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Seed tuber treatments with Pseudomonas spp. to reduce potato common scab incidence and severity

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Biology

SEED TUBER TREATMENTS WITH *PSEUDOMONAS* SPP. TO REDUCE POTATO COMMON SCAB INCIDENCE AND SEVERITY

(Thesis format: Monograph)

by

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Abstract

Potato common scab is a tuber- or soil-borne disease caused by several species in the genus *Streptomyces*. The objective of this study was to study the treatment of seed tubers with *Pseudomonas* spp. as a means to reduce common scab incidence and severity, and improve seed tuber quality and marketability. All four *Pseudomonas* spp. tested inhibited *Streptomyces* SS-1; PEI-1 and AL-1 *in vitro*.

In laboratory trials, treatments of seed tubers with *Pseudomonas* strains A25, A145, and A153, significantly reduced in the number of pathogenic *Streptomyces* in comparison to untreated tubers within five weeks ($P<0.05$). Seed tuber treatments with A25 and A153 significantly reduced scab incidence and severity in Russets Burbank and Prospect, respectively. This study also demonstrated the use of TaqMan qPCR detecting the *txtAB* gene analysis as a novel and practical method to quantify pathogenic *Streptomyces* in tissues of scabby and apparently clean potato seed tubers.

Keywords: Common scab, *Streptomyces scabies*, *Pseudomonas*, biocontrol, TaqMan qPCR, *txtAB*, seed tuber treatments, scab incidence, scab severity.

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Abbreviations

AL-1: Pathogenic *Streptomyces* isolated from scabby tubers obtained from Alliston, Ontario.

Ct value: Cycle threshold value

cv.: Cultivar

DAPG: Diacetylphoroglucinol

LB: Luria Broth

m/s: meters per second

NA: Nutrient Agar

OBA: Oat Bran Agar

PDA: Potato Dextrose Agar

PCA: Phenazine-1-Carboxylic Acid

PEI-1: Pathogenic *Streptomyces* isolated from scabby tubers obtain from Prince Edward Island.

POX: Peroxidase

PAL: Phenylalanine Ammonia Lyase

PVA: Polyvinylacetate

qPCR: Quantitative real-time Polymerase Chain Reaction

SS-1: *Streptomyces scabies* obtained from A&L Biologicals culture collection

v/v: Volume to volume ratio

w/v: Weight to volume ratio

Chapter 1

Introduction

Plant root exudates create a nutrient rich environment for pathogenic and beneficial microorganisms that constitutes one of the influential forces on plant growth and health. The number and diversity of pathogenic and beneficial microorganisms depend on the quantity and quality of plant exudates and the microbial interactions that occur within the rhizosphere (Raaijmakers *et al*., 2009). Soilborne plant pathogens include fungi, nematodes and few bacteria that are notoriously difficult to control once they establish in soil. Late blight and common scab are two pathogens that are of major concerns to many potato growers worldwide. Potato is the third largest global food crop after wheat and rice (Taehtjaerv *et al*., 2013) and is vulnerable to a variety of pathogenic fungi and bacteria that can greatly affect its marketability, yield or both. Late blight, caused by *Phytophtora infestans*, results in 16% loss of potato yield globally (Taehtjaerv *et al*., 2013). *Phytophthora* and pathogenic strains of *Streptomyces* produce resistant spores that can survive from one season to the next on potato tubers or in soil. *Phytophthora infestans* destroys the entire plant in only a few days, limiting production of tubers. Infected tubers spread the infection to neighboring healthy tubers, turning them into a smelly rotten mass, destroying potential food sources. A late blight outbreak Ireland in the 1840s, it resulted in a mass food shortage during which time millions to die of starvation and many million others emigrated (Taehtjaerv *et al*., 2013). Unlike *P. infestans,* pathogenic Streptomyces can survive in the soil on decaying organic matter and do not require living plant tissue in order to propagate, increasing the complexity of its control (Keinath and Loria, 1989).

The most effective management strategy to control late blight and common scab is to avoid introducing spores into the field by planting healthy certified seed potatoes, planting resistant varieties. Late blight can also be controlled by chemical fungicides but there are no effective pesticides to control the scab pathogen. However, planting certified seed tubers is does not guarantee that the seed is pathogen-free. Resistant varieties are not available for common scab. The use of chemical fungicides can increase the risk of

developing resistant strains and poses risk to human health. Chloropicrin, a broad spectrum pesticide, was used to control common scab with restricted use of 2.4 g/m^3. However, the product has health concerns and is relatively expensive (Dees and Wanner, 2012).

1.1 Potato common scab

In Canada, the potato industry is worth about $6.4 billion and potatoes account for 32 % of all vegetable farm cash receipts (Statistics Canada, 2006-2007). Potato common scab is one of the main concerns for many farmers after potato late blight and bacterial soft rot (Loria *et* al., 1997) Common scab-causing *Streptomyces* target a wide range of root crops, such as radish, beet, carrot, and potatoes. The unsightly symptoms reduce the marketability of potatoes by producing superficial, raised, or deep pitted lesions that can coalesce to cover large areas of the tuber (Wang and Lazarovits, 2005; Wanner 2006). Potatoes processed for French fries and chips are most frequently peeled, so lesion-free tubers with at most with superficial lesions are usually preferred (Wanner *et al*, 2013).

The disease is usually soil-borne but can be transferred to new locations via infested seed potato tubers. Wilson *et al.* (1999) confirmed that the severity of the disease in harvested potatoes is directly proportional to the severity of the disease in seed tubers. Similarly, Wang and Lazarovits (2005) demonstrated that the population of pathogenic *Streptomyces* is highest in soils immediately surrounding the seed tuber and declines with distance. Furthermore, they found that planting visually scab free seed tubers resulted in higher disease coverage in progeny tubers than when seed tubers were disinfected and planted in pathogen-free soil (Wang and Lazarovits, 2005). These findings suggest that the disease can also spread to new locations from infested seed tubers. This can be problematic because Canada has been a world leader in the production of seed potatoes for more than 90 years, with 150 potato varieties registered for the production of seed tubers (Statistics Canada, 2012-2013).

Unfortunately, little information is available on the prevalence and severity of scab incidence in Canada. According to a survey conducted by Hill and Lazarovits (2005), 82 % of the potato growers surveyed indicated that scab was a problem on their farms.

Losses due to grade-out of scabby tubers were estimated to results in a loss of $7500-$8500 per farm and a total economic loss of $15 to $17 million was due to common scab. The farm gate value of potato crops was about a1 billion dollars in Canada. Efforts have been made to control the disease using common cultural practices such as crop rotation, irrigation, and soil amendments, but results were inconsistent. Unfortunately, there are no safe and effective pesticides currently available to control common scab of potato (St-Onge et al. 2011).

1.2 Disease transmission and control

Streptomyces spp. are a gram-positive bacteria with a filamentous growth form similar to fungi. When sufficient nutrients are available they form a complex mycelial network that aids in scavenging organic compounds in the rhizosphere (Chater, 1984). When nutrient levels are low the vegetative mycelium separates into fragments to form spores. The vast majority of *Streptomyces* spp. play a major role in nutrient cycling in the soil due to their ability to produce a wide variety of catabolic enzymes that degrade cellulose and chitin (Wanner, 2006). However, only a small proportion of *Streptomyces* spp. are known to be plant pathogens. As pathogens, common scab-causing *Streptomyces* (which can also survive as saprotrophs) are limited to infecting expanding tissue during tuber formation, and necrosis is usually the first symptom of the disease (Loria *et al*., 1997).

Four pathogenic *Streptomyces* spp. are known to cause common scab in North America: *S. scabies*¸ *S. acidiscabies*, *S. turgidiscabies*, and *S. ipomoeae* (Signhai *et al*., 2011; Wanner, 2009). However, *S. scabies* is the major and common cause of common scab in many potato-growing fields worldwide (Dees and Wanner, 2012; Wanner, 2006).

Disease incidence and severity may vary from region to region, field to field within regions and from year to year depending on potato susceptibility (Goth *et al*., 1993, Lambert *et al*., 2006), pathogen severity, and environmental conditions (Wanner, 2009). Once established, these bacteria can survive in the soil for years as saprophytes on plant debris and organic matter (Hiltunen *et al.*, 2009). This complicates efforts to control the disease. Efforts have been made to manage the disease through chemical fumigation, crop rotation, irrigation, and soil amendments, but results varied from year to year and field to

field. However, disease suppression even though the pathogen is present can occur naturally due to the presence of antagonistic microorganisms (Meng *et al.,* 2009; Meng *et al.*, 2012). The use of antagonistic microorganisms may be an effective and economically feasible strategy in managing potato common scab.

1.3 Pathogen detection and quantification

Despite differences in phenotypic and genotypic traits, all common scab causing *Streptomyces* have similar mechanisms for pathogenicity. Traditionally, pathogenic *Streptomyces* were characterized based on pathogenicity, thaxtomin production (Conn *et al*., 1998), and morphological and physiological characteristics (Wanner, 2004). Melanin production has been used as a diagnostic characteristic of *S. scabies*, but a small proportion of non-pathogenic *Streptomyces* also produce melanin (Wanner, 2004).

Common scab causing *Streptomyces* are reliably differentiated from non-pathogenic strains only through the production of a family of phytotoxins known as thaxtomin. Lawrence *et al.* (1990) reproduced symptoms typical of common scab disease by aseptically inoculating cultured minitubers with cell free extracts from scab lesions of field-grown and cultured tubers that were infected with *S. scabies*. They further identified one of the active components of cell free extracts as thaxtomin A. Thaxtomin A inhibits the cellulose synthesis pathway and induces cell hypertrophy and necrosis on expanding host tissues in a concentration dependent manner (Fry and Loria, 2002; Scheible *et al*., 2003; Tegg *et al*., 2005). Its production begins at the onset of aerial mycelium growth and peaks during sporulation and is intimately involved in disease development (St-Onge *et al.*, 2010). During tuber formation high levels of cellobiose (one of the main building blocks of cellulose) accumulate in the rhizosphere inducing the thaxtomin biosynthetic pathway. Two thaxtomin synthetases are involved: TxtA and TxtB, encoded by *txtA* and *txtB* genes that are upregulated in the presence of cellobiose (Loria *et al.* 2008*;* Lerat *et al*,. 2009).

Conn *et al*. (1998) developed a semi-selective medium (oatmeal medium) for screening large numbers of *Streptomyces* from soil samples for thaxtomin production. However this procedure can be time consuming. It requires an initial incubation of homogenized soil

samples for two weeks on semi-selective culture (STR) medium before screening for thaxtomin A production can be initiated.

With the advent of molecular technologies recent studies have used polymerase chain reaction (PCR)-based detection as it provides high sensitivity and specificity and is a relatively simple technique (Flores-Gonzáles *et al*., 2008; Qu *et al*., 2008). Flores-Gonzáles *et al*. (2008) were able to detect common scab causing *Streptomyces* directly from tuber lesions by designing primers that would amplify a fragment of the *txtA* and *txt*B genes, considered to be the pathogenicity determinants in *S. scabies*. With this technique they were able to detect and isolate pathogenic *Streptomyces* from 70 samples taken from 84 infected potato samples across Western Europe. Qu *et al*. (2008) developed a SYBR Green quantitative real-time PCR assay that amplifies the *txtAB* gene to quantify pathogenic *Streptomyces* populations in potatoes and soil. They found that the real-time PCR assay was highly specific with a detection limit of 10 fg of target DNA. In addition, with this technique they were able to quantify pathogenic *Streptomyces* ranging from 10^1 to 10^6 pg in 1 g of scabby potato peel.

1.4 Biocontrol

Biological control of plant disease is the artificial introduction of living microorganisms into soil to control a pathogen (Höfte and Altier, 2010). Naturally suppressive soils against common scab have been identified and the reduction of disease, even though the pathogen is present, has been related to the presence of antagonistic micro-organisms (Meng *et al*., 2009; Meng *et al*., 2012; Rosenzweig *et al*., 2012). These micro-organisms, commonly denoted as plant-growth promoting rhizobacteria, reduce disease incidence by either competitively colonizing plants or repressing pathogenic micro-organisms by antibiotic production. Microbial composition in these suppressive soils revealed that several species of non-pathogenic *Streptomyces*, *Bacillus* spp. and *Pseudomonas* spp. inhibit the growth of *S. scabies* (Meng *et al.,* 2009; Meng *et al.,* 2012). However, the most widely studied agents associated with suppressive soils are members of the genus *Pseudomonas*.

The application of biological control through *Pseudomonas* spp. has gained much attention in recent years as a potentially effective management tool for several soil borne pathogens including bacteria (Keel *et al*., 1992; St-Onge *et al.*, 2011; Mavrodi *et al*., 2011). Leeman *et al.* (1995) investigated the effect of repeated seed application with *Pseudomonas fluorescens* WCS374 on Fusarium wilt disease yield of radish, in successive radish crops in a commercial greenhouse. Over a 4-year period, seed-treatments with WCS374 significantly reduced disease incidence by 42.6% on average and increased yield by 44.7% compared to control seed tubers treated with polyvinyl acetate (PVA).

However, the effectiveness of biological control in the field can vary depending on the efficiency of *Pseudomonas* spp. to be present on the roots in sufficient numbers in order have beneficial effects on the plant. To protect plants from *Gaeumannomyces graminis* var. *tritici*, the fungus causing take-all of wheat, *Pseudomonas* spp. need to establish a population of 10^5-10^6 CFU (colony forming units) g^{-1} (Haas and Keel, 2003). In addition, interactions with the host plant (Meyer *et al.*, 2010), environmental conditions, and the microbial community can affect the outcome of disease suppression (Wanner *et al*., 2013).

1.5 *Pseudomonas* spp. potential as biocontrol agents

The United States Environmental Protection Agency listed four *Pseudomonas* spp. among the fourteen registered bacterial biocontrol agents (Fravel, 2005). Their potential use in agriculture is steadily increasing as they can be used in rotation with chemical pesticides in order to reduce the development of pathogenic resistance. Such biological control agents offer an attractive way to replace harmful chemicals and pesticides (Höfte and Altier, 2010; Bhattacharyya and Jha, 2012).

De Souza *et al*. (2003) found high frequency of 2,4-diacetylphoroglucinol (DAPG)-producing *Pseudomonas* spp. that play a key role in two naturally suppressive Dutch soils to take-all disease of wheat. Mazurier *et al*. (2009) assessed the role of DAPG and phenazine producing fluorescent *Pseudomonas* spp. in naturally suppressive and conductive soils to *Fusarium oxysporum* by comparing densities, diversity and

antimicrobial activity. They found that DAPG producing *Pseudomonas* from suppressive and conductive soils were equally effective in suppressing Fusarium wilt, either alone or in combination with non-pathogenic *F. oxysporum.* Mutant strains that do not produce DAPG were equally as effective, suggesting that DAPG does not play a key role in suppression.

In contrast, they only detected phenazine producing *Pseudomonas* spp. in suppressive soils, but these isolates did not suppress Fusarium wilt on their own and acted synergistically in combination with non-pathogenic *F. oxysporum*.

1.6 Effect of *Pseudomonas* seed tuber treatments on common scab

Root-colonizing pseudomonads are able to protect crop plants against a variety of soil borne plant pathogens, notably by secreting antimicrobial secondary metabolites into the rhizosphere. *Pseudomonas fluorescens* and *P. chlororaphis* have gained much attention in recent studies for suppression of both bacterial and fungal plant diseases. Both produce wide range of antibiotics such as phenazine carboxylic acid (PCA) and DAPG that have broad spectrum activity against bacteria and fungi. St. Onge *et al*. (2011) presented results that suggest that PCA produced by *Pseudomonas* sp. LBUM 223 repressed the expression of *txtAB* and *txtC* that mirrored the decrease in thaxtomin A quantity and consequently the inhibition of *S. scabies* growth.

Similarly, induction of systemic resistance in plants is another mechanism of plant protection by directly suppressing soil borne pathogens through activation of defence genes such as peroxidase (POX) and phenylalanine ammonia lyase (PAL). Singhai *et al*. (2011) found that coating potato tubers with *Pseudomonas* R1 rapidly induced POX and PAL enzyme specific activities in leaves and peels especially when challenged with an inoculation with *S. scabies.* Both POX and PAL activities peaked at 48 h in both peels and leaves, but activity levels were higher in peel than in leaves. Furthermore, they found that progeny tubers of seed tubers coated with *Pseudomonas* R1 were still marketable with lower scab incidence and index by 10 % and 12 %, respectively, in comparison to control treatments.

1.7 Hypothesis

The objective of this study is to reduce scab incidence and severity by reducing the initial number of pathogenic *Streptomyces* that can establish on newly forming potato tubers. Preliminary experiments to quantify common scab causing *Streptomyces* from scabby tubers using semi-selective media proved to be time consuming. Melanin production is an imperfect indicator of pathogenicity, whereas all common scab causing *Streptomyces* carry the *txtAB* gene (Wanner and Lazarovits, 2005). In this study preliminary experiments showed strong linear correlation between the concentration of *txtAB* amplicon (copies/mg of scabby tissue) detected by qPCR was and PEI-1 CFU (colony forming units) per mg of tissue ($y=x+4.8$; $R^2=1$). Therefore, I hypothesize that TaqMan qPCR targeting the *txtAB* gene will provide with an accurate, simple, specific, and reproducible method in quantifying the number of pathogenic *Streptomyces* from scabby tissue samples compared to the traditional plating method using semi-selective media.

In this study, *Pseudomonas* strains obtained from A&L Biologicals Inc. were tested as seed tuber treatments on seed tubers and analyzed for the number of pathogenic *Streptomyces* after five weeks. tubers treated with *Pseudomonas* A25, A145 and A53 had significantly lower number of pathogenic *Streptomyces*. Wilson *et al.* (1999) demonstrated that the severity of the disease in harvested potatoes is directly proportional to the severity of the disease in seed tubers. Wanner (2004) investigated the effect of different inoculum concentrations on scab severity in radish. Most pathogenic strains produced superficial to raised lesions less than 10mm in diameter, at concentrations ranging from 10^4-10^5 CFU/g, respectively. Lesion severity increased with higher inoculum density but reached a plateau at 10^6 CFU/g. I hypothesize that treating seed tubers with *Pseudomonas* A25, A145 and A153 will reduce scab incidence and severity in harvested tubers by reducing the number of pathogenic *Streptomyces.*

Chapter 2

Methods and Materials

2.1 Isolation of common scab-causing *Streptomyces* from scabby tissue

Strains of pathogenic *Streptomyces* spp. (see Table 2-2) were isolated from scabby tubers obtained from Prince Edward Island (PEI) and Alliston, Ontario as described by Wang and Lazarovits (2004) with few modifications. Tubers were gently washed with distilled water to remove adhering soil particles then air dried. Scabby lesions of 200 mg were excised at a depth of 0.5-1 mm from the surface using 1.2 cm cork borer. To optimize isolation, the parameters tested included the size of glass beads used (0.5, 1.0 and 2.5 mm; BioSpec Products, Inc.) and the speed settings used for homogenization with the FastPrep system (4.5, 5.0, or 5.5, or 6.0 m/s for 20, 30, 40, 50 or 60 seconds). Based on the results of these tests, tissue samples were homogenized in 2 mL tubes containing three 2.5 mm glass beads and 500 μL of 0.1 % (w/v) water agar, using the FastPrep system (FastPrep®-24 Instrument, MP Biomedicals) at speed of 4.5 m/s for 50 seconds.

Homogenized suspensions were serially diluted and 100 μL aliquots of the homogenate plated onto 2 % (w/v) water agar. Plates were incubated at 29-30 ^{o}C for at least 10 days. To control fast growing bacteria, homogenized samples were heat-treated for 3 minutes at 55 ^{o}C before plating. Under sterile conditions, white, powdery colonies were selected and re-streaked onto Potato Dextrose Agar (PDA) (Sigma-Aldrich) to determine the production of dark, brown diffusible pigment, indicating melanin production – a characteristic feature of *S. scabies*. For long term storage, spore suspensions were stored in 20 % glycerol at -80 ^{o}C.

2.2 DNA extraction from pure culture and tissue samples

Two different commercially available kits: GeneElute^{TM} bacterial genomic DNA extraction Kit (Sigma-Aldrich) and Norgen Kit Soil DNA isolation Kit (Norgen Biotech Corp., Ontario, Canada) were tested to optimize DNA extraction from pure *Streptomyces* culture and scabby potato tissue

From a pure culture, 2-3 sporulating colonies were scooped using a sterile loop and placed into 2 mL bead tubes provided by both kits. Following the protocol provided by the manufacturer, GeneElute extraction kit provided with higher concentrations of DNA but with $A_{260/280}$ of 0.97. This however, did not affect the qPCR quality where detection and quantification of the *txtAB* occurred from samples obtained from scabby potato peel. This procedure proved to be too time consuming in comparison to using the Norgen Kit. However, combining some steps provided in the purification process provided in both kits, a time efficient and easy DNA extraction method was developed for recovery of DNA with higher concentration and better quality. The protocol provided by the Norgen kit manufacturer was modified as follows. An additional heating step at 65 ^{o}C for 10 minutes was included before samples were beat-beaten at 6.5 m/s for 1 minute using the FastPrep system. Before DNA samples were eluted, samples were washed with 70 % (v/v) ice cold ethanol to increase DNA precipitation. The addition of these steps to the original Norgen Kit protocol helped increase the final concentration of DNA eluted from 10 ng/μL to 50 ng/μL on average with $A_{260/280}$ of 1.78. Similarly, DNA extraction from scabby tissue samples (250 mg) was done using the modified protocol of the Norgen kit.

2.3 Biocontrol strains

Three *Pseudomonas* strains were obtained from A&L Biological collection *Pseudomonas* A25, A145, and A153, to test their inhibitory affect against pathogenic *Streptomyces in vitro*. Based on 16S rDNA analysis conducted by Dr. Amy Turnbull (A&L Canada Laboratories, London), strains A25, A145, and, A153 were identified to be closely related to *P. monteilii*, *P. chlororaphis*, and *P. brassicacearum*, respectively (Table 2-1).

Table 2-1 Bacterial strains used in this study

Species	**Strain number**	**Isolation Source**	**Genbank Accession of Best-matching 16S sequence**
Pseudomonas brassicacearum	A153	A&L Biologicals, soil	NR024950
P. chlororaphis	A145	A&L Biologicals, soil	NR_044974
P. monteilii	A25	A&L Biologicals, soil	CP006978
P. monteilii	A33	A&L Biologicals, soil	CP006979
Pathogenic *Streptomyces*	AL-1	Alliston, ON, scabby potato peel	D63863
Pathogenic *Streptomyces*	PEI-1	PEI, scabby potato peel	D63862
Streptomyces scabies	SS-1	A&L Biologicals, scabby potato peel	FN554889

2.4 Antibiosis assay

The antibiosis assay is a technique used to test the potential of biocontrol agent to inhibit the growth a pathogen *in vitro*. Four *Pseudomonas* strains: *Pseudomonas* A25, A33, A145, and A153 were obtained from A&L Biological culture collection (Table 2-1). Each strain was inoculated into Nutrient broth (NB) (Sigma-Aldrich) and incubated overnight with constant shaking at 25 ^{o}C. Cultures of *S. scabies* SS-1 obtained from A&L Biological culture collection, and *Streptomyces* AL-1 and PEI-1 isolated from tubers obtained from Alliston, Ontario and a field from PEI, respectively, were streaked onto PDA 10-11 days until sporulation occurred. For the antibiosis assay 1-2 sporulating colonies were selected and placed into 1 mL of 0.1 % (w/v) water agar. The spores were dislodged using the FastPrep bead beater (2 mL capped tubes with 2.5 mm glass beads) at 4.5 m/s for 50 seconds. Spore suspensions were adjusted to 0.3 (OD at 600 nm wavelength) and then plated onto Nutrient Agar (NA) (Sigma-Aldrich). Twenty microliters of overnight cultures of *Pseudomonas* spp. adjusted to 0.8 (OD at 600 nm wavelength) were then spotted onto a lawn of *Streptomyces* growing on NA. After 1-2 days of incubation at 29-30 ^{o}C, zones of inhibition of the developing *Streptomyces* lawn became obvious with bacteria producing antibiotic compounds. All positive experiments were repeated three times.

2.5 16S rDNA sequence

Pathogenic *Streptomyces* PEI-1 and Al-1 were grown on PDA for 10 days at 29-30 ^{o}C. Two to three sporulating colonies were scooped using sterile loop and placed into 2 mL bead tubes containing 2 % (w/v) water agar. Samples were heated for 10 minutes at 65 ^{o}C then bead beaten at 6.5 m/s for 1 minute using the FastPrep system. DNA was extracted from these samples using the Norgen Kit Soil DNA isolation Kit. DNA eluted was adjusted to 30 ng/μL and was used as a template for the PCR reaction.

To perform the PCR reaction, the following master mix was used: 37.5 μL ddH_2O; 5 μL 10X Buffer solution (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3 at 25 ˚C; New England BioLabs Inc.); 2 μL of 2mM $MgCl_2$; 1.25 μL BSA (20 mg/mL; New England BioLabs); and 1 μL of 4 mM dNTP, 20 mM of each Bact27-F and Bact1492-R

(Table 2-2), 1 μL of template, and 0.25 μL of Taq DNA polymerase (5 units/μL; New England BioLabs Inc.). PCR reactions were carried out in a Bio-Rad thermocycler with an initial activation step at 95 °C for 30 seconds, followed by 30 cycles at 95 °C for 15 seconds, 50 °C for 15 seconds, 68 °C for 1 minute, and 72 °C for 5 minutes. After the PCR reaction 1 μL of the product was run on a 1 % (w/v) agarose gel at 110 V for 30 minutes to check for product presence and size. The rest of the successful PCR products were purified for sequencing using the Zymo kit (DNA Clean and Concentrator^{Tm}, Zymo Research). For 50 μL of PCR product, 250 μL of binding solution was added and mixed well then centrifuged for 1 minute through a spin column filter. The sample was washed twice with 250 μL of washing buffer and then eluted with 15 μL of DNA free H_2O. The DNA samples were checked with a spectrophotometer at 68:2 (TB buffer:DNA sample) ratio to check for purity and concentration of DNA, then adjusted to 30 ng/μL and 10 μL samples with 5 μL of 20 mM Bact1492 reverse primer added were submitted to London Regional Genomic Center, Robarts Research Institute, University of Western Ontario, for sequencing. Sequences received were then identified by comparison with the most similar named reference sequence in GenBank using BLASTn (Altschul *et al*., 1998)

2.6 Secondary metabolite characterization

Overnight cultures of *Pseudomonas* strains were serially diluted to obtain 10^4 CFU/mL. Twenty microliters of each strain were spotted onto PDA plates and incubated for one week at 29-30 ^oC. The production of green crystals within the growing culture was considered positive for phenazine-1-carboxylic acid (PCA).

2.7 Detection of pathogenic *Streptomyces* from scabby potato peel using TaqMan qPCR

The real-time PCR protocol was already available at A&L Biologicals including the designs of the forward primer, (Strep F), the reverse primer, (Strep R), and the TaqMan probe (Strep 2Q) designed by Dr. Keri Wang (A&L Canada Laboratories, London; see Table 2-2). The qPCR assay was initially optimized using DNA extracted from pure culture of *S. scabies* SS-1. Parameters tested included concentrations of $MgCl_2$ (2.0-6.0 mM), primers (0.1-1 μM), probe (0.1-1 μM) and volume of 30 ng/μL DNA templates (1-

3 µL). The results of these tests were similar to those found by Qu *et al.* (2011). Based on these results qPCR amplification henceforth was performed in a 10 µL reaction mixture containing 1X PCR buffer (10 mM Tizma-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001 % (w/v) gelatin, pH 8.3 at 25 °C) (Applied Biosystems), 6 mM $MgCl_2$, 200 µM of each dNTP, 0.1 µM of each primer, 0.3 µM of probe Strep 2Q and 0.625 U AmpliTaq Gold DNA polymerase (Applied Biosystems). Three microliters of *S.* scabies DNA (30 ng/µL) was used as template per reaction. Quantitative real-time PCR was carried out in Qiagen/Corbett Rotor-Gene® 6000 with an initial activation step at 94 °C for 15 seconds, followed by 45 cycles at 63 °C for 30 seconds, and 72 °C for 30 seconds. PCR efficiency was determined from the slope of a calibration curve by the equation $E = 10^{[-1/slope]}$. A standard of 10,000 copies/µL was used for further quantification of the *txtAB* gene.

To evaluate the sensitivity limit and quantification of TaqMan real-time PCR assay serial dilutions of *S. scabies* SS-1 genomic DNA ranging from 10 ng to 1 fg were performed. A standard curve was constructed by plotting Ct values versus the log concentration of *S. scabies* SS-1 genomic DNA.

2.8 Quantification of pathogenic *Streptomyces*: Standard curve

A standard curve correlating Ct value with CFU/mL was created -to quantify the number of pathogenic *Streptomyces* present in a tissue sample. Homogenized scabby tissue excised from Prospect tubers obtained from PEI, were serially diluted and plated onto 2 % (w/v) water agar to calculate the number of *Streptomyces* colony forming units (CFU) per mL. The number obtained was used as the initial concentration. Using $C_1V_1=C_2V_2$ the expected/predicted CFU/mL in each serial dilution was calculated. DNA was then extracted from each serial dilution and analysed for the *txtAB* amplification. Ct values obtained from the qPCR reaction and the calculated CFU/mL were then used to construct the standard curve. Tissue samples from visually clean Russet potatoes were initially tested for the detection of *S. scabies.* Tubers that were negative for *S. scabies* in qPCR assay were used as negative controls. Two hundred milligrams of *S. scabies* free potato peel was spiked with 1mL of *S. scabies* PEI-1 at each serial dilution. DNA extraction was performed as described earlier and a second standard curve was constructed by plotting

the Ct value against CFU/mL. Furthermore, concentrations of the *txtAB* amplicon calculated by the qPCR program were plotted against the CFU/mL to investigate the relationship between both concentrations.

2.9 Seed tuber treatments

Seed tuber treatment preparation

Seven seed tuber treatments were prepared for laboratory and field experiment: control, A25, A145, A153, and a mixture of these three *Pseudomonas* strains (1:1:1), liquid broth and ethanol. Treating with autoclaved water served as a control. On the other hand, Liquid broth is thought to increase the number of pathogenic *Streptomyces* that already inhabit potato surface whereas treatment with ethanol would reduce their numbers. *Pseudomonas* strains A25, A145, and A153 cultures were prepared by inoculating 1 mL of overnight bacterial culture into 500 mL freshly made LB and incubated for 5 days with shaking at 25 ˚C to promote antibiotic production. Ten Prospect tubers of similar size (250 g) were selected and washed gently with distilled water to remove adhering soil and left to dry over night before applying seed tuber treatments.

After 5 days growth each *Pseudomonas* culture was suspended in LB to obtain 10^7 CFU/mL. Each culture was then mixed with 1 % (w/v) carboxymethyl cellulose (CMC). Each of the seed tuber treatments were then poured into 7 different autoclaved 500 mL beakers under aseptic conditions. Tubers were then dipped into bacterial cultures for 5 minutes and air dried. Tubers were also dipped into liquid broth and 80 % (v/v) ethanol. For control treatments tubers were dipped into autoclaved water. Treated tubers were then placed under sterile conditions in autoclaved magenta boxes and stored at room temperature.

To analyze the effect of seed tuber treatments on the number of pathogenic *Streptomyces* three samples of scabby potato peel (250 mg) were excised from each tuber 0.5-1 mm from the surface using a 1.2 cm cork borer one and two weeks after seed tuber treatments. DNA was then extracted from each tissue sample and analyzed for the *txtAB* gene

fragment using TaqMan qPCR. The number of pathogenic *Streptomyces* was then calculated using the standard curve created in section 2.5.

Field set-up, experimental design, and site characteristics

Three different cultivars of different scab susceptibility were used to test the effectiveness of *Pseudomonas* seed tuber treatments in the field: Yukon Gold (moderately susceptible), Russet Burbank (moderately resistant), and Prospect (susceptible) (Figure 2-1) The field experiment was conducted at the Ecologistics Research Services Center farm, London, ON, with planting on 4 June 2013. The site soil was loamy sands with pH 7.2, ideal for potato cultivation. The plot was 19.8 by 8.2 m in size and divided into seven rows where each row represented one treatment. Each row was then divided into three blocks (4.6 by 0.9 m in size).

Due to not having enough seed tubers for planting they were cut in half and treated with various pseudomonads (A25, A145, A153, and the consortium), liquid broth, ethanol, and control (autoclaved water) and stored under room conditions until planting fifteen seed-pieces per block. The experimental set-up was three cultivars x 4 potential *Pseudomonas* seed tuber treatments x 15 replicates (tubers planted), planted in a completely randomized design.

Germination readings were taken on 19 July 2013. After four months the plants had died down and the middle ten plants were harvested separately and used to compute the number of tubers per plant, weight of tubers per plant, scab incidence and index, scab lesion size, and the number of pathogenic *Streptomyces* per mg of tissue.

Scoring common scab: At the end of the season, tubers from the ten middle plants were harvested where each plant was placed into separate bags. Disease incidence was expressed as the percentage of tubers with common scab symptoms in each block. Tubers weighing greater than 5 g were scored based on percent surface covered with scab for on a scale of 0-6, where 0 = no scab, 1 =trace-5 %, 2 =6-15 %, 3 = 16-25 %, 4 = 26-35 %, 5 = 36 -60%, 6 = 61-100 % (Wang and Lazarovits, 2005). Lesion sizes were scored according to Wanner *et al.* (2013) on a scale 0-3, with 0=no symptoms, 1=superficial

lesions, 2=raised lesions, and 3=pitted lesions. Scab severity was measured as [Σ (Percentage coverage by lesions × predominant lesion type × number of tubers with these scores)/(total number of experimental replicates × total number of potato tubers evaluated)] × 100 (Wanner *et al*., 2013)

Detection and quantification of pathogenic *Streptomyces*: Ten tubers with similar scab score, index, and scab lesion size were selected from each cultivar and treatment for quantifying the number of pathogenic *Streptomyces*. From each tuber three visually clean and three scabby tissue samples were excised 0.5-1 mm from the surface using a 1.2 cm cork borer. DNA was then extracted from each tissue sample and analyzed for the *txtAB* amplification using TaqMan real-time PCR. The number of pathogenic *Streptomyces* was calculated using a standard curve generated by plotting the CT-value against log CFU/mg.

2.10 Data analysis

Common scab incidence and severity from the field experiment was subjected to analysis of variance (ANOVA), and means were separated using Fisher's least significant difference test (R-statistic software version 3.0.2). Pairwise comparison tests were used to analyse the impact of different treatments and cultivars on lesion size and number of pathogenic *Streptomyces*.

Table 2-2 Primers and probe used in the study.

Primer/ Probe	Sequence 5'-3'	Target gene	Product size	Reference or Gene Accession
Bact 27-F	AGAGTTTGATCCTGGCTCAG	Bacterial 16S		Edwards *et al*., 1989
Bact 1492-R	GGTTACCTTGTTACGACTT	Universal		Lane, 1991
Strep F	5'-GCAGGACGCTCACCAGGTAGT-3'	*txtAB*	71	AF255732
Strep R	5'-ACTTCGACACCGTTGTCCTCAA-3'			AF255732
Strep 2Q	5'-CAL Fluor Red610-TCGGTGATCCAGTACTTTCCGTCGGC-BHQ-2-3'			AF255732

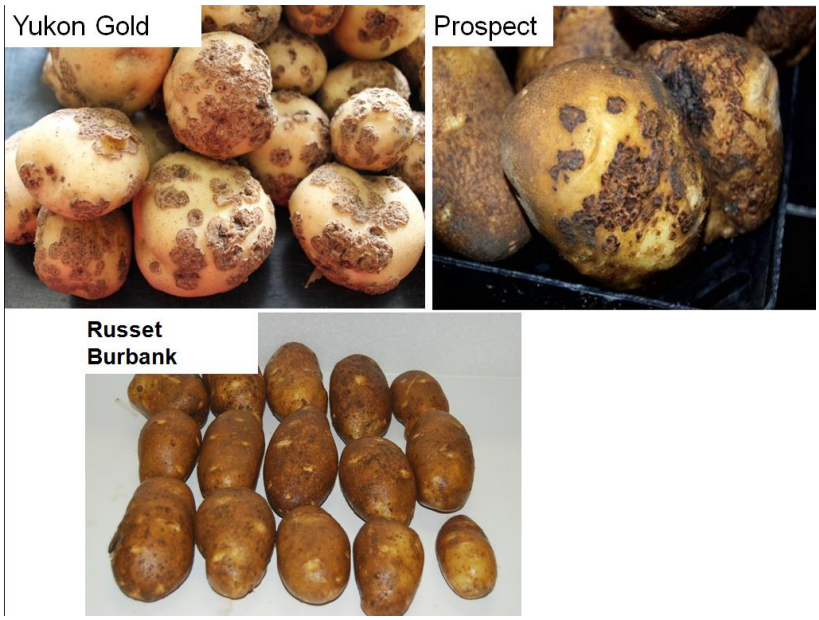

Figure 2-1 Three cultivars with different scab susceptibility were used to test the effect of seed tuber treatments with different *Pseudomonas* spp. in the field. Yukon Gold and Prospect seed tubers were 30-40 % covered with scab lesions whereas Russet Burbank seed tubers were visually clean.

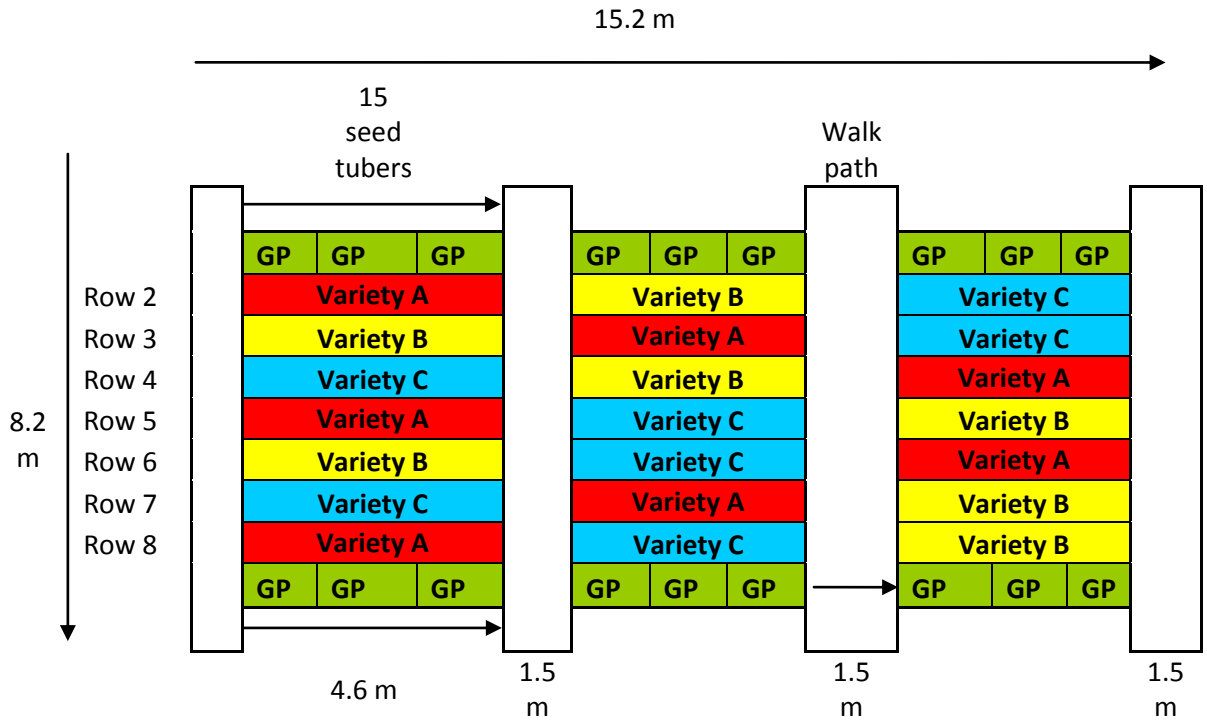

Figure 2-2 Field plan used for testing of seed tuber treatments at Ecologistics research center, London, Ontario. Russet Burbank (Variety A), Yukon Gold (Variety B), and Prospect (Variety C). Seed tuber treatments were included control (Row 1), A153 (Row 2), Ethanol (Row 3), A145 (Row 4), the consortium (Row 5), A25 (Row 6) and liquid broth (Row 7). Guard plants (GP) were planted around the plot.

Chapter 3

Results

3.1 Isolation of common scab-causing *Streptomyces* from scabby tubers obtained from PEI and Alliston, Ontario.

Streptomyces spp. isolated from infested tubers obtained from a grower in PEI did not grow well on *Streptomyces* selective media (STR). Even though STR favours the growth of actinomycetes, it was difficult to enumerate the total number of *Streptomyces* spp. isolated from scabby tissue because of contamination by fast-growing bacteria. To overcome this, the protocol from Wanner and Lazarovits (2004) was used with few a modifications. Homogenates of scabby tissues were plated onto 2 % (w/v) water agar and incubated at 29-30 ^{o}C for 10 days until white, powdery colonies formed and number of CFU per milligram of tissue was calculated (Figure 3-1a). These colonies were re-streaked on PDA (Potato dextrose Agar) to determine the production of dark, brown pigmentation, indicating melanin production characteristic of *S. scabies* (Figure 3-1b). In addition, heating bead beaten potato peel at 55 ^{o}C for 3 minutes reduced the number of fast-growing bacteria (Figure 3-2).

3.2 Antibiosis assay

When PEI-1 strain isolated from infected potatoes obtained from PEI was cultured in the presence of *P. monteilii* A25, *P. monteilii* A33, *P. chlororaphis* A145 and *P. brassicacearum* A153, inhibition of mycelium growth was observed around the paper disc (Figure 3-3). The inhibition zone indicates mycelium and sporulation inhibition, with *P. brassicacearum* A153 showing larger inhibition zone where as *P. monteilii* A33 only showed weak inhibition. Further strains of *Pseudomonas* were tested against *S. scabies* SS-1 and newly isolated *Streptomyces* strains from potatoes obtained from Alliston, ON, and PEI. *Pseudomonas* isolates showed broad inhibition to different *Streptomyces* isolates (Table 3-1)

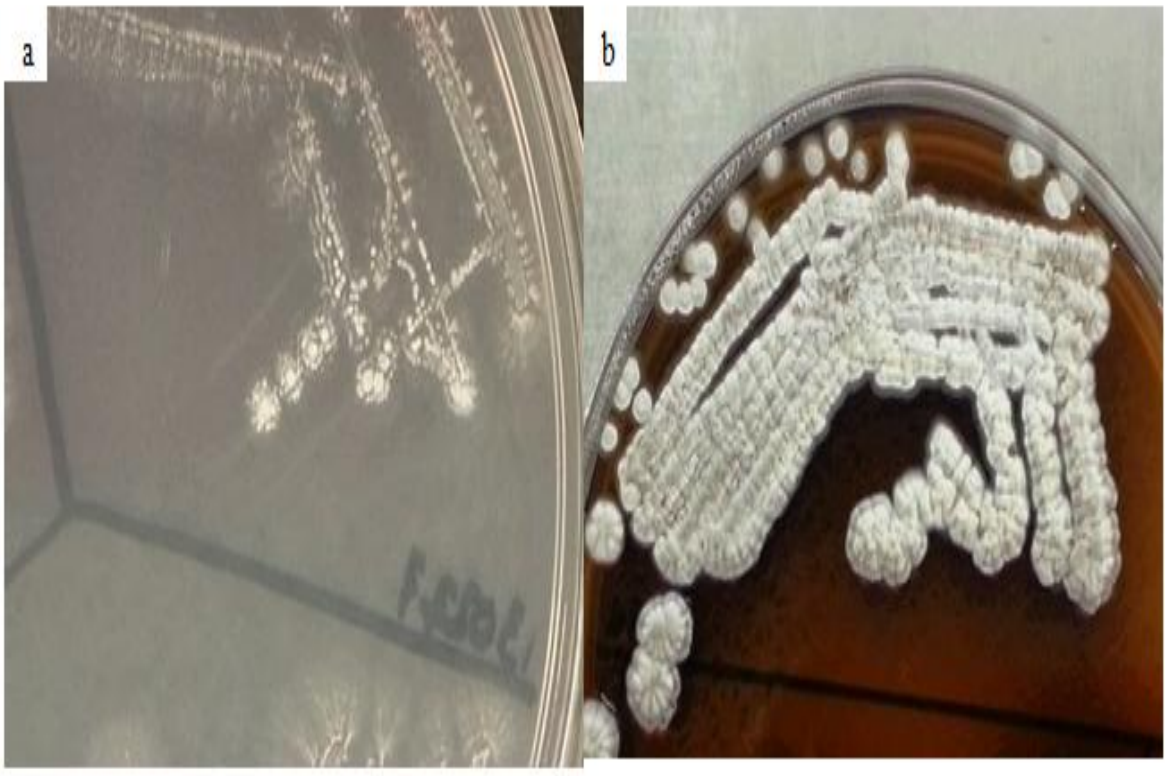

Figure 3-1 Morphological characteristics of *Streptomyces* spp. isolated from scabby Prospect tubers on 2 % (w/v) water agar (a) and PDA (b).

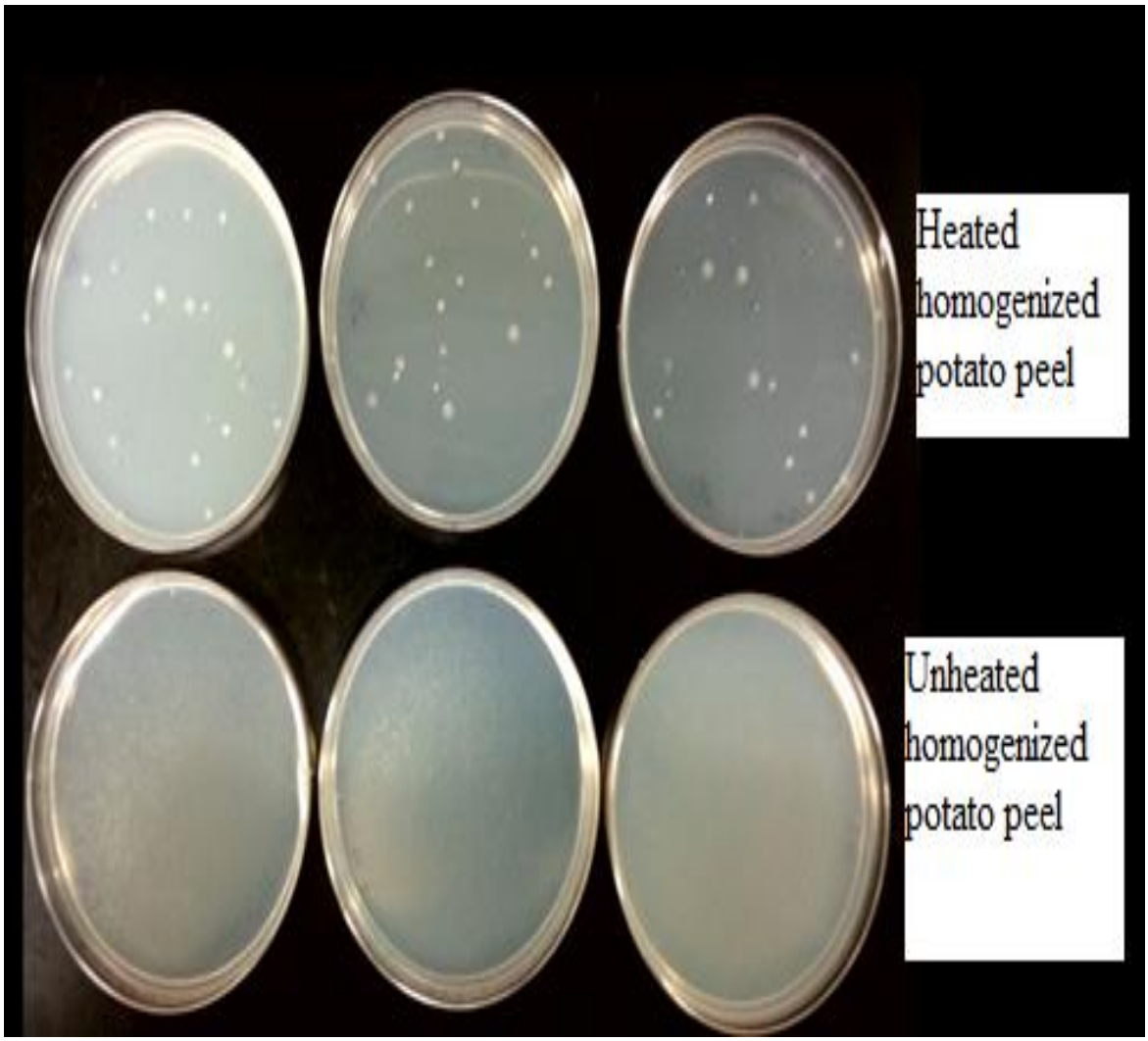

Figure 3-2 Heat treatment of PEI potato peel at 55 ˚C for 3 minutes before plating helped in reducing the number of fast-growing bacteria growing on 2 % (w/v) water agar present as an invisible film on the surface of the lower row of plates.

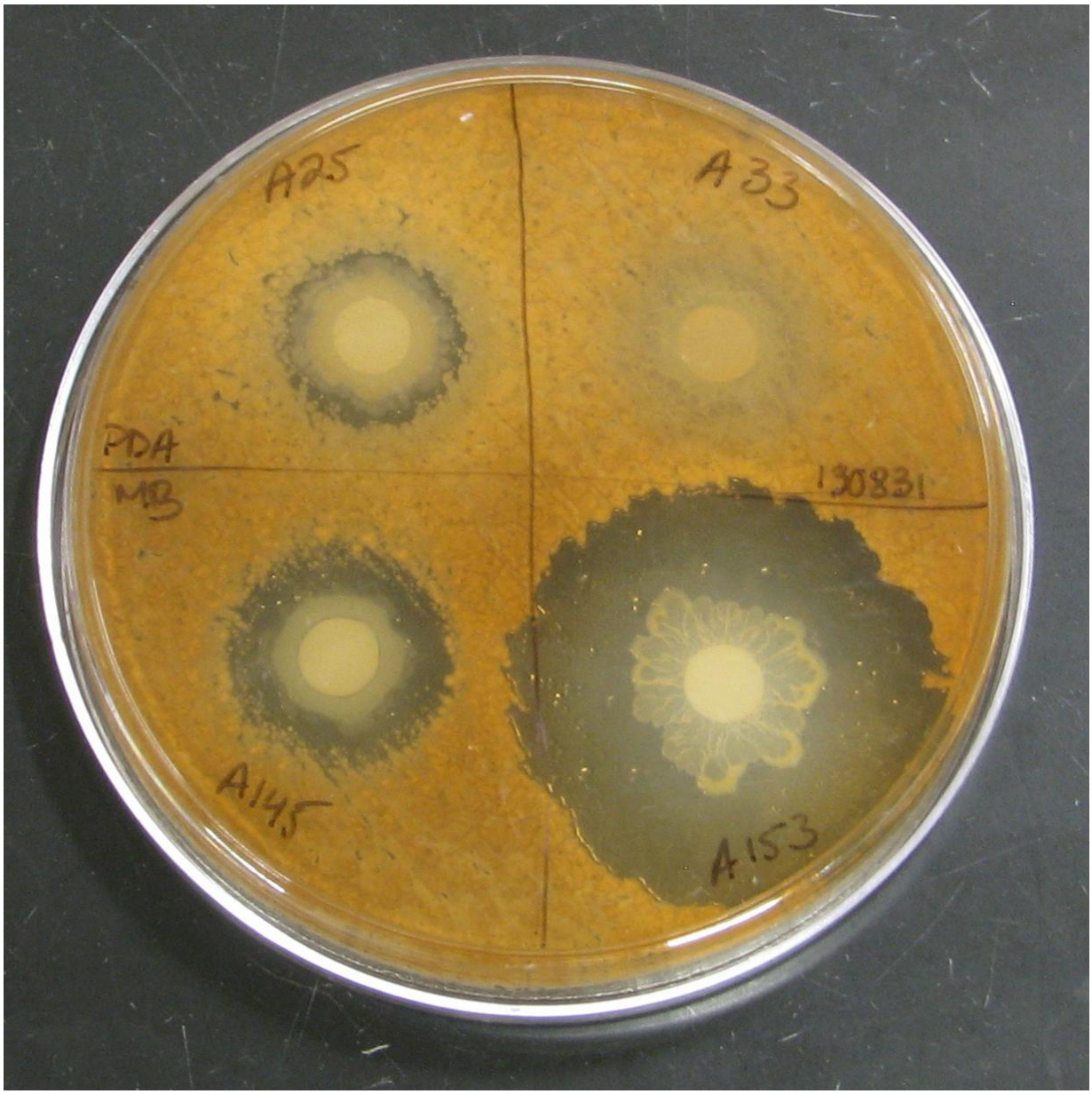

Figure 3-3 Antibiosis assay against *Streptomyces* strain PEI-1. On Potato Dextrose Agar *Pseudomonas* strains A25, A33, A145 and A153 were plated against PEI-1 isolated from scabby Prospect tubers obtained from Prince Edward Island.

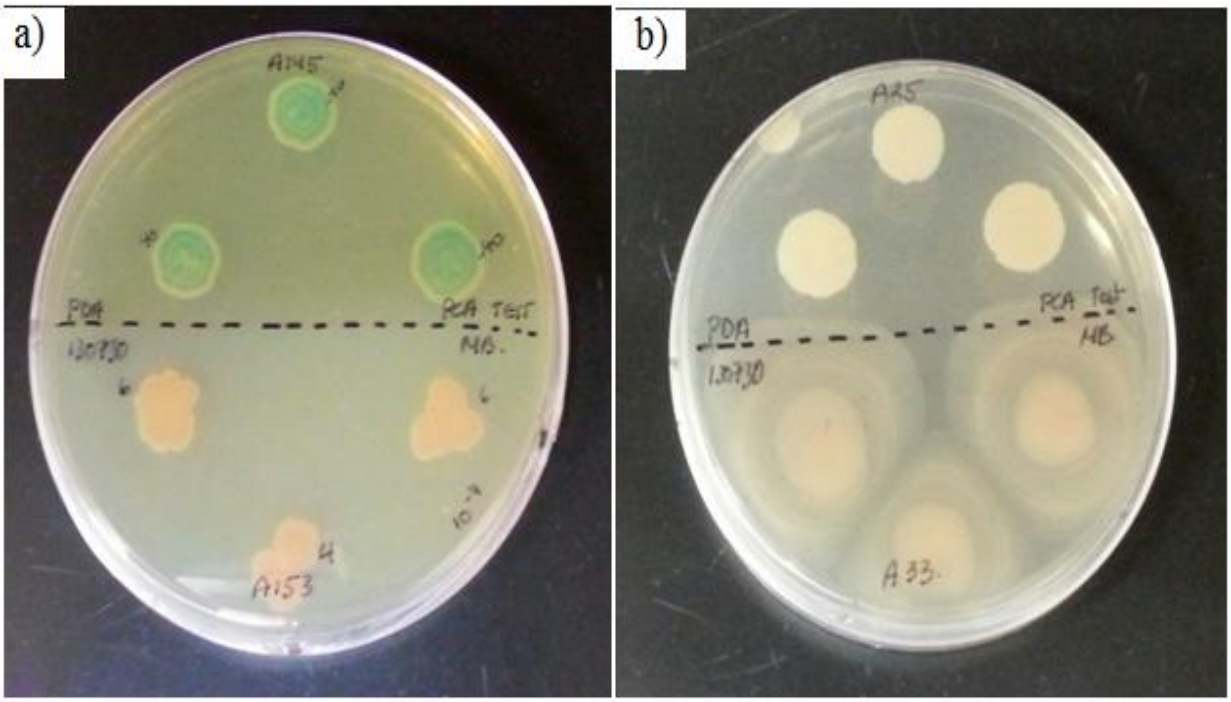

Figure 3-4 Production of secondary metabolites by *Pseudomonas* strains. (a) Strains A145 and A153. Green crystals forming between colonies is indicative of Phenazine-1-carboxylic acid (PCA) production (b) Strains A25 and A33, negative for PCA production.

3.3 Secondary metabolites produced by *Pseudomonas* A25, A33, A145, and A153

Phenazine compounds and their derivatives such as phenazine-1-carboxylic acid (PCA) are produced by *Pseudomonas* spp. In the rhizosphere, they have a broad-spectrum antifungal activity but I was not able to find any information as to their antibacterial activity. To test for PCA, *P. monteilii* A25 and A33, *P. chlororaphis* A145, and *P. brassicacearum* A153 were grown to reach 10^3 CFU ml^{-1}. After incubation at 29 ^oC for one week *P. chlororaphis* A145 showed green crystals forming between colonies (Figure 3-4a). This is was positive for PCA. Other strains showed negative results for PCA.

3.4 Detection of pathogenic *Streptomyces* from scabby PEI tubers

Modifying the Norgen kit protocol by preheating at 65 ^oC after the addition of lysis buffer to tissue samples from infested PEI significantly increased the concentration of DNA detected from no detection at all to 21, 496 ± 2,269 copies/reaction. Similar results were found when using GeneElute with 22,087± 2,244 copies/reaction. Therefore, the use of the NorgenKit with the addition of an extra step by heating the samples for 10 minutes at 65 ^oC was efficient in extracting over 60 samples within 8 hours. This protocol can be cost effective as it can be used for detecting *Streptomyces* from soil or tissue samples.

The detection limit and quantification range of TaqMan real-time PCR using primer and probe: Strep 2Q and Strep 2, respectively, was determined by using serial dilutions of *S. scabies* PEI-1 genomic DNA. From the three replicates 10 pg was determined to be the reliable lower limit detected. At 1 pg Ct values were variable and were not included in the analysis. A standard curve was constructed by plotting the threshold cycle (Ct) values against the serially diluted genomic DNA (log ng) extracted from *S. scabies* PEI-1 (Figure 3-5). The standard curve showed a linear relationship with high correlation coefficient (R^2= 0.99). The real-time PCR efficiency was found to be E= 0.97 using the formula E= $10^{[-1/slope]}$, indicating that the assay could be used to quantify bacterial DNA.

3.5 Quantification of pathogenic *Streptomyces* per milligram of scabby tissue

Based on three replicates, Ct values from serially diluted homogenized scabby potato peel showed a linear relationship with CFU/mg of scabby tissue (y= -1.7366x+41.956; R^2= 0.907; Figure 3-6). Similarly, spiking showed a linear relationship (y= -2.83+ 46.4; R^2=0.99). Furthermore, the concentration of *txtAB* amplicon calculated by the qPCR program was directly proportional to CFU/mL (y=x+4.8; R^2=1).

3.6 Seed tuber treatments

Lab trial

All seed tuber treatments, except those treated with ethanol and A145, significantly increase in the number of pathogenic *Streptomyces* by two fold compared to untreated tubers within the first week of incubation (P=0.67; Figure 3-7). Treatment with 80 % (v/v) ethanol decreased the number of pathogenic *Streptomyces* detected from 10^5 to $10^{3.8}$ CFU/mg whereas A145 was not significantly different from those detected from untreated tubers.

Within two weeks, treatment with 80 % ethanol maintained the number detected ($10^{3.8}$ CFU/mg) (P<0.05; Figure 3-7). However, the number of pathogenic *Streptomyces* detected from tubers treated with liquid broth and A153 were higher than those detected from untreated tubers but it was not significant (P=0.35; Figure 3-7). Similarly, tubers treated with A25 decreased the number of pathogenic *Streptomyces* but it was not significanlty compared to untreated tubers (P=0.25). The addition of industrial sand mixed with 5 % (w/w) peat moss, for 5 weeks resulted significant lower number of pathogenic *Streptomyces* by 84 %, 95 %, 99.7 %, and 99.9 % when tubers were treated with A145, liquid broth, A25, and A153, respectively (P<0.001; Figure 3-8). However, treatment with A25 resulted in the decay of 50% of the tubers. All tubers that were treated with 80 % (v/v) ethanol decayed and were not included in the analysis.

Field trial

The spring was cold and extremely wet. Germination of the cultivars was slow and plants remained quite small over the season. Germination of seed tubers was measured after one and four months after planting (Table 3-1). Yukon Gold seed tubers completely failed to germinate, likely due to decay of the cut pieces in the cold moist soil. Russet Burbank seed tubers treated with *Pseudomonas* A25, A145, A153, and the consortium had 67 %, 73 %, 73 %, and 93 % of seed tubers germinated within the first 30 days. Seed tubers treated with 80 % (v/v) ethanol had 93 % germination. Germination of Prospect one month after planting was better than of Russet Burbank and 80 %, 80 %, 87 % of the seed tubers treated with *Pseudomonas* A25, A145, and A153, produced plants while seed tubers treated with the consortia, the liquid broth and untreated seed tubers had 100 % germination (Table 3-1). In this case Prospect seed tubers treated with ethanol failed to germinate. By season's end all seed tubers, except for those treated with *P. monteilii* A25 or ethanol had 100 % germination.

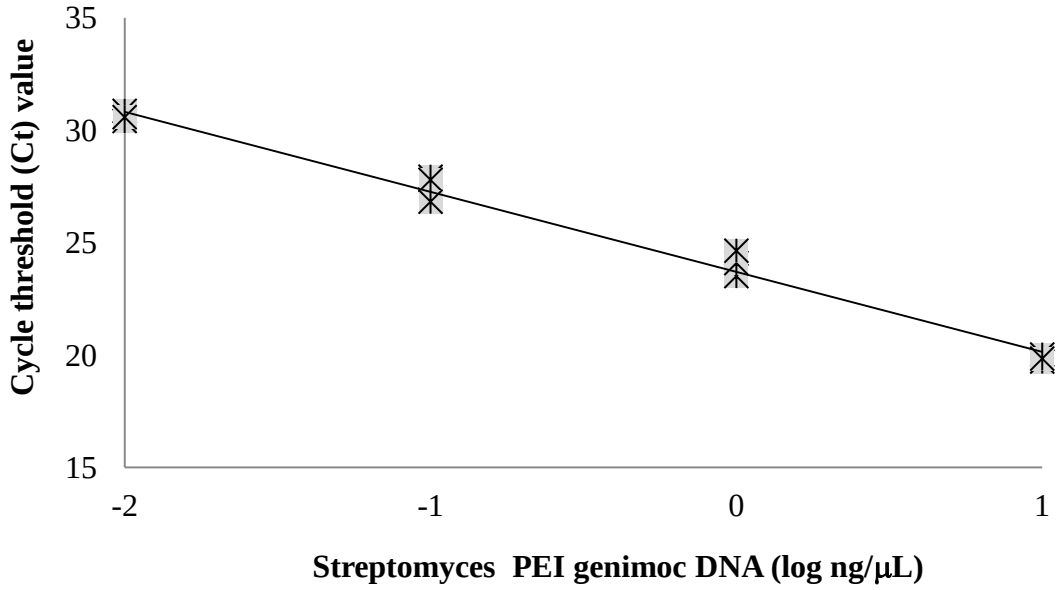

Figure 3-5 qPCR sensitivity range for *txtAB* detection obtained by plotting the extracted DNA from *S. scabies* PEI-1. Means ±SE shown are from three separate real-time PCR analysis.

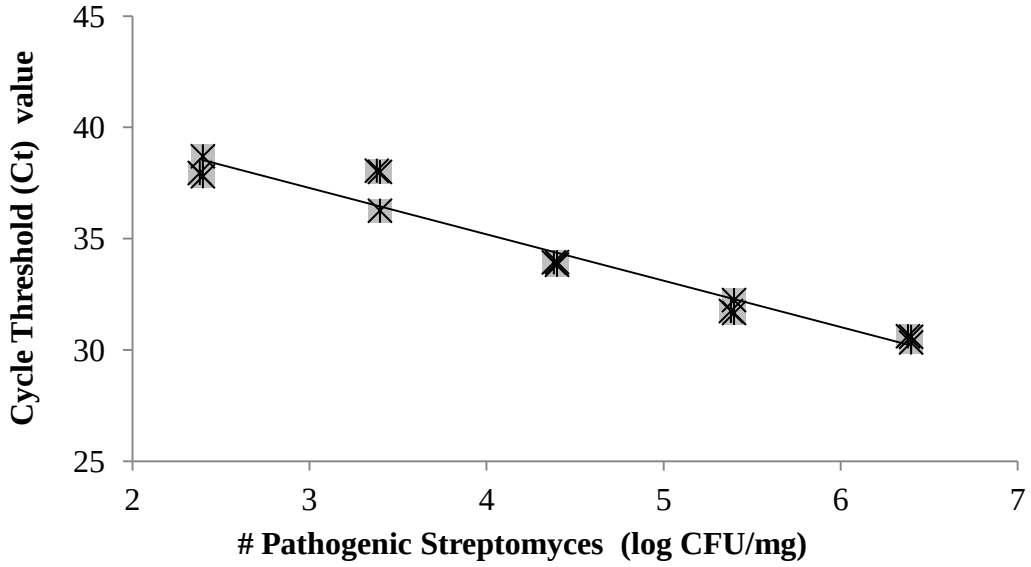

Figure 3-6 Standard curve for quantifying the number of pathogenic *Streptomyces* obtained by plotting the Ct value against number of pathogenic *Streptomyces* (log CFU/mg). Means ±SE shown are from three repeated experiments.

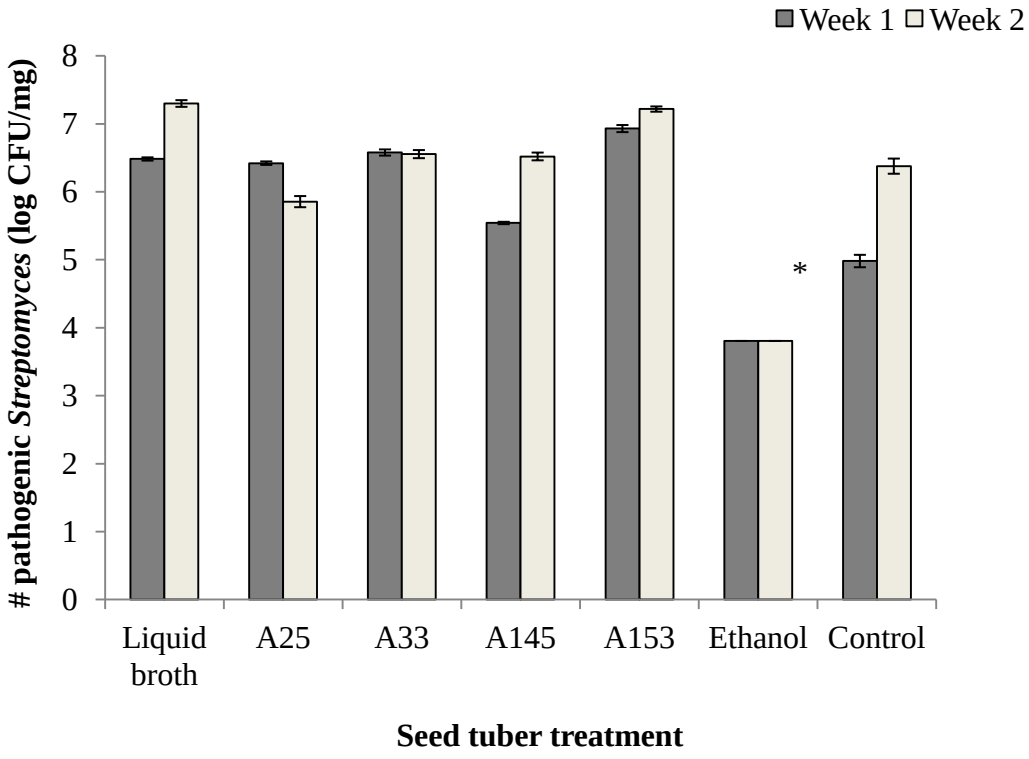

Figure 3-7 Number of pathogenic *Streptomyces* (log CFU/mg) from excised Prospect scabby tissue one and two weeks after application of seed tuber treatments. Means ±SE with significant differences are illustrated with an asterisk according to the LSD-test (*P*=0.05).

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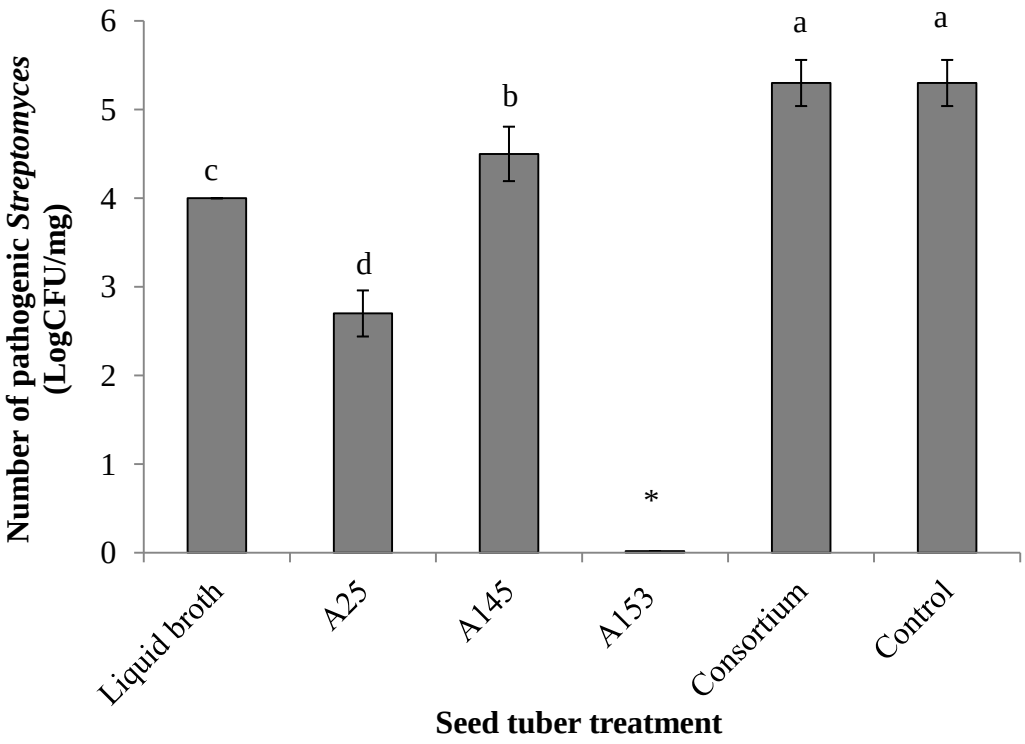


Figure 3-8 Number of pathogenic *Streptomyces* (log CFU/mg) from excised Prospect scabby tissue five weeks after application of seed tuber treatments. * Detection of the *txtAB* amplicon was below Ct threshold for pathogenic *Streptomyces.* Means ±SE with significant differences are illustrated with different letters according to the LSD-test (*P*=0.05).

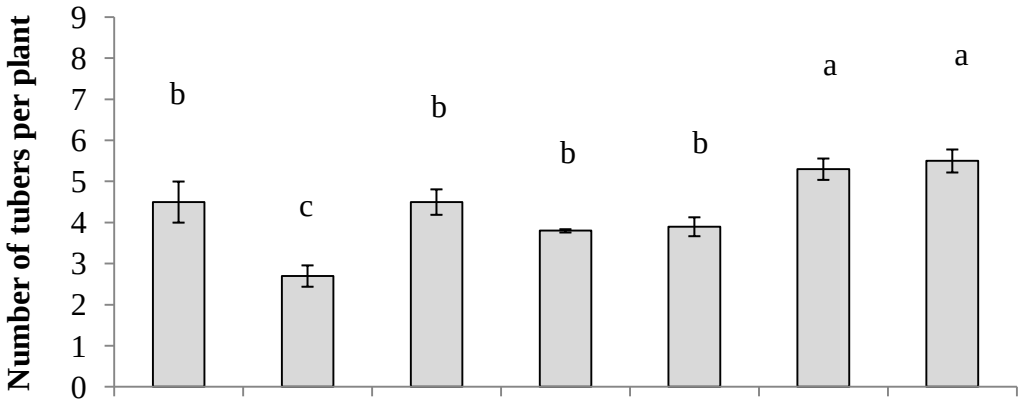
Table 3-1 Germination (number out of 15 seed tubers planted) of Yukon Gold, Russet Burbank and Prospect one and four months after planting.

	Seed tuber treatment													
	Liquid broth		A25		A145		A153		Ethanol		Consortium		Control	
#months	1	4	1	4	1	4	1	4	1	4	1	4	1	4
Yukon Gold	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	12	15	10	15	11	15	11	13	14	15	14	14	15	15
Prospect	15	15	12	13	12	15	13	15	0	0	15	15	15	15

Effect of seed tuber treatments on number of daughter tubers produced per plant: Russet Burbank seed tubers treated with A25 resulted in lowest numbers of tubers produced per plant, followed by A145, A153, liquid broth and 80 % (v/v) ethanol whereas those treated with the consortia of all three and untreated tubers yielded nearly 5 tubers per plant, respectively ($P<0.05$; Figure 3-9a). Prospect tubers untreated, treated with liquid broth, *P. brassicacearum* A153 and in consortia yielded approximately 7 tubers per plant. Those treated with A145 yielded 5 tubers per plant, and those treated with A25 only yielded an average of 2 tubers per plant. However, surface sterilization with 80 % (v/v) ethanol of symptomatic Prospect seed tubers killed all plants ($P<0.001$; Figure 3-9b).

Effect of seed tuber treatments on yield: Seed tuber treatments of Russet Burbank with A25 resulted in a decrease in yield by 61 % in comparison to untreated tubers ($P<0.001$, Figure 3-10a). None of the other treatments adversely affected yield. In comparison to untreated Prospect seed tubers, A25 significantly decreased yield by 68 % followed by those treated with A145 and A153 where yield was lower by 37 %, and 29 %, respectively ($P<0.001$; Figure 3-10b).

a)



b)

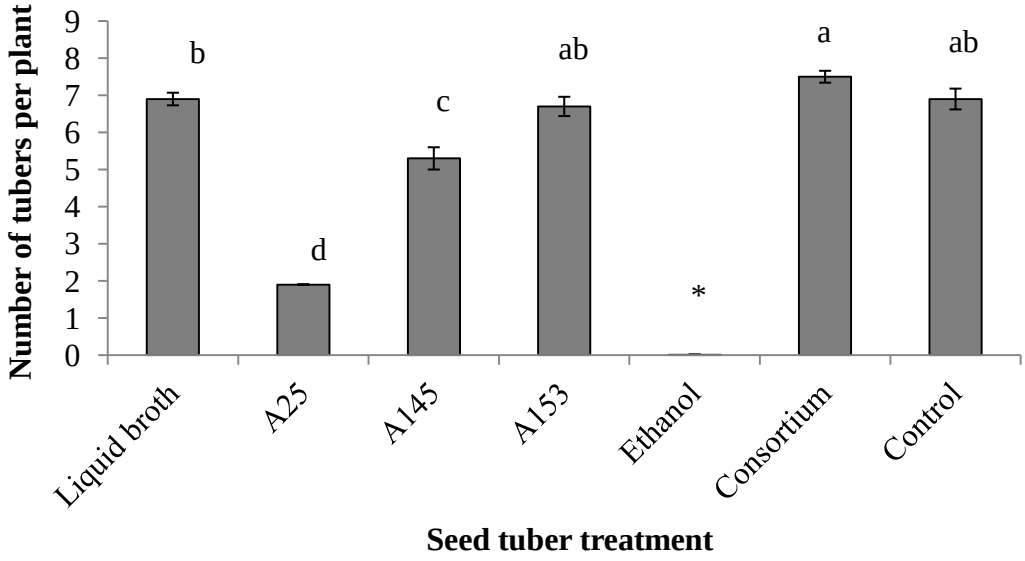


Figure 3-9 Number of Russet Burbank (a) and Prospect (b) progeny tubers after seed tuber treatments with A25, A145, A153, surface sterilization with 80 % (v/v) ethanol, and in consortia (1:1:1). Means ±SE with significant differences are illustrated with different letters as determined by LSD-test (P=0.05). * Surface sterilization with 80 % (v/v) ethanol killed all plants.

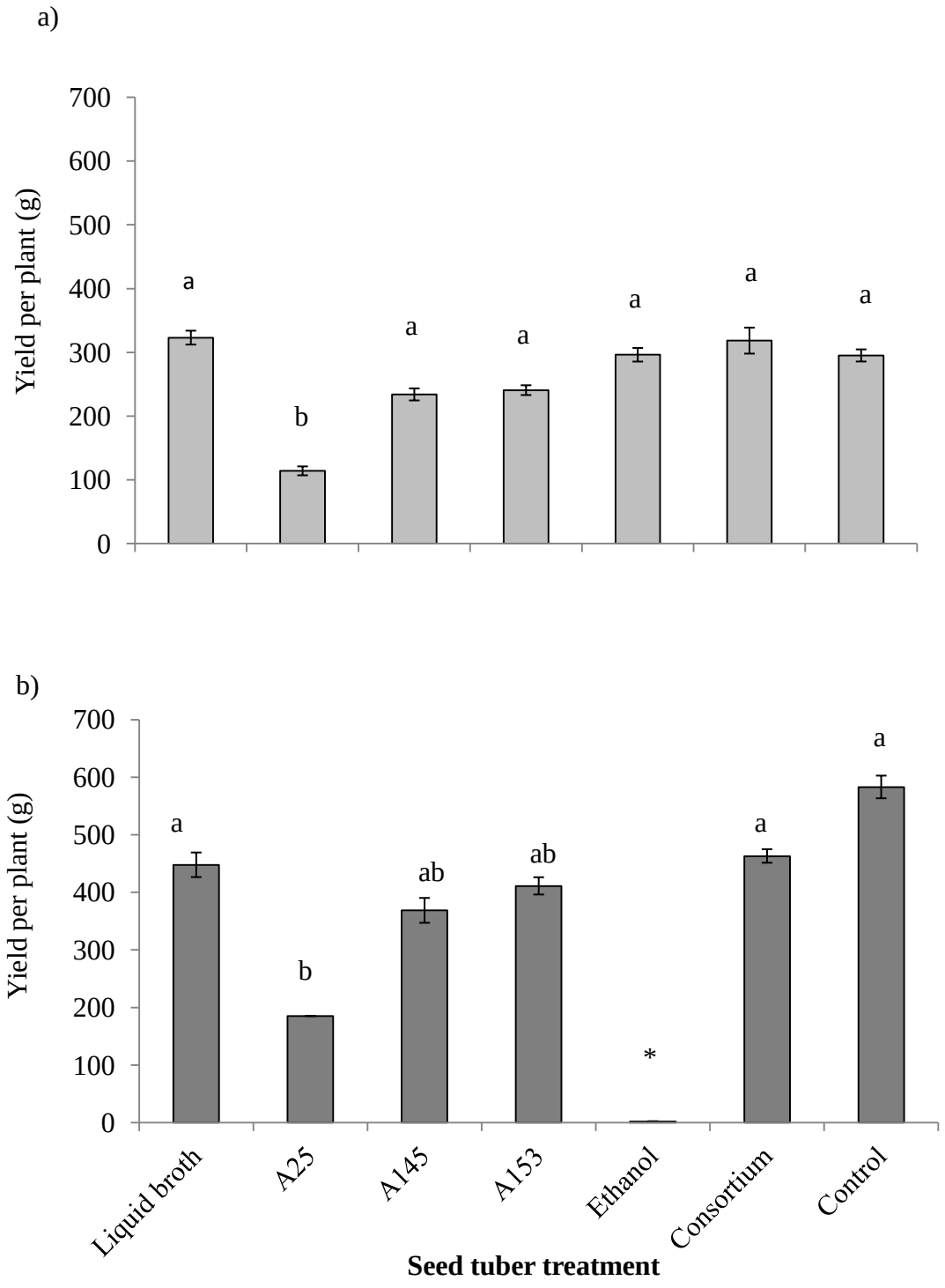


Figure 3-10 Yield of tubers per plant (g) from Russet Burbank (a) and Prospect (b) after seed tuber treatments with liquid broth, A25, A145, *P.* A153, consortium of all three (1:1:1) and untreated seed tubers. Means ±SE with significant differences are illustrated with different letters according to the LSD-test (*P*=0.05).* Surface sterilization with 80 % (v/v) ethanol killed all plants and this treatment was not included in the analysis.

Effect of cultivar on scab incidence and severity: The effect of cultivar on both scab incidence and severity was significant in the field trial (Table 3-2). In control treatments, analysis on the cultivar mean common scab incidence and severity showed that both Russet Burbank and Prospect tubers were equally susceptible to common scab.

Effect of seed tuber treatments on scab incidence and severity: Treating Russet Burbank seed tubers with A145 and in consortia resulted in 12.7±1 % and 26.1±4 % scab incidence whereas untreated seed tubers had 78.7±3 % scab incidence (Table 3-3). Progeny tubers of A25 treatment had scab incidence of less than 1 % and would be considered marketable. Surface sterilization resulted in 57.2±4 % scab incidence. However, A153 did not decrease scab incidence. Treatment of Prospect seed tubers with A25, A145, in consortia and A153 resulted in 10 %, 18 %, 28 % and 29 % scab incidence in comparison to 59 % scab incidence in progeny tubers from untreated seed tubers. Furthermore, Russet Burbank and Prospect tubers of control treatments were predominantly covered with deep pitted lesions (Figure 3-11**).**

Effect of seed tuber treatments on lesion size (mm): Liquid broth, A153 and the consortia of all three *Pseudomonas* strains significantly increased scab lesion size by 1.4 mm in comparison to untreated progeny tubers. However, A25 progeny tubers had average lesions size of 1 mm in comparison to untreated tubers ($P<0.001$; Figure 3-12). Prospect seed tubers treated with liquid broth and A25, resulted in a significant decrease in mean lesion size from 8.3±0.3 mm to 5.5±0.12 and 2.3±0.1 mm, respectively ($P<0.01$; Figure 3-12). In addition, A145, A153 and the consortia of all three strains resulted in were visually clean progeny tubers.

Effect of seed tuber treatments on the number of pathogenic *Streptomyces* per milligram of tissue: Cultivar had a significant effect on the number of pathogenic *streptomyces* detected in visually clean and scabby tissue (Figure 3-13 and Figure 3-14, respectively). Russet Burbank and Prospect tubers treated with various *Pseudomonas*A145, A153 and the consortium had significantly lower number of pathogenic *Streptomyces* per milligram of visually clean tissue compared to untreated tubers ($P<0.001$; Figure 3-13).

Russet Burbank treated with A25 significantly decreased the number of pathogenic *Streptomyces* from 125 to 16 CFU/mg of scabby tissue. On the other hand Prospect tubers treated with *Pseudomonas* A153 had significantly fewer the number of pathogenic detected from visually clean and scabby tissue ($P<0.001$, Figure 3-14).

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Table 3-2 ANOVA of common scab incidence (%) and severity between Russet Burbank and Prospect cultivars control treatments.

	Incidence (%)	**Severity (%)**
Cultivar		
Russet Burbank	78.8±28.4a†	48±23.5a
Prospect	59±18.0a	43±18.2a

†Means followed by the same letter are not significantly different at *P*=0.05 as determined by Fisher's least significant difference (*P*=0.05).

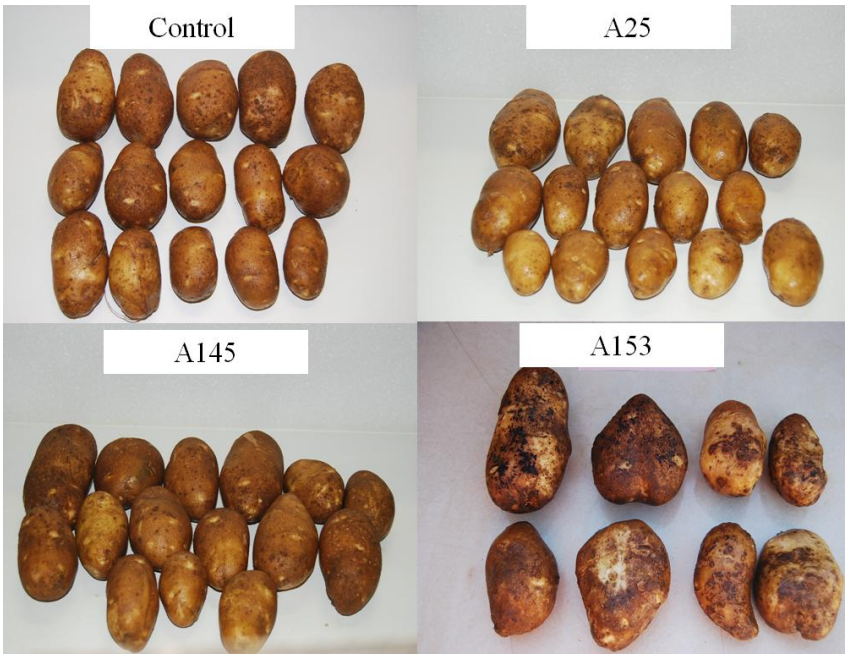
Table 3-3 Mean common scab incidence (%) and severity (%) for each cultivar-seed tuber treatment

	Incidence (%)		**Severity (%)**	
	Russet Burbank	Prospect	Russet Burbank	Prospect
Seed tuber treatment				
Control	79a‡	59a	48b	43a
Liquid broth	93a	38b	79a	34a
A25	<1c	10e	29c	11b
A145	13c	18d	18c	33a
A153	87b	29c	49b	9b
Consortia	26bc	28c	27c	12b

†A25: Seed tuber treatment with *P. monteilii* A25, A145: *P. chlororaphis* A145, A153: *P. brassicacearum* A153, and consortium of all three strains mixed together (1:1:1)

‡Means followed by the same letter are not significantly different as determined by Fisher's least significant difference (*P*=0.05).

a)



b)

Figure 3-11 Russet Burbank (a) and Prospect (b) progeny tubers harvested 21 September, 2013 and scored for percent surface covered with scab.

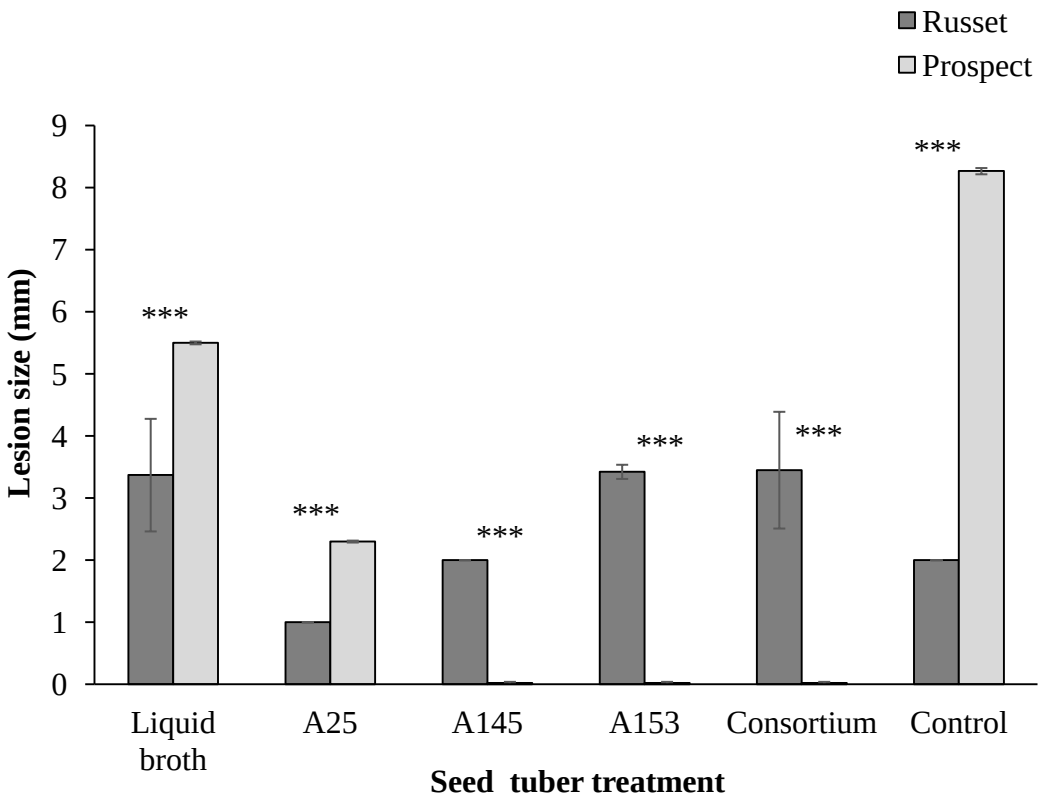


Figure 3-12 Average lesion size (mm) on progeny Russet Brubank and Prospect tubers after seed tuber treatments with liquid broth, A25, A145, *P.* A153, consortium of all three (1:1:1) and untreated seed tubers. Means ±SE with significant differences are illustrated with *, **, and *** at *P*=0.05, *P*= 0.01, and *P*=0.001, respectively, as determined by pairwise t-test.

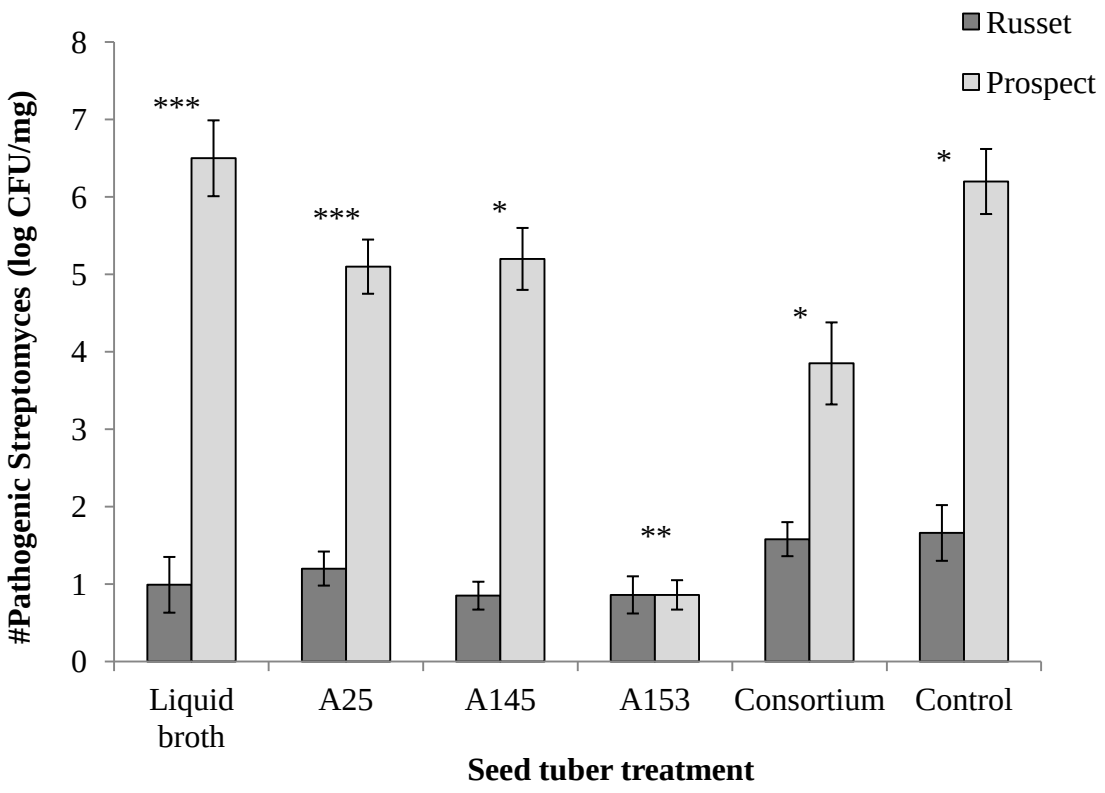


Figure 3-13 Number of pathogenic Streptomyces (log CFU/mg) quantified from visually clean tissue samples excised from Russet Burbank and Prospect progeny tubers. Means ±SE with significant differences are illustrated with *, **, and *** at *P*=0.05, *P*= 0.01, and *P*=0.001, respectively, as determined by pairwise t-test.

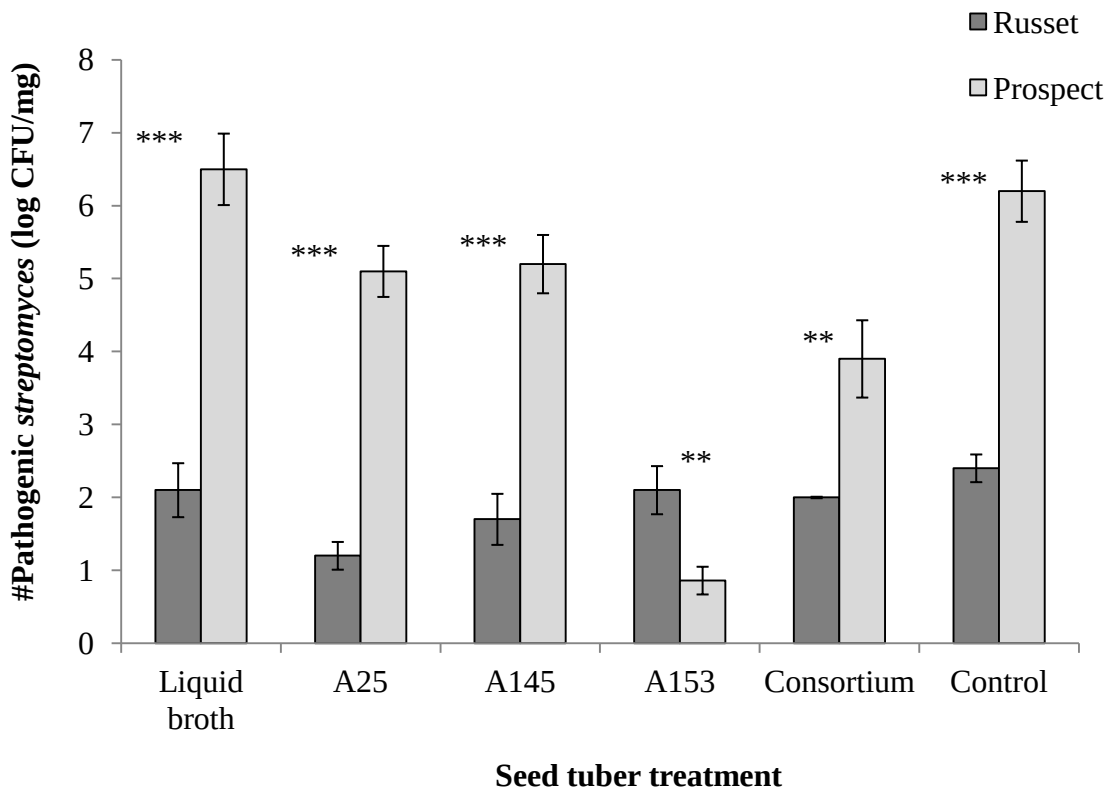


Figure 3-14 Number of pathogenic *Streptomyces* (log CFU/mg) from scabby tissue samples excised from Russet Burbank and Prospect progeny tubers. Means ±SE with significant differences are illustrated with *, **, and *** at P=0.05, P= 0.01, and P=0.001, respectively, as determined by pairwise t-test.

Chapter 4

Discussion

The primary objective of this study was to treat potato seed tubers with *Pseudomonas* spp. to reduce common scab incidence and severity, and improve seed quality and marketability. Three potential *Pseudomonas* spp. that inhibited the growth of three pathogenic *Streptomyces* isolates *in vitro* were selected for this study. Two *Pseudomonas* species have been previously documented for their biological control: *P. chlororaphis* and *P. brassicacearum*. *P. chlororaphis* PCL1391 controlled tomato root rot caused by *F. oxysporum* by colonizng tomato roots and through the production of PCA (Chin-A-Woeng *et al.*, 1998). There is a commercially available strain of *P. chlororaphis* marketed as "Atze" used to treat crops, vegetables and ornamental plants in green-houses against *Fusarium* and *Rhizoctonia* (Fravel, 2005). Wheat seed tubers treated with *P. brassicacearum* significantly decreased disease incidence by increasing plant density compared to non-treated controls infested with *Microdochium nivale* and *Fusarium* spp. (Levenfors *et al.*, 2008). However, no studies have looked at the potential use of these strains to control potato common scab.

Three potato cultivars were used in the field experiment, including common scab-moderately resistant cultivar "Russet Burbank", susceptible cultivar "Prospect", and "Yukon Gold" which was expected to fall in between. All three cultivars are registered potato varieties in Canada and used in the fresh market industry and processing of French fries. Yukon Gold did not germinate including control plants probably due to seed quality and not because of the seed tuber treatments. On the other hand over 50 % of Russet Burbank and Prospect seed tubers germinated within the first month. Treating both cultivars with A25 significantly decreased yield and the number of tubers in comparison to untreated seed tubers. Limited information is available on the effect of *Pseudomonas* on tuber formation but Blom *et al*. (2011) found that *P. fluorescens* CHA0, *P. chlororaphis* subsp. a*ureofaciens* and *P. aeruginosa* showed high quantities of hydrogen cyanide (HCN) production that led to plant death or drastic growth reduction.

It was expected that Russet Burbank would show reduced susceptibility to common scab. However, assessment of scab incidence and severity showed that in control treatments both cultivars were equally susceptible to common scab. Wanner *et al.* (2013) observed similar shifts in susceptibility in Yukon Gold planted in Michigan field plot between 2009 and 2010. Yukon gold cultivar was expected to fall in between Katahdin, considered being most susceptible and least susceptible cultivar, Atlantic. In 2009, analysis of common scab incidence and severity confirmed that susceptibility of Yukon Gold did fall in between, however, its susceptibility shifted from being less susceptible than 'Atlantic' but more susceptible than Katahdin in 2010. In both years, weather conditions did vary with average month temperature being below-normal and above-normal precipitation in 2009, but temperature and precipitation being above-normal in 2010.

Both untreated cultivars were predominantly covered with deep pitted lesions but Prospect tubers had lesions that were double in size. It is not well understood why scab lesions range from few superficial lesions to raised or deep-pitted lesions (Wanner *et al.*, 2013). Potatoes processed for French fries are most often peeled, so a shift away from deep-pitted or raised lesions to superficial or scab-free tubers is usually preferred. The effectiveness of *Pseudomonas* spp. in decreasing the size of scab lesions differed in the field trial.

As expected, variations in disease suppression are explained by the differences in host and *Pseudomonas* species. *Pseudomonas* A25 significantly decreasing lesion size when applied to both Russet Burbank and Prospect. The other *Pseudomonas* treatments did not have an effect on lesion size when applied Russet Burbank but resulted in significantly smaller lesions when applied to Prospect. It was hypothesized that the use of TaqMan qPCR would provide with a more rapid and accurate technique in enumerating the number of pathogenic *Streptomyces* compared to the conventional plating. However, Prospect tubers treated with A145, A153, and the three in consortium did not significantly decrease the number of pathogenic *Streptomyces* compared to untreated tissue samples even though progeny tubers had lesions that were almost negligible. This could be due to the quantification of dead cells. Hierro *et al.* (2006) found reverse

transcription-QPCR (RT-QPCR) had a lower detection limit and was more accurate than qPCR in detecting yeast cells responsible for wine spoilage. This assay was fast and accurate in enumerating the total viable yeast cells during industrial wine fermentation and controlling the risk of wine spoilage.

Pathogenic *Streptomyces* need to establish 10^4 CFU/g in order to cause scab lesions (Wanner, 2004). Therefore reducing the number could be a key in improving seed quality. Russet Burbank treated with A25 resulted in 88 % less pathogenic *Streptomyces* detected from scabby tissue of progeny tubers. On the other hand, Prospect tubers treated with A153 significantly decreased the number of pathogenic *Streptomyces* where only 9 and 7 CFU/mg were detected from visually clean and scabby tissue, respectively. Reduction in lesion size when seed tuber treatments were applied may be due to the ability of *Pseudomonas* spp. to kill pathogenic *Streptomyces* through antibiotic production, or protect plants from *Streptomyces* infection by strengthening the epidermal and cortical cell walls by up-regulating callose formation (Singhai *et al*., 2011). In addition, *Pseudomonas* spp. can reduce scab severity by inhibiting the thaxtomin biosynthetic pathway. St-Onge *et al*. (2011) illustrated that *Pseudomonas* LBUM 223 and *Pseudomonas* LBUM 300 inhibited *Streptomyces* growth on OBA medium but only the former lowered the transcription level of *txtAB* by approximately 46 %.

Plant disease results from the interaction of a pathogen, a susceptible host plant, and environmental conditions such as soil conditions (moisture, pH, and temperature), climate and weather, and soil microbial community (Wanner *et al*., 2014). The effectiveness of *Pseudomonas* seed tuber treatments in suppressing common scab differed when applied to Russet Burbank and Prospect seed tubers.

Variation in biocontrol activity between both cultivars may be due to Russet Burbank or Prospect having greater capacity in sustaining interactions with *Pseudomonas* spp. (Meyer *et al*., 2010). Root morphology and root exudates may also vary between cultivars and may have affected successful root-colonization by A25, A145, and A153 (Meyer *et al*., 2010). All seed tuber treatments significantly decreased scab incidence when applied to Prospect seed tubers but only three reduced scab incidence > 50 % when

applied to Russet Burbank. Results from this study supported the hypothesis that seed tuber treatments with *Pseudomonas* spp. would have lower number of pathogenic *Streptomyces* compared to those found on the surface of untreated tubers. However, A153 did not have the same effect on the number of pathogenic *Streptomyces* when applied to both cultivars. Further studies looking into the interaction of *Pseudomonas* and different potato cultivars would be helpful in improving the quality and effectiveness of the seed tuber treatment.

The potato French fries and fresh market industry is heavily dependent on scab-free or visually clean tuber. Significant improvement of common scab severity and incidence could therefore have great industrial importance and aid in lowering the risk of high economic losses due to common scab.

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