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Rhizosphere bacteria antagonistic to soybean cyst (*Heterodera glycines*) and root-knot (*Meloidogyne incognita*) nematodes: Identification by fatty acid analysis and frequency of biological control activity

JOSEPH W. KLOEPPER, RODRIGO RODRÍGUEZ-KÁBANA, JOHN A. McINROY and REBECCA W. YOUNG
Department of Plant Pathology and Alabama Agricultural Experiment Station, Auburn University, Auburn, AL 36849-5409, USA

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Abstract

Rhizosphere bacteria were isolated from roots of young and mature plants with known antagonism to phytopathogenic nematodes, including velvet bean (*Mucuna deeringiana*), castor bean (*Ricinus communis*), sword bean (*Cannavalia ensiformis*), and Abruzzi rye (*Secale cereale*). Isolates from antagonistic plants were compared to soybean isolates for the frequency of antagonism to the root-knot (*Meloidogyne incognita*) and soybean cyst (*Heterodera schachtii*) nematodes in a disease assay with soybean. Bacterial isolates were identified using fatty acid analysis, and isolates which exhibited a significant reduction in incidence of soybean damage from both nematodes were characterized physiologically. The bacterial taxa associated with antagonistic plants were markedly different from soybean bacteria. Isolates from soybean were predominantly *Bacillus* spp., while those from antagonistic plants included more coryneform and Gram-negative genera. *Pseudomonas cepacia* and *Pseudomonas gladioli* were predominant among Gram-negative bacteria on antagonistic plants but were not isolated from soybean. Four to six times the number of bacteria from antagonistic plants, compared to soybean, significantly reduced disease incidence of both nematodes. No single pattern of physiological reactions was common among all these bacteria, suggesting that multiple mechanisms accounted for the observed biological control. The results suggest that rhizospheres of antagonistic plants may be useful sources of potential biological control agents for phytopathogenic nematodes.

Introduction

Historically, research on biological control of nematodes has focussed on the use of organic amendments to stimulate soil microorganisms antagonistic to nematodes (Rodríguez-Kábana and Morgan-Jones, 1987) and on the use of predatory or parasitic microorganisms (Jatala, 1986; Sayre and Starr, 1988). As recently as 1989, Dropkin stated that in nematology, 'the term biological control refers specifically to parasites and predators' (Dropkin, 1989). Although promising levels of control have been realized with amendments and parasites and predators, both approaches have disadvantages for widespread use in commercial agriculture. Many organic amendments must be incorporated into soil at levels of several tonnes per hectare. Predatory or parasitic microorganisms often decline after introduction in soils due to competition from indigenous soil microorganisms.

One alternative approach toward nematode biocontrol involves the use of rhizobacteria as seed treatments. Rhizobacteria are those root-zone bacteria which colonize roots in the presence of the indigenous soil microflora (Schroth and Hancock, 1982). Rhizobacteria which exert beneficial effects on plant development through growth promotion and/or biological control are termed plant growth-promoting rhizobacteria (PGPR) (Kloepper et al., 1990). Select strains of PGPR have been reported as biological control agents against several soil-borne plant-pathogenic fungi (Kloepper, 1991).

Two recent reports demonstrate that PGPR may reduce plant damage caused by phytopathogenic nematodes. Becker et al. (1988) reported a reduction in gall number caused by *Meloidogyne incognita* on cucumber in a soil-less assay following treatment with select strains of rhizobacteria. Oostendorp and Sikora (1989) isolated strains of *Pseudomonas fluorescens* which reduced early root infection of sugar beet by *Heterodera schachtii* in field trials.

We have begun investigations on PGPR as biological control agents with the long-term goal of assessing their practical value in commercial agriculture. The first step toward this goal involves isolations of antagonistic bacteria from sources where nematode populations are adequate to cause disease, but where disease is lacking. In a previous study (Kloepper et al., 1991), we reported that rhizosphere bacteria from antagonistic plants were physiologically distinct from those of soybean, suggesting that specific groups of microorganisms have become adapted to rhizospheres of antagonistic plants. The objective of this study was to further investigate rhizospheres of antagonistic plants by examining them as sources for bacterial biological control agents for phytopathogenic nematodes. An additional objective was to classify representative bacteria from rhizospheres of antagonistic plants and soybean using fatty acid analysis (Jantzen and Hofstad, 1985; Moss et al., 1982; Rosoamananjara et al., 1986), thereby identifying specific bacterial taxa associated with nematode antagonism. The results will be used to plan strategies for isolation of candidate biocontrol rhizobacteria.

Materials and methods

Isolation and physiological characterization of bacteria

Rhizosphere bacteria were isolated from roots of young and mature plants of velvet bean (*Mucuna deeringiana*), castor bean (*Ricinus communis*) sword bean (*Cannavalia ensiformis*), and Abruzzi rye (*Secale cereale*), all of which are antagonistic to phytoparasitic nematodes (Grandados-Alvarez, 1989; Lear, 1959; Pedersen and Rodríguez-Kábana, 1991; Rodríguez-Kábana et al., 1990; Rodríguez-Kábana et al., 1988; Vincente and Acosta, 1987), and from soybean (*Glycine max*) cv. 'Davis' (regarded as susceptible to root knot and soybean cyst nematodes) using methods previously described (Kloepper et al., 1991). Isolations for this study were done on 5% tryptic soy agar (TSA) (Difco, Detroit, MI). After incubation at 28°C for 48 hr, 50 bacterial colonies were randomly selected from each plant sample at both plant ages.

Selected colonies were purified on TSA prior to placing in ultra-cold storage. For storage, bacteria were grown 24–48 hr in tryptic soy broth (TSB) (Difco), centrifuged at 6,000 rpm for 5 min, rinsed with 20 mM phosphate buffer pH 7.0 (PB), resuspended in TSB amended with 20% sterile glycerol, and frozen in vials at −80°C. All bacterial isolates were physiologically characterized for chitinolytic activity, gelatin and starch hydrolysis, phenol oxidation, antibiosis toward *Pythium ultimum* and *Rhizoctonia solani*, and siderophore production using methods previously reported (Kloepper et al., 1991).

Bacterial identification

Identification of all bacterial isolates from young and mature antagonistic plants and soybean was based on analysis of fatty acid methyl-esters (FAMEs) (De Boer and Sasser, 1986; Gitaitis and Beaver, 1990; Rizzo et al., 1987). In preparation for FAME analysis, frozen bacterial isolates were streaked on TSA, incubated 24–48 h at 28°C, and rechecked for purity. A 4-mm-diameter loopful (40 mg) of each isolate was placed in the bottom of a glass tube.

Saponification was conducted by adding to each tube 1.0 mL of saponification reagent (45 g sodium hydroxide, 150 mL methanol, 150 mL deionized H_2O), vortexing for 10 sec, heating for 5 min in a 100°C water bath, vortexing 10 sec, and reheating for 25 min. Tubes were then cooled in room-temperature water. Methylation was accomplished by adding 2.0 mL of methylation reagent (325 mL 6.00 *N* HCl, 275 mL methanol), vortexing for 10 sec., and heating for 10 min in a water bath at 80°C. After cooling to room temperature, FAMEs were removed from the acidic aqueous phase and transferred to an organic phase by adding 1.25 mL of extraction solvent (200 mL hexane, 200 mL methyl-tert butyl ether [MTBE]), mixing end-over-end in a laboratory rotator for 10 min., and discarding the lower aqueous phase with a Pasteur pipette. The organic phase was washed in 3.0 mL of base wash (10.8 g NaOH, 900 mL d H_2O) for 5 min with end-over-end mixing. Two -thirds of the upper solvent phase was removed for FAME analysis. Prepared samples were stored at −20°C until analysis in the gas chromatograph.

FAMEs were analysed with a Hewlett-Packard series II gas chromatograph model 5890 equipped with a 25-m × 0.2-mm × 0.33 μ phenyl methyl silicone capillary column. Samples were processed with the Microbial Identification System (MIS) of MIDI (Newark, DE) which calibrated the gas chromatograph with a commercial FAME mixture (MIDI) prior to running samples and after every tenth sample. FAME peaks were named by the MIS software, and bacterial isolates were identified using the MIS 'Aerobe Library' (October, 1990 update). Bacterial strains identified by Aerobe Library as the closely related species *Pseudomonas cepacia* or *P. gladioli* were examined further for the ability to use levulinate as the sole carbon source, since *P. cepacia* has this ability, while *P. gladioli* does not (Palleroni, 1984), using the protocol of Hildebrand et al. (1988).

Biological control activity against phytopathogenic nematodes

Field soil naturally infested with the root-knot and soybean cyst nematodes was passed through a 1-mm screen, and the numbers of *M. incognita* and *H. glycines* juveniles were determined using the 'salad bowl' technique (Rodríguez-Kábana and Pope, 1981). Juvenile populations were adjusted by mixing sand to have a final population of 50—100 juveniles 100 cm^{-3} soil. Soybean seed (cultivar 'Davis') was planted in a container (a plastic cylindrical tube, 4 cm diam × 20 cm height) containing the nematode-infested soil/sand mixture and a glass-wool plug at the bottom. Four replicate plants were used per treatment. The experimental design was a completely randomized block with treatments consisting of different rhizobacteria strains and controls consisting of nontreated and aldicarb treatment. Roots were evaluated after 6 wk growth in the greenhouse for both gall and cyst severity using a 1—10 scale (10 = maximum galls or cysts; 0 = no galls or cysts) (Zeck, 1971). Data were analysed using SAS analysis, and treatment means from tests with a significant F value were evaluated for statistical difference to control means using LSD at $p = 0.05$.

Bacterial inocula were prepared by streaking from −80°C storage onto TSA and evaluating for purity after 48 hr incubation at 28°C. Single colonies were transferred to 15 mL TSB and incubated with shaking for 48 hr. Cultures were centrifuged; pellets were rinsed in PB; and cells were resuspended in 15 mL PB, which resulted in an average population of log 9 cfu mL^{-1}. Bacterial suspensions were applied to seeds immediately prior to covering with soil by pipetting 100 μL suspension per seed.

Results

Bacterial identification

Pronounced differences were found in the bacterial taxa associated with plants antagonistic to phytopathogenic nematodes in comparison to soybean (Table 1). Forty of 43 identifiable isolates from young soybean and 40 of 41 from mature soybean were identified as Gram-positive genera. In comparison, Gram-negative isolates occurred at higher frequencies from antagonistic plants, representing 13 and 29 isolates from

young and mature velvet bean, 18 and 37 from young and mature castor bean, 22 and 19 from young and mature Abruzzi rye, and 37 and 29 from young and mature sword bean. Of the Gram-positive isolates from young soybean, 36 were species of *Bacillus*, and only four were identified as the coryneform genus *Arthrobacter*. No coryneform types were identified from ma-

Table 1. Identification of bacteria from rhizospheres of antagonistic plants and soybean[a]

Bacterial group	Taxon	No. of isolates from rhizospheres of indicated plants									
		Young plants[b]					Mature plants[b]				
		VB	C	AR	SW	SB	VB	C	AR	SW	SB
Gram-positive											
Coryneform	*Arthrobacter* sp.	5	3	6	3	4	0	0	2	1	0
	Aureobacterium sp	5	0	0	0	0	0	0	1	1	0
	Clavibacter michiganese	0	0	0	0	0	5	0	5	2	0
	Corynebacterium aquaticum	0	0	1	2	0	1	1	6	5	0
	Curtobacterium flaccumfaciens	0	0	0	0	0	2	1	0	0	0
Others	*Bacillus laterosporus*	0	0	4	1	0	0	0	0	0	3
	B. megaterium	10	20	12	0	7	0	0	5	0	17
	B. pabuli	0	0	0	0	5	0	0	0	1	0
	B. polymyxa	0	0	0	0	2	0	0	1	0	0
	B. pumulis	7	0	1	2	16	3	0	0	0	13
	B. sphaericus	0	0	0	0	3	0	0	0	1	3
	B. thuringiensis	3	4	0	0	2	0	0	0	0	4
	Microbacterium sp.	0	0	0	0	1	1	1	2	2	0
	Subtotal	30	27	24	8	40	12	3	22	13	40
Gram-negative	*Acinetobacter johnsonii*	0	0	0	2	0	0	0	0	0	0
	Agrobacterium radiobacter	0	0	0	0	0	0	1	0	1	0
	Alcaligenes sp.	0	0	0	5	0	0	0	0	2	0
	Aquaspirillum autotrophicum	0	0	0	0	0	0	0	0	1	0
	Enterobacter asburiae	0	3	0	0	0	0	0	0	0	0
	Erwinia herbicola	0	0	0	0	0	0	0	0	0	1
	Flavobacterium sp.	0	2	0	2	2	0	3	0	2	0
	Hydrogenophaga flava	0	3	0	0	0	1	0	2	0	0
	Klebsiella sp.	0	2	0	0	0	0	0	0	1	0
	Phyllobacterium rubiacearum	0	0	0	0	1	3	4	2	3	0
	Proteus vulgaris	0	0	0	0	0	0	0	0	1	0
	Subtotal	0	10	0	9	3	4	8	4	11	1
	Pseudomonas aureofaciens	8	0	10	9	0	0	0	0	0	0
	P. cepacia	0	0	0	15	0	15	11	7	7	0
	P. fluorescens	0	0	5	0	0	0	0	0	0	0
	P. gladioli	5	0	0	0	0	9	10	3	8	0
	P. pickettii	0	0	0	0	0	0	2	4	0	0
	P. putida	0	8	5	4	0	0	0	0	0	0
	P. solanacearum	0	0	0	0	0	1	1	1	0	0
	P. syringae	0	0	1	0	0	0	0	0	0	0
	Serratia marcescens	0	0	0	0	0	0	4	0	0	0
	Sphingobacterium sp.	0	0	0	0	0	0	0	0	1	0
	Xanthomonas maltophilia	0	0	1	0	0	0	1	0	2	0
	Unknown[c]	7	5	4	6	7	9	10	9	8	9
	Subtotal	20	12	26	33	7	34	39	24	26	9
	Grand total	50	50	50	50	50	50	50	50	50	50

[a] Figures represent the number of isolates of 50 tested which were identified to each taxon using fatty acid methyl-ester (FAME) analysis.
[b] VB = velvet bean; C = castor bean; AR = Abruzzi rye; SW = sword bean; SB = soybean.
[c] 'Unknown' stains are those with no match to strains in the FAME library.

ture soybean. In contrast, with antagonistic plants, larger numbers of isolates were identified as coryneform bacteria from all mature plants, and from all young plants except castor bean.

The closely related species *Pseudomonas cepacia and P. gladioli* represented the dominant group of isolates from mature plants of all antagonistic plants (Table 1). These two species increased in frequency with castor bean, for example, from no strains from young plants to 21 strains from mature plants. None of the isolates from young or mature soybean was identified as *P. cepacia* or *P. gladioli*.

The overall diversity of bacterial genera in rhizospheres was greatest for mature sword bean (16 genera) and lowest for mature soybean (2 genera). Diversity decreased with age for soybean but increased or remained the same for all antagonistic plants.

Some bacterial genera were only isolated from one of the test plants. This crop-specific association was noted for Acinetobacter, Alcaligenes, Aquaspirillum, Proteus, and Sphingobacterium from sword bean and for *Enterobacter* and *Serratia* from castor bean. Some bacteria demonstrated an age-specific association with the host plant. *Pseudomonas aureofaciens* and *Bacillus megaterium* were isolated from 3 of the 4 young antagonistic plants but from none and one of the mature plants, respectively.

Biological control activity against phytopathogenic nematodes and physiological characterization of bacteria

The frequency with which bacterial isolates exhibited biological control activity toward nematodes was affected by the source plant of bacteria (Table 2). Seed treatment with 35 of 50 bacterial isolates from soybean induced a significant reduction in gall formation by *M. incognita*, while only 11 to 14 isolates from antagonistic plants induced similar reductions. With *H. glycines*, the results were completely different. Only three isolates from soybean significantly reduced cyst numbers ($p = 0.05$), while 16 to 31 isolates from antagonistic plants inhibited cyst development. Four to six times the number of bacteria from antagonistic plants significantly reduced numbers of galls and cysts compared to the number with similar activity from soybean.

The identification and physiological characterization of those bacteria which significantly reduced numbers of both galls and cysts are shown in Table 3. Some bacteria with dual biological control activity were unique to each source plant. *Hydrogenophaga flava* and *Pseudomonas pickettii* biological control agents were only found on Abruzzi rye; Alcaligenes, Klebsiella, Aureobacterium, and Xanthomonas biological control agents were only found on sword bean;

Table 2. Frequency of antagonism to *Meloidogyne incognita* and *Heterodera glycines* among bacteria isolated from rhizospheres of plants showing nematode antagonism in comparison to soybean

	No. of isolates[a] which induced a significant reduction ($p = 0.05$) in mean symptom ratings compared to nontreated controls for the indicated nematode on soybean				
Plant from which bacteria were isolated[b]	*M. incognita*	Range of control[c]	*H. glycines*	Range of control[c]	Both nematodes
Velvet bean	14	30–65%	16	43–71%	8
Castor bean	11	30–100%	27	43–100%	10
Abruzzi rye	12	30–100%	31	43–100%	12
Sword bean	14	30–100%	25	43–100%	11
Soybean	35	30–87%	3	43–100%	2

[a] Of 50 tested isolates.
[b] All listed plants except soybean are antagonistic to nematodes.
[c] The percentage reduction in symptom rating, compared to nontreated controls.

Table 3. Profiles of physiological reactions for rhizosphere bacterial strains which significantly ($p = 0.05$) reduced soybean damage to root-knot and cyst nematodes

Host plant	Strain no.	Identification[b]	Physiological characterization[a]						
			Chitinolytic activity	Gelatin hydrolysis	Starch hydrolysis	Phenol oxidation	Antibiosis on TSA toward Pythium	Antibiosis on TSA toward Rhizoctonia	Siderophore production
Velvet bean	1230	*Pseudomonas gladioli*	−	+	−	−	+	+	+
	1231	*Phyllobacterium rubiacearum*	−	−	−	+	−	−	−
	1410	*Pseudomonas cepacia*	−	−	−	−	+	+	+
	1416	*Pseudomonas cepacia*	−	−	−	−	+	+	+
	1419	*Pseudomonas gladioli*	−	−	−	+	−	+	+
	1420	*Phyllobacterium rubiacearum*	−	−	−	+	−	−	−
	1539	Unknown	−	−	−	+	−	−	−
	1563	*Pseudomonas gladioli*	−	−	−	−	+	+	+
Castor bean	1619	*Corynebacterium aquaticum*	−	−	−	−	−	−	−
	1674	*Pseudomonas cepacia*	−	−	−	+	+	−	+
	1675	Unknown	−	−	−	+	−	−	−
	1676	Unknown	−	−	−	+	−	−	−
	1677	*Pseudomonas gladioli*	−	−	−	+	−	−	−
	1680	Unknown	−	−	−	+	−	W	−
	1681	*Pseudomonas gladioli*	−	−	−	+	−	−	+
	1682	*Agrobacterium radiobacter*	−	−	−	+	−	−	−
	1684	*Pseudomonas cepacia*	−	−	+	−	+	+	+
	1687	*Pseudomonas gladioli*	−	−	−	+	−	−	+

Abruzzi rye	1736	*Hydrogenophaga flava*	–	–	–	+	–	–	–
	1746	*Pseudomonas pickettii*	–	–	–	–	–	–	+
	1758	*Bacillus megaterium*	–	–	+	+	+	+	–
	1760	*Corynebacterium aquaticum*	–	–	–	+	+	+	–
	1761	*Clavibacter michiganense*	–	–	+	–	–	–	–
	1764	*Corynebacterium aquaticum*	–	–	–	+	–	–	–
	1769	Unknown	–	–	+	–	–	–	–
	1774	*Clavibacter michiganense*	–	–	–	+	–	–	+
	1776	Unknown	–	–	+	–	–	–	–
	1778	Unknown	–	–	–	–	–	–	–
	1785	*Pseudomonas cepacia*	–	–	–	+	+	+	+
	1786	Unknown	–	–	–	–	–	–	–
Sword bean	1470	*Alcaligenes* sp.	–	–	–	–	–	–	–
	1471	Unknown	–	–	–	–	–	–	–
	1478	*Klebsiella terrigena*	–	–	–	–	–	W	–
	1481	*Pseudomonas gladioli*	–	–	–	+	–	–	+
	1705	*Aureobacterium barkeri*	–	–	+	+	–	+	–
	1707	*Clavibacter michiganense*	–	–	+	–	–	–	–
	1710	*Pseudomonas cepacia*	–	–	–	–	+	+	+
	1712	*Xanthomonas maltophilia*	+	–	–	W	–	–	–
	1715	*Pseudomonas cepacia*	–	–	–	+	–	–	+
	1722	*Pseudomonas cepacia*	–	–	–	–	+	+	+
	1724	*Corynebacterium aquaticum*	–	–	–	+	–	–	–
Soybean	163	*Bacillus pumilus*	–	+	–	–	–	+	–
	165	*Bacillus* sp.	–	+	–	–	–	+	–

[a] W = weakly positive.
[b] Identification was performed using fatty acid methyl-ester (FAME) analysis. 'Unknown' strains are those with no match to strain in the FAME library.

Phyllobacterium rubiacearum was only found on velvet bean; and *Agrobacterium radiobacter* was only found on castor bean. Bacteria with dual biological control activity which were isolated from more than one plant included *P. cepacia* from all 4 antagonistic plants, *Corynebacterium aquaticum* and *P. gladioli* from 3 of 4 antagonistic plants, and *Clavibacter michiganense* from two antagonistic plants.

No single pattern of physiological reactions was common among all bacteria with dual biological control activity (Table 3). The following four reaction profiles were most common among these bacteria: starch hydrolysis alone, phenol oxidation alone, antibiosis toward fungi plus a positive reaction in at least one other test, and no positive reactions in any test.

Discussion

One hypothesis to explain how antagonistic plants reduce damage to nematodes is that the antagonistic plants may directly affect nematodes by the production of toxic compounds (Alam et al., 1990; Rich et al., 1989). Alternatively, the plants may indirectly affect nematodes by selecting for rhizosphere microorganisms with deleterious effects on nematodes. If the indirect method is contributing to reduction in nematode damage, then rhizospheres of antagonistic plants should support a distinct microflora from nonantagonistic plants. Further, microorganisms from rhizospheres of antagonistic plants should have a higher frequency of inhibition to nematodes than those from nonantagonistic plants. The results of this study support the conclusion that the indirect mechanism is operable for the tested antagonistic plants.

Identification of bacteria by fatty acid analyses indicated that antagonistic plants supported a greater diversity of genera than soybean. Four coryneform genera and 12 Gram-negative genera were isolated only from antagonistic plants and not from soybean (Table 1). The same trend was observed for species within the genus Pseudomonas where the predominant group of pseudomonads isolated from antagonistic plants was the *P. pseudomallei* DNA homology group within the rRNA homology Group II of Palleroni et al. (1973) which consists of *P. cepacia*, and *P. gladioli*. None of the members of this group was isolated from soybean. Similarly low frequencies of fluorescent pseudomonads have been observed in previous studies in our laboratory from soybean and cotton collected from fields in Alabama. Hence, plants which exhibit antagonism to phytopathogenic nematodes supported a distinct rhizosphere microflora compared to soybean. This same conclusion was reached in a previous study based on physiological characterization of bacteria from the same antagonistic plants (Kloepper et al., 1991).

A higher frequency of bacteria from antagonistic plants than from soybean exhibited biological control activity against nematodes based on the number of strains which reduced significantly the symptoms for both root-knot and soybean cyst nematodes (Table 2) and the number which reduced symptoms of soybean cyst alone. The same trend was not observed for bacterial effects on root-knot nematode alone, where the frequeucy of inhibitory bacterial strains was highest for soybean. While this study does not elucidate the reasons for this difference, one possible explanation is that the relatively low diversity of genera from soybean may result in less microbial inactivation of broad-spectrum antibiotics produced by the predominant *Bacillus* spp. (Norris et al., 1981). This low diversity of rhizosphere bacteria and the predominance of *Baccillus* spp. have been observed previously in our laboratory with soybeans from Alabama and may relate to high soil temperatures during early stages of plant growth.

The rRNA homology Group II of Palleroni et al. (1973) (consisting of *P. cepacia* and *P. gladioli*) was the most frequent bacterial taxonomic group containing isolates with dual activity against nematodes. Rhizosphere strains of *P. cepacia* have been reported to colonize roots and increase root hair development (de Freitas and Germida, 1990), to produce wide-spectrum antifungal metabolites (Lambert et al., 1987), and to protect onion seedlings from damping-off disease caused by *Fusarium oxysporum* f. sp. *cepae* (Kawamoto and Lorbeer, 1976). Hence, this group should be further examined as a potential source of phytopathogenic nematode biological control agents, a task which would be

facilitated by the use of TB-T medium (Hagedorn et al., 1987) which was developed for isolation and enumeration of *P. cepacia* from soil. In contrast, very few of the total Bacillus isolates demonstrated dual activity against nematodes. Therefore, although bacilli are frequently encountered in rhizospheres in the southern US, this group may not be a good focus for future phytopathogenic nematode biocontrol investigations.

The in vivo assay for biological control activity was chosen instead of an in vitro assay since the latter would predispose for selection of bacteria which exhibit antagonism based on antibiosis, while the in vivo assay would allow selection of multiple mechanisms of antagonism. Analysis of the physiological test profiles of rhizobacteria which significantly antagonized both the root-knot and cyst nematodes (Table 3), supports the conclusion that multiple mechanisms likely acconnt for the observed biological control activity, as no single pattern of physiological reactions was common among all bacteria. It is interesting that only 10 of 42 rhizobacteria with dual biological control activity exhibited antibiosis to both Pythium and Rhizoctonia, which suggests that broad-spectrum antibiotic-like compounds are not the predominant mechanism for biological control by microorganisms from antagonistic plants. Thirteen rhizobacteria were positive only for phenol oxidation, suggesting that this trait may relate to a commonly occurring mechanism for biological control. How phenol oxidation may relate to biological control is unclear and should be investigated further. Of particular interest is that five rhizobacteria had negative reactions in all physiological tests, showing that phenotypes which account for antagonism in vivo may not relate to phenotypes which can be assessed in vitro. One hypothesis to account for how such strains reduce nematode development would be that they increase host resistance (i.e. induced resistance).

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