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Characterization of Rhizobacteria Associated with Weed Seedlings†

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Rhizobacteria were isolated from seedlings of seven economically important weeds and characterized for potential phytopathogenicity, effects on seedling growth, and antibiosis to assess the possibility of developing deleterious rhizobacteria as biological control agents. The abundance and composition of rhizobacteria varied among the different weed species. For example, fluorescent pseudomonads represented from 11 to 42% of the total rhizobacterial populations from jimsonweed and lambsquarters, respectively. Other bacteria frequently isolated were nonfluorescent pseudomonads, *Erwinia herbicola*, *Alcaligenes* spp., and *Flavobacterium* spp. Only 18% of all isolates were potentially phytopathogenic, based on an *Escherichia coli* indicator bioassay. However, the proportion of isolates that inhibited growth in seedling assays ranged from 35 to 65% depending on the weed host. Antibiosis was most prevalent among isolates of fluorescent *Pseudomonas* spp., the activity of which was due to siderophore production in over 75% of these isolates. Overall, rhizobacterial isolates exhibited a complex array of properties that were inconsistent with accepted definitions for plant growth-promoting and deleterious rhizobacteria. It is suggested that for development of effective biological control agents for weed control, deleterious rhizobacteria must be screened directly on host seedlings and must possess several properties including high colonizing ability, specific phytotoxin production, and resistance or tolerance to antibiotics produced by other rhizosphere microorganisms, and they must either synthesize or utilize other bacterial siderophores.

Weed seedlings emerge yearly from the vast reservoir of viable weed seeds in soils cultivated to crops. The resulting weed infestations require repeated weed control treatments with chemical herbicides over a period of years for successful weed management. Although notable advances in chemical weed control have been made since the 1940s, it is estimated that weeds still reduce yields of all crops grown in the United States by about 12% annually (1).

In recognition of the inadequacies of chemical herbicides, efforts have been developed for exploiting microorganisms for biological weed control (32). One such approach is to select microorganisms that specifically inhibit weed seedling development, thereby hindering the establishment of a weed population competing with crop plants for light, water, and nutrients. In previous studies with crop plants, the colonization of roots by certain bacteria was found to be detrimental to plant development and was implicated as a significant factor in limiting crop growth (7, 10, 28, 31). Many of these deleterious rhizobacteria (DRB) have been identified as members of the genera *Pseudomonas*, *Enterobacter*, *Flavobacterium*, *Citrobacter*, and *Achromobacter* (28, 31). This group of bacteria likely induces damage through the production of phytotoxins that are absorbed by the plant roots. DRB, overlooked in the past due to their nonparasitic and relatively subtle nature of attack on plants, are now considered an important group of bacterial phytopathogens (28, 31). Methods of manipulating the rhizosphere to minimize or eliminate the effects of DRB and benefit crop growth are under development (3, 33).

Suslow and Schroth (31) suggested that DRB are ubiquitous and likely common to all plant root systems. Therefore, such bacteria may well exist of the roots of economically important weeds. Selection for rhizobacteria that are specifically detrimental to weed seedling growth could benefit agriculture by contributing to increased crop yields, by minimizing weed competition, and by reducing the use of chemical herbicides. The presence of soil-borne phytopathogens or rhizobacteria resembling DRB on weed plants has been surveyed (2, 22, 23, 27); however, the potential of these bacteria as biotic agents for weed control was not investigated. There have been only two reports suggesting the use of DRB as a weed management strategy; a preliminary report involving dicotyledonous weeds (17) and a study specifically addressing DRB effects on the grass downy brome occurring in winter wheat fields (4). The present study provides more information about the occurrence and characteristics of bacteria associated with rhizospheres of weeds and the possible importance of selected rhizobacteria regarding their potential as biocontrol agents.

The objectives of this investigation were to (i) identify and characterize naturally occurring rhizobacteria colonizing selected dicotyledonous weed seedlings under field conditions in Missouri, (ii) determine the effects of isolated rhizobacteria on the growth of weed seedlings, and (iii) screen for antibiotic and siderophore activities for evaluating microbial relationships in the rhizosphere.

MATERIALS AND METHODS

Isolation of rhizobacteria from seedling roots. Intact seedlings of seven economically important weeds were collected from soybean, maize, and grain sorghum production fields in Boone and Osage Counties in Missouri periodically during the growing seasons in 1985 and 1986. The weed seedlings sampled were common cocklebur, common lambsquarters, jimsonweed, morning glory, Pennsylvania smartweed, redroot pigweed, and velvetleaf. The plants were kept in sterile

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plastic bags on ice until processed in the laboratory. Roots were removed from plants, shaken vigorously to remove loose soil, and washed twice in sterile distilled water (200 ml) to remove adherent soil particles. The washed roots were placed in milk dilution bottles (160 ml) containing 100 ml of 0.01% Tween 40 and shaken on a rotary shaker at 500 rpm for 10 min. Serial 10-fold dilutions were made in sterile phosphate-buffered saline (10 mM K_2PO_4-KH_2PO_4, 0.14 M NaCl [pH 7.2]) and plated on the medium of Sands and Rovira (SR medium [25]) and tryptic soy agar (Oxoid Ltd.). After 24 h of incubation at 28°C, bacterial colonies were counted; representative colonies were selected from both media and subcultured by streaking onto tryptic soy agar, and then single-colony isolates were characterized. The selection of bacteria was based on distinct types observed according to culture plate morphological characteristics including pigment; colony form, elevation, and margin; texture; and opacity (29). Although SR medium is selective for fluorescent pseudomonads (25), we were able to isolate species of several other genera of gram-negative bacteria. Roots were dried at 60°C for 48 h for root dry weight determinations.

Characterization of rhizobacteria. Isolates of rhizobacteria were characterized based on standard procedures (29) to include tests for Gram stain, oxidase reaction, motility, and morphology. Gram-negative rods were identified by standard procedures (19).

Antimetabolite production. Production of potentially phytotoxic antimetabolites by rhizobacteria was assayed by using the indicator technique of Gasson (11). Freshly grown colonies of rhizobacteria were transferred to a minimal agar medium (11) containing 10^8 cells of *Escherichia coli* B by stabbing the agar with a needle containing inoculum. A clear zone of inhibition of *E. coli* growth after 48 h at 27°C indicated antimetabolite production. The reactions of all isolates tested were compared were that of known cultures of the phytopathogen *Pseudomonas syringae* pv. *pisi*.

Antibiotic production. Rhizobacterial isolates were tested for in vitro antibiosis against *Erwinia herbicola* and *Fusarium oxysporum*. *E. herbicola* was cultured in nutrient broth (Oxoid) for 48 h at 27°C. Broth culture samples were incorporated in SR medium at 10^8 cells per ml for agar plate assays. *F. oxysporum* was cultured on potato-dextrose agar (Difco Laboratories) for 10 days at 27°C. Spore suspensions were obtained by flooding fungal growth on plates with sterile 0.05% Tween 40. Spore suspensions (0.5 ml) were spread-plated on SR agar medium (without antibiotic amendment) and allowed to dry for 2 to 3 h. Rhizobacterial isolates were stabbed in quadrants of agar plates containing the indicator organisms and incubated for 48 h (*E. herbicola*) or 7 days (*F. oxysporum*) at 27°C. Antibiotic production against the indicator organisms was observed as a zone of growth inhibition around the agar stabs of the rhizobacterial isolates.

Involvement of siderophores produced by rhizobacteria was also tested. Antagonistic activity of rhizobacteria classified as pseudomonads was tested as the ability to inhibit the growth of *E. herbicola* and *F. oxysporum* on SR and SR-Fe^{3+} (20 μg of $FeCl_3$ ml^{-1}). Presumptively, rhizobacteria able to inhibit the test microorganism on SR but not SR-Fe^{3+} produce extracellular iron-chelating siderophores, which efficiently complex environmental iron making it less available to certain competing microorganisms (14). Positive siderophore production was identified as suppression of antibiotic activity toward the test organisms on Fe^{3+}-supplemented medium.

Effects of rhizobacteria on weed seedling growth. Seeds of velvetleaf, pigweed, jimsonweed, morning glory, and cocklebur were surface sterilized by immersing in 1.25% sodium hypochlorite for 4 min, rinsing in sterile water, immersing in 70% ethanol for 2 min, rinsing five times in sterile water, and blotting on autoclaved filter paper. Effectiveness of surface sterilization was assessed as previously described (18). Seeds of all weeds except cocklebur were transferred to sterilized plastic growth pouches (Northrup King) containing filter-sterilized nutrient solution (30). Growth pouches were not adequate for the growth of cocklebur seedlings, which were considerably larger than the other weed hosts. Thus, cocklebur were tested in pots (7.5-cm diameter) containing autoclaved vermiculite. Due to poor seed germination of lambsquarters and smartweed, these weed hosts could not be tested.

Two-day-old cultures of each rhizobacterial isolate on SR plates were suspended in 10 ml of phosphate-buffered saline, from which 1 ml (ca. 10^8 cells) was used to inoculate seeds of their respective host weed per pouch or pot. Five pouches or pots were inoculated for each isolate and were placed in growth chambers maintained at 28°C during 16-h light and at 21°C during 8-h dark periods. Light was supplied by fluorescent and incadescent lamps at a photon flux density of 250 $\mu mol\ m^{-2}\ s^{-1}$. After 14 days, seedling shoots and roots were examined for disease symptoms and tap root lengths and shoot dry weights were measured.

RESULTS

Distribution and characterization of weed seedling rhizobacteria. The average densities of rhizobacteria on seedling roots were similar, ranging from 14×10^6 to 62×10^6 CFU g of $root^{-1}$ for lambsquarters and cocklebur, respectively. The presence of bacteria on root surfaces was supported with more direct evidence obtained by scanning electron microscopy (M. F. T. Begonia, R. J. Kremer, and L. Stanley, unpublished data), which indicated that rhizobacteria often associated with root surfaces in characteristic patterns of colonization. Rhizobacteria isolated from all weed seedling roots were comprised primarily of fluorescent and nonfluorescent pseudomonads, *E. herbicola*, *Flavobacterium* spp., and *Alcaligenes* spp. (Table 1). The proportion of these groups for each seedling remained relatively constant regardless of time of sampling during the growing season. The fluorescent isolates were predominantly *Pseudomonas fluorescens*, *P. putida*, and *P. syringae*. Nonfluorescent pseudomonads were mainly *P. cepacia*, *P. maltophilia*, and *P. stutzeri*. Additional gram-negative bacteria found infrequently included representatives of the genera *Acinetobacter*, *Citrobacter*, *Serratia*, and *Xanthomonas*. Gram-positive bacteria comprised less than 1% of all isolates. All weed species except jimsonweed possessed high proportions (>25%) of fluorescent pseudomonads. Jimsonweed, pigweed, cocklebur, lambsquarters, and smartweed had high proportions of nonfluorescent pseudomonads. All weed species except pigweed and smartweed had levels comprised of >20% *E. herbicola*.

Antimetabolite production. Presumed antimetabolites detected with the *E. coli* indicator technique were associated with isolates from all of the major groups of rhizobacteria (Table 2). The frequency of toxin-producing isolates among fluorescent and nonfluorescent pseudomonads was similar for those originating from velvetleaf and cocklebur. The highest proportions of toxin producers were associated with *E. herbicola* isolates from cocklebur, velvetleaf, pigweed,

TABLE 1. Composition of the bacterial population detected on roots of weed seedlings sampled during 1985 and 1986

Bacteria	No. of bacteria of indicated species (% of total) found in roots of:						
	Cocklebur	Morning glory	Velvetleaf	Jimsonweed	Pigweed	Lambsquarters	Smartweed
Acinetobacter spp.	0 (0)	8 (2.4)	0 (0)	0 (0)	5 (2.3)	1 (1.0)	0 (0)
Alcaligenes spp.	8 (3.3)	54 (15.9)	26 (6.7)	12 (10.5)	10 (4.6)	1 (1.0)	10 (16.1)
Bacillus spp.	0 (0)	2 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Citrobacter freundii	0 (0)	0 (0)	0 (0)	3 (2.6)	0 (0)	2 (2.0)	0 (0)
Erwinia herbicola	74 (30.8)	70 (20.6)	92 (23.6)	27 (23.7)	28 (13.1)	20 (19.8)	12 (19.4)
Flavobacterium spp.	10 (4.2)	54 (15.9)	24 (6.2)	8 (7.0)	26 (12.2)	8 (7.9)	3 (4.8)
Pseudomonas fluorescens	28 (11.7)	64 (18.8)	72 (18.4)	9 (7.8)	36 (16.8)	31 (30.7)	13 (21.0)
P. putida	30 (12.5)	10 (3.0)	54 (13.8)	4 (3.5)	18 (8.4)	1 (1.0)	0 (0)
Pseudomonas: other fluorescent spp.[a]	12 (5.0)	13 (3.9)	22 (5.6)	0 (0)	2 (0.9)	11 (10.8)	5 (8.1)
P. maltophilia	10 (4.2)	3 (0.8)	14 (3.6)	14 (12.3)	9 (4.2)	0 (0)	0 (0)
Pseudomonas: other nonfluorescent spp.[b]	66 (27.5)	56 (16.5)	70 (18.0)	34 (29.8)	73 (34.1)	26 (25.7)	16 (25.8)
Serratia spp.	2 (0.8)	0 (0)	4 (1.0)	0 (0)	0 (0)	0 (0)	0 (0)
Xanthomonas spp.	0 (0)	5 (1.5)	12 (3.1)	3 (2.6)	7 (3.2)	0 (0)	3 (4.8)

[a] Species identified in this group included *P. aeruginosa*, *P. aureofaciens*, *P. syringae*, and *P. viridiflava*.
[b] Species identified in this group included *P. alcaligenes*, *P. cepacia*, and *P. stutzeri*.

and smartweed; *P. fluorescens* from velvetleaf; and nonfluorescent pseudomonads from cocklebur, jimsonweed, velvetleaf, and pigweed. Isolates from cocklebur and velvetleaf had a higher overall incidence of toxin producers than did isolates from the other weed species. However, only 18% of the total rhizobacterial collection from all weed hosts produced antimetabolites.

Effects of rhizobacteria on seedling growth. The proportion of rhizobacterial isolates that inhibited seedling growth of their respective weed hosts in growth pouch and pot assays ranged from 35% for isolates originating from cocklebur to 65% for those from jimsonweed (Table 2). The greatest proportions of inhibitory rhizobacteria were associated with the nonfluorescent pseudomonads and *E. herbicola*. This is consistent with the distribution of DRB reported for other plants (7, 10, 13, 31).

An array of effects on seedling root length and shoot dry weight was observed for all weed hosts inoculated with rhizobacteria. Representative results of growth chamber experiments indicated typical plant growth responses caused by inoculation of host seedlings with selected rhizobacterial isolates. Several isolates strongly inhibited both root and shoot growth, whereas some were either neutral or stimulatory toward growth of their host seedlings. Symptoms observed on foliar growth induced by inhibitory isolates ranged from general growth retardation to various types of leaf chlorosis, mottling, and distortions. Occasionally, root growth inhibition was exemplified as stunting and discoloration and poor lateral root development; however, most rhizobacteria reduced plant growth without obvious plant cell damage. This is similar to previous reports first describing DRB, which attributed reduced plant growth to rhizobacterially produced toxins that were absorbed by roots (8, 9, 28, 31). In general, the effects of rhizobacteria on root length were proportional to effects observed on shoot growth as measured by dry weight. All seedling growth studies were repeated at least once with similar results.

Consistent relationships between seedling growth inhibition (illustrated with root length measurements) and *E. coli* inhibition by rhizobacterial isolates did not occur. Only 6 of 10, 7 of 10, and 6 of 10 representative isolates strongly inhibiting *E. coli* (zones, >5 mm in diameter) significantly reduced ($P < 0.05$) seedling growth of velvetleaf, morning glory and pigweed, respectively, by 21 to 62% of the controls (Fig. 1). However, 4 of 10, 5 of 10, and 5 of 10 isolates weakly or not inhibiting *E. coli* were able to inhibit seedling growth of velvetleaf, morning glory, and pigweed, respectively, by 16 to 70%. All representative isolates from jimsonweed that strongly inhibited *E. coli* also significantly reduced ($P < 0.05$) seedling root growth, yet 9 of 10 representative isolates that caused weak or no inhibition of *E. coli* strongly inhibited seedling growth. Interestingly, although jimsonweed had a high proportion (65%) of inhibitory rhizobacteria based on growth pouch assays, a relatively low proportion (16%) inhibited *E. coli* (Table 2). It is possible that test conditions for toxin production by isolates that were not inhibitory to *E. coli* were inadequate; however, the present results suggest that in vitro assays for potential phytotoxicity with *E. coli* as an indicator are not valuable as the sole criterion for screening weed seedling DRB.

Antibiosis potentials of rhizobacteria. The antibiosis potentials of rhizobacterial isolates were determined because these properties may aid in understanding the interrelationships among the different types of rhizobacteria and fungi in weed seedling rhizospheres. An isolate of *E. herbicola* was used as a test for antibacterial activity, since previous work with plant growth-promoting rhizobacteria from crop plants indicated that growth promotive effects may be due in part to elimination of DRB (i.e., *Erwinia* spp.) through antibiotic production (15). Likewise, *F. oxysporum* isolated from a velvetleaf seedling root was tested, since plant growth-promoting rhizobacteria may also inhibit development of fungal pathogens in the rhizosphere (31). Similar interactions likely occur in weed seedling rhizospheres.

Antibiotic production was observed for the majority of fluorescent pseudomonads randomly selected from the seven host weed species. Antibacterial activity against *E. herbicola* was exhibited by 76% of the isolates, whereas 79% were antifungal against *F. oxysporum* (Table 3). The proportion of isolates from each weed host that were antibiotic producers varied. Antibiotic activity against both *E. herbicola* and *F. oxysporum* was exhibited by 85% or more of the fluorescent pseudomonads originating from morning glory, cocklebur, and lambsquarters, yet only about 50% of the isolates from jimsonweed were active. Activities from over 60 and 75% of isolates producing antibiotics against *E. herbicola* and *F. oxysporum*, respectively, were inhibited by Fe^{3+}, indicating that a majority of fluorescent pseudomo-

TABLE 2. Proportion of selected rhizobacteria from weed seedlings inhibiting *E. coli* and significantly inhibiting ($P < 0.05$) growth of respective host seedings[a]

Bacteria	Cocklebur			Morning glory			Velvetleaf			Jimsonweed			Pigweed			Lambsquarters			Smartweed		
	Total no. tested	% Inhibiting:		Total no. tested	% Inhibiting:		Total no. tested	% Inhibiting:		Total no. tested	% Inhibiting:		Total no. tested	% Inhibiting:		Total no. tested	% Inhibiting		Total no. tested	% Inhibiting
		E. coli	Seedling growth		*E. coli*	Seedling growth		*E. coli*	Seedling growth		*E. coli*	Seedling growth		*E. coli*	Seedling growth		*E. coli*			*E. coli*
Alcaligenes spp.	8	0	0	50	0	60	26	23	50	12	0	16	10	0	30	0			10	0
E. herbicola	74	43	56	70	14	62	90	33	73	25	12	80	28	25	42	20	20		12	25
Flavobacterium spp.	10	0	20	50	12	66	24	25	50	8	0	75	25	24	48	8	12		3	33
P. fluorescens	25	24	20	62	10	48	70	30	33	9	0	33	35	0	23	30	13		13	0
P. putida	25	20	16	10	10	50	52	25	27	4	0	25	18	0	22	0			0	
Nonfluorescent pseudomonads	66	33	30	55	20	71	70	28	67	30	30	83	70	27	68	25	8		15	0

[a] Seedling growth determined by measuring shoot dry weight for cocklebur or root length for morning glory, velvetleaf, jimsonweed, and pigweed. Seedling growth was not determined for lambsquarters or smartweed due to poor seed germination.

nads were antibiotic due to siderophore or other Fe-regulated activity (14). Isolates exhibiting antibiosis in the presence of Fe^{3+} apparently produced antibiotics other than siderophores. Some weed species had higher proportions of fluorescent pseudomonads with nonsiderophore antibiotic production (i.e., lambsquarters), whereas the proportion for others was low or nonexistent (i.e., jimsonweed). Only 26 and 27% of all nonfluorescent isolates produced antibacterial and antifungal activity, respectively. The highest proportion of nonfluorescent bacteria exhibiting antibiotic activity occurred with those originating from pigweed. Of the nonfluorescent bacteria producing antibiotics, approximately 35% were inhibited by Fe^{3+}.

DISCUSSION

The distribution and characteristics of rhizobacteria associated with weed seedlings is in agreement with the general observation that rhizospheres of many plants provide favorable environments for gram-negative bacteria, most of which are motile and chromogenic (5). The consistent occurrence of both fluorescent and nonfluorescent pseudomonads and *E. herbicola* in rhizospheres of weed seedlings reported here (Table 1) has also been documented for a number of plant species (7, 10, 13, 20, 31). The limited research reported for weeds indicated that pseudomonads (4, 27) and *Erwinia* spp. (2, 22, 23) are often dominant inhabitants of the rhizosphere. The high incidence of yellow-pigmented rhizobacteria (*E. herbicola*, *Flavobacterium* spp. *P. maltophilia*, *Xanthomonas* spp.) on jimsonweed, morning glory, and cocklebur compared with those of the other weed species indicated that the composition of rhizosphere bacteria may be influenced by individual plant species. Differences in both abundance and composition of microorganisms in rhizospheres of plant species and even varieties within species have been reported (5, 6, 21). Distinctive rhizosphere bacteria observed for different plant species may be influenced by specific root exudates or other factors (5), possibly controlled by specific genes in the plant (6, 21, 24). Indeed, the prevalence of *Erwinia* spp. on roots of diverse weed plants was found to vary considerably depending on the host plant (2, 22, 23).

The presence of an array of rhizobacteria that caused various growth effects on seedling roots of weeds is consistent with previous reports of rhizobacteria associated with several crop and horticultural plants (8, 15, 28, 31). Even though growth-inhibiting rhizobacteria were detected along with beneficial bacteria as part of the normal microflora of the rhizosphere, their effects on plant growth are usually subtle and likely escape notice, especially under field conditions (28, 31). Growth-inhibitory bacteria present on roots may be potential pathogens under conditions where their colonization and multiplication could be promoted. Research to define these conditions may contribute to development of growth-inhibiting rhizobacteria as effective biotic agents in weed control.

Results from growth pouch tests (Table 2) demonstrated the great potential of many bacteria in the rhizosphere to interfere with growth of weed seedlings. The ability of an organism to live on and colonize plant roots certainly should increase its capability to affect the plant in various ways (12). Effects of plant-rhizobacterium combinations that lasted the duration of the study indicated the ability of selected bacteria to successfully proliferate in the rhizosphere.

The inconsistent relationships between seedling growth effects in growth pouches and *E. coli* indicator assays (Fig. 1) are in contrast to those reported by Fredrickson and

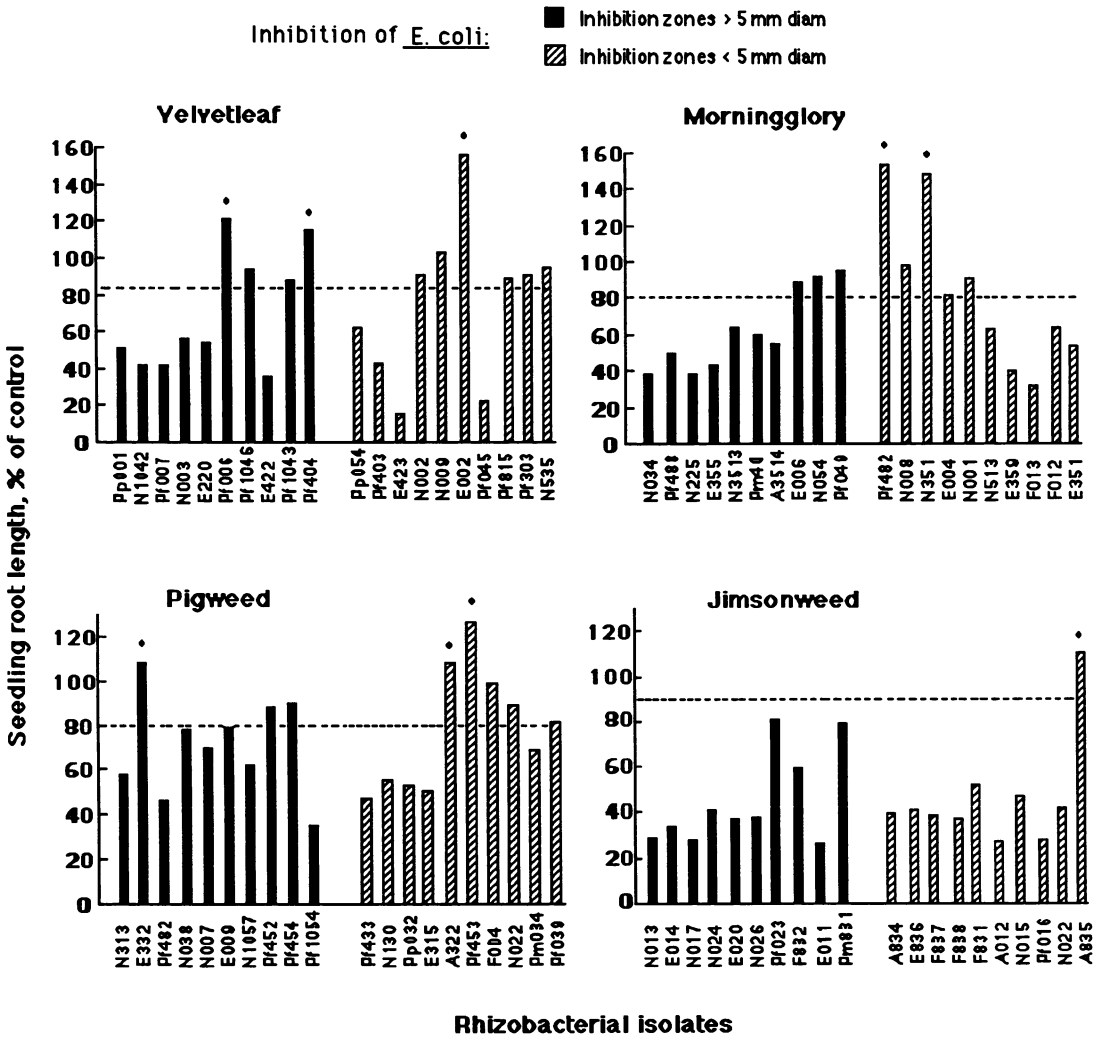

FIG. 1. Activity of selected rhizobacterial isolates with strong (zones >5 mm in diameter) and weak or no inhibition (zones <5 mm in diameter) of *E. coli* toward growth of four weed seedlings. Significant ($P < 0.05$) root growth inhibition is indicated as a percentage of the control value below the dashed line for each weed species. Significant ($P < 0.05$) seedling growth stimulation is indicated with an asterisk. Codes for each identified isolate include the following designations: A, *Alcaligenes* sp.; E, *E. herbicola*; F, *Flavobacterium* sp.; N, nonfluorescent pseudomonad; Pf, *P. fluorescens*; Pm, *P. maltophilia*; Pp, *P. putida*.

Elliott (8), who showed that DRB isolates on winter wheat seedling roots inhibiting *E. coli* also significantly inhibited root growth in seedling bioassays. The inconsistency between the two assays in our study may be due to several factors, including the production of separate but similar toxins active in seedling growth inhibition and *E. coli* antibiosis. Also, rhizobacterial toxins may only be produced or become active in the rhizosphere, where plant root-derived materials serve as precursors of microbial metabolites or enhance their production (26).

A correlation between antibiotic producers (Table 3) and distribution of the various groups of rhizobacteria among the weed hosts (Table 2) could not be found. However, results suggest that antibiotic production may be mediated by undefined factors distinctive for individual plant rhizospheres (5). The proportion of antibiotic-producing bacteria in the rhizosphere might be influenced and under genetic control by the host plant (5, 24). Gardner et al. (10) reported that over 90% of fluorescent bacteria from citrus rhizospheres possess siderophore activity. The existence of fluorescent pseudomonads with typical plant growth-promoting properties including antibacterial and antifungal activities, as previously defined (31), indicates that weeds also possess microorganisms with growth promoting activity. Thus, these bacteria may lend a competitive edge to weed seedlings by providing protection against potential rhizosphere phytopathogens.

The rhizobacterial isolates possessed a range of diverse properties. Several isolates appear to be typical DRB or plant growth-promoting rhizobacteria, based on previous descriptions for these groups (26, 28). However, many isolates exhibited properties that deviated from the accepted definitions. We observed isolates that inhibited *E. coli* yet significantly stimulated seedling growth and other isolates noninhibitory to *E. coli* but detrimental to seedling growth (Fig. 1). Also, several fluorescent pseudomonads that appeared to promote plant growth based on antibiotic-siderophore activity inhibited seedling growth. These results are in agreement with previous observations regarding the complex properties of rhizobacteria (5, 15). Based on our obser-

TABLE 3. In vitro antibiotic activity of rhizobacteria isolated from various weed seedlings[a]

Weed host	Isolate type	Total tested	No. (%) of isolates with:			
			Antibacterial activity		Antifungal activity	
			Control	$+Fe^{3+}$	Control	$+Fe^{3+}$
Velvetleaf	Fluorescent	48	37 (77)	7 (15)	31 (65)	5 (10)
	Nonfluorescent	44	19 (43)	14 (32)	12 (27)	10 (22)
Morning glory	Fluorescent	38	32 (85)	8 (21)	36 (95)	6 (16)
	Nonfluorescent	36	5 (14)	4 (11)	11 (30)	9 (25)
Cocklebur	Fluorescent	40	34 (85)	16 (40)	35 (88)	15 (50)
	Nonfluorescent	30	4 (13)	3 (10)	2 (6)	2 (6)
Pigweed	Fluorescent	23	16 (70)	9 (39)	21 (91)	7 (30)
	Nonfluorescent	28	13 (46)	9 (33)	14 (50)	11 (40)
Lambsquarters	Fluorescent	20	19 (95)	12 (60)	19 (95)	8 (40)
	Nonfluorescent	15	3 (20)	2 (13)	2 (13)	2 (13)
Smartweed	Fluorescent	20	14 (70)	10 (50)	16 (80)	0 (0)
	Nonfluorescent	14	4 (28)	2 (14)	2 (14)	2 (14)
Jimsonweed	Fluorescent	30	15 (50)	0 (0)	16 (53)	0 (0)
	Nonfluorescent	26	4 (15)	2 (8)	10 (38)	5 (19)

[a] Formation of inhibition zones >5 mm in diameter by test isolates considered positive for antibiotic activity.

vations, we suggest that simple bioassays for potential growth effects of rhizobacteria should be used with caution and that screening on the intended test seedlings must be conducted.

Research on indigenous rhizosphere bacteria on weed seedlings as potential biocontrol agents has previously been inadequately addressed. This may be due perhaps to intensified efforts directed toward foliar mycoherbicides rather than those directed at weed seedlings (1, 32). In the present study the characterization of rhizobacteria associated with various weed seedlings provides a significant basis for selecting and evaluating potential biocontrol agents for weed management systems. The diverse properties of the bacteria isolated from weed seedling roots illustrate the complex nature of microorganism-rhizosphere associations that must be considered in the development of biotic agents aimed at weed seedlings. The microbial composition of some crop and horticultural plants can be manipulated to favor a predominance of beneficial rhizobacteria to obtain growth-promoting effects (28, 33). Therefore, weed seedling rhizospheres might also be manipulated to host a majority of specific deleterious rhizobacteria needed to obtain detrimental effects for controlling weeds. Based on our results, the successful biotic agent must have high colonizing ability, produce specific phytotoxin(s) against its host weed(s), not be negatively affected by antibiotics or siderophores from competing rhizosphere bacteria, and either synthesize or utilize other bacterial siderophores. An effective biotic agent will not be possible, however, until dynamics of the interactions of the agent, weed seedling rhizosphere, and soil environment are fully understood.

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