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K. Toyota · K. Ikeda

Relative importance of motility and antibiosis in the rhizoplane competence of a biocontrol agent *Pseudomonas fluorescens* MelRC2Rif

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Abstract Mutants defective in motility or antibiotics production were obtained by Tn5 mutagenesis of a biocontrol agent *Pseudomonas fluorescens* MelRC2Rif (wt). Tomato or melon seeds were co-inoculated with a Tn5 mutant and wt in a 1:1 ratio and then grown in soil for 10 days. There was no change in ratios of Tn5 mutants defective in antibiosis to wt in the process of rhizoplane colonization, suggesting little contribution of in vitro antibiosis to the rhizoplane competence of *P. fluorescens* MelRC2Rif. Similar results were also obtained when seeds treated with bacteria were planted in soil artificially infested with fungal pathogens. In contrast, ratios of Tn5 mutants defective in motility to wt significantly decreased, suggesting the contribution of motility to the rhizoplane competence of this bacterium. When a non-motile Tn5 mutant and wt were co-inoculated into soil at a matric potential of pF 2.3 (–20 kPa) and plants were then grown, there was no change in the ratio in rhizoplane colonization, suggesting that motility might have a role in the movement along roots but an insignificant role in the movement from bulk soil towards roots. When they were co-inoculated into 0.2% water agar (WA) instead of soil, a remarkable decline in ratios was detected. Thus it was soil structure that hindered the efficiency of motility. Time course enumeration of rhizoplane colonization of tomatoes grown in WA revealed that motility was an important means of movement towards and/or along roots rather than the multiplication on roots.

Key words *Pseudomonas fluorescens* · MelRC2Rif · Motility · Antibiosis · Rhizosphere

K. Toyota (✉) · K. Ikeda
Laboratory of Soil Biology and Chemistry,
School of Agricultural Sciences, Nagoya University,
Nagoya 464-01, Japan
Tel: +81 (52) 789 5323; Fax: +81 (52) 789 4012;
e-mail: ktoyota@nuagr1.agr.nagoya-u.ac.jp

Introduction

The development of biocontrol agents against soil-borne plant pathogens has been attempted by many investigators. There have been, however, few commercially successful examples in the field, except for *Agrobacterium radiobacter* (Kerr 1980). As Deacon (1991) pointed out, most attempts to develop biological control agents in the root environment have failed because the organisms were selected from in vitro antagonism and were ecologically unsuited to the natural environment of the pathogens. One of the major constraints is the inconsistent colonization of roots by biocontrol agents, where the agents presumably protect plants from the pathogens. Therefore, knowledge about the factors that contribute to root colonization is required.

Root colonization is defined as the process whereby introduced bacteria become distributed along roots in nonsterile soil, multiply and survive for several weeks in the presence of indigenous soil microbiota (Weller 1988). Root colonization involves two phases: phase I is the attachment to roots, and phase II is the multiplication on roots (Howie et al. 1987). Rhizosphere competence describes the relative root-colonizing ability of a rhizobacterium (Weller and Thomashow 1994).

According to Weller (1988) and Weller and Thomashow (1994), bacterial traits that contribute to rhizosphere competence are mostly unknown, but some that may be important are categorized into three classes. Class I involves cell surface polysaccharides (Anderson et al. 1988; Chao et al. 1988; Tari and Anderson 1988), fimbriae (Vesper 1987), flagella (De Weger et al. 1987) and chemotaxis toward seed or root exudates (Heinrich and Hess 1985; Scher et al. 1985). Misaghi et al. (1992) reported that non-motile isolates were poorer root colonizers than motile isolates. These factors are important in phase I of root colonization. Class II involves growth rate (Parke 1991) and ability to utilize complex carbohydrates (Ahmad and Baker 1987a,b) that are important in phase II, the multiplication on the root. Class III involves osmotolerance, which is necessary for survival (Loper et al. 1985).

Mazzola et al. (1992) demonstrated that the production of phenazine antibiotics contributes to the colonization of roots by *Pseudomonas fluorescens* 2–79.

We have investigated the relative importance of motility and antibiosis in the rhizoplane competence of *P. fluorescens* MelRC2Rif by using different inoculation methods for this bacterium: (1) seed and soil inoculation to estimate the effect of soil structure on motility, and (2) inoculation into soil infested with pathogens to which MelRC2Rif shows in vitro antibiosis, in order to estimate the effect of antibiosis on the rhizosphere colonization.

Materials and methods

Soil and strains used

A culture soil [a mixture of 32% kaolinite, 10% sand, 8% vermiculite, 30% farmyard manure and 20% peat moss; total C 8.2%, total N 0.27%, pH(H_2O) 6.6, maximum water-holding capacity (MWHC) 80.8%] was used. The soil was sieved (2 mm) and kept at 40 °C before use.

Strain *P. fluorescens* MelRC2Rif was used in this experiment. It was a rifampicin-resistant spontaneous mutant of strain MelRC2, which was obtained from melon roots. MelRC2Rif was tentatively identified as *P. fluorescens* biovar III, based on quinon type (Q9), fluorescent pigment production (+), substrate utilization pattern, cellular fatty acid composition etc. Bacterial inocula were prepared by overnight culture with shaking (75 rev min^{-1}) at 28 °C in 10^{-1} strength nutrient broth (1/10 NB: Eiken Chemical Co. Ltd.).

Rhizoctonia solani, *Pythium aphanidermatum*, *Fusarium oxysporum* f. sp. *melonis*, *Verticillium dahliae*, and *Penicillium notatum* were used as model soil fungi and they were maintained on potato dextrose agar (PDA: Eiken Chemical Co. Ltd.). *Pseudomonas* (*Burkholderia*) *solanacearum* was also used as a model bacterial pathogen and maintained on 10^{-1} strength nutrient agar (1/10 NA: Eiken Chemical Co. Ltd.)

Isolation of Tn5-induced mutants

Tn5-induced mutants were obtained by the method of Simon et al. (1983). Briefly, *Escherichia coli* S17-1 harboring pSUP10141 was mated with *Pseudomonas fluorescens* MelRC2Rif for 1 day at 28 °C on NA. Transconjugants were selected on 1/10 NA containing rifampicin (100 μg ml^{-1}) and kanamycin (50 μg ml^{-1}). Colonies were screened for motility on motility plates containing 1/10 NB solidified with 0.2% agar (Scher et al. 1985). After spot inoculation, motility was judged after 24-h-incubation at 28 °C. The colonies were also screened for non-antibiotics-producing mutants on PDA inoculated with *R. solani*. Each plate contained 10–15 transconjugants. After 2–4 days of incubation at 28 °C, mutants showing no clear zone were selected and then their antagonistic abilities against *Pythium aphanidermatum*, *F. oxysporum* f. sp. *melonis* and *Pseudomonas* (*Burkholderia*) *solanacearum* were further tested on PDA, King's medium B (KMB; King et al. 1954) and KMB supplemented with 2% of glucose (KMBGlu). Volatile inhibitors produced by MelRC2Rif were detected in a split plate experiment. Bacteria were grown for 2 days on KMB with 2% of glycin (KMBGly) in one side of a split plate, then fungal pathogens were inoculated on the other side of the plates. Radial growth of the pathogens was measured for 2–4 days. Cyanide production was detected with a picric acid assay (Castric 1975).

Effects of in vitro antibiosis on the rhizoplane competence of MelRC2

Melons (*Cucumis melo* L., cv. Amus) were surface-sterilized in 70% ethanol for 2 min, rinsed with sterile distilled water (SDW) and preemerged on 1/10 NA at 28 °C. Two-day-old seeds were soaked for 20 min in 2 ml of a 0.5% suspension of methylcellulose (Sigma) containing two bacteria mixed in a 1:1 ratio (each ca. 1×10^{11} cfu ml^{-1}) of MelRC2Rif (wt) and an antibiotics-production negative mutant. As a result, a volume of 2.5 μl and 1.0 μl of bacterial suspension adsorbed onto a melon and tomato seed, respectively. The treated seeds were grown in test tubes (ϕ 26 mm×260 mm) containing 70 g of the soil sample, the water content of which was adjusted to 50% MWHC (water content = 28.8%), corresponding to ca. pF 2.3 (–20 kPa) according to the measurement with tensiometer (HM type DIK-3130, Daiki Rika Kogyo Co., Tokyo), in a greenhouse (daytime: 28 °C for 16 h; night-time: 20 °C for 8 h). Plants were watered every day with SDW to maintain a constant weight of the test tubes. After 10 days incubation, when the roots had reached the bottom of the test tubes, the roots were taken out from soil and washed in SDW to remove adhering soil materials. Populations of inoculated bacteria on the root were counted with 1/10 NA amended with Rif (100 μg ml^{-1}) after the roots had been macerated in a sterile mortar and pestle. Three or four whole root systems were separately macerated for counts and three replicate plates were used for each dilution. The numbers of total culturable bacteria were also determined on 1/10 NA. The results were expressed as cfu per gram of fresh root. Over 100 colonies that appeared on 1/10 NA with Rif were transferred onto 1/10 NA with kanamycin (50 μg ml^{-1}) for separating Tn5 mutants from wt. Indigenous bacteria resistant to Rif (100 μg ml^{-1}) were negligible on the roots (less than 1.8×10^{3} cfu g^{-1} of root).

To further examine the role of antibiotics production in rhizoplane colonization, melon seeds treated with antagonists as mentioned above were planted in soil heavily infested by several soil fungi. The infested soil was prepared by the following way: wheat bran sterilized by autoclaving was inoculated separately with *R. solani*, *F. oxysporum* f. sp. melonis, *V. dahliae* or *Penicillium notatum* and incubated for 10 days at 28 °C. These fungi, grown in the bran, were mixed together and inoculated into the soil sample, at 4% (w/w), in which melon seeds treated with antagonists were transplanted. After 10 days incubation, populations of Tn5 mutant and wt were determined as mentioned above.

Tn5 mutants and wt were co-inoculated in a 1:1 ratio into 1/10 NB at an initial density of 4×10^{6} cfu ml^{-1} and incubated with shaking (75 rev min^{-1}) at 28 °C. After 24 h, when the density reached 4×10^{8} cfu ml^{-1}, the numbers of Tn5 mutants and wt were determined as mentioned above.

Effects of motility on the rhizoplane competence of MelRC2Rif

Seed inoculation

Melon and tomato (*Lycopersicon esculentum* cv. Odoriko) seeds treated with antagonists were planted in the soil sample. After 10 days incubation, washed roots were separated into halves and macerated and populations of wt and non-motile mutants (M2 and M6) were enumerated. The procedures were the same as those for seeds treated with antibiotics-production negative mutants.

Soil inoculation

Sterile melon and tomato seeds were grown in the soil sample inoculated with wt and non-motile mutant M2 in a 1:1 ratio at a concentration of 3×10^{6} cfu g^{-1}. After 10 days incubation, roots were separated into halves and macerated and populations of wt and non-motile mutants were enumerated as mentioned above.

Inoculation into agar medium

To avoid the effects of soil physical structure, tomato seeds surface-sterilized in 70% ethanol for 2 min, rinsed with SDW and preemerged on 1/10 NA at 28 °C for 2 days were aseptically planted in 0.2% water agar containing wt and non-motile mutant M2 in a 1:1 ratio at a concentration of 6×10^{6} cfu ml^{-1}. After 10 days incubation, roots were macerated and populations of wt and non-motile mutants were

enumerated as above mentioned. To investigate the time course of colonization on roots by wt and a non-motile mutant, tomato plants grown in 0.2% water agar for 10 days in the greenhouse are deliberately taken out, washed with SDW and transferred into fresh 0.2% water agar containing MelRC2Rif and non-motile mutant M2 in a 1:1 ratio at a concentration of 10^6 cfu ml^{-1}. Roots were periodically taken out and populations of wt and a non-motile mutant were enumerated as above mentioned.

Results

Isolation of Tn5 mutants of MelRC2Rif

Putative Tn5 mutants were obtained at a frequency of 10^{-7} following more than 10 independent matings between MelRC2Rif and *E. coli* S17-1 (pSUP10141). Of the 3390 Km^r transconjugants, 11 (0.3%) mutants were motility-negative on 1/10 NA with 0.2% agar and 10 mutants (0.3%) were antibiotics-production negative on PDA against *R. solani*. Out of these, two mutants M2 and M6 were selected as motility-negative mutants on the basis of similar growth rates, colony morphologies, siderophore production and antibiotics production to the parental strain and not auxotrophs. Both M2 and M6 lost flagella (data not shown). Mutants K6 and K8 were selected as antibiotics-production negative mutants on the basis of similar growth rates, similar colony morphologies, siderophore production and motility to the parental strain and not auxotrophs.

Effects of in vitro antibiosis on rhizoplane colonization of MelRC2Rif

MelRC2Rif (wt) inhibited growth of *Pythium aphanidermatum*, *R. solani* and *F. oxysporum* f. sp. *melonis* on PDA, KMB and KMBGlu and the inhibition zones were ca. 2 times greater on PDA than KMB and KMBGlu, suggesting antibiotics production other than siderophores (data not shown). But wt did not inhibit the growth of the three pathogens on the split plate for the enumeration of volatile inhibitors, although cyanide production was detected. Both non-antibiotics-producing mutants K6 and K8 showed no inhibition to the three pathogens on any media tested, although cyanide production was still detected, suggesting little contribution of cyanide production to the in vitro antibiosis observed.

The role of antibiotics production in the process of rhizoplane colonization of wt was assessed comparing wt and a Tn5 mutant K6 or K8 defective in antibiotics production. There was no significant ($P<0.05$) change in ratios of K6:wt following rhizoplane colonization, which were 58% when melon seeds were dipped in bacterial suspension and 51% after 10 days of cultivation. Conversely, the ratio of K8:wt increased significantly ($P<0.05$) after rhizoplane colonization: 51% at time 0 and 59% after 10 days. To challenge the role of antibiotic production, melon seeds were grown in soil heavily infested by *R. solani*, *F. oxysporum* f. sp. *melonis*, *V. dahiae* and *Penicillium notatum*. There was still no significant ($P<0.05$) decrease in ratio of K6:wt in rhizoplane colonization: 51% at time 0 and 57% on the roots grown for 10 days. When wt and K6 were co-incubated in 1/10 NB for 24 h, the ratio of K6:wt did not differ significantly ($P<0.05$) between time 0 (53%) and 24 h after inoculation (59%).

Effects of motility on rhizoplane colonization of MelRC2Rif

Seed inoculation

When melon or tomato seeds were co-inoculated in a 1:1 ratio with wt and a Tn5 mutant M2 or M6 defective in motility, a significant ($P<0.05$) reduction of ratios of M2 or M6 to wt was observed in rhizoplane colonization (Table 1). This reduction was greater in lower parts of roots than in upper parts (Table 1). When wt and M2 were co-incubated in 1/10 NB for 24 h, there was no significant ($P<0.05$) change in ratio of M2 to wt: 49% at time 0 and 50% at 24 h.

Table 1 Change in the ratio of a non-motile mutant: the parental strain *Pseudomonas fluorescens* MelRC2Rif (wt) in the process of rhizoplane colonization when applied onto seed or into soil (*ND* not determined)

Method of inoculation	Plants	Combination	Parts of root	Populations on rhizoplane (log cfu g^{-1} root)		Ratio (%) of mutant:wt		
				Mutant + wt	Culturable bacteria	Time 0	10 days after	LSD ($P<0.05$)
Seed	Melon	M2:wt	Upper Lower	5.89±0.05 5.81±0.11	8.23±0.15 8.21±0.03	45.1±0.1	39.0±2.4 18.8±4.1	3.9
Seed	Melon	M6:wt	Upper Lower	ND ND	ND ND	42.7±0.5	20.4±4.4 11.5±6.3	6.3
Seed	Tomato	M2:wt	Whole	5.81±0.22	ND	44.7±0.2	31.2±6.4	6.4
Soil	Melon	M2:wt	Upper Lower	5.54±0.07 6.27±0.06[a]	ND ND	45.1±0.1	54.6±1.0 45.0±3.8	4.5
Soil	Tomato	M2:wt	Whole	5.45±0.22	8.12±0.02	44.7±0.2	44.6±5.5	5.5

[a] Significantly different between upper and lower parts

Soil inoculation

When sterile melon or tomato seeds were planted in soil inoculated with wt and M2 in a 1:1 ratio, there was no significant ($P < 0.05$) decrease in ratios of M2:wt in rhizoplane colonization (Table 1).

Inoculation into agar medium

When sterile tomato seeds were planted in 0.2% water agar inoculated with wt and M2 in a 1:1 ratio, a drastic decline in ratios of M2:wt was observed following rhizoplane colonization, especially in lower parts of roots (Table 2). When 10-dayold tomato seedlings were planted in 0.2% water agar containing wt and M2, wt colonized tomato roots more rapidly than M2, especially in the first 6 h, resulting in a more rapid decline of ratios of M2:wt for the first 6 h (Table 3). In contrast, the ratio of M2:wt in 0.2% water agar at 10 days remained significantly unchanged ($P < 0.05$) (Table 3).

Table 2 Change in the ratio of a non-motile mutant M2 to the parental strain *Pseudomonas fluorescens* MelRC2Rif (wt) on tomato roots grown in 0.2% water agar into which bacteria were inoculated. Values in a column followed by the same *letter* are not significantly different ($P<0.05$)

	Parts of root	Populations (M2+wt) on rhizoplane (log cfu g^{-1} root)	Ratio (%) of M2:wt
Time 0			42.8±0.2a
After 10 days			
	Upper	6.67±0.08a	16.4±1.7b
	Lower	5.35±0.21b	3.6±3.1c
LSD ($P<0.05$)		0.22	2.9

Table 3 Time course enumeration of rhizoplane colonization of tomato roots grown in 0.2% water agar by *Pseudomonas fluorescens* MelRC2Rif (wt) and a non-motile mutant M2. Values in a column followed by the same *letter* are not significantly different ($P<0.05$)

Incubation time (h)	Parts of roots	Populations (M2+wt) on rhizoplane (log cfu g^{-1} root or ml^{-1}	Ratio (%) of M2:wt
0	Agar	6.79±0.01	48.3±0.2a
1	Whole	6.23±0.16a	44.8±1.3a
2	Whole	6.41±0.22a	39.3±3.0b
6	Whole	6.77±0.14b	27.0±3.5c
24	Whole	7.15±0.11c	24.7±7.8c
240	Whole	7.67±0.02d	16.0±3.8de
	Upper	7.56±0.24d	18.7±3.1d
	Lower	7.70±0.18d	12.7±3.6e
	Agar	6.47±0.04	46.0±2.0a
LSD ($P<0.05$)		0.20	5.5

Discussion

This work reports the estimation of the relative importance of motility and antibiotics production in the rhizoplane competence of *Pseudomonas fluorescens* MelRC2Rif, using on non-motile and non-antibiotics-producing mutants. These mutants were obtained by mutagenesis with Tn5, a transposon that is known to rarely insert more than one site per genome (Berg and Berg 1983).

When melon seeds were coinoculated with a Tn-5 mutant K6 or K8, both of which were defective in in vitro antibiosis, and the parental strain *P. fluorescens* MelRC2Rif (wt) in a 1:1 ratio, there were no changes in ratios of K6 or K8 to wt in rhizoplane colonization, suggesting little contribution of antibiotics production in the rhizosphere competence of MelRC2Rif. Similar results were obtained even when melon seeds were planted in soil heavily infested with fungal pathogens, where antibiotics production could have been a powerful means for colonization. Mazzola et al. (1992) reported that phenazine antibiotics production contributes to the ecological competence of *P. fluorescens* in the rhizosphere of wheat, and that short-term experiments might be insufficient for assessing the importance of a particular trait. In contrast, many other studies have reported that the loss of antibiotic-producing ability did not alter the ability of *Pseudomonas* spp. to colonize the rhizosphere (Kloepper and Schroth 1981; Thomashow and Weller 1988; Howie and Suslow 1991; Fenton et al. 1992; Pierson and Thomashow 1992; Carroll et al. 1995). Thus, antibiotics production may have little relevance to the rhizosphere competence of *Pseudomonas* strains, at least in an early stage of rhizoplane colonization.

Co-inoculation of a non-motile mutant and wt revealed that motility might contribute to the rhizoplane colonization when these strains were inoculated onto seeds. This effect was greater in the lower parts of roots, i.e. more distant from the inoculation sites (Table 1). But this was not the case when these strains were co-inoculated into soil instead of seeds (Table 1). These results suggested that motility might have a role in the movement along roots and probably does not operate in the movement from bulk soil towards roots. Arora and Gupta (1993) also reported that bacteria showed no chemotactic responses to fungal propagules in soil at a matric potential of –15 and –20 kPa, although significant responses were observed in soil at greater than –10 kPa. This was supported by the experiments using 0.2% water agar instead of soil: when a non-motile mutant M2 and wt were co-inoculated into 0.2% water agar, there was a drastic reduction in ratios of M2:wt in rhizoplane colonization, suggesting that motility had an advantage in rhizoplane colonization especially under conditions where no structual hinderance existed (Table 2). In addition, time course enumeration of rhizoplane colonization by M2 and wt revealed that motility was an important means in the movement towards and/or along roots rather than the multiplication on roots, since a drastic decline in the ratio of M2:wt was observed in the first 6 h (Table 3). Consequently, motility can potentially enhance

rhizoplane competence both in terms of the movement from bulk soil to roots as well as along roots. However, soil structure may hinder the former advantage in the real world.

Contrary to our findings, many other studies have reported that motility of *P. fluorescens* did not affect its root colonization when it was applied by either seed or soil inoculation (Howie et al. 1987; Scher et al. 1988; Bowers and Parke 1993; Boelens et al. 1994), except for De Weger et al. (1987) who found that bacterial motility was required for potato root colonization by *P. fluorescens*. One possibility that may explain the differences between our study and others is that in this study a parental strain and its mutants were simultaneously inoculated, thereby enabling sensitive estimation of the effect.

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References

Ahmad JS, Baker R (1987a) Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77:182–189

Ahmad JS, Baker R (1987b) Competitive saprophytic ability and cellulolytic activity of rhizosphere-competent mutants of *Trichoderma harzianum*. Phytopathology 77:358–362

Anderson AJ, Habibzadegah-Tari P, Tepper CS (1988) Molecular studies on the role of a root surface agglutinin in adherence and colonization by *Pseudomonas putida*. Appl Environ Microbiol 54:375–380

Arora DK, Gupta S (1993) Effect of different environmental conditions on bacterial chemotaxis towards fungal spores. Can J Microbiol 39:922–931

Berg DE, Berg CM (1983) The prokaryotic transposable element Tn5. Biotechnology 1:417–435

Boelens J, Woestyne MV, Verstraete W (1994) Ecological importance of motility for the plant growth-promoting rhizopseudomonas strain ANP15. Soil Biol Biochem 26:269–277

Bowers JH, Parke JL (1993) Colonization of pea (*Pisum sativum* L.) taproots by *Pseudomonas fluorescens*: effect of soil temperature and bacterial motility. Soil Biol Biochem 25:1693–1701

Carroll H, Moenne-Loccoz Y, Dowling DN, O'Gara F (1995) Mutational disruption of the biosynthesis genes coding for the antifungal metabolite 2,4-diacetylphloroglucinol does not influence the ecological fitness of *Pseudomonas fluorescens* F113 in the rhizosphere of sugarbeets. Appl Environ Microbiol 61:3002–3007

Castric PA (1975) Hydrogen cyanide, a secondary metabolite of *Pseudomonas aeruginosa*. Can J Microbiol 21:613–618

Chao WL, Li RK, Chang WT (1988) Effect of root agglutinin on microbial activities in the rhizosphere. Appl Environ Microbiol 54:1838–1841

Deacon JW (1991) Significance of ecology in the development of biocontrol agents against soil-borne plant pathogens. Biocontrol Sci Technol 1:5–20

De Weger LA, Vlugt CIM van der, Wijfjes AHM, Bakker PAHM, Schippers B, Lugtenberg B (1987) Flagella of a plant-growth-stimulating *Pseudomonas fluorescens* strain are required for colonization of potato roots. J Bacteriol 169:2769–2773

Fenton AM, Stephens PM, Crowley J, O'Callagham M, O'Gara F (1992) Exploitation of gene(s) involved in 2,4-diacetylphloroglucinol biosynthesis to confer a new biocontrol capability to a *Pseudomonas* strain. Appl Environ Microbiol 58:3873–3878

Heinrich D, Hess D (1985) Chemotactic attraction of *Azospirillum lipoferum* by wheat roots and characterization of some attractants. Can J Microbiol 31:26–31

Howie WJ, Suslow TV (1991) Role of antibiotic biosynthesis in the inhibition of *Pythium ultimum* in the cotton spermosphere and rhizosphere by *Pseudomonas fluorescens*. Mol Plant Microbe Interact 4:393–399

Howie WJ, Cook RJ, Weller DM (1987) Effects of soil matric potential and cell motility on wheat root colonization by fluorescent pseudomonads suppressive to take-all. Phytopathology 77:286–292

Kerr A (1980) Biological control of crown gall through production of agrocin 84. Plant Dis 64:25–30

King EO, Ward MK, Raney DE (1954) Two simple media for the demonstration of pyocyamin and fluorescin. J Lab Clin Med 44:301–307

Kloepper JW, Schroth MN (1981) Relationship of *in vitro* antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. Phytopathology 71:1020–1024

Loper JE, Haack C, Schroth MN (1985) Population dynamics of soil pseudomonads in the rhizosphere of potato (*Solanum tuberosum* L.) Appl Environ Microbiol 49:416–422

Mazzola M, Cook RJ, Thomashow LS, Weller DM, Pierson LS III (1992) Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. Appl Environ Microbiol 58:2616–2624

Misaghi IL, Olsen MW, Billotte JM, Sonoda RM (1992) The importance of rhizobacterial mobility in biocontrol of bacterial wilt of tomato. Soil Biol Biochem 24:287–293

Parke JL (1991) Root colonization by indigenous and introduced microorganisms. In: Keister DL,Cregan PB (eds) The rhizosphere and plant growth. Kluwer, Dordrecht, pp 33–42

Pierson LS III, Thomashow L (1992) Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens* 30–84. Mol Plant Microbe Interact 5:330–339

Scher FM, Kloepper JW, Singleton CA (1985) Chemotaxis of fluorescent *Pseudomonas* spp. to soybean seed exudates *in vitro* and in soil. Can J Microbiol 31:570–574

Scher FM, Kloepper JW, Singleton C, Zaleska I, Laliberte M (1988) Colonization of soybean roots by pseudomonas and serratia species: relationship to bacterial motility, chemotaxis, and generation time. Phytopathology 78:1055–1059

Simon R, Priefer U, Pühler A (1983) A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram negative bacteria. Biotechnology 1:784–791

Tari PH, Anderson AJ (1988) Fusarium wilt suppression and agglutinability of *Pseudomonas putida*. Appl Environ Microbiol 54:2037–2041

Thomashow L, Weller DM (1988) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. J Bacteriol 170:3499–3508

Vesper SJ (1987) Production of pili (fimbriae) by *Pseudomonas fluorescens* and correlation with attachment to corn roots. Appl Environ Microbiol 53:1397–1403

Weller DM (1988) Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu Rev Phytopathol 26:379–407

Weller DM, Thomashow LS (1994) Current challenges in introducing beneficial microorganisms into the rhizosphere. In: O'Gara F, Dowling DN, Boesten B (eds) Molecular ecology of rhizosphere microorganisms. VCH, Weinheim, pp 1–18