Selection and Performance of Bacterial Strains for Biologically Controlling Fusarium Dry Rot of Potatoes Incited by *Gibberella pulicaris*

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ABSTRACT

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The microbiota from 29 different agricultural soils were individually transferred to separate samples of γ irradiation-sterilized field soil enriched with potato periderm. After incubation, the samples (chemically, physically, and nutritionally similar but microbiologically dissimilar) were assayed for biological suppressiveness to Fusarium dry rot using a whole-tuber/infested-soil assay. Over 350 isolates of bacteria, yeasts, and actinomycetes were recovered from tubers and soil associated with the five most suppressive soil samples. In three of four soils assayed, periderm amendment increased suppressiveness over that in the same soil without the amendment. In a whole-tuber assay, 18 bacterial strains consistently suppressed dry rot incited by three different strains of *Gibberella pulicaris*, including one resistant to thiabendazole. Strains effective in biological control included members of the genera *Pseudomonas, Enterobacter*, and *Pantoea*. The time of isolation after initiating the whole-tuber/infested-soil assay and the isolation medium influenced the number of effective strains recovered. The majority of the 18 superior biological control strains were recovered from two of the five suppressive soil samples.

Fusarium dry rot, caused by *Gibberella pulicaris* (Fr.:Fr.) Sacc. (anamorph: *Fusarium sambucinum* Fuckel), is a disease of worldwide importance that affects potatoes (*Solanum tuberosum* L.) both in the field and in storage (3). Greater than 60% of tubers in storage can be infected (6). Although comprehensive figures are lacking, average annual crop losses attributed to dry rot have been estimated at 6–10%, with reports of losses as high as 60% (7,41). In addition to destroying tuber tissues, *G. pulicaris* can produce trichothecene toxins that have been implicated in mycotoxicoses of humans and animals (12,37). Resistance to thiabendazole (TBZ), the only registered postharvest chemical effective against dry rot, is widespread (10,17,40) and likely to persist in the field due to the prevalence of vegetative compatibility among North American strains of *G. pulicaris* and the apparent fitness of resistant strains (10). All commonly grown potato cultivars are susceptible to the disease (24).

Biological control of postharvest diseases of fruits and vegetables has proved feasible in numerous studies (43). Biological control measures have not been developed against dry rot even though the etiology of this disease is well suited to the use of microbial antagonists. *G. pulicaris* requires a wound in order to penetrate tubers. Depending on the potato cultivar, tuber age, and storage environment, potato wounds heal in 5 days to several weeks via the formation of wound periderm (18). Thus, agents of biological control need only remain viable and effective during the relatively brief period when wounded potatoes are susceptible. Additionally, storage conditions (high relative humidity and temperatures between 5 and 15 C) are favorable for the growth of many microbial agents.

The initial objective of this study was to identify soil samples containing microbial communities suppressive to Fusarium dry rot and to selectively isolate microorganisms from those communities. Our second objective, as part of an ongoing program to develop strategies and organisms for the biological control of Fusarium dry rot, was to assay the strains of microorganisms isolated and identify those effective in protecting potato tubers from infection by *G. pulicaris*. A preliminary report has been made on this topic (36).

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MATERIALS AND METHODS

Assay for suppressive microbial communities. Soil samples obtained primarily from fields cropped to potatoes and with a low incidence of disease initiated by *G. pulicaris* were collected from 27 different sites in Wisconsin and two in Illinois. Soils were sieved through 2-mm screens and stored for not more than 5 mo at 4 C in loosely closed plastic bags. Just prior to use, soils were again sieved through 2-mm screens. Slightly moist field soil samples (approximate soil water potential = −0.5 MPa); powdered, heat-sterilized periderm of potato cultivar Russet Burbank; and γ irradiation-sterilized (5 megarads minimum) silty loam field soil (Dakota silty loam; pH 6.8, 1:1 water:soil, 1.3% organic matter) were then combined (5:2:93, w/w/w, respectively). Such mixtures allow microbial communities from the small proportion of live soil in each mixture to establish in chemically and physically similar, periderm-enriched soil backgrounds. Four randomly selected field soils were also used to construct mixtures without potato periderm to determine if enrichment influenced soil suppressiveness. The water potential of each mixture was adjusted to approximately −0.1 MPa by misting with sterile deionized water, and the mixtures were incubated in plastic bags for 1 wk at 15 C, with periodic shaking. Highly concentrated aqueous suspensions of 7-day-old conidia of *G. pulicaris* isolate R-6380, produced as described elsewhere (12), were then added by misting conidial suspensions over each soil mixture to obtain a final concentration of 1×10^6 conidia per gram (dry weight equivalent) of soil. Bags containing inoculated soil mixtures were shaken thoroughly and incubated for 2 days at 15 C.

Russet Burbank tubers, obtained from the University of Wisconsin Lelal Starks Elite Foundation Seed Potato Farm, Rhinelander, were used for assays of soil suppressiveness. Tubers were stored in darkness at 10 C and several hours before use were brought to room temperature, gently washed with sterile distilled water, and allowed to air-dry. Tubers were then wounded at the stem and apical ends with a board containing four blunted nails (4 mm long, 2 mm in diameter) positioned at each corner of a 1×1 cm square. Aqueous pastes of each soil were then immediately applied to each end of wounded tubers. Controls consisted of uninoculated wounded potatoes and wounded potatoes inoculated with a paste of sterile field soil. Four replicate tubers per treatment were arranged in trays in a completely randomized design. Trays were enclosed in large plastic bags and incubated in darkness at 15 C for 4 wk. This incubation temperature was chosen because it represents the temperature at which biocontrol agents would be expected to perform in commercial tuber storage houses.

To evaluate the extent of tissue colonization by *G. pulicaris* R-6380, tubers were cut in half widthwise and then each

half was cut twice lengthwise, with each parallel cut passing 1 mm to the side of two of the four wounds. The total depth and width of necrotic colonized tissue were then recorded for each side of each potato wedge, excepting those that had been excised with toothpicks. Values were log-transformed to normalize data prior to one-way analysis of variance. In this and all other assays, necrotic tissues were randomly sampled, surface-disinfected, and placed on nutrient media to confirm the association of *G. pulicaris* with tissues expressing symptoms of disease.

Isolation of microorganisms. Five days after tuber inoculation, two wounds from one tuber per treatment were excavated with sterile toothpicks, and the cell matter obtained was streaked onto one-tenth strength tryptic soy broth agar (TSBA/10; Difco Laboratories, Detroit, MI) + cycloheximide (0.05 g/L) and acidified YME agar containing, per liter, 3.0 g yeast extract, 3.0 g malt extract, 5.0 g peptone (type III), and 0.1 g chloramphenicol, acidified with 1 M HCl to pH 3.7 after autoclaving. Bacteria and yeasts were preferentially isolated on TSBA/10 and YME, respectively. Samples were streaked in the event that some primary colonists active in suppressing dry rot could be more readily recovered soon after wounding rather than after determining the extent of dry rot at assay harvest 4 wk after tuber inoculation. Plates were incubated for 24 hr at 15 C and then stored in plastic bags for 4 wk at 4 C until the data from the suppressive soil assay were analyzed. Immediately after the suppressive soil assay was harvested, data were analyzed to determine superior suppressive soils. Microorganisms were then isolated from two disease-free wedges chosen from tubers harvested from each selected suppressive treatment. A 2-mm square piece of tissue was cut from around each wound of a selected wedge, the fresh weight determined, and the tissue pieces macerated with a sterile scalpel. The macerate was then diluted in 0.1% water agar and agitated for 2 min in a vortex mixer, and serial dilutions were prepared in 0.004% phosphate buffer (pH 7.2) with 0.019% $MgCl_2$ (PO_4 buffer; Aid-Pack USA, Gloucester, MA). Dilutions were spread on plates of acidified YME + chloramphenicol, TSBA/10 + cycloheximide, and one-quarter strength potato-dextrose agar (PDA/4, Difco) + 0.05 g/L cycloheximide. After incubation for 3 days at 27 C, colony counts of bacteria were made, and several isolates of each morphologically distinct colony from each medium were isolated in pure cultures. For the five superior treatments from the suppressive soil assay, additional putative antagonists were isolated from the streak plates made from tissues excavated from potato wounds 5 days after initiating the assay. Isolates were streaked for purity and stored at −80 C in 10% glycerol until needed.

Assay of efficacy of bacterial isolates against *G. pulicaris* R-6380. A total of 353 isolates were tested in single replication assays using whole potato tubers to evaluate the efficacy of isolates in controlling *G. pulicaris*. Only those isolates that permitted slight or no disease development were selected for further testing. Just prior to use, slightly turbid suspensions (absorbance of 0.170 at 620 nm; corresponding cell count of approximately 1×10^8 cfu/ml) in PO_4 buffer were prepared from 18-hr-old cultures grown on TSBA/5 agar. Russet Burbank tubers, obtained from commercial sources, were prepared as described above except that four individual wounds were made at equal distances around the tuber circumference. Conidia of *G. pulicaris* (1×10^6 conidia per milliliter, prepared as described previously) and a microbial suspension were then combined 1:1 and thoroughly mixed in a microtiter dish, and 5 μl was inoculated into one wound per microbial treatment. Controls consisted of tubers inoculated with PO_4 buffer or suspensions of macroconidia only. Tubers were placed in trays and incubated as previously described. Tubers were harvested after 3 wk, and the extent of disease was evaluated by quartering the tubers longitudinally through each of the four wounds and measuring the total depth and width of exposed necrotic tissue at a wound site.

Forty-four superior strains from the previous assay were then evaluated in a replicated experiment. Tubers, wounds, and microbial suspensions were prepared as described above. Four wounds on four different tubers were inoculated for each strain assayed (four replicates). An additional treatment, *Pseudomonas fluorescens* strain 2-79, was included as a biological control agent with known disease control capabilities (42). For control treatments, a total of 16 wounds on four potatoes were inoculated with conidia of *G. pulicaris* alone, eight wounds were inoculated with a solution containing 5×10^5 heat-killed conidia of *G. pulicaris* per milliliter, and eight wounds were inoculated with PO_4 buffer only. Inoculated tubers were incubated for 3 wk, then harvested and evaluated for disease as described previously. In a second experiment, the best 19 strains from this trial were similarly tested. Values from both experiments were log-transformed to normalize data prior to one-way ANOVA. Means were separated from controls using Fisher's protected LSD.

Efficacy of superior strains against additional strains of *G. pulicaris*. Assays to determine the efficacy of the best 18 bacterial strains against two strains of *G. pulicaris*, RN-1 (TBZ-resistant) (10) and RN-5 (TBZ-sensitive), were performed as described above. Antagonist strain S09:T:16 was not tested because its morphology, DNA restriction fragment pattern analysis (32), and growth rate in liquid culture were virtually identical to those of strain S09:T:14, obtained from the same soil. No other strain pairs exhibited identity. The experiment was repeated.

Bacterial identification. The 18 bacterial strains that limited disease development were subjected to gas-chromatographic analysis of phospholipid fatty acids according to the method of Sugimoto et al (38) except that version 3.6 of the aerobic library was used. Strains were further identified with Biolog GN Microplates (version 3.0, Biolog, Inc., Hayward, CA). Traits of presumptively identified strains were then compared with known specimens and published descriptions of genera and species (22). Biochemical and physiologic tests of taxonomic utility were also run on all strains (22). Several Enterobacteriaceae and Pseudomonadaceae strains were sent to the American Type Culture Collection, Rockville, Maryland, and the LMG Culture Collection, Ghent, Belgium, respectively, for confirmatory identification.

RESULTS

Assay for suppressive microbial communities. In three of four soils assayed, amendment with potato periderm decreased the amount of disease compared with that in the same soil without periderm amendment. For these paired soil treatments, diseased tissue measurements averaged 0.52 cm for soils with periderm and 0.66 cm for soils without periderm (overall effect not significantly different, paired t test, $P = 0.54$). The top five selected soils included four with periderm amendment and one without. For all soils, the range of treatment means for diseased tissue was 0.18–0.94 cm.

Isolation of microorganisms. The number of bacteria recovered from serial dilutions of macerated excised pieces of wounded tuber tissue ranged from 7.8×10^8 to 9.5×10^7 (cfu/g fresh weight) on TSA/10, 5.9×10^8 to 8.5×10^7 on PDA/4, and 4.8×10^6 to none on YME. The number of pure cultures obtained from dilution plates was 273, whereas 80 cultures were obtained from streak plates of tissues excised from potato wounds.

Assay of efficacy of bacterial isolates against *G. pulicaris* R-6380. Of 353 isolates tested in the initial single replication assays, 44 provided complete or nearly complete control and therefore warranted further testing. In the first replicated experiment, 15 strains significantly inhibited disease development, whereas *P. fluorescens* strain 2-79 did not, compared with the controls inoculated with conidia of *G. pulicaris* R-6380 ($P = 0.05$ or 0.01) (Table 1). In the second experiment with the 19 best antagonists from experiment 1, all 19 bacterial strains

tested significantly inhibited disease development ($P = 0.01$) (Table 1), as did control strain 2-79. Bacteria + pathogen treatments did not significantly differ from the wound only or wound + heat-killed conidia controls ($P = 0.05$). Limited browning around the wounds in control tubers was due to suberization of adjacent tissues.

Efficacy of superior strains against additional strains of *G. pulicaris*. Compared with control tubers inoculated with *G. pulicaris* strain RN-1 (TBZ-resistant) or RN-5 (TBZ-sensitive), all bacterial strains controlled disease, frequently against both fungal strains ($P = 0.05$ and $P = 0.01$) (Table 2). *P. fluorescens* strain 2-79 also provided control. In some cases, bacteria + pathogen treatments were indistinguishable from the wound only or wound + heat-killed conidia controls.

Bacterial identification. Bacteria effective in inhibiting disease development incited by *G. pulicaris* strains R-6380, RN-1, and RN-5 were identified as members of the genera *Pseudomonas, Enterobacter*, and *Pantoea* (Table 3). The strains have been deposited in the NRRL patent culture collection (Table 3).

Origins of effective bacterial strains. Seventeen of the 18 effective bacterial strains (S series) were recovered from day 28 dilution plates, whereas only one effective strain (P series) was recovered from day 5 streak plates (Table 4). Effective strains were recovered primarily from two of the five soils that rated the highest in Fusarium dry rot suppressiveness (Table 4). Isolation media influenced the percentage of strains recovered that were effective in controlling disease ($P = 0.10$, chi-square test of arcsine-transformed values) (Table 4).

Table 1. Effect of bacterial strains on symptom development in potato tubers colonized by *Gibberella pulicaris* strain R-6380

Strain	Colonized tissue (mm)[a] Expt. 1	Expt. 2
P22:Y:05	0.5** [b]	0.0**
S09:P:06	0.0**	0.2**
S09:P:14	0.5**	0.8**
S09:T:12	0.8**	0.5**
S09:T:14	1.0*	0.2**
S09:T:16	1.0*	0.8**
S09:Y:08	1.5	1.0**
S09:P:08	1.0*	0.5**
S09:T:04	1.8	0.5**
S09:T:10	1.0*	1.0**
S11:P:12	0.8**	0.5**
S11:P:14	2.8	1.0**
S11:P:08	0.8**	1.2**
S11:T:06	1.2	0.2**
S11:P:02	1.0*	0.8**
S11:T:04	1.0*	0.0**
S11:T:07	0.7**	0.8**
S22:T:04	1.0*	0.5**
S22:T:10	0.5**	0.5**
2-79	2.8	0.8**
None (control)	6.0	21.2

[a] Tubers were quartered 21 days after inoculation by making longitudinal cuts perpendicular to the tuber surface and through previously inoculated wounds. The sum of the depth and width of the exposed darkened, dry-rotted tissue was then determined. Means are based on four replicates for strain treatments, 16 replicates for the experiment 1 control, and 39 replicates for the experiment 2 control.

[b] Within a column, values followed by one or two asterisks are significantly different from the control based on Fisher's protected LSD ($P = 0.05$ and $P = 0.01$, respectively). One-way ANOVA and mean comparisons were performed on log-transformed data.

Table 2. Potato tuber colonization by *Gibberella pulicaris* strains RN-1 (thiabendazole-resistant) and RN-5 (thiabendazole-sensitive) as influenced by selected bacterial strains

Strain	Colonized tissue (mm)[a] RN-1 Expt. 1	RN-1 Expt. 2	RN-5 Expt. 1	RN-5 Expt. 2
P22:Y:05	1.2** [b]	15.8**	12.5**	8.2**
S09:P:06	1.1**	2.2**	2.1**	3.5**
S09:P:14	0.4**	ND	2.4**	ND
S09:T:12	7.9**	19.5**	11.4*	9.8**
S09:T:14	0.6**	3.5**	9.4**	5.0**
S09:T:16	ND	ND	ND	ND
S09:Y:08	4.1**	12.0**	10.5*	13.8**
S09:P:08	10.5**	33.2	8.1*	23.5
S09:T:04	3.0**	11.2**	3.6**	19.0
S09:T:10	7.2**	36.0	6.0**	27.2
S11:P:12	22.2*	ND	16.2	ND
S11:P:14	17.2**	ND	8.8**	ND
S11:P:08	12.5**	ND	4.9**	ND
S11:T:06	17.0**	ND	5.9**	ND
S11:P:02	3.6**	2.0**	1.5**	5.8**
S11:T:04	3.0**	12.8**	1.8**	10.5**
S11:T:07	1.5**	17.8*	5.1**	4.8**
S22:T:04	3.0**	13.5*	4.0**	20.0*
S22:T:10	4.0**	3.5**	6.8**	5.5**
2-79	11.0**	4.8**	4.2**	10.8**
None (+RN-1 control)	39.2	33.7	NA	NA
None (+RN-5 control)	NA	NA	25.0	36.2

[a] Tubers were quartered 21 days after inoculation by making longitudinal cuts perpendicular to the tuber surface and through previously inoculated wounds. The sum of the depth and width of the exposed darkened, dry-rotted tissue was then determined. Means are based on four replicates for strain treatments and 20 replicates for each control treatment.

[b] Within a column, values followed by one or two asterisks are significantly different from the control based on Fisher's protected LSD ($P = 0.05$ and $P = 0.01$, respectively). One-way ANOVA and mean comparisons were performed on log-transformed data. ND = not determined, NA = not applicable.

Table 3. Identity of bacterial strains that control Fusarium dry rot disease development in potato tubers

Strain	NRRL accession number	Identification
P22:Y:05	B-21053	*Pseudomonas fluorescens* biovar V[a]
S09:P:06	B-21049	*Pseudomonas corrugata*[a]
S09:P:14	B-21105	*Pseudomonas corrugata*
S09:T:12	B-21104	*Pantoea* sp.
S09:T:14	B-21051	*Pseudomonas corrugata*[a]
S09:Y:08	B-21128	*Pseudomonas* sp.[b,c]
S09:P:08	B-21129	*Pseudomonas corrugata*
S09:T:04	B-21103	*Enterobacter* sp.
S09:T:10	B-21101	*Enterobacter* sp.[d]
S11:P:08	B-21132	*Enterobacter* sp.[d]
S11:P:12	B-21133	*Pseudomonas* sp.[c]
S11:P:14	B-21134	*Pseudomonas* sp.[c]
S11:T:06	B-21135	*Pseudomonas* sp.[c]
S11:P:02	B-21136	*Pseudomonas corrugata*
S11:T:04	B-21048	*Pantoea agglomerans*[e]
S11:T:07	B-21050	*Enterobacter cloacae*[e]
S22:T:04	B-21102	*Pseudomonas* sp.[b,c]
S22:T:10	B-21137	*Pseudomonas* sp.[c]

[a] Identification confirmed by the LMG Culture Collection, Ghent, Belgium, using auxanographic analysis and confirmatory physiologic and biochemical tests.

[b] Misidentified as *Pseudomonas corrugata* using Biolog GN microplates.

[c] Produces fluorescent pigment.

[d] Identified as *Enterobacter taylorae* using Biolog GN microplates, but species identification not confirmed.

[e] Identification confirmed by the ATCC, Rockville, Maryland.

DISCUSSION

A large number of bacterial strains were isolated that were extremely effective in suppressing dry rot disease incited by three different strains of *G. pulicaris*. This is the first report of biological control of Fusarium dry rot of potatoes. Symptoms on tubers coinoculated with an effective antagonist and *G. pulicaris* were sometimes reduced to levels equivalent to those of uninoculated control tubers. Control provided by the bacterial strains isolated in this study was comparable to or better than that provided by the proven bacterial biological control strain *P. fluorescens* 2-79 (42). Biological control of pathogens that incite diseases of tuber seed pieces has also been reported (2,5,9,13,19,20,39,44). Our study supports the feasibility of biologically controlling an important postharvest pathogen of stored potatoes.

A high percentage of field isolates of *G. pulicaris* are resistant to TBZ (10, 17,40), the only chemical registered for postharvest use on potato tubers. Additionally, all commonly grown potato cultivars are susceptible to Fusarium dry rot (24). The consistent high level of effectiveness of these antagonists against a variety of strains of *G. pulicaris*, including one resistant to the chemical TBZ, represents a promising first step in the development of a biological control product that could augment or replace TBZ as TBZ-resistance continues to spread in field populations of *G. pulicaris*.

A large number of the strains effective in controlling *G. pulicaris* were identified as *Pseudomonas corrugata* (Table 3). Strains of *P. corrugata* also biologically control take-all disease in cereal grains (16) and *Pythium aphanidermatum* (Edson) Fitzp. on hydroponically grown cucumber (27). The *P. corrugata* taxon is known to contain strains that can incite a stem and pith necrosis of tomatoes (*Lycopersicon esculentum* Mill.), especially on greenhouse-grown plants. Whether any of the current strains are pathogenic on tomato is not known. We have seen no evidence of pathogenicity of our strains on potato tubers. It is a curiosity worth mentioning that *P. corrugata* strains were regularly recovered from soils previously cropped to potatoes, a species allied to tomato. Whether exudates or volatiles from these solanaceous crops specifically enrich for *P. corrugata* is unknown. Metabolites and cells of *P. corrugata* elicit the biosynthesis of phytoalexin in *Trifolium repens* L. (14) and cells induce resistance to *Pythium* in cucumber (45). A similar elicitation of rapid phytoalexin production in potato tubers could be a mechanism of biological control employed by some of the *P. corrugata* strains in the present study. Fluorescent *Pseudomonas* spp. (26,29,30) and *Enterobacter cloacae* strains (31) have a history of demonstrated biological control efficacy. Reports of such activity being associated with strains of *Pantoea (Enterobacter) agglomerans* are limited (15).

The large number of effective bacterial strains acquired in this study necessitates reducing the number of strains selected for detailed research. The rate and other growth characteristics of these strains differ considerably in liquid culture (P. J. Slininger, *unpublished*). Growth characteristics of microbial strains in liquid culture significantly impact the cost of commercial scale biomass production (28). Final selection of strains for further development will, therefore, consider both strain efficacy and amenability to economical production in liquid culture (36).

The mechanism(s) of suppression exhibited by these strains is not known. Most of the strains produce at least one antifungal compound in vitro (K. D. Burkhead, *unpublished*). Preemptive colonization of wounds might also account for the activity of some strains (30), as would inciting induced resistance as previously discussed. Biological control activity would also be anticipated if a strain inhibited the ability of *G. pulicaris* to detoxify the potato phytoalexins rishitin and lubimin (11). Combining strains based on differing mechanisms of activity may also prove fruitful in the development of an effective biological control product.

A variety of techniques and strategies have been suggested for isolating microbial antagonists active against plant pathogens (1,8,21,25,35). Selection techniques such as those applied in the current study should have considerable application in other programs aimed at isolating putative antagonists. Transferring soil microbial components to a standardized sterile soil background assures that suppressive soils identified are biologically suppressive rather than suppressive because of the chemical or physical nature of the soil. Additionally, large numbers of microbial communities can be screened for pathogen-suppressive activity, with only the most active selected for the labor-intensive step of dilution plating and screening of individual isolates for activity. A further advantage of this technique is that it does not include a selection based on a petri plate antagonism assay. Enrichment with potato periderm positively influenced the suppressiveness of three of four soils tested. Enrichment with wheat roots, provided by continuous cropping to wheat over successive years, is apparently partially responsible for the development of soils suppressive to take-all (8).

For the suppressive soil assay, only 1% of bacterial isolates recovered from day 5 isolations of wound tuber tissues were among the 18 superior suppressive strains, compared with 6% of day 28 isolates. Primary colonists with effective temporary protection against tuber infection by *G. pulicaris* but with limited competitive ability may have been the predominant microbial type recovered at day 5. Full pathogen suppressiveness and associated microbial populations might not have reached high levels by day 5 (14 days after microbial introduction to sterilized soil). Aerated-steam pasteurized soils can require more than 12 days to reach stationary levels of pathogen suppressiveness (34). Interestingly, none of the 18 superior suppressive strains were obtained from the suppressive soil without periderm amendment (soil S4A, Table 4), although the microbial component of this soil was one of the most highly suppressive of all the soils tested. Some suppressive soils apparently result from the concerted contribution of a complex of microorganisms and a unique soil type. This type of soil suppressiveness does not necessarily contribute strains that individually possess high levels of suppressive activity (4,33). No effective isolates were obtained from soil 19, which was the least suppressive of

Table 4. Influence of time of isolation, soil of origin, and isolation medium on the recovery of bacterial strains effective in controlling Fusarium dry rot of potatoes[a]

	Time of isolation		Soil of origin[b]					Isolation medium[b]		
	Day 5	Day 28	S9	S11	S19	S22	S4A	PDA/4	TSA/10	YME
Effective isolates[c]	1	17	8	7	0	2	0	6	10	1
Isolates assayed	80	273	64	72	51	47	39	103	112	58
Percent effective	1.2	6.2	12	10	0	4.3	0	5.8	8.9	1.7

[a] Time of isolation, soil of origin, and isolation medium impacted the number of effective isolates recovered ($P = 0.10$, $P = 0.05$, and $P = 0.10$, respectively, chi-square test).

[b] Only isolates from day 28 are included because of the limited recovery of effective isolates from day 5.

[c] Number of isolates that significantly controlled disease incited by *Gibberella pulicaris* strains R-6380, RN-1, and RN-5 in at least one of two experiments.

the five soils chosen for study.

Previous research has demonstrated that dry rot is more effectively controlled by chemical application at harvest when potatoes are entering storage than at grading (6,23). Studies to determine the ability of effective strains to colonize and maintain populations on tubers should help determine how to most effectively utilize bacterial strains to control dry rot development. Concern over pesticide residues is especially relevant when considering chemical treatments of harvested fruits and vegetables. With the favorable climate for the development of biological control alternatives to disease control, the effectiveness of the strains described in the present study, and the lack of effective alternative control measures, continued research of this biological control system is warranted.

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