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Biological Control of Pathogens with Rhizobacteria [and Discussion]

B. Schippers, J. M. Lynch, P. Neuenschwander, J. W. Deacon, B. C. Hemming, J. A. Lucas and G. Defago

Phil. Trans. R. Soc. Lond. B 1988 **318**, 283-293
doi: 10.1098/rstb.1988.0010

Phil. Trans. R. Soc. Lond. B **318**, 283–293 (1988)
Printed in Great Britain

Biological control of pathogens with rhizobacteria

By B. Schippers

Willie Commelin Scholten Phytopathological Laboratory, Department of Plant Pathology, State University of Utrecht, State University of Amsterdam; 3742 *CP Baarn, The Netherlands*

Present knowledge on the biological control of soil-borne plant pathogens by rhizosphere inhabiting bacteria, especially fluorescent *Pseudomonas* spp., is discussed. Attention is paid to the use of molecular biological techniques to analyse the mechanism(s) of antagonism (competition for iron, antibiosis) and the population dynamics of the antagonist(s). Special attention is given to the biological control of a new class of pathogen that does not obviously damage the host, except by stunting its growth and yield. The need for more information on mechanisms of root colonization and survival of antagonists to improve their use as biocontrol agents is emphasized.

1. Rhizobacteria and pathogens

Bacteria isolated from the rhizosphere and belonging to a wide variety of genera, have the potential to suppress diseases caused by a diversity of soil-borne plant pathogens (table 1). Some of these, especially *Pseudomonas* spp. and *Bacillus* spp., significantly suppress disease and increase yield of crops in field trials.

Most progress in understanding the mechanisms of control and of successes and failures in the field has come from recent studies on the role of fluorescent *Pseudomonas* spp. in promoting plant growth by suppressing deleterious rhizosphere microorganisms (DRM) (Burr & Caesor 1984; Schippers *et al.* 1987*a*, *b*; Suslow 1982), and on their role in soils naturally suppressive to diseases caused by *Fusarium oxysporum* in a variety of crops (Baker *et al.* 1986), by *Gaeumannomyces graminis* in wheat (Weller & Cook 1986*a*) and by *Thielaviopsis basicola* in tobacco (Ahl *et al.* 1986).

Disease-suppressive soils are attractive model systems for exploring the potentials of rhizosphere microorganisms to control disease because in these soils, the antagonists also function in modern agricultural practices and can give even better results than do pesticides.

Plant growth promotion by rhizobacteria is another attractive model system for research, because it operates with a variety of crops (table 1) by enhancing root function and growth, thereby improving the uptake of nutrients and water from soil.

Experience with *Pseudomonas* spp. as biocontrol agents in these model systems has shown that even within this pre-eminent group of rhizobacteria, interaction with the target organism varies from inhibition of germination and germ tube growth (*F. oxysporum*) in the rhizosphere (Baker *et al.* 1986) to restriction of the pathogenic activity (*G. graminis*) inside infected and necrotic root tissue (Weller & Cook 1986*a*). In addition, the mechanisms controlling activity of the pathogens are diverse and include competition for Fe^{3+}, antibiosis and induction of host resistance. Competition for iron seems to be a major factor in some and to be involved in other mechanisms of biocontrol of diseases caused by microbial plant pathogens.

Table 1. Recent reports on genera of rhizobacteria with biocontrol potential

bacteria	*pathogen*	host	reference
Alcaligenes	*Fusarium oxysporum*	carnation	Yen & Schroth (1986)
Arthrobacter†	*Fusarium oxysporum*	carnation	Sneh (1981)
Bacillus†	*Gaeumannomyces graminis*	wheat	Capper & Campbell (1986)
Bacillus†	*Phytophthora cactorum*	apple	Gupta & Utkhede (1986)
Bacillus	*Sclerotium cepivorum*	onion	Utkhede & Rahe (1983)
Enterobacter	*Phytophthora cactorum*	apple	Gupta & Utkhede (1986)
Hafnia	*Fusarium oxysporum*	carnation	Sneh *et al.* (1985)
Pseudomonas	*F. oxysporum*	carnation, flax	Baker *et al.* (1986)
Pseudomonas†	*Erwinia carotovora*	potato	Xu & Gross (1986)
Pseudomonas†	*Erwinia carotovora*	potato	Rhodes & Logan (1986)
Pseudomonas†	*Gaeumannomyces graminis*	wheat	Weller & Cook (1986*a*)
Pseudomonas†	*Pythium* spp.	wheat	Weller & Cook (1986*b*)
Pseudomonas	*Pythium* spp.	cotton	Howell & Stipanovic (1979)
Pseudomonas	*Rhizoctonia solani*	cotton	Howell & Stipanovic (1980)
Pseudomonas	*Thielaviopsis basicola*	tobacco	Ahl *et al.* (1986)
Pseudomonas†	deleterious microorganisms	potato	Kloepper *et al.* (1980)
Pseudomonas†	deleterious microorganisms	potato	Schippers *et al.* (1986)
Pseudomonas	deleterious microorganisms	beet	Suslow (1982)
Rhizobium	*Phytophthora megasperma*	soybean	Tu (1978)
Serratia	*Fusarium oxysporum*	carnation	Sneh *et al.* (1985)

† Significant control was also obtained in field trials.

2. Competition for iron as a mechanism of disease control

Iron plays a central role in the energy metabolism of aerobic and semi-anaerobic microorganisms. Its availability in soil for microorganisms and plants drops dramatically with increasing pH above pH6. Microorganisms compete for iron by releasing siderophores (S) which are small proteins with a high binding affinity to Fe^{3+} (Neilands 1981; Leong 1986; Schippers *et al.* 1987*a*,*b*). The affinity for Fe^{3+} of the many different microbial siderophores varies widely and is *ca.* ten times greater for the catechol-hydroxamate type siderophores of pseudomonads, than for the hydroxamate type siderophores of fungi. Competition for Fe^{3+} can also occur among different strains of *Pseudomonas* spp. It probably depends, for example, on differences in siderophore production, in affinity of their siderophores for Fe^{3+}, and also on the specificity of their receptor proteins for their own siderophores. The receptor proteins are located in the outer cell membrane of bacterial cells.

The ability, or inability of microorganisms to use each others Fe^{3+}-siderophore complexes seems to be important for their ability to colonize the rhizosphere (Bakker *et al.* 1986*b*). Our plant growth promoting (PGP) *Pseudomonas putida* WCS358 can use siderophores of strains of many other fluorescent *Pseudomonas* spp. isolated from the rhizosphere of potato plants. However, the use of its own siderophore by other strains of *Pseudomonas* spp. is limited. PGP *Pseudomonas fluorescens* WCS374 can use the siderophores of only a few other strains of *Pseudomonas* spp., but its own siderophore can be used by many other strains. Strain WCS358 can use the siderophore of WCS374, but WCS374 cannot use the siderophore of WCS358.

The significance of these differences for the colonization of the rhizosphere was demonstrated in pot experiments. Mutants of WCS358 and WCS374, which had lost their ability to produce siderophores but not their receptors, were obtained by *Tn5* transposon mutagenesis (Marugg *et al.* 1985). When roots that had just emerged from potato sprouts were inoculated before planting with the siderophore-negative mutant (S^-) of either WCS358 or WCS374 and planted in soil containing the siderophore producing parent strain (S^+) of either WCS358 or

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WCS374, populations of S⁻ mutants on the roots developed differentially (Bakker *et al.* 1986*b*). WCS358 S⁻ was significantly stimulated in the presence of its parent strain and of WCS374, but WCS374 S⁻ only increased in the presence of its parent strain and not in the presence of WCS358 (figure 1). These observations strongly suggest not only that siderophores of

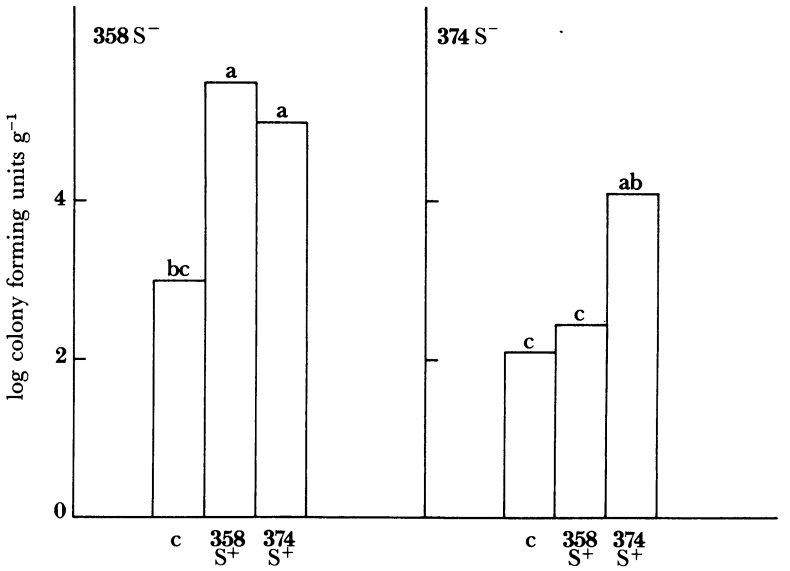

FIGURE 1. The significance of siderophores (S) for root colonization by *Pseudomonas* spp. Root colonization from soil of a transposon *Tn5* mutant of *P. putida* 358 (358 S⁻), which has lost its ability to produce siderophores, is significantly enhanced by the presence on the roots of its siderophore-producing parent strain 358 S⁺, or of *P. fluorescence* 374 S⁺, siderophores of which can be used by 358. Root colonization from soil by *P. fluorescence* 374 S⁻ is only enhanced by its siderophore-producing parent strain 374 S⁺, but not by 358 S⁺, siderophores of which can not be used by 374. Roots were inoculated with *Pseudomonas* strain 358 S⁺ or strain 374 S⁺ before planting in soil inoculated with 358 S⁻ or 374 S⁻. Values with the same letter on top of a bar are not significantly different at $p = 0.01$ based on Student's *t*-test. Control treatment of roots (c).

Pseudomonas spp. are being produced in the rhizosphere but also demonstrate the possible impact of siderophore-mediated competition for iron among rhizosphere pseudomonads on their population dynamics in the rhizosphere.

3. PLANT GROWTH PROMOTION AND DELETERIOUS RHIZOSPHERE MICROORGANISMS

Siderophores produced by strains of fluorescent *Pseudomonas* play a key role in growth promotion of a variety of crops in pot experiments (Geels & Schippers 1983; Kloepper *et al.* 1980; Leong 1986; Schippers *et al.* 1986; Suslow 1982). Potato tuber treatment with *P. putida* WCS358 improved seed tuber yield by 13%. Its *Tn5* transposon S⁻ mutant, however, had no effect in these field experiments (Bakker *et al.* 1986*a*). This shows that *Pseudomonas* spp. siderophores operate in plant growth promotion and in yield increases in the field.

The growth promotion is attributed to suppression of ill-defined rhizosphere inhabiting microorganisms that impair root function (Kloepper & Schroth 1981; Suslow 1982; Schippers *et al.* 1986, 1987*a*, *b*). In our potato-field experiments they seem to be involved in the progressive decrease in tuber yield with increasing frequency of potato cropping.

We hypothesize that in soils frequently cropped to potato, hydrocyanic acid production by

deleterious rhizosphere pseudomonads impairs the energy metabolism of potato root cells thereby decreasing uptake of nutrients from soil. The HCN production of microorganisms probably is then suppressed by the PGP *Pseudomonas* spp. strains (Bakker & Schippers 1988). This hypothesis is supported by the following observations *in vitro*, and in pot experiments (figure 2):

(1) more than 50% of potato rhizosphere *Pseudomonas* spp. isolates can produce HCN;

(2) potato root cytochrome oxidase respiration is suppressed by less than 5 μM HCN;

(3) the potato root exudate components glycine and proline enhance HCN production by pseudomonads;

(4) HCN production by *Pseudomonas* spp. depends highly on availability of iron and can be suppressed by PGP *Pseudomonas putida* WCS358 or its purified siderophore pseudobactin 358.

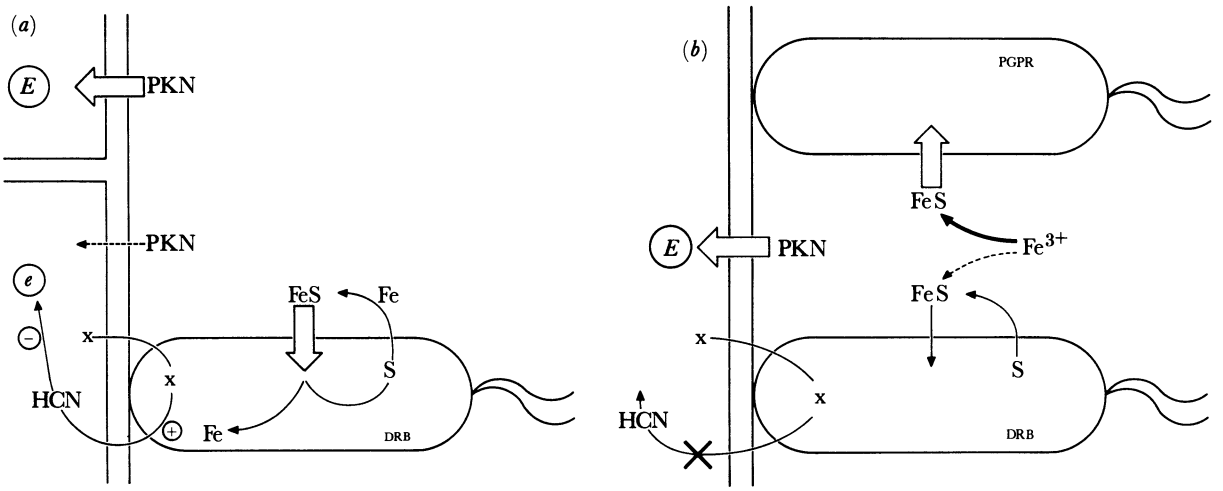

FIGURE 2. Diagram of hypothetical interactions between plant growth promoting rhizobacteria (PGPR), deleterious rhizobacteria (DRB) and the plant root cell. (*a*) Inhibition of potato root cell function by hydrocyanide-producing DRB. The uptake of iron by siderophores of DRB (S) from the soil enhances their HCN production from the root exudate component 'x'. HCN inhibits the energy metabolism (*e*) of the root cell and thus decreases uptake of phosphorus, potassium and nitrogen (PKN). (*b*) Competition for iron by the siderophores of a PGPR inhibits the HCN production by DRB, thereby increasing the available energy (*E*) of the root cell for uptake of PKN.

Microbial HCN production in the rhizosphere is difficult to demonstrate because HCN is rapidly inactivated in soil. It may, however, occur inside healthy root tissue because for many crops, *Pseudomonas* populations have been demonstrated in these microsites (Schippers *et al.* 1987*a*, *b*).

Whether the interactions shown in figure 2 function in the field needs further experimental evidence. So far they have attracted little attention. They may, however, have a large impact on crop yields.

4. SPONTANEOUS DISEASE SUPPRESSION BY RHIZOBACTERIA

According to Baker *et al.* (1986) the spontaneous suppression of wilt diseases caused by *formae speciales* of *Fusarium oxysporum* in fine sandy loam soils in the Salinas Valley of California, is most likely to be caused by siderophore-mediated competition for iron with certain *Pseudomonas*

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putida strains. No evidence for the participation of pseudomonads in parasitism, antibiosis, competition for carbon sources or in inducing plant resistance in suppressiveness was obtained. The biocontrol seems to be due particularly to inhibition of chlamydospore germination and of germ-tube growth in the saprophytic phase of the fungus in rhizosphere soil.

Cook and co-workers provided evidence that both siderophore production and production of antibiotic phenazin compounds by certain strains of fluorescent *Pseudomonas* spp. are involved in suppression of take all disease caused by the fungus *G. graminis* in continuous wheat culture in the Pacific Northwest of the U.S.A. Protection is thought to be primarily the result, of interference with the pathogen in its parasitic phase. Antibiotic negative mutants obtained by *Tn5* mutagenesis were significantly less suppressive of disease development than was the parent strain (Cook *et al.* 1988; Weller & Cook 1986*a*).

Défago *et al.* (1987) selected a *P. fluorescens* strain CHAo producing siderophores, hydrocyanic acid and several antibiotics, which they consider to be responsible for natural suppression in certain Swiss soils, of black root rot of tobacco caused by the fungus *Thielaviopsis basicola* (Ahl *et al.* 1986). The strain is also highly suppressive of take all in wheat (Défago *et al.* 1987). Its siderophores are not considered to be toxic not because they deplete the environment of Fe^{3+}, but because they increase the Fe^{3+} concentration to the point where it becomes toxic to the many fungi tested (Ahl *et al.* 1986). Hydrocyanic acid production by the *P. fluorescens* strain CHAo is supposed to be produced particularly inside the initially healthy root tissue, and to induce resistance to the pathogen. Mutants obtained by transposon mutagenesis, which had lost their ability to produce hydrocyanic acid, also had lost their ability to suppress disease (G. Défago, personal communication).

These examples demonstrate the rapid increase in our knowledge of biocontrol by rhizosphere pseudomonads, the diversity of the mechanisms and of the sites of disease suppression and the contributions of molecular and cell biological techniques to elucidating these mechanisms.

Interesting information has also recently been obtained on induction of suppression by rhizosphere pseudomonads of seedling diseases in wheat caused by *Pythium* spp. (Weller & Cook 1986*b*), in cotton by *Pythium* spp. and *Rhizoctonia solani* (Howell & Stipanovic 1979, 1980) and on decreasing infection of potato daughter tubers by *Erwinia carotovora* (Xu & Gross 1986; Rhodes & Logan 1986).

5. Field trials: successes and failures

Significant control of diseases and consequent increases in plant development and yield have been obtained for a variety of soil-borne pathogens and crops in field trials (table 1). Biocontrol of *Pythium* spp. in wheat with *Pseudomonas* spp. was similar to control with the fungicide metalaxyl (Weller & Cook 1986*b*). Treatment of potato tubers with *P. putida* WCS358 increased yields of seed tuber of potatoes in short rotations to those of potatoes in long rotations (Bakker *et al.* 1986*a*).

The major problem, however, is the failure to repeat these results consistently on different soils or in different years in naturally contaminated fields and to make biological control of soil-borne pathogens competitive with chemical control (Capper & Campbell 1986; Geels *et al.* 1986). The factors involved are various and of physical, chemical and biological origin. Insufficient root colonization seems to be a major factor. With spontaneous or natural

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suppression of *Fusarium* wilts and of root rot caused by *Thielaviopsis basicola*, the function and perpetuation of the microbial antagonism seem to depend on soil properties such as the absence (illite) or presence (vermiculite) of particular clay minerals (Stutz *et al.* 1985), iron availability and soil pH (Baker *et al.* 1986). The presence of the pathogen may also be a factor in selectively stimulating and perpetuating the antagonistic *Pseudomonas* populations.

6. Root colonization and survival of rhizobacteria

Antibiotic resistance labelling of rhizobacteria, either by selection of spontaneous mutants or by transposon mutagenesis, has recently provided much information on their distribution and survival in field soils. When applied to seed or seed tubers, labelled strains of *Pseudomonas* spp. could be reisolated from all parts of potato and wheat plants throughout the summer or winter (Bahme & Schroth 1987; Geels *et al.* 1986; Bakker *et al.* 1986*a*). Their distribution over the root system, however, is lognormal (Loper *et al.* 1984*b*, 1985) and parts of roots or even complete roots may not be colonized at all (Bahme & Schroth 1987). Population sizes and distribution were recently shown to be much affected by soil types differing in pore sizes, adsorbing clay particles, organic colloids and water movement, especially along roots (Bahme & Schroth 1987).

Detailed sampling of underground parts of potato plants in the field throughout the season in California and in The Netherlands revealed that population densities of introduced strains of *Pseudomonas* spp. sufficient to protect against pathogens occur primarily on plant roots within ± 15 cm from treated seed tubers, depending on soil type (Bahme & Schroth 1987; P. A. H. M. Bakker, A. W. Bakker & B. Schippers, unpublished results). According to Bahme & Schroth (1987), greatest protection therefore is most likely to occur in the crown area against pathogens attacking juvenile tissue, including seed-decaying bacteria, fungi such as *Pythium* spp. and ill-defined deleterious rhizosphere microorganisms. The distribution of antagonistic strains of *Pseudomonas* spp. that develop inside the root tissue, however, may be different and less affected by environmental factors.

Whether strains of *Pseudomonas* spp. that keep pace with growth of roots through soil can be obtained or constructed seems questionable as their generation times are too slow. Differences in abilities to survive in soil and to colonize parts of young roots growing through the soil, however, are worth exploring. Bahme & Schroth (1987) noticed that their *Pseudomonas* strain A1-B persisted in non-rhizosphere soil throughout the season. In our field experiments with fluorescent *Pseudomonas* spp. strain WCS292G, antagonistic to the take-all pathogen of wheat *G. graminis*, improved protection of roots was obtained if the pseudomonads were introduced in the preceding year (J. G. Lamers & B. Schippers, unpublished results).

7. Genetic manipulation

Selective elimination of the production of microbial metabolites such as siderophores, antibiotics or hydrocyanic acid by transposon mutagenesis has enhanced the possibility of understanding mechanisms of antagonism *in vivo*. Similarly, genetic manipulation will be a valuable tool in detecting the traits for microbial survival and root colonizing abilities. This may lead to increased efficacy and consistency of biological control by management of

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antagonistic rhizobacteria. Analysis of the molecular genetics of siderophore biosynthesis by strains of *Pseudomonas* spp. with potential as biocontrol agents is in progress for three isolates: *P. fluorescens/putida* B10 (Moores *et al.* 1984), *P. syringae* pv. *syringae* JL 2000 (Loper *et al.* 1984*a*) and *P. putida* WCS358 (Marugg *et al.* 1985). These studies may show how to manipulate production of siderophore or siderophore receptors in new, improved strains for use as biocontrol agents.

This approach does, however, raise questions about the risks of introducing microorganisms obtained by recombinant DNA into the environment.

8. Conclusions

Several genera of rhizobacteria have the potential to be used successfully for the biological control of soil-borne plant pathogens. The site of their interactions with the different pathogens and their hosts and the mechanisms of the interactions are diverse even for the genus *Pseudomonas*. The slow development of commercial use of rhizobacteria is due largely to the inconsistency of results in the field. To improve this situation, more fundamental knowledge is needed on the biotic and abiotic factors affecting the population dynamics, survival and antagonistic activity of the rhizobacteria *in situ*, particularly by the use of molecular biological techniques. More research also is needed on the technology of using rhizobacteria, especially in formulating commercial products and manipulation of the environment in which they will be used.

Biological control with rhizobacteria is only one of the many biocontrol mechanisms that operate as part of a complex system in nature. The real challenge is to integrate it with other systems of control such as (induced) resistance, other mechanisms of microbial antagonism and control of the environment.

I thank my co-workers Peter A. H. M. Bakker, Albert W. Bakker and Patrick Geels, whose work I have cited; the Netherlands Technology Foundation (STW) for support of the work and Dr R. Harling for his critical reading of the text.

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Discussion

J. M. Lynch (*Glasshouse Crops Research Institute, Littlehampton, U.K.*). Would Professor Schippers please comment on (1) the observation that pseudobactin, the siderophore produced by rhizosphere bacteria, can inhibit the uptake of iron by plants and (2) the relative affinity constants of plant and microbial siderophores?

B. Schippers. (1) Becker *et al.* (1985) showed that addition of pseudobactin to a nutrient solution can inhibit Fe^{3+} assimilation by maize and pea plants. Such a treatment probably leads to a decrease of iron availability over the entire root system because of binding of ferric ions in the nutrient solution by pseudobactin. In soil, pseudobactin will only be produced locally by *Pseudomonas* bacteria in micro-sites. Moreover, other microbial siderophores may enhance Fe^{3+} assimilation by plants. Dicotyledonous plants can react to iron deficiency by releasing protons from the roots. This reaction will create micro-environments with a low pH unfavourable for growth of pseudobactin-producing pseudomonads or pseudobactin production or both. Thus pseudomonads in the rhizosphere do not necessarily interfere with iron acquisition by the plant.

(2) Affinity constants ($\log_{10}Kf$) of microbial siderophores for ferric ion vary from 22.9 (aerobactin) to 52 (enterobactin).

Phytosiderophores such as aminohydroxylcarboxylates (in root washings of graminaceous plants) have a $\log_{10}Kf$ equal to 18. Affinity constants of phytosiderophores seem not to be so high as those of bacterial and fungal siderophores.

References

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P. Neuenschwander (*International Institute of Tropical Agriculture, Ibadan, Nigeria*). May I ask a general question as an entomologist to pathologists working in biological control?

Do they do, or can they do, studies in population dynamics? And if so, do they consider such studies relevant?

B. Schippers. Yes, we can do studies in population dynamics. Gilligan (1985) has an introductory chapter and his own chapter on disease progress of soil-borne plant pathogens. He points out that modelling crop disease is a rapidly expanding discipline, but one with a relatively short history compared with the study of insect population dynamics. He nominates work in the 1960s as the beginning of the present mathematical phase (see, for example, Van der Plank 1963).

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D. Bouhot & Joannes (1983) have produced a simple deterministic model in population dynamics in soil that is an application of the logistic equation to two competing populations (*Pythium* spp. v. microflora in his bioassay).

For many years there has been much interest in inoculum and infection, which has necessitated knowing about populations of propagules and this can be traced back to Gregory's (1948) multiple infection transformation and beyond. R. Baker's interest in models of root infection go back to the early 1960s (Baker 1965). D. Hornby (1981) tried to quantify inoculum of the take-all fungus.

References

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J. W. Deacon (*Department of Microbiology, Edinburgh University, U.K.*). If, as Professor Schippers, suggests, some deleterious rhizobacteria produce cyanide, which depends on an adequate supply of iron, and if this benefits these bacteria by making root cells more 'leaky', then I would expect these bacteria to develop effective siderophores and siderophore-uptake mechanisms. In such circumstances it is difficult to see how these deleterious bacteria would be controlled by siderophore-producing beneficial rhizobacteria. In any case, I would expect such control to be only temporary, because it would select for effective siderophore-producers among the deleterious bacteria.

B. Schippers. Our hypothesis is that by frequent cropping of potato, the production of hydrocyanide by rhizosphere inhabiting pseudomonads increases either by increasing availability of iron for these particular pseudomonads and/or by increasing the availability in soil of precursors of microbial HCN production. The increasing availability of iron for HCN-producing pseudomonads could for example originate in accumulation of *Pseudomonas* spp. Fe^{3+}-siderophores, that are recognized and utilized by the HCN-producing pseudomonads. The introduction in the rhizosphere of (plant growth promoting) pseudomonads, the Fe^{3+}-siderophores of which cannot be recognized by HCN-producing pseudomonads (such as that of our strain WCS358), then reduces the availability of Fe^{3+}. I agree that this may select for deleterious (HCN-producing) bacteria that can use the competitive (WCS358) siderophore, as also stated in Schippers *et al.* (1986) and Schippers *et al.* (1987*a*).

B. C. Hemming (*Monsanto Life Services Research Center, St Louis, U.S.A.*). Because our *in vitro* experiments have demonstrated that HCN production by fluorescent pseudomonads is medium- or substrate-dependent, what evidence exists for production of HCN *in situ* (i.e. on plants or in soils) by bacterial isolates?

The creation of insertional mutants by Dr Défago, and their evaluation, certainly implicates involvement of HCN in the processes observed. Increased root hair extension and development of epidermal transfer cells has, however, also been shown to occur under plant iron stress.

B. Schippers. We do not have evidence yet, for HCN production *in situ* by bacterial isolates; however, we recently have demonstrated that growth of potato plants can be decreased significantly by concentrations of HCN below the limits of detection. HCN was produced by bacterial isolates inoculated on sterile squashed potato roots on a mineral nutrients agar plate.

J. A. Lucas (*Department of Botany, University of Nottingham, U.K.*). I was wondering about this suggested phenomenon of induced resistance in roots. Is it being proposed that these rhizobacteria somehow trigger a host response in root tissues? If so, what mechanisms are envisaged, and how do these bacteria differ from others in the resident rhizosphere microflora that do not induce resistance?

B. Schippers. Yes, indeed it is hypothesized that specific *Pseudomonas* spp. strains trigger a host response in root tissue. Their HCN production inside the root tissue, by suppressing the cytochrome oxidase respiration pathway and stimulating the alternative respiration pathway, possibly triggers the production of secondary metabolites, toxic to the pathogen.

These bacteria may differ from others in the resident rhizosphere microflora by their strong HCN production and by their ability to develop inside the healthy root tissue.

G. Défago (*Swiss Federal Institute of Technology, Zürich, Switzerland*). In answer to the question [not printed] about resistance in tobacco to *Thielaviopsis basicola* induced by a selected wild-type strain of *Pseudomonas fluorescens* there are the following points: (1) the wild-type strain produces HCN when colonizing roots; HCN is known to induce in roots an alternative respiratory pathway that is associated with the synthesis of phytoalexins; (2) the strain increases the production of phenolic compounds in the roots; (3) it greatly stimulates the formation of root hairs; and (4) it suppresses disease.

In contrast, an HCN^- mutant induced by *Tn5* insertion: (1) did not stimulate formation of root hairs; and (2) did not suppress disease. Also the wild-type strain does suppress disease in soil with vermiculite clay minerals but does not in soil with illite clays. CN bound to vermiculite clays induces root hair formation whereas CN bound to illite clays does not. Finally, a 'rough' mutant that did not suppress disease was confined to root surfaces whereas the effective wild-type strain penetrated and grew inside roots. From these facts it may be concluded that the wild-type strain suppresses disease by growing inside roots and by producing HCN which then alters the physiology of roots in ways that make them resistant to the pathogen.

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