and by its purified siderophore pseudobactin 358 in batch cultures (A. W. Bakker, unpublished results). Interestingly, all attempts to detect HCN production by the potato growth–promoting strains *P. putida* WCS358 and *P. fluorescens* WCS374 failed (2). The development and distribution over the potato root system of populations of HCN-producing fluorescent pseudomonads, as affected by strain WCS358, are presently being investigated in the field in short (1 : 3) and long (1 : 6) potato rotations.

Many secondary metabolites of fluorescent pseudomonads, and possibly of other rhizosphere microorganisms, may have the potential to affect root cell physiology and root growth negatively. Those metabolites (such as syringotoxin; 32), whose production, like that of HCN, depends on availability of iron in the environment, deserve special attention. Siderophore-mediated plant growth promotion does not necessarily imply a decrease in numbers of DRMO but could equally well derive from a decrease in production of the deleterious metabolite (or metabolites).

Three of three hundred HCN-producing fluorescent *Pseudomonas* spp. isolates from potato rhizospheres and also strain WCS361 were capable of utilizing the Fe(III) pseudobactin of PGPR strain WCS358 (A. W. Bakker & P. A. H. M. Bakker, unpublished results). We therefore may not exclude the possibility that certain DRMO that can use the Fe(III) siderophore complex of a particular PGPR could increase either in numbers, in deleterious activity, or both after bacterization, thereby nullifying the initial beneficial effect of the PGPR. Such a process may be involved in failures of PGPR in the field.

FAILURES OF PGPR AND POSSIBLE IMPROVEMENTS

Although significant increases in yields by seed tuber inoculation with PGPR have been demonstrated in the field, results vary from field to field and from year to year in the same field (4, 30, 44). Failures seem to be due to unfavorable environmental factors, resulting in inadequate distribution and establishment of introduced rhizobacterial strains or failure of their antagonistic activity towards DRMO.

The availability of Fe(III) is limited in alkaline and neutral soils, and increases with increasing soil acidity. One might therefore expect more (iron-dependent) deleterious activity and less suppression of DRMO by PGPR with increasing soil acidity. Certain clay minerals seem to have a pronounced effect on siderophore-mediated microbial activity. For example, the presence or absence of particular clay minerals has been correlated with the suppressiveness of certain soils to soilborne fungal diseases (74, 75). The presence of illite inhibited the antagonistic activity of fluorescent *Pseudomonas* spp. isolates towards the root pathogen *Thielaviopsis basicola*. By contrast, the antagonistic activity was favored by vermiculite (76). The respiration of the human-pathogenic fungus *Histoplasma capsulatum* in broth culture was reduced by montmorillonite. This inhibition appeared in part to result from the clay's interference with the iron nutrition of the fungus. The iron-transporting siderophore, desferric coprogen B, was apparently absorbed by the clay (48). The frequency and relative proportion of specific clay minerals in field soils may therefore also be involved in failures of PGPR.

Besides the direct effects of environmental factors on activity of DRMO and PGPR, the environment may also affect the rate of exudation by plant roots and the composition of the exudate (73). This effect may influence plant–microbe as well as microbe–microbe interactions indirectly, e.g. by changing microbial growth conditions and/or the availability of precursors of toxic secondary microbial metabolites in the rhizosphere.

In our field experiments performed over five successive years (1981–1985), tuber bacterization increased yields of seed tubers (harvested early) but seldom of ware potatoes harvested one to two months later (4, 30). While this

difference might be explained by the decrease of the relative numbers of introduced pseudomonads later in the season (Table 3) (4), it is also possible that the harmful rhizosphere *Pseudomonas* spp. are favored by the changes in root-exudate composition of the aging potato plants. The amino acid proline, which stimulates HCN production by pseudomonads in vitro, increased dramatically in potato plants in response to stress conditions (51), and thus may also increase in root exudates.

Pseudomonads with the potential to produce HCN and to use the Fe(III) siderophore complex of PGPR may be of no importance in the early season. However, their multiplication and activity may have been stimulated by strain WCS358 and may be partly responsible for the lack of significant yield increase in response to this strain but measured at the end of the season. Finally, potato yield depressions caused by microbial factors fluctuate from year to year (Figure 1), which possibly correlates with fluctuating numbers or activity of DRMO. In years when the numbers or activity of DRMO is low, PGPR cannot be expected to increase yields.

In our field experiments, the treatment of seed tubers with *P. putida* WCS358 increased yield by 12% in a 1:3 potato rotation during the wet summer of 1985, but had no effect in the very dry summer of 1986. In both years, however, significant yield reductions occurred in the 1:3 potato rotation. The DRMO, thus, were apparently active in both the dry and wet season. Possibly the low availability of water in the dry summer made it impossible for strain WCS358 to colonize the developing root system adequately. However, the DRMO survive on root residues of the preceding crops distributed through soil, and thus are always available for colonizing young roots. It is also possible that in a dry season other DRMO (possibly *Streptomyces* spp.) that are less sensitive to PGPR are active.

The inadequate colonization of roots after treatments of seed or seed tubers seems to be one of the major factors restricting commercial application of PGPR at this time. Selection of PGPR strains that adequately colonize roots under a wide range of environmental conditions, including low soil water availability, or introducing genes into such strains that encode for the production of compounds involved in microbial antagonism, e.g. of particularly effective siderophores such as pseudobactin 358, may improve the effectiveness of PGPR.

In our field experiments with fluorescent *Pseudomonas* spp. strains antagonistic to the take-all pathogen of wheat (*Gaeumannomyces graminis* var. *tritici*), we obtained evidence that the pseudomonads introduced in one year survived on root residues until the next year and prohibited take-all disease (J. Lamers & B. Schippers, unpublished results). This approach should therefore be considered for the introduction of PGPR in other crops. Also, the use of inoculant carriers for the slow release of PGPR (8) into soil needs attention.

Finally, various plant cultivars may differ in their sensitivity to DRMO or in the exudation of compounds that stimulate their deleterious activity. Cultivar rotation, then, could be considered as a means to limit yield losses due to high frequency of that crop species.

CONCLUDING REMARKS

Considerable retardation in plant growth and decreased crop yield may result from the deleterious activity of saprophytic rhizosphere microorganisms. This activity increases with certain cropping practices such as growing the same crop frequently in the same field. The deleterious effects seem to be due to microbial metabolites that affect physiological processes in root cells. Evidence is accumulating that microbial cyanide inhibits the energy metabolism of potato root cells in high-frequency potato cropping. Increase in production of HCN with increasing cropping frequency possibly derives from the accumulation of HCN precursors in soil. HCN production may also be due to increased availability of iron in soil for the deleterious *Pseudomonas* strains, which is required for HCN production. The deleterious effect is host specific. Certain components of potato root exudate that facilitate HCN production, therefore, seem to be involved. If so, potato cultivars lacking these components may not be affected.

The deleterious effects of microbial metabolites, the production of which also depends on availability of iron, may be particularly amenable to control by siderophore-mediated iron competition by PGPR. The actual production in the rhizosphere of the siderophore of *P. putida* strain WCS358, which counteracts yield depressions in sites cropped frequently to potato, was demonstrated by complementation experiments using Tn*5*-induced Sid^- mutants in combination with their wild types (5, 68). The key function of the siderophore was demonstrated in the field by the inability of the Tn*5* Sid^- mutants to increase yields in a 1:3 potato cropping frequency (4). The actual production of HCN in situ has yet to be demonstrated.

A major factor in failures of PGPR to increase yields seems to be inadequate root colonization. The presence or gradual increase of DRMO that can use the Fe(III)-siderophore complex of particular PGPR may also be involved. Some HCN-producing pseudomonads have this ability.

The negative influence of saprophytic rhizosphere microorganisms on crop production is in need of greater research effort in plant pathology. Fundamental research on the mechanisms involved is needed to control this particular and important group of plant pathogens intelligently, either by plant breeding, cropping practices, or use of PGPR.

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