Salisbury
UNIVERSITY

Honors College

Honors Thesis

An Honors Thesis Titled

Bdellovibrio bacteriovorus as a Biocontrol Agent against Blackleg Disease in Potatoes

Submitted in partial fulfillment of the requirements for the Honors Designation to the

Honors College

of

Salisbury University

in the Major Department of

Biological Sciences

by

Rebecca Paulis

Date and Place of Oral Presentation: SUSRC April 28, 2017
Salisbury, MD

Signatures of Honors Thesis Committee

	Signature	Print
Mentor:	Elizabeth Emmert	Dr. Elizabeth Emmert
Reader 1:	M F Frana	Dr. Mark Frana
Reader 2:	Leanne Wood	Dr. Leanne Wood
Director:		JAMES J. BUCK

Abstract:

Blackleg disease is a condition that occurs in potatoes when bacteria such as *Erwinia carotovora* infect the tuber. The pathogenic bacteria enter the host plant through natural pores or wounds and cause the plant tissue to macerate, the foliage to wilt, and the stems to turn dark brown/black. Symptoms of blackleg disease commonly develop while in storage and at temperatures above 25 degrees Celsius. As much as 5% of the crop production in North-Western Europe may be lost annually due to blackleg disease (Sharga and Lyon 1998). A possible method for preventing blackleg disease may be implementing biocontrol agents such as predatory bacteria. *Bdellovibrio bacteriovorus* is a gram negative, uniflagellated, motile, and parasitic bacterium that preys on other gram negative bacteria. Extensive research has shown that *B. bacteriovorus* is effective in reducing pathogenic bacteria on the host. In this study, this predatory bacterium will be inserted into potatoes to prevent blackleg disease or reduce its symptoms; we hypothesize that *B. bacteriovorus* will prey on the bacterium that causes blackleg disease in potatoes, reducing disease severity. Potato slices were inoculated with treatments including pathogenic *E. carotovora* alone, the combination of *E. carotovora* and *B. bacteriovorus,* and control treatments. The zone width of maceration was measured daily for three days per trial and there was a total of seven trials in this study. There was a significant difference in the zone width of maceration between the potato slices inoculated with the pathogen and the potato slices inoculated with the pathogen and the predatory bacterium ($p=0.005$). This suggests that *B. bacteriovorus* may be an effective treatment for reducing blackleg disease severity caused by the pathogen *E. carotovora*.

Introduction:

The potato, *Solanium tuberosum*, is a staple vegetable crop in many countries around the world and has a production record of 325 million tons in 2007 (Lahbabi et al. 2012). A portion of this crop production is lost annually, as much as 5% in North-western Europe, due to soft rot diseases such as blackleg disease (Sharga and Lyon 1998). Soft rot diseases, caused by the genus *Erwinia*, are characterized by the pathogen's ability to cause extensive maceration of plant tissue and the breakdown of the host's cell wall (Starr 1983). Blackleg disease is seed borne and occurs in potatoes when bacteria such as *Erwinia carotovora* subsp. *carotovora* or *Erwinia carotovora* subsp. *atroseptica* infect the tuber of the potato plant and cause the foliage to wilt and the plant tissue to macerate (Duarte et al. 2004). Infection can occur in the field when contaminated seed tubers allow the pathogen to spread through the soil to infect progeny tubers or during handling when the pathogen can be transmitted through wounds (Toth et al. 2003). The pathogenic bacteria penetrate the potato plant through natural pores or mechanical wounds and may spread throughout the entire plant through its vascular vessels (Raoul des Essarts et al. 2015). The pathogens produce extracellular enzymes such as pectate lyases, cellulases, and proteases that degrade the cell wall of the host plant and cause soft rot symptoms or tissue maceration (Lahbabi et al. 2012).

The pathogenicity of blackleg bacteria is temperature dependent because temperature directly affects enzyme expression which is the most important virulence factor (du Raan et al. 2016). The pathogenic bacteria optimally grow between 15 and 24 degrees Celsius but they can survive up to 37 degrees Celsius (du Raan et al. 2016).

Symptoms of blackleg disease commonly develop while in storage at temperatures above 25 degrees Celsius (Duarte et al. 2004). Other environmental conditions are required for blackleg disease to occur such as soil moisture. Specifically, moist and cool environments are best for disease (Perombelon 2000). The focus of this study was to use predatory bacteria, specifically *Bdellovibrio bacteriovorus*, to prevent or lessen symptoms of blackleg disease in potatoes.

Predatory bacteria can be used as biocontrol agents against diseases in various hosts. *Bdellovibrio* and like organisms (BALOS) are effective against diseases caused by complex microbial communities (Allen et al. 2014). *Bdellovibrio bacteriovorus* is a well-known BALO and it can be characterized as a gram-negative, motile, uniflagellated, and parasitic bacterium that preys on other gram negative bacteria (Banwart 1989). Genomic sequencing has shown that *Bdellovibrios* contain a large complement of hydrolytic enzymes which is what allows them to attack and lyse a range of prey cells (Sockett and Lambert 2004). Extensive research done on *B. bacteriovorus* has shown that it is effective in reducing pathogenic bacteria on the host (Dashiff and Kadouri 2011). The predatory lifecycle of *B. bacteriovorus* lasts about three to four hours and occurs in various stages (Sockett and Lambert 2004). In the attack phase, the motile cells are able to swim at high speeds because they are propelled by active flagellar motility and they locate prey cells by randomly bumping into them and using chemotaxis (Sockett and Lambert 2004). Once a *B. bacteriovorus* cell collides with a prey cell, *B. bacteriovorus* attaches to the cell wall of the prey bacterium and enters the cell through penetration. Once inside, *B. bacteriovorus* grows into a long spiral form and cleaves individual copies

of itself and in the process, the prey cell wall is enzymatically remodeled (Sockett and Lambert 2004). The new *B. bacteriovorus* cells are released once the prey cell is destroyed and move on to other prey cells to repeat this process resulting in the destruction of the pathogenic bacteria (Scherff 1972).

There are two phases in the life cycle of *B. bacteriovorus* including the free-living attack phase and the intra-periplasmic growth phase. The intra-periplasmic growth phase takes place in the periplasm of bacterial prey cells and as a whole this is called the bdelloblast (Markelova 2010). The main stage in their life cycle is as a bdelloblast which provides a form of protection against unfavorable growth and reproductive conditions (Markelova 2010). The growth of *B. bacteriovorus* is dependent upon the nutrients inside their prey and once the nutrients are used up, new *B. bacteriovorus* cells are released and the prey cell is lysed (Lambert and Sockett 2008). The unique predacious qualities that *B. bacteriovorus* possess give it the potential to be an environmental water clean-up agent, a clinical therapeutic agent, a crop protection agent (Sockett 2009), or an alternative to antibiotics (Allen et al. 2014).

Antibiotics are commonly used to treat diseases in animal agriculture and human medicine but with the rise of antibiotic resistant pathogens, there is a need for suitable alternatives to antibiotics. Antibiotics often have unintended consequences such as affecting gut microbiota, which results in irritable bowel disorder and infection by intestinal pathogens (Allen et al. 2014). Alternatives to antibiotics can be targeted to specific bacteria which avoids selecting for resistance of non-targeted bacteria (Allen et al. 2014). Among methods like phage therapy and bacteriocins, predatory bacteria such

as *B. bacteriovorus* can be used as a possible alternative to antibiotics because they predate gram negative bacteria for their own energy and nutrients (Allen et al. 2014). In addition to being a substitute for antibiotics, *B. bacteriovorus* has been shown to prevent disease in organisms by ingesting pathogenic bacteria.

Research done on *B. bacteriovorus* has shown its ability to prevent or lessen symptoms of periodontitis (Daschiff and Kadouri 2011), bacterial blight in soybeans (Scherff 1972), and vibriosis in whitetail shrimp (Cao et al. 2015). Dashiff and Kadouri studied *B. bacteriovorus* and its ability to prey on oral pathogens that cause periodontitis (Daschiff and Kadouri 2011). They determined that *B. bacteriovorus* can remove biofilms in the presence of saliva as well as detach metabolically inactive biofilms (Daschiff and Kadouri 2011). However, *B. bacteriovorus* did not consume the entirety of its prey, so antimicrobial agents were needed to remove surface-attached cells (Daschiff and Kadouri 2011). The results of this study indicate that *B. bacteriovorus* was able to prey on all *Aggregatibacter actinomycetemcomitans* serotypes tested with and without being incubated in saliva (Daschiff and Kadouri 2011). Scherff conducted a study that focused on *B. bacteriovorus* and its ability to control bacterial blight in soybeans (Scherff 1972). Soybean blight is similar to blackleg disease in that it is a bacterial disease that turns foliage yellow-brown and occurs in cool and moist conditions. This study showed evidence of *B. bacteriovorus* inhibiting the development of local and systemic symptoms of soybean bacterial blight (Scherff 1972). Sherff isolated *B. bacteriovorus* cells from the rhizosphere of soybean roots and inoculated them onto soybean roots along with *Pseudomonas glycinea* in 9:1 and 99:1 ratios (Scherff 1972). The ability of *B.*

bacteriovorus to inhibit soybean blight symptoms was highly correlated with cell burst size of the particular isolate (Scherff 1972). The *B. bacteriovorus* cells that released the most progeny cells were more effective in preventing the development of bacterial blight than the *B. bacteriovorus* cells that released fewer cells. Cao et al.'s (2015) study demonstrates the ability of *B. bacteriovorus* cells to lyse pathogenic cells that cause vibriosis in whitetail shrimp, *Penaeus vannemai*. Vibriosis is caused by *Vibrio cholerae* and is characterized by a yellowing of the tail. It is a major economic concern in the freshwater cultured shrimp industry (Cao et al. 2015). Cao et al.'s study shows that *B. bacteriovorus* significantly reduced the pathogenic cell density after inoculation (2015). In addition, BALOs other than *Bdellovibrio,* have been shown to prevent or lessen symptoms of polymicrobial infections in cystic fibrosis patients and *Proteus* sp. biofilm infections in catheters (Allen et al. 2014).

Studies on the prevention of blackleg disease have used other types of bacteria as biocontrol agents as well as salt compounds as a treatment method. *Pseudomonas* biocontrol strains have reduced the virulence of *Dickeya dianthicola* on potato plants and tubers (Raoul des Essarts et al. 2015). Like *Erwinia*, *D. dianthicola* can cause blackleg disease in potatoes. Crop losses due to stem and tuber rot caused by *Erwinia* and *Dickeya* are major causes for the rejection of potatoes during the potato seed certification process (Raoul des Essarts 2015). The results in this study indicate a slower development of blackleg symptom appearance in potatoes inoculated with a mixture of three *Pseudomonas* strains (Raoul des Essarts et al. 2015). Another study showed how treatments of *Bacillus subtilis* prior to the inoculation of pathogenic bacteria on potato

plants show reduced symptoms of soft rot (Sharga and Lyon 1998). The cut surface of a potato tuber was pressed up against a culture lawn of *B. subtilis* and this allowed a bacterial mat to form that inhibited the establishment of *Erwinia carotovora* (Sharga and Lyon 1998). However, a high concentration of applied *B. subtilis* or the antibiotic produced by *B. subtilis* is required to completely prevent soft rot symptoms in cut potato tuber (Sharga and Lyon 1998).

Endophytic bacteria have been studied and there is evidence that endoplant bacteria may be effective in pathogen defense (Reiter et al. 2002). One of the mechanisms for disease resistance is the interaction between a host plant and its endophytic bacteria (Reiter et al. 2002). Microbial endophytes in potato plants may contribute to the resistance of blackleg disease through outcompetition of the pathogen, induction of systemic resistance, and the production of antibiotics (Reiter et al. 2002). Disease is inhibited by a diverse endophytic bacterial community because it keeps the pathogenic bacteria at a population level low enough to inhibit development of disease symptoms (Reiter et al. 2002). Another study shows how Moroccan actinobacterial isolates, such as *Streptomyces*, can lessen rotting symptoms induced by the pathogens *E. carotovora* and *E. atroseptica* (Lahbabi et al. 2012). This particular study shows promising evidence for the use of biological control agents as part of an integrative control program against soft rot bacteria (Lahbabi et al. 2012). The ability of actinobacteria to produce antibiotics and cell-wall degrading enzymes is important in its potential for use as biocontrol of soil borne pathogens (Lahbabi et al. 2012). Salt compound treatments are an alternative to the previously used treatment methods for

blackleg disease in potatoes (Mills et al. 2006). Salt compounds have been shown to prevent cabbage soft rot and various types of bacterial infections in potatoes and apples (Mills et al. 2006). Certain salt compounds may be effective in controlling blackleg disease in potatoes during storage (Mills et al. 2006). Heat treatments are effective in treating blackleg pathogens but this method is high in energy costs (Mills et al. 2006). Post-harvest sanitation using chlorine bleach is also an effective treatment but this method is environmentally damaging (Mills et al. 2006).

There is abundant evidence indicating that *B. bacteriovorus* can prevent or lessen symptoms of disease in various organisms and there have been numerous methods studied for treating or preventing blackleg disease in potatoes. The aim of this study was to use the predatory bacterium *B. bacteriovorus* as a biocontrol agent against blackleg disease in potatoes caused by *Erwina carotovora.* We hypothesized that the use of *B. bacteriovorus* would prevent or lessen symptoms of blackleg disease in potatoes.

Methods:

Making *B. bacteriovorus* and *E. carotovora* liquid cultures

To make *B. bacteriovorus* liquid culture, *Escherichia coli* from a Tryptic Soy Agar (TSA) plate was put into a sterile test tube along with 2 mL of YT broth and was placed in the shaker incubator overnight at 30°C. The next day, 3 mL of Ca/HEPES buffer was placed in a sterile test tube and 200 µL of the *E. coli* liquid culture was added. When adding *B. bacteriovorus* from a YPSC (Yeast extract-peptone-sulfate-cysteine) plate, the edges of the clearing zone were scraped with a sterile stick and added to the new *E. coli* culture. When adding *B. bacteriovorus* from a previous liquid culture, 50 µL

were pipetted directly into the test tube. The tubes were incubated overnight at 30°C and microscopically observed frequently to look for zippy *B. bacteriovorus* cells. The protocol for maintaining *B. bacteriovorus* was based on the protocol described by Lambert and Sockett (2008). To make *E. carotovora* liquid culture, 2 mL of Luria broth (LB) broth was pipetted into a sterile test tube and *E. carotovora* was added from a previously streaked TSA plate. It was then placed in the shaking incubator at 30°C overnight to allow the bacteria to grow. Each of these cultures were made a day in advance of each trial to ensure fresh cultures.

Using *E. carotovora* to cause blackleg disease on potatoes

Several potatoes of roughly the same size and the same brand were rinsed off with water and dried with a paper towel before they were cut into roughly 1 cm slices and placed into sterile petri dishes. With a borer and tweezers that have been sterilized in 95% ethanol and passed through a flame in between each potato slice, small wells with a diameter of 8 mm were created in the center of each potato slice to hold the bacterial inoculations. The control treatment was inoculated with 20 µL of sterile LB broth and the experimental treatment was inoculated with 10 µL of sterile LB broth and 10 µL of *E. carotovora* liquid culture. Each treatment of inoculated potato slices was placed into sealed Ziploc containers with damp paper towels surrounding the petri dishes to ensure moist conditions. The Ziploc containers were placed in the incubator at 27°C and the potato slices were observed and the width of the zone of maceration was measured once a day for three days. Two trials with a sample size of three potato slices per treatment and five trials with a sample size of six potato slices per treatment were conducted.

Using *B. bacteriovorus* to control blackleg disease symptoms

Potato slices were prepared as described earlier. The first control group was inoculated with 20 µL of sterile LB broth and the second control treatment was inoculated with 10 µL of sterile LB broth and 10 µL of *B. bacteriovorus.* The first experimental treatment was inoculated with 10 µL of sterile LB broth and 10 µL of *E. carotovora.* The final experimental treatment was inoculated with 10 µL of *E. carotovora* and 10 µL of *B. bacteriovorus.* Incubation and disease progression was as described earlier. Three trials with a sample size of three potato slices per treatment were done. Four trials were done with a sample size of six potato slices per treatment where the pathogen to predatory bacteria cultures were mixed in equal ratios and the length of the trial was three days. The data were analyzed using a two-sample t test in Excel.

Results:

Out of the seven trials completed in this study, three of the trials had a significant difference between the maceration zone width in the potatoes inoculated with the pathogen and the potatoes inoculated with the pathogen plus predatory bacteria. Four of the trials did not have a significant difference. However, when the seven trials were combined and the data were analyzed, there was a significant difference between the maceration zone width in the two potato treatments. The following figures represent the day three average maceration zone width in potato slices inoculated with sterile LB broth plus pathogenic *E. carotovora* and potato slices inoculated with *E. carotovora* plus *B. bacteriovorus*. The error bars represent the standard error of the mean. The sample size in figures 1, 2, and 3 is three potato slices and the sample size in figures 4, 5, 6, and 7 is six

potato slices. The control groups (just sterile LB broth and sterile LB broth plus *B. bacteriovorus*) for each trial showed no maceration zone width (data not shown).

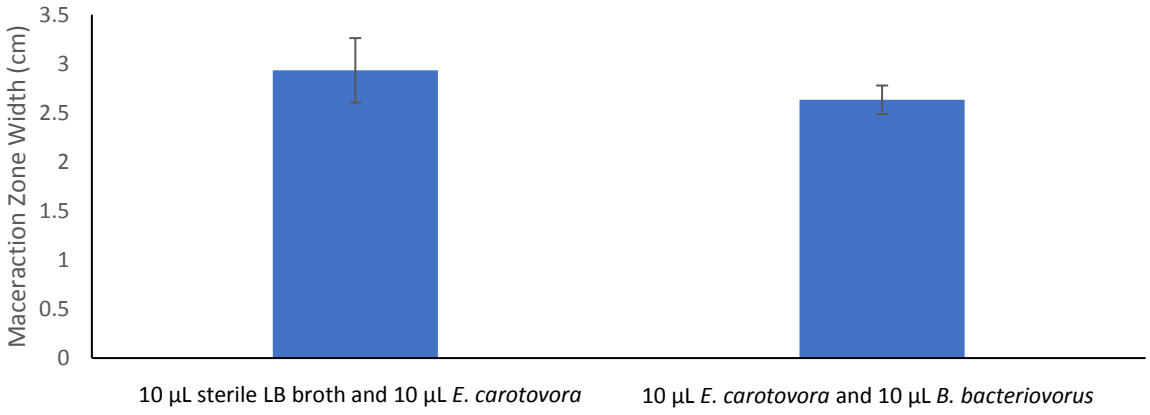

Figure 1. Comparison of maceration caused by pathogen alone with maceration in the presence of both the pathogen and biocontrol agent. Zone width measured three days after inoculation. Inoculation day was March 5, 2016. N=3 and p=0.464.

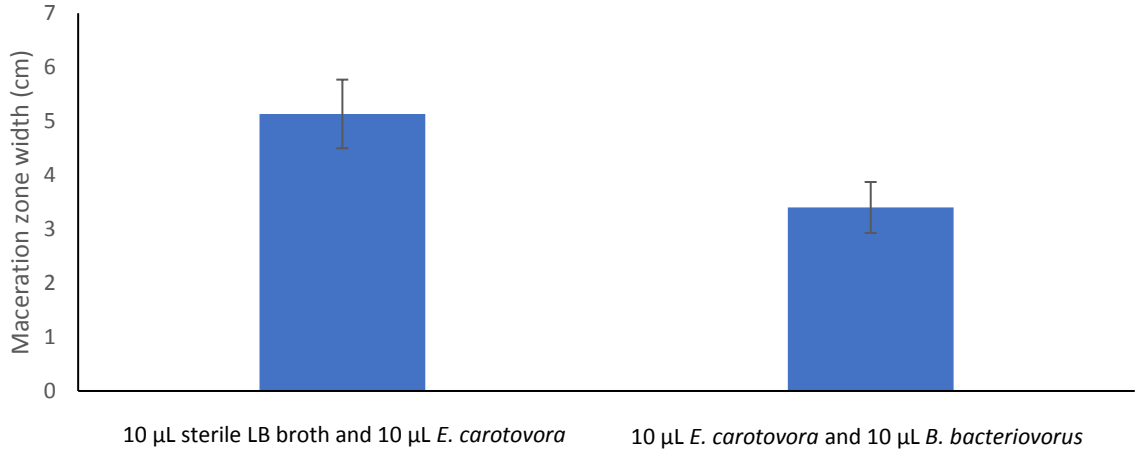

Figure 2. Comparison of maceration zone width caused by pathogen alone with maceration in the presence of both the pathogen and predatory bacteria. Zone width measured three days after inoculation. Inoculation day was March 8, 2016. N=3 and p=0.09.

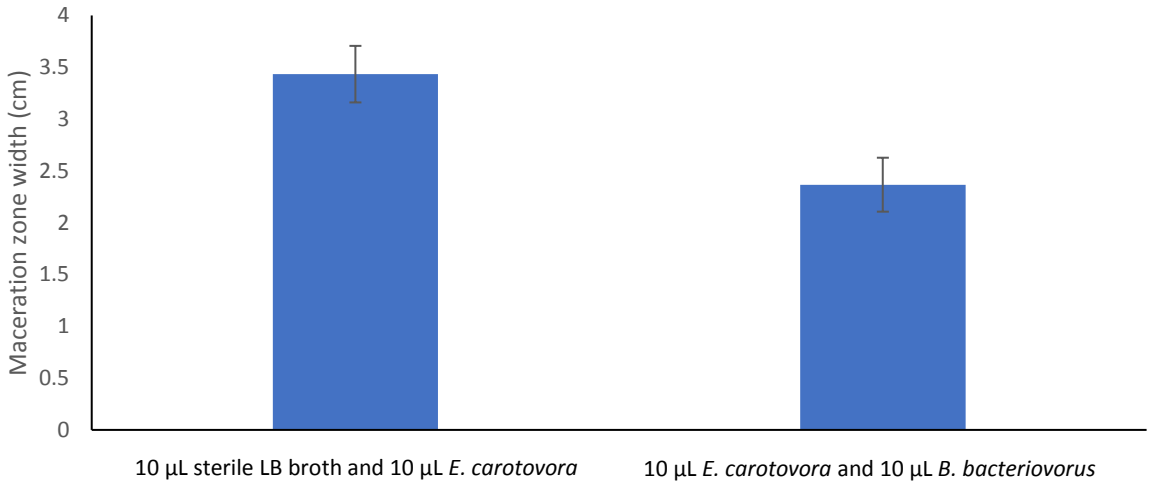

Figure 3. Comparison of maceration caused by pathogen alone with maceration in the presence of both the pathogen and biocontrol agent. Zone width measured three days after inoculation. Inoculation day was March 24, 2016. N=3 and p=0.047.

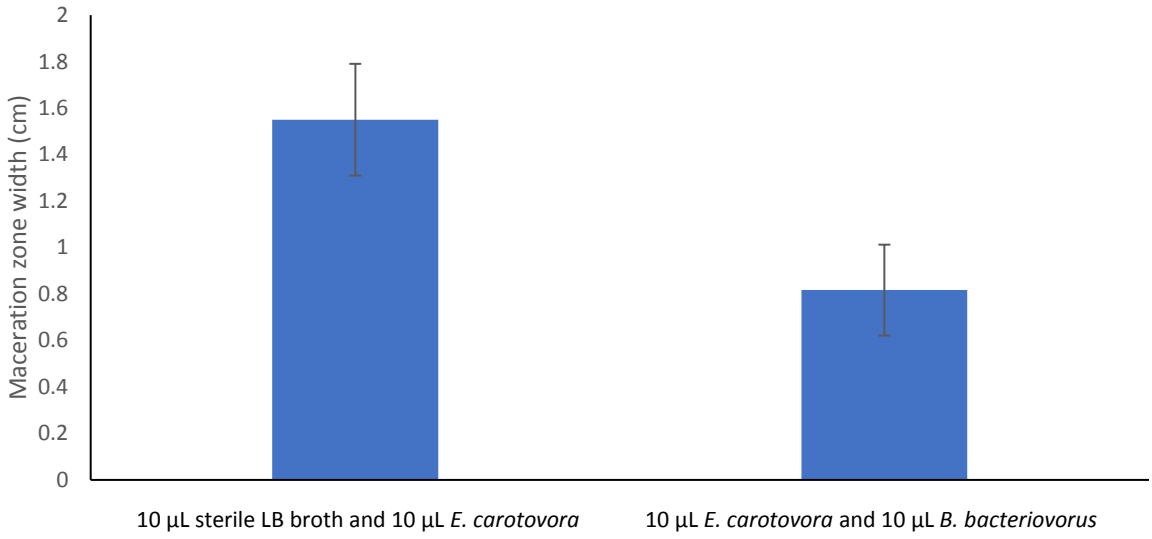

Figure 4. Comparison of maceration caused by pathogen alone with maceration in the presence of both the pathogen and predatory bacteria. Zone width measured three days after inoculation. The inoculation day was November 7, 2016. N=6 and p=0.039.

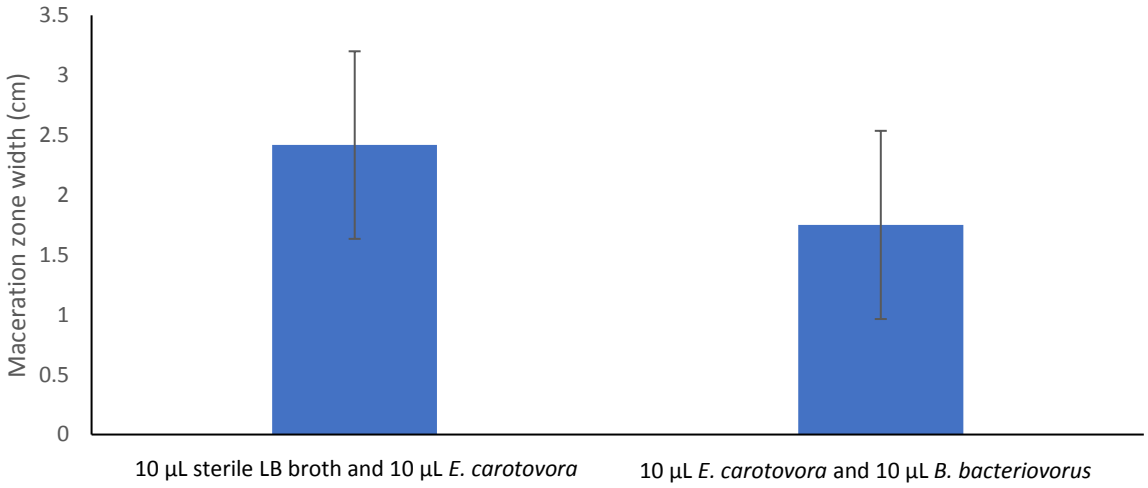

Figure 5. Comparison of maceration zone width caused by pathogen alone with maceration in the presence of both the pathogen and biocontrol agent. Zone width measured three days after inoculation. Inoculation day was November 18, 2016. N=6 and p=0.561.

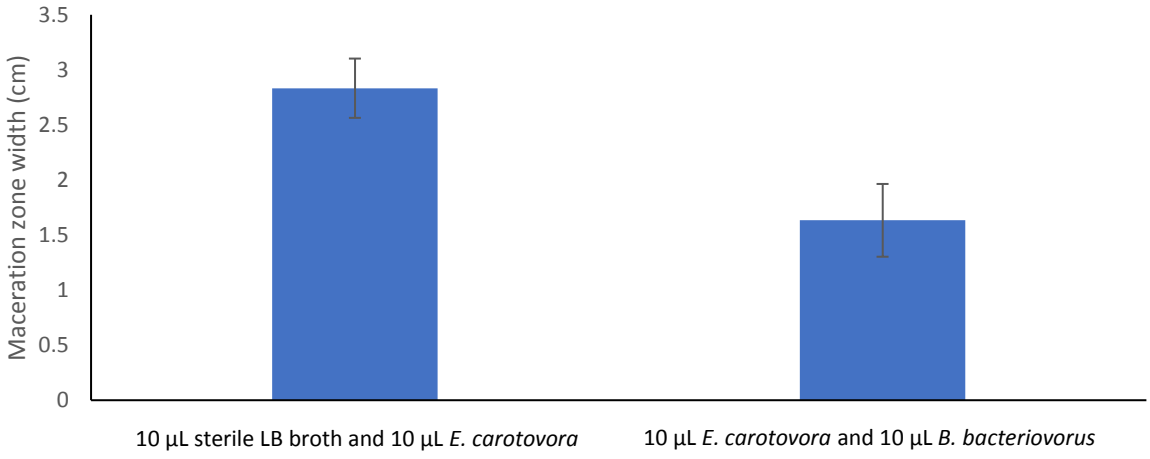

Figure 6. Comparison of maceration caused by pathogen alone with maceration in the presence of both the pathogen and biocontrol agent. Zone width measured three days after inoculation.The inoculation day was November 29, 2016. N=6 and p=0.018.

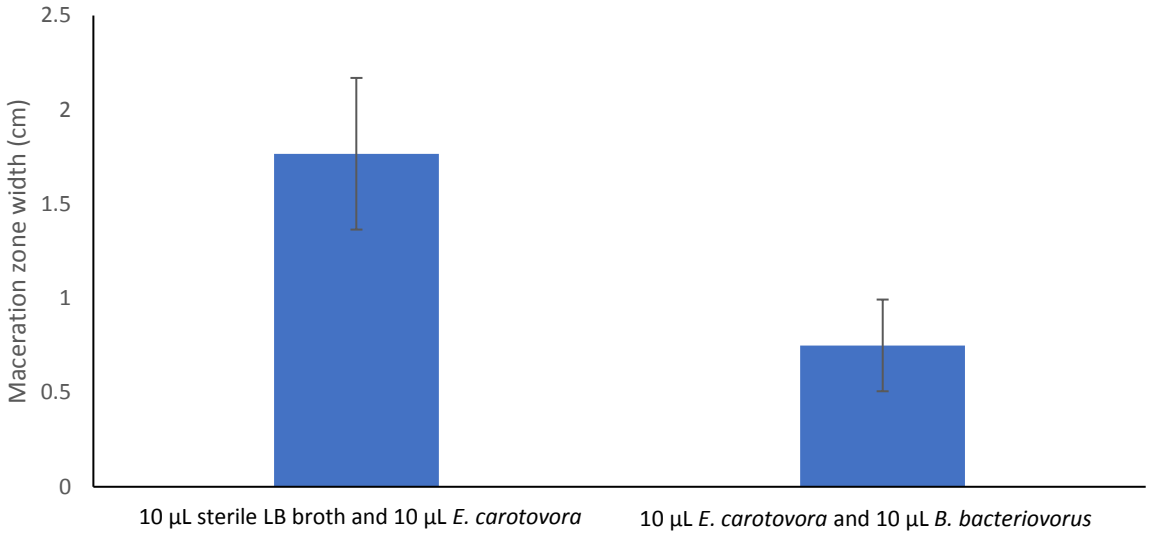

Figure 7. Comparison of maceration caused by pathogen alone with maceration in the presence of both the pathogen and predatory bacteria. Zone width measured three days after inoculation. Inoculation day was December 3, 2016. N=6 and p=0.062.

The data in figures 1, 2, 5, and 7 did not indicate a significant difference between the maceration zone width in the two potato treatments ($p>0.05$ for each trial). The data in figures 3, 4, and 6 did indicate a significant difference between the maceration zone widths in the two potato treatments ($p<0.05$ for each trial). When the all the data in the seven trials were combined, and analyzed as one single trial, there was a significant difference between the maceration zone width in the potato slices inoculated with just the pathogen and the potato slices inoculated with the pathogen and the predatory bacteria (figure 8, $p<0.05$). The sample size for the combined analysis was thirty-three potato slices for *E. carotovora* only and thirty-three potato slices for *E. carotovora* plus *B. bacteriovorus.*

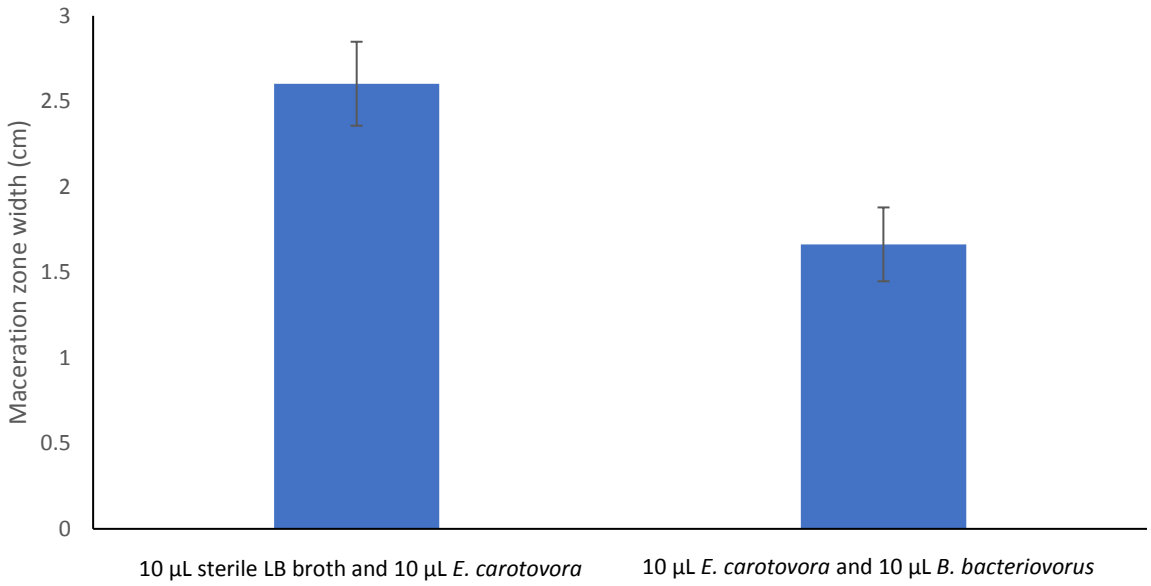

Figure 8. Comparison of maceration zone width caused by pathogen alone with the maceration in the presence of both the pathogen and biocontrol agent. Zone width measured three days after inoculation. The average of all seven trials. N=33 and p=0.005.

Discussion:

Out of the seven trials, only three showed a significant difference between the potato slices inoculated with the pathogen and the potato slices inoculated with the pathogen and the predatory bacteria which means that four of the trials did not show a significant difference. The average maceration zone width for the potato slices inoculated with just the pathogen varied among the trials from 1.5 cm to 6.0 cm. This indicates that *E. carotovora* did not show consistent symptoms of blackleg disease among the different trials. However, the average maceration zone widths of the potato slices inoculated with the pathogen and the predatory bacteria were consistently smaller than the potato slices

inoculated with just the pathogen even though four of the trials indicated insignificant differences.

The disease severity caused by *E. carotovora* itself varied possibly because of the nature of pathogens, the possible variation in the age of potatoes used, and the complex interactions between the host, the pathogen, and other organisms that may be present in this system. The zone of maceration in the potato slices inoculated with just the pathogen was the largest in the earlier trials and generally decreased in size in later trials. The idea of pathogen evolution leading to reduced virulence, severity of disease, could possibly explain the variability in disease severity. The evolution of pathogen virulence includes trade-offs that involve pathogen virulence and this leads to a decrease in disease severity caused by the pathogen over time (Gandon et al. 2001). The potatoes used in this study came from the same store and were the same brand but they sometimes came from different bags. The potatoes may have been harvested at various times and this means that the potatoes that came from different bags may have different ages. This could have affected the ability of *E. carotovora* to cause disease. Also, there may be endophytic bacteria or other organisms that are present in the system even though we were careful to not introduce outside organisms. These organisms may be interacting with *E. carotovora* and affecting the virulence.

There were several things we did to increase disease severity, including adding moisture (damp paper towels), increasing the temperature (incubator at 27°C), and changing the ratios of the treatments. The disease severity was decent at first, but as the trials progressed, the severity of disease decreased. In order to get more consistent

disease, such as constant widths of the zone of maceration, future studies could look at how much *E. carotovora* is required in order to get constant virulence. Increasing the amount of pathogen inoculated onto the potato slices may increase the virulence. Also, there are other environmental factors that could be changed, such as temperature and moisture, that may induce consistent virulence but it is important to remember that the environmental conditions must also be optimal for *B. bacteriovorus*. The rescue of disease by *B. bacteriovorus* cannot be measured without disease.

When the results were analyzed together there was a significant difference between the width of the maceration zone among the potato slices inoculated with the pathogen and the potato slices inoculated with the pathogen and the predatory bacteria. More specifically, the width of the zone of maceration in the potato slices inoculated with the pathogenic bacteria was bigger than the width of the zone of maceration in the potato slices inoculated with the pathogen and the predatory bacteria. This may suggest that *B. bacteriovorus* can lessen blackleg disease severity caused by *E. carotovora* in potatoes. These results agree with my hypothesis and with other studies in that *B. bacteriovorus* can lessen symptoms of disease. However, further research is required to study how *B. bacteriovorus* affects other symptoms of this disease such as the wilting of the foliage, the discoloration of the stems, and the destruction of the cell wall. Also, further research would help determine the implications of these results.

The predacious interactions between *B. bacteriovorus* and *E. carotovora* makes this system a good contender for being used as biocontrol for blackleg disease. According to Mcspaden Gardener and Fravel (2002), biocontrol is suppressing pest or pathogenic

organisms with another organism. In order for *B. bacteriovorus* to act as a biocontrol agent it is important that the environmental conditions are optimal for both organisms and that is what makes this difficult to incorporate into a real-world application. Future research would have to focus on how to get this system to work effectively in the conditions that are normal for potato crop production. The interrelationships between changing environmental variables and the complex relationships between different organisms and their environment make biocontrol effective. Based on recent studies, there has been interest among conventional and organic growers in using biocontrol agents meaning that the market demand for biocontrol products will increase in the coming years (Mcspaden Gardener and Fravel 2002). Applications of biologically sound biocontrol programs have been successful in greenhouse conditions where the environment and experimental design can be carefully controlled (Mcspaden Gardener and Fravel 2002).

Previous studies have used *B. bacteriovorus* as a biocontrol agent but they are different than this study regarding the feasibility of application. For example, Cao et al. (2015) studied biocontrol using *B. bacteriovorus* in an aqueous system with the whiteleg shrimp. It is easier to apply *B. bacteriorvorus* to the tank water than it is to apply *B. bacteriovorus* to potatoes in storage. Recep et al. (2009) studied biological control of potato dry rot using *Fusarium* species and PGPR (Plant Growth Promoting Rhizobacteria) species. *Burkholderia cepacian*, a PGPR strain, showed immense potential to be used as a biocontrol agent against potato dry rot caused by *Fusarium* during storage (Recep et al. 2009). Cell suspensions of biocontrol agents were

individually assayed against the pathogens by co-inoculating individual wounds (Recep et al. 2009). This method of biocontrol application may not be suitable for large scale application because of how much time it takes to inoculate each individual wound.

In conclusion, the results of this study suggest that *B. bacteriovorus* can be used as a biocontrol agent against blackleg disease in potatoes caused by *E. carotovora.* The predacious qualities that *B. bacteriovorus* possess gives this system potential for reduction of blackleg disease severity in potato plants. Future studies should focus on how to get consistent disease, how the biocontrol agent affects other symptoms of blackleg disease, and how the results of this study could possibly be applied in the potato crop production industry.

References:

Allen, H. K., J. Trachsel, T. Looft, and T. A. Casey. 2014. Finding alternatives to antibiotics. *Annals of the New York Academy of Sciences* 1323: 91-100.

Banwart, G. J. *Basic Food Microbiology*. New York: Van Nostrand Reinhold, 1989.

Cao, H., J. An, W. Zheng, and S. He. 2015. *Vibrio cholerae* pathogen from the freshwater-cultured whiteleg shrimp *Penaeus vannemai* and control with *Bdellovibrio bacteriovorus. Journal of Invertebrate Pathology* 130:13-20.

Dashiff, A., and D.E. Kadouri. 2011. Predation of oral pathogens by *Bdellovibrio bacteriovorus* 109J. *Molecular Oral Microbiology* 26: 19-34.

Duarte, V., S. H. Boer, L. J. Ward, and A. M. R. de Oilveira. 2004. Characterization of atypical *Erwinia carotovora* strains causing blackleg of potato in Brazil. *Journal of Applied Microbiology* 96: 535-545.

Du Raan, S., T. A. Coutinho, J. E. van der Waals. 2016. Cardinal temperature differences, determined in vitro, between closely related species and subspecies of pectinolytic bacteria responsible for blackleg and soft rot on potatoes. *European Journal of Plant Pathology* 144: 361-369.

Gandon, S., M. J. Mackinnon, S. Nee, and A. F. Read. 2001. Imperfect vaccines and the evolution of pathogen virulence. *Nature* 414: 751-755.

Lahbabi, D., M. Baz, S. Samri, F. Val, G. Hamelin, I. Madore, K. Bouarab, C. Beaulieu, M.M. Ennaji, and M. Barakate. 2012. Control of potato soft rot caused by *Pectobacterium carotovorum* and *Pectobacterium atrosepticum* by Moroccan

actinobacteria isolates. *World Journal of Microbiology & Biotechnology* 28.1: 303-11.

Lambert, C. and R.E. Sockett. 2008. Laboratory maintenance of *Bdellovibrio. Current Protocols of Microbiology* 7B.2.1-7B.2.13.

Markelova, N.Y. 2010. Predacious bacteria, *Bdellovibrio* with potential for biocontrol. *International Journal of Hygiene and Environmental Health* 213: 428-431.

Mcspaden Gardener, B.B. and D.R. Fravel. 2002. Biological control of plant pathogens: research, commercialization, and application in the USA. *Plant Health Progress* 1-18.

Mills, A.A.S., H.W. (Bud) Platt, and R.A.R. Hurta. 2006. Sensitivity of *Erwinia* spp. to salt compounds in vitro and their effect on the development of soft rot in potato tubers in storage. *Postharvest Biology and Technology* 41: 208-214.

Perombelon, M. C. M. 2000. Blackleg risk potential of seed potatoes determined by quantification of tuber contamination by the causal agent and *Erwinia carotovora* subsp. *atroseptica:* a critical review. *Scottish Crop Research Institute* 30: 413-420.

Raoul des Essarts, Y, J. C., A. Queta-Laurent, A. Caron, E. Munier, A. Beury-Cirou, V. Helias, and D. Faure. 2015. Biocontrol of the potato blackleg and soft rot diseases caused by *Dickeya dianthicola*. *Applied and Environmental Microbiology* 82: 268-278.

Recep, K., S. Fikrettin, D. Erkol, E. Cafer. 2009. Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. *Biological Control* 50: 194-198.

Reiter, B., U. Pfeifer, H. Schwab, and A. Sessitsch. 2002. Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *atroseptica*. *Applied and Environmental Microbiology* 68: 2261-2268.

Scherff, R.H. 1972. Control of bacterial blight of soybean by *Bdellovibrio bacteriovorus*. *Phytopathology* 63: 400-402.

Sharga, B.M., and G.D. Lyon. 1998. *Bacillus subtilis* BS 107 as an antagonist of potato blackleg and soft rot bacteria. *Canadian Journal of Microbiology* 44: 777-783.

Sockett, R. E. 2009. Predatory lifestyle of *Bdellovibrio bacteriovorus. Annual Review of Microbiology* 63: 523-39.

Sockett, R.E. and C. Lambert. 2004. *Bdellovibrio* as therapeutic agents: a predatory renaissance? *Nature Reviews Microbiology* 2: 669-675.

Starr, M. *Phytopathogenic Bacteria.* New York: Springer-Verlag, 1983.

Toth, I. K., L. Sullivan, J.L. Brierley, A.O. Avrova, L.J. Hyman, M. Holeva, L. Broadfoot, M.C.M. Perombelon, and J. McNicol. 2003. Relationship between potato seed tuber contamination by *Erwinia carotovora* ssp. *atroseptica*, blackleg disease development and progeny tuber contamination. *Plant Pathology* 52: 119-126.