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Biocontrol of *Fusarium sambucinum*, dry rot of potato, by *Serratia plymuthica* 5–6

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Serratia grimesii 4–9 and *Serratia plymuthica* 5–6, isolated from the rhizosphere of pea, *Pisum sativum* (L), were evaluated for their potential to suppress growth of *Fusarium sambucinum in vitro* and to reduce Fusarium dry rot in stored potatoes (*Solanum tuberosum* L). *In vitro* studies indicated that these bacterial isolates suppressed growth of *F. sambucinum* by 60% or more at both 15 and 25°C. In a potato tuber slice assay the number of infection sites in potato slices exposed to *F. sambucinum* and treated with *S. grimesii* 4–9 and *S. plymuthica* 5–6 was reduced by 96 and 97%, respectively, at 15°C. The diameter (mm) of the infection sites was reduced 91 and 96%, respectively, when compared to slices treated with *F. sambucinum* alone. Studies with Fusarium-infected whole potato tubers also showed significant reduction in dry rot formation following treatment with the bacterial isolates or the fungicide thiabendazole. When applied simultaneously with the pathogen, *S. grimesii* 4–9 and *S. plymuthica* 5–6 suppressed development of Fusarium dry rot by 60 and 77%, respectively, at 15°C and by 63 and 84%, respectively, at 25°C compared to tubers inoculated with the pathogen alone. Thiabendazole suppressed development of Fusarium dry rot by 66 and 81% at 15 and 25°C, respectively, compared to tubers inoculated with the pathogen alone. These studies demonstrate the potential of soil bacteria as biofungicides for managing post-harvest crop diseases. Due to the potential risks to human health associated with *S. grimesii* 4–9, *S. plymuthica* 5–6 is recommended for further study for biofungicide development.

Keywords: Fusarium dry rot; potato; *Fusarium sambucinum*; biocontrol; biofungicide

Introduction

The fungal pathogen, *Fusarium sambucinum* (*Gibberella pulicaris* (Fries)), is the primary causal agent of Fusarium dry rot, a severe type of dry rot or black rot in potatoes, *Solanum tuberosum* L, throughout temperate regions. Other *Fusarium* spp. including *F. avenaceum*, *F. solani* and *F. coeruleum* are also known to cause dry rot in potatoes (Boyd 1972; Sadfi, Chérif, Hajaoui, and Boudabbous 2002). Fusarium dry rot is characterized by shrunken, collapsed diseased tissues that are usually dry. It is an economically important disease worldwide, which affects tubers in storage and may also damage seed pieces, reducing crop stand. Fusarium dry rot accounts for losses of up to 25% of potatoes in storage (Slininger, Schisler, and Bothast 1996; Slininger, VanCauwenberge, Shea-Wilbur, and Bothast 1998; Lui and Kushalappa 2002; Slininger, Schisler, Burkhead, and Bothast 2004). Thompson

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and Waterer (2000) estimated that from 5 to 40% of the Saskatchewan potato crop is lost to Fusarium dry rot each year.

Potato tubers are infected by *F. sambucinum* via wounds incurred at harvest or during handling and these tubers manifest disease during storage. Fusarium spores can survive in soil for several years and can infect the cut surfaces of seed potatoes in the spring. Curing of wounds via deposition of polyphenolic and polyaliphatic compounds in cell walls at the wound site reduces desiccation and blocks infection by post-harvest pathogens like Fusarium (Lulai and Suttle 2004). The optimal temperature for curing potato tubers is from 10 to 15°C; however, this temperature is high enough to support development of Fusarium. Fusarium dry rot progresses slowly at the lower temperatures of 3–5°C, recommended for long-term storage of seed and table potatoes, but progresses more rapidly at the higher temperatures of 8–10°C, required for storing processing potatoes. Fusarium dry rot may also proceed rapidly as the potatoes pass through retail channels and to the consumer as potatoes are rarely refrigerated in these situations.

Growers have few chemical control options to manage development of Fusarium dry rot in stored potatoes. Registered fungicides include thiabendazole (TBZ) and imazalil. TBZ, which has been available since the 1970s, is applied to tubers as they are loaded into storage. Repeated use of this fungicide has led to the selection of TBZ-resistant strains of *F. sambucinum* (Kawchuk, Holley, Lynch, and Clear 1994; Shinners-Carnelley, Bains, McLaren, and Thomson 2003). The spread of these resistant strains by seeding with infected potatoes has decreased the efficacy of TBZ, leading to more widespread losses to Fusarium dry rot (Hide, Read, and Hall 1992; Desjardins, Christ-Harned, McCormick, and Secor 1993; Kawchuk et al. 1994; Holley and Kawchuk 1996; Schisler, Burkhead, Slininger, and Bothast 1998). New chemical fungicides entering the market are subject to intense scrutiny for social, environmental, and economic reasons (Bruhn et al. 1992; Dunlap and Beus 1992; Magnusson and Cranfield 2005). Alternative forms of post-harvest disease control such as hydrogen peroxide solution (Oxidate, Biosafe Systems Inc., Glastonbury, CT) have been developed as reduced risk options for control of Fusarium dry rot (MacPhail 2007). However, these products have proven to be of limited efficacy in commercial storage facilities (Waterer, personal communication, 2008).

Biological control is the use of naturally occurring antagonists to control pests and pathogens (Cook 2000). Phytopathogen antagonists, such as rhizosphere bacteria, can decrease disease and indirectly increase plant health by: (i) suppressing the activity of phytopathogens via the production of antagonistic metabolites, i.e. antibiotics; (ii) competing for nutrients on the tuber surface, essential for growth and survival of phytopathogens; and (iii) inducing the host plant's systemic and localized defence systems (Glick 1995; Whipps 1997, 2001; Haas and Keel 2003; Haas and Defago 2005).

Bacterial antagonists including isolates from the Enterobacteriaceae, Pseudomonadaceae and Bacillaceae families, have been shown to suppress dry rot formation by *Fusarium* spp. on potato (Kiewnick and Jacobsen 1997; Schisler, Slininger, Kleinkopf, Bothast, and Ostrowski 2000; Sadfi et al. 2002; Slininger et al. 2003). Bacterial metabolites such as phenylacetic acid, indole-3-acetic acid and tyrosol identified in the culture supernatant of *Enterobacter cloacae* S11:T:07 were implicated in providing biofungicidal activity (Slininger et al. 2004). Efficacy studies with potatoes dipped in bacterial suspensions in the laboratory (Kiewnick and Jacobsen 1997; Schisler and Sliningler 1994), greenhouse, field, cold storage (Sadfi et al. 2002) and commercial storage (Schisler et al. 2000) have consistently shown significant reduction in Fusarium dry rot of potato equal to or better than the fungicide TBZ, demonstrating the potential of bacterial antagonists for managing this post harvest disease. Eukaryotic microorganisms have also been studied for biocontrol

of Fusarium dry rot. Niemira, Hammerschmidt, and Safir (1996) reported biocontrol of Fusarium dry rot in potato minitubers by arbuscular mycorrhizal fungi. Several strains of yeasts were examined for biocontrol of Fusarium dry rot of potatoes, however, bacterial agents proved more effective (Schisler, Kurtzman, Bothast, and Slininger 1995). Despite the apparent value, no products based on bacterial antagonists to Fusarium dry rot are available to growers. There are several reasons that may account for this including: the potential risk to human health of the putative biocontrol agents, the cost of production, inconsistent efficacy, poor shelf-life and the inability to deliver the biocontrol agent in a user-friendly formulation (Hynes and Boyetchko 2006).

The objective of this study was to evaluate the potential of rhizosphere bacteria isolated from Canadian soils to suppress dry rot of potato caused by *F. sambucinum*. Two bacterial isolates, *Serratia grimesii* 4–9 and *S. plymuthica* 5–6, with broad spectrum *in vitro* antifungal activity against *Fusarium avenaceum*, *Rhizoctonia solani* CKP7 and *Pythium* spp. p88-p3 (Hynes, Leung, Hirkala, and Nelson 2008), were selected for this study. *In vitro* and *in vivo* experiments were conducted to examine the potential of *S. grimesii* 4–9 and *S. plymuthica* 5–6 to control growth of *F. sambucinum* and the onset of Fusarium dry rot in potato.

Materials and methods

Fusarium sambucinum *culture*

Fusarium sambucinum was isolated from diseased potato tubers obtained from the University of Saskatchewan and maintained on potato dextrose agar, 39 g/l dH$_2$O, (PDA, Difco, Becton-Dickinson, MD, USA) at 4°C. The race of *F. sambucinum* selected for the study was chosen based on its virulence to potato variety Cal red and not resistance or sensitivity to TBZ. *F. sambucinum* was periodically inoculated into potato and re-isolated to ensure its virulence. When suspensions of *F. sambucinum* conidia were required, fungal plugs were transferred to PDA and incubated at 27°C. After 3 weeks of incubation conidia were scraped off the agar plates into 10 ml of sterile distilled water and used immediately. The yield of conidia of *F. sambucinum* was log$_{10}$ 5/ml, determined using a Petroff Hauser counter.

Bacterial cultures

The bacterial antagonists used in this study were selected from a collection of bacteria isolated from the rhizosphere of pea, *Pisum sativum* L., grown on farms near Rosetown and North Battleford, Saskatchewan (Hynes et al. 2008). *Serratia grimesii* 4–9 suppressed the growth of fungal pathogens isolated from diseased lentil (*Fusarium avenaceum*) and chickpea (*Rhizoctonia solani* CKP7), while *Serratia plymuthica* 5–6 suppressed the growth of the above pathogens, as well as *Pythium* sp. p88-p3 isolated from pea (Hynes et al. 2008).

Identification of *S. grimesii* 4–9 and *S. plymuthica* 5–6 was achieved by sequencing PCR amplifications of the 16S rRNA gene using whole cell lysates from pure cultures. Each 16S gene sequence was queried for similarities with BLAST (Altschul, Gish, Miller, Meyers, and Lipman 1990) and with the Ribosomal Database Project (RDP) Similarity Rank and Chimera Check programs (Maidak et al. 2000). The nucleotide sequences were aligned using the CLUSTAL X program (Thompson, Higgins, and Gibson 1994) applying default parameters.

Bacterial cultures were started from stock cultures maintained at –70°C in 20% glycerol. They were streaked onto half strength tryptic soy agar (TSA, Difco, Becton-Dickinson), g/l dH$_2$O: pancreatic digest of casein 8.5, enzymatic digest of soybean meal 1.5, NaCl 2.5, K$_2$HPO$_4$ 1.3, dextrose 1.3 g, agar 15 g, and following confirmation of culture purity two to three colonies were transferred into 100 ml of half strength tryptic soy broth, as above without agar, in a 250 ml Erlenmeyer flask. Cultures were placed on a shaker at 150 rpm at 23°C. After 48 h. bacterial cultures were diluted 100 times in dH$_2$0 and approximately log$_{10}$ 7 colony forming units per millilitre (CFU/ml) were applied to potatoes as described below.

Potatoes

The potato variety Cal Red was used for the bioassay slice experiment and tuber experiments. This variety does not possess any significant level of resistance to Fusarium dry rot (Anonymous 2001).

Chemical control

Thiabendazole (TBZ, Mertect, produced by Syngenta) is the most commonly used fungicide for Fusarium control in stored potato. TBZ inhibits fungal mitosis. The recommended rate for treating potatoes is 8 l Mertect/120 l water (19 g TBZ/l) with 2 l of this solution sprayed onto 1000 kg of freshly harvested potatoes as they are loaded into storage. Due to concern about uneven and incomplete application of the chemical achieved using spray equipment, the potatoes in this study were rolled in a solution of TBZ equivalent to the above recommended rate.

In vitro *bioassay*

Growth suppression or antagonism of *F. sambucinum* by the putative biocontrol agents was initially studied using a dual culture technique on one-fifth strength PDA. An agar plug of *F. sambucinum*, taken from close to the margin of a growing culture, was placed in the centre of a Petri dish containing PDA. Bacterial isolates, *S. grimesii* 4–9 and *S. plymuthica* 5–6, were applied in a 40-mm long straight line approximately 30 mm from the *F. sambucinum* plug. Control treatments contained the fungal plug in the absence of the putative biocontrol agents. Four replicate plates per treatment were incubated at 15 or 25°C. The 15°C treatment was representative of the temperatures maintained during potato wound curing while the 25°C treatment simulated conditions encountered during marketing of potatoes.

After 20 days of incubation at 15 and 25°C, growth of *F. sambucinum* was measured. Percent inhibition was determined as 1 minus (the ratio of the radius of the Fusarium colony in the presence of the rhizobacteria to that of the control without rhizobacteria) times 100.

In vivo: *Fusarium dose–response*

Potatoes free of obvious symptoms of Fusarium dry rot were scrubbed with a plastic brush under tap water and then surface sterilized in 50% ETOH. Each potato was sliced on a clean, dry paper towel with a knife sterilized with 95% ETOH. The potato slices were about 5 mm thick. The slices were rinsed with distilled water to remove any alcohol residue and

then placed in petri dishes with sterile filter paper (Ray and Hammerschmidt 1998). Limited mycelial growth of unidentified fungi was seen on the outer surface of the potato slices at the end of the incubation period.

Four rates, $\log_{10}$ 1.3–$\log_{10}$ 4.4, of *F. sambucinum* conidia were applied to the surface of each potato slice. Fifteen 13-μl drops of each of the four rates of *F. sambucinum* or dH$_2$O (control) were applied to a potato slice. The initial propagule suspension was prepared as described above and diluted as required. Ten replicate slices were evaluated for each level of inoculum. The inoculated slices were incubated at 25°C in the dark for 14 days. The minimum dosage which consistently produced two to three infection pockets was used in the subsequent trials.

In vivo: *co-inoculation study*

The potatoes were selected, washed, disinfected and sliced as described above. Potato slices were inoculated with 15 13-μl drops of a bacterial inoculant ($\log_{10}$ 7.3/slice) and/or the fungal pathogen ($\log_{10}$ 2.18 conidia/slice,) and/or distilled water such that each slice received 390 μl of liquid. Treatments tested were: (i) sterile distilled water, (ii) *S. grimesii* 4–9, (iii) *S. plymuthica* 5–6, (iv) *S. grimesii* 4–9+*F. sambucinum*, (v) *S. plymuthica* 5–6+ *F. sambucinum*, and (vi) *F. sambucinum*. Ten potato slices per treatment were prepared as indicated. Five to 10 min were allowed between applications of each component, allowing the liquid to be fully absorbed into the potato. Potato slices were incubated in plastic storage containers ($5 \times 18 \times 29$ cm), one container per treatment, and placed randomly within the 15 and 25°C controlled temperature rooms.

Rating of in vivo *disease suppression*

The diameter of each Fusarium dry rot infection was measured, along with the number of infections after 18 days incubation at 15 or 25°C. These measurements were then used to calculate the total surface area of the Fusarium dry rot infection. To obtain percent (%) control of the pathogen, the average of the total surface area affected by Fusarium for each treatment was compared to the corresponding *Fusarium* alone-inoculated control.

In vivo: *tuber bioassay*

Unwashed, bruise-free potatoes were given four identical wounds using a 3×8 mm (wide $\times$ long) nail in a board. Wounds were inflicted around the middle of the potato (Platt 1992). Each potato tuber was then rolled and coated in a treatment solution containing *S. grimesii* 4–9 or *S. plymuthica* 5–6, $\log_{10}$ 7.3 cfu/ml, and/or *F. sambucinum* $\log_{10}$ 2.18 conidia/ml. Treatments solutions included: (i) sterile distilled water, (ii) *S. grimesii* 4–9, (iii) *S. plymuthica* 5–6, (iv) TBZ, (v) *F. sambucinum*, (vi) *S. grimesii* 4–9+*F. sambucinum*, (vii) *S. plymuthica* 5–6+*F. sambucinum*, and (viii) TBZ+*F. sambucinum*. TBZ treatment was prepared and applied as previously described. The tubers were then placed in a sealed plastic containers, $16.5 \times 38 \times 30$ cm, with a damp paper towel, one container per treatment, and randomly placed in 15 or 25°C, temperature controlled storage rooms. After 24–48 h the lids were loosened to allow for air circulation and to produce drier conditions – otherwise bacterial soft rot was induced, leading to the loss of the tuber. Experiments were carried out in triplicate, with five tubers per treatment in each experiment.

Rating of Fusarium tuber bioassay

The colonization and severity of tissue destruction by *F. sambucinum* was determined by measuring the diameter of the wound after 26–32 days of incubation. Treatment effects were determined by comparison with controls.

Statistical analysis

The data reported are pooled means from replicate trials. The dose–response experiment was carried out twice, whereas the *in vivo* potato slice and potato tuber experiments were each carried out three times. The data were statistically analyzed using a completely randomized one-way ANOVA with a 5% significance level and the *post-hoc* LSD and Duncan's Multiple Range tests in SPSS (SPSS Inc. Chicago, IL).

Results

In vitro: *growth challenge bioassay*

Fusarium sambucinum grew 23% faster at 25°C than at 15°C. *Serratia grimesii* 4–9 and *S. plymuthica* 5–6 suppressed growth of *F. sambucinum* in dual culture bioassay at both 15 and 25°C. In the presence of *S. grimesii* 4–9, growth of *F. sambucinum* at 15 and 25°C was suppressed by 58 and 67%, respectively, relative to *F. sambucinum* alone. *S. plymuthica* 5–6 suppressed growth of *F. sambucinum* by 60 and 67% at 15 and 25°C, respectively, compared to *F. sambucinum* alone. The standard error of measurements ranged from 3 to 7% of the data means.

In vivo: *dose–response for* F. sambucinum

Infection pocket development increased with increased dosage, $\log_{10} 1.2$ to $\log_{10} 4.4$, of *F. sambicinum* conidia/ml (Figure 1). One of the potato slices in the control treatment, 0 conidia, was accidentally infected; however, the remaining slices of this treatment showed
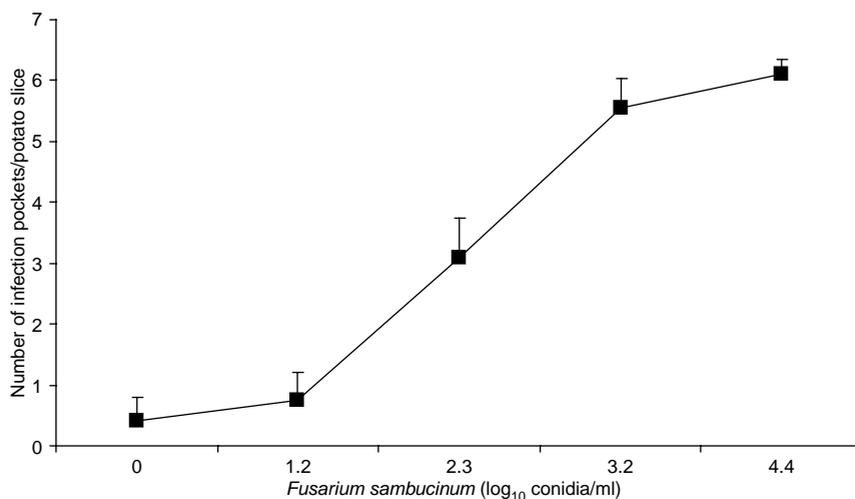

Figure 1. Effect of dosage of *F. sambucinum* conidia on the development of disease infection pockets on potato slices. The bars indicate the standard error of the mean.

the typical light brown colour discolouration from exposure to air. A consistent number of infection pockets of 3 was achieved with the application of $\log_{10}$ 2.3 conidia/potato slice (Figure 1). This application rate was used for subsequent experiments.

In vivo: *potato slice bioassay*

Potato slices treated with water or *S. grimesii* 4–9 or *S. plymuthica* 5–6 without *F. sambucinum* showed very little discolouration of the tuber slice surface, indicating that the presence of the rhizobacteria was not detrimental to internal potato tissue. After 18 days of incubation at 25°C the potato slices treated with *F. sambucinum* alone were completely covered in dry rot pockets. Potato slices incubated at 15°C showed 582 mm^2 of infected area or 73% less disease than at 25°C. Inoculation with *S. grimesii* 4–9 suppressed *F. sambucinum* infection site development by 96% and the size of the infection sites by 91% at 15°C; inoculation with *S. plymuthica* 5–6 suppressed *F. sambucinum* infection site development by 97% and the size of the infection sites by 96% at 15°C (Table 1). Inoculation with *S. grimesii* 4–9 or *S. plymuthica* 5–6 reduced the area infected by *F. sambucinum* by 99% at 15°C and by 87 and 96%, respectively, at 25°C.

In vivo: *potato tuber bioassay*

When applied simultaneously with the pathogen, *S. grimesii* 4–9 reduced development of Fusarium dry rot in whole tubers by 60% at 15°C and by 63% at 25°C compared to *F. sambucinum* alone (Figure 2). Inoculation with *S. plymuthica* 5–6 decreased Fusarium dry rot by 77% at 15°C and by 84% at 25°C. TBZ reduced development of Fusarium dry rot by 66% at 15°C and 81% at 25°C compared to *F. sambucinum* alone. The level of control achieved by the application of the bacterial biocontrol agents was comparable to that achieved using the fungicide TBZ (Figures 2 and 3). In the absence of *F. sambucinum*, inoculation of the potato tubers with the bacterial isolates did cause some discolouration, but no more than treatment with water or TBZ.

Discussion

In this study *Serratia grimesii* 4–9 and *S. plymuthica* 5–6 were identified as potential biofungicides for control of Fusarium dry rot of potato. They were chosen for study based

Table 1. Effect of *S. grimesii* 4–9 and *S. plymuthica* 5–6 on the number and size of infection sites initiated by *F. sambucinum* in *in vivo* potato slice bioassays at 15 and 25°C.

Treatments	Number of infection sites		Diameter of infection sites (mm)	
	15°C	25°C	15°C	25°C
S. grimesii 4–9+*F. sambucinum*	0.3 B[1]	5.9 A	0.5 B	4.0 A
S. plymuthica 5–6+*F. sambucinum*	0.2 B	2.2 B	0.2 B	2.3 AB
F. sambucinum	8.6 A	See note[2]	5.4 A	See note[2]

[1]Values followed by the same letter within each column do not differ significantly according to Duncan's Multiple Range test at $P < 0.05$. [2]At the end of the incubation time 100% of the potato slices surface were infected and we were unable to distinguish the number and size of infection sites.
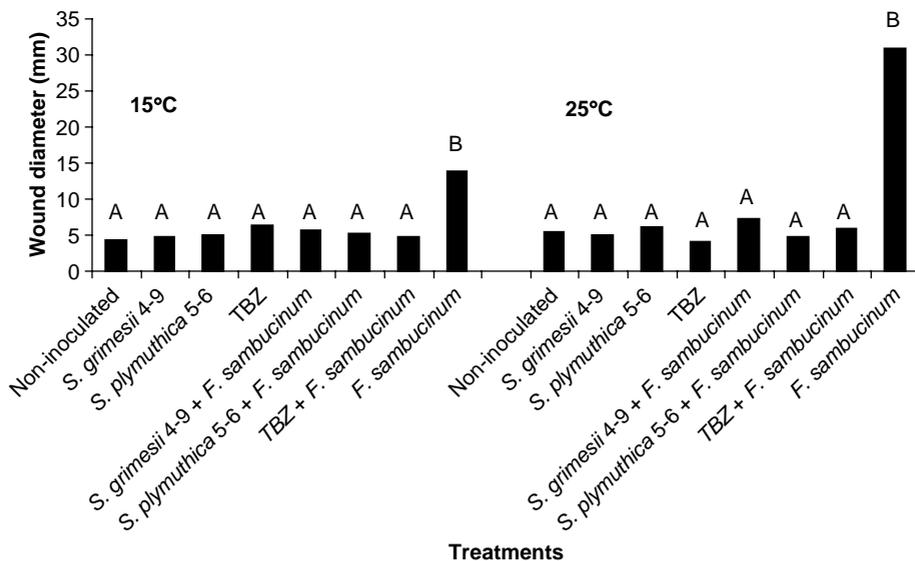
Figure 2.   Effect of the potato biocontrol agents *S. grimesii* 4–9, *S. plymuthica* 5–6 and the fungicide Thiabendazole (TBZ) on wound diameter of potato tubers infected with *F. sambucinum* after incubation at 15 and 25°C. Values with the same letter from each incubation temperature do not differ significantly according to Duncan's Multiple Range test at $P < 0.05$.

on their antagonism to phytopathogens of lentil, chickpea and field pea: *Fusarium avenaceum*, *Rhizoctonia solani* CKP7 and *Pythium* sp. p88-p3, respectively (Hynes et al. 2008). Both isolates showed significant control of growth of *F. sambucinum* on artificial medium, tuber slices and whole potato tubers at temperatures similar to those encountered during curing and marketing of potatoes. The biocontrol agents had no observable negative effects on tuber quality. The mode(s) of action of *S. grimesii* 4–9 and *S. plymuthica* 5–6 remain to be determined; however, (i) production of antifungal metabolites, (ii)
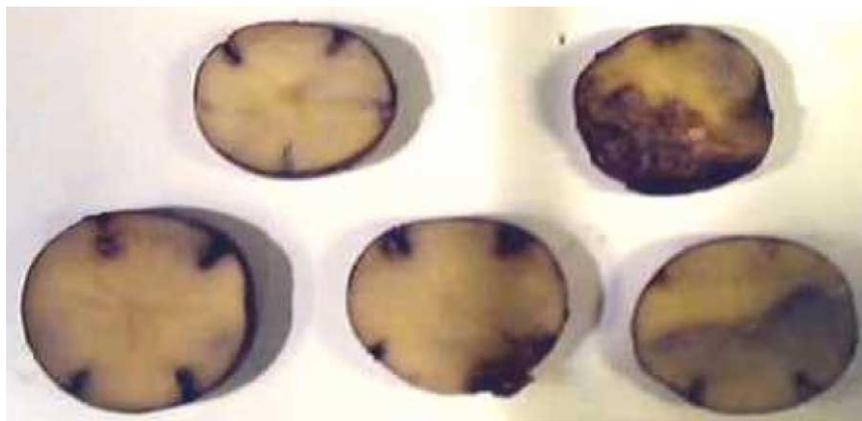

Figure 3.   Effect of the potato biocontrol agents *S. grimesii* 4–9, *S. plymuthica* 5–6 and the fungicide TBZ on disease onset in whole tubers inoculated with *F. sambucinum*. Treatments, upper left to right: control (non-inoculated) and *F. sambucinum* alone; lower left to right *S. grimesii* 4–9+*F. sambucinum*, *S. plymuthica* 5–6+*F. sambucinum* and TBZ+*F. sambucinum*.

competition for nutrients and space, and (iii) induction of resistance mechanisms in the host may account for the observed suppression of *F. sambucinum* (Whipps 2001). Levenfors, Hedman, Thaning, Gerhardson, and Welch (2004) reported that *Serratia plymuthica* A 153, isolated from the roots of winter wheat, produces antifungal metabolites including chlorinated macrolides and haterumalide (NA, B, NE and X), which suppressed apothecial formation in *Sclerotinia sclerotiorum* and slowed spore germination of several filamentous fungi. *S. plymuthica* also produces pyrrolnitrin and 1-acetyl-7-chloro-1-H-7-chloro-1-H-indole which suppress fungal spore germination. *Enterobacter cloacae* S11:T:07 produces the anti-fungal metabolites phenylacetic acid, indole-3-acetic acid and tyrosol and has been reported to suppress dry rot on potato (Schisler et al. 2000; Slininger et al. 2004). *S. grimesii* 4–9 and *S. plymuthica* 5–6 produce Fe-chelating siderophores; however, neither produce indoles, including indole-3-acetic acid, nor do they possess ACC deaminase (Hynes et al. 2008).

Fusarium sambucinum and the putative biocontrol agents were applied to potato within 20 min of each other in the experiments reported here. This method of application ensured equal opportunity for both the pathogen and isolate to colonize the wound sites. Commercial producers strive to keep the time interval between harvest and application of post-harvest disease control treatment to 2 h (Slininger et al. 1996). The relationship between time of infection versus time of application of biocontrol agents needs to be further examined.

Several genera of soil bacteria including *Pseudomonas*, *Enterobacter*, *Pantoea* (Schisler and Slininger 1994) and *Bacillus* (Sadfi et al. 2002) have been identified as potential biocontrol agents of *F. sambucinum*. In commercial storage bin trials, Schisler et al. (2000) reported that control of Fusarium dry rot by *Pseudomonas fluorescens* P22:Y:05 and *Enterobacter cloacae* S11:T:07 was comparable to that achieved using TBZ fungicide. In this study, *Serratia grimesii* 4–9 and *S. plymuthica* 5–6, both members of the Enterobacteriaceae family, also suppressed Fusarium dry rot as effectively as TBZ. The high level of disease suppression demonstrated by these and other bacterial isolates indicates the potential for biological control of Fusarium dry rot and should interest industry for product development (Sliningler et al. 2007).

Serratia plymuthica are frequently isolated from the rhizosphere of plants (De Vleesschauwer and Höfte 2007). Several isolates of *S. plymuthica* have been reported as being highly effective biocontrol agents for a variety of plant diseases including *S. plymuthica* HRO C48 against *Verticillium dahliae* Kleb. on strawberry (Kurze, Bahl, Dahl, and Berg 2001; Scherwinski, Wolf, and Berg 2007) and *S. plymuthica* A21-4 against *Phytophthora capsici* blight on pepper (Shen, Piao, Lee, and Park 2007). Several other *S. plymuthica* isolates and disesases controlled by these isolates are listed by De Vleesschauwer and Höfte (2007). In field studies suppression of weeds (*Chenopodium album*, Galeopsis speciosa, *Polygonum convolvulus* and *Stellaria media*) by *S. plymuthica* A153 was reported (Weissmann, Uggla, and Gerhardson 2003).

Serratia plymuthica is classified as a member of risk group 1 (as is *Bradyrhizobium japonicum*, a microbial symbiont of soybean) by the German Collection of Microorganisms and Cell Cultures (DSMZ). This species of *Serratia* is unlikely to pose an inadvertent threat to human health (De Vleesschauwer and Höfte 2007). *Serratia grimesii* and *Enterobacter cloacae* are classified into risk 2 group, as they pose greater risks to human health. Although both *S. plymuthica* 5–6 and *S. grimesii* 4–9 were effective and had no effects on the host plant, *S. plymuthica* 5–6 may be more promising for future product development as a biocontrol agent of Fusarium dry rot in potato, because it poses fewer potential health risks to applicators or handlers.

Serratia plymuthica 5–6 is a potential candidate for commercial development of a biofungicide for dry rot of potato. This microorganism is not fastidious and grows readily on inexpensive media, it has no adverse effects on potato, its efficacy is similar to that of TBZ and it is identified as being in the lowest risk group for microorganisms. Additional studies are required to examine efficacy of *S. plymuthica* 5–6 with spray application, the method typically used to apply fungicides.

Over 70 microbial agents are registered with the Environmental Protection Agency including the biofungicides *Bacillus subtilis*, *Pseudomonas syringae* and *Streptomyces griseoviridis* (US EPA 2007). As the database on environmental fate of microbial products expands and adoption and user comfort level of this technology increases, the full potential of pest control by microbial agents such as *S. plymuthica* 5–6 will be recognized.

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