CENTER for PRODUCE SAFETY

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FINAL PROJECT REPORT

Project Title
Genomic elucidation of the physiological state of enteric pathogens on pre-harvest lettuce

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Principal Investigator
Martin Wiedmann
Department of Food Science
Cornell University
607-254-2838
martin.wiedmann@cornell.edu

Co-Principal Investigator
Teresa Bergholz
Department of Veterinary and Microbiological Sciences
North Dakota State University

Objectives
1) *Identify the physiological state of Salmonella and EHEC on lettuce plants.*
2) *Determine if pre-harvest environmental conditions affect the physiological state of Salmonella and EHEC on the surface of lettuce plants.*
3) *Determine if the physiological state of Salmonella and EHEC on lettuce plants influences subsequent survival during decontamination washes as well as during gastric passage.*

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Abstract

Contamination of produce with enteric pathogens can occur in the pre-harvest environment, and many produce associated outbreaks have been traced back to the pre-harvest environment. Once contamination of produce in the field occurs, the enteric foodborne pathogens enterohemorrhagic *E. coli* (EHEC) and *Salmonella* are capable of surviving on the plant surface over long periods of time, yet little is known about the physiological state of the pathogen in this environment, and how pre-harvest environmental factors influence the physiological state of the pathogen. Understanding the physiological state of foodborne pathogens is important as physiological state can affect the ability of the pathogens to (i) survive post-processing decontamination procedures, such as a chlorine wash, and to (ii) survive and cause disease in the host (for example, physiological state may affect the ability of a pathogen to survive gastric acidity). The physiological state of enteric pathogens on pre-harvest produce can also significantly impact the ability to detect these pathogens in this environment. Our works here evaluated the physiological state of 4 EHEC and 4 *Salmonella* on lettuce under different environmental conditions, using both RNA-sequencing and phenotypical characterization. We found that different strains within the same EHEC or *Salmonella* serotype can show considerable variation in survival on lettuce plants and that EHEC and *Salmonella* associated with pre-harvest lettuce are experiencing osmotic and oxidative stresses, as indicated by the changes in gene expression during incubation on lettuce. We also found that at least some produce associated EHEC strains may show increased chlorine resistance after 3 and 5 days adaptation to lettuce, suggesting that validation studies on post-harvest interventions and specifically chlorine washes may not correctly estimate the effectiveness of these washes, unless challenge strains have not been adapted to lettuce for at least 3 days. The complete RNA transcript level data created here for 4 EHEC and 4 *Salmonella* grown on lettuce also provide a future resource for identification of RNA targets for novel more rapid and sensitive detection methods for these pathogens.

Background

Contamination of produce with enteric pathogens can occur in the pre-harvest environment (in the field) as well as during post-harvest handling. While some produce associated outbreaks have been traced back to post-harvest contamination (e.g. packing houses), many produce associated outbreaks have been traced back to the pre-harvest environment, most notably the 2006 *E. coli* O157:H7 outbreak in spinach (CDC 2006, Jay et al. 2007). Numerous studies have shown that enteric pathogens are present in the pre-harvest environment (Bolton et al. 2011, Micallef et al. 2012), and can come into contact with produce in the field via a number of different routes. Key enteric pathogen of concern for produce are *Salmonella* and enterohemorrhagic *E. coli* (EHEC) O157:H7; both of these are among the top 5 foodborne pathogens with the highest numbers of annual hospitalizations (Scallan et al. 2011), and a number of outbreaks associated with these pathogens have been attributed to consumption of fresh produce (Rangel et al. 2005, Hanning et al. 2009). The transmission of EHEC and *Salmonella* to produce is complex, and involves poorly-understood transmission processes and unknown environmental reservoirs. Many physical environmental factors can affect dispersal, growth and survival of foodborne pathogens in the pre-harvest environment, such as free moisture in the soil, wildlife, and seasonal precipitation (Cooley et al. 2007).

Once contamination of produce in the field occurs, the pathogens are capable of surviving on the plant surface for varying lengths of time, dependent on the method of contamination as well as environmental conditions. For example, Moyne et al. showed that *E. coli* O157:H7 spray-inoculated onto field grown lettuce was detected by direct plating for 7 days, and was detected by enrichment 4 weeks after inoculation (Moyne et al. 2011). Another study found that *E. coli* O157:H7 could be recovered from spray-inoculated field grown lettuce for up to 27 days (Erickson et al. 2010). *Salmonella* Typhimurium inoculated onto lettuce and parsley via contaminated compost and water was detected on plants in the field up to 60 days after inoculation (Islam et al. 2004). Taken together, these data indicate that EHEC and *Salmonella* can survive on the plants in the field over long periods of time, yet little is known about the physiological state of the pathogen in this environment, and how pre-harvest environmental factors influence the physiological state of the pathogen.

The impact of specific pre-harvest variables on survival of enteric pathogens has been assessed, and, in some cases, pre-harvest variables play a significant role in the survival of pathogens on pre-harvest produce. For example, the method of irrigation (drip or spray) has been shown not to play a role in survival of EHEC O157:H7 on lettuce or baby greens (Moyne et al. 2011, Tomas-Callejas et al. 2011). Humidity levels on the other hand have been shown to play a significant role in survival of enteric pathogens on produce, but the effects are dependent on the specific pathogen. For example, *Salmonella* enterica survived better on lettuce under high humidity compared to low humidity, while EHEC O157:H7 survived better on lettuce under low humidity compared to high humidity (Stine et al. 2005). The amount of UV exposure also significantly influences survival of pathogens on plant surfaces; studies have shown that pathogens inoculated onto the lower, or shaded, side of leaves have increased survival compared to when inoculated onto the upper side of leaves (Stine et al. 2005, Erickson et al. 2010). EHEC and *Salmonella* are capable of surviving on pre-harvest lettuce over time, and the extent of their survival can be influenced by environmental conditions in the field.

Environmental stresses, such as changes in temperature, moisture, and UV exposure, as well as plant defense mechanisms and native plant bacteria, impose physical, chemical, and/or biological stress on pathogens present on produce plants. Pathogens are able to manage these stresses and survive, leading to the presence of these organisms in the food supply. One of the first steps that allow bacteria to adapt to a new environmental situation is their ability to alter gene expression patterns, typically initiated by transcriptional regulators stimulated by cell systems that sense environmental changes (Rodriguez-Romo et al. 2005). Modulation of gene expression in response to stress indicates activation or repression of a specific physiological response (Brul et al. 2005). The physiological state of bacteria and their capabilities for stress resistance can be assessed by identifying genome-wide changes in gene expression (Bergholz et al. 2009, Bergholz et al. 2012). Little is known about the physiological state of EHEC or *Salmonella* on the surface of pre-harvest produce. Recent genomics-based efforts have focused on identifying the responses of *S.* Typhimurium internalized in tomatoes (Noel et al. 2010) and gene expression of *Salmonella* and *E. coli* internalized in lettuce (Teplitski et al. 2012), which poses different stresses to the pathogen compared to on the surface of plants. While use of whole-genome expression profiling tools to understand the genetic response of foodborne pathogens inoculated onto post-harvest produce has been applied (Carey et al. 2009, Kyle et al. 2010, Fink et al. 2012, Parker et al. 2012), transcriptional profiling techniques also have tremendous potential for improving our ability to understand the physiological state of pathogens in the pre-harvest environment.

Pathogens associated with pre-harvest produce are exposed to a variety of environmental stresses during post-harvest processing and handling. For lettuce and other leafy greens, this typically includes a decontamination treatment. Post-harvest decontamination techniques are used for fresh produce to reduce the risk of foodborne infections as well as to reduce the risk of microbial spoilage of the product (Gomez-Lopez et al. 2009). Many studies have focused on evaluating the efficacy of chlorine-based decontamination methods to reduce EHEC and *Salmonella* on lettuce and other fresh produce (Lang et al. 2004, Mahmoud et al. 2008, Luo et al. 2012). These studies commonly inoculate post-harvest produce with bacterial cultures grown under optimal laboratory conditions, which are not indicative of pathogens that have experienced pre-harvest stresses on the produce plants. The need for a standardized approach for comparing the effectiveness of decontamination treatments has been identified (Gil et al. 2009). We propose that the pre-harvest physiological state of the pathogens should be taken into account as part of a standardized approach for evaluating physical and chemical decontamination treatments. If pre-harvest environmental stresses induce bacterial stress responses, they could be more resistant to decontamination treatments. In addition, the physiological state can be used to validate new treatments as they are developed, such as low X-ray irradiation (Jeong et al. 2010) or chlorine dioxide (Mahmoud et al. 2008) for reducing EHEC O157:H7 and *Salmonella* on lettuce.

The physiological state of enteric pathogens on pre-harvest produce can also significantly impact the ability to detect these pathogens in this environment. Bacteria can enter a viable but non-culturable (VBNC) state, and while the VBNC phenomenon is not well understood, it does comprise a distinct physiological state for enteric pathogens (Dinu et al. 2009). The potential for cells in the VBNC state has been reported for *Salmonella* Thompson associated with cilantro under low moisture conditions (Brandl et al. 2002). Factors that are associated with induction of the VBNC state in enteric pathogens include low nutrients, temperature, solar radiation, osmotic stress, and pH stress (Dinu et al. 2009), many of these stresses would be experienced by enteric pathogens in the pre-harvest environment. Pathogens in the VBNC state will be unlikely to be detected via culture-based methods, and molecular based detection methods need to be able to discriminate between cells that are alive or dead. Therefore, it is critical to understand the physiological state of enteric pathogens on pre-harvest produce for development and assessment of appropriate detection methods. In addition, an understanding of the physiological state of bacterial cells on plants, including an understanding of RNA levels for different genes found in bacteria present on plants, also has considerable implications for the development of faster detection methods for target foodborne pathogens. As RNA levels for specific target genes can be 100 to 1,000 fold higher than DNA levels, targeting some of these genes for detection will allow for considerably more rapid detection methods.

It is unknown what stress responses are induced in pathogens on growing plants in the pre-harvest environment. As EHEC and *Salmonella* have been transmitted to humans via produce, it is clear that these pathogens are capable of surviving in the pre-harvest environment as well as during post-harvest handling and processing. The physiological state of the pathogen plays a significant role in its ability to survive subsequent stresses, such as decontamination treatments and those stresses encountered during distribution. Understanding how these pathogens survive on plants is the first step in mitigating the presence of these pathogens in the food supply. The goal of this research project was to determine how pre-harvest conditions impact the ability of EHEC and *Salmonella* to survive in the food chain, providing insights for

developing effective post-harvest treatments to reduce consumer exposure to these pathogens on produce.

Research Methods and Results

The original objectives of this proposal were:

Objective 1: Identify the physiological state of *Salmonella* and EHEC on lettuce plants.

Objective 2: Determine if pre-harvest environmental conditions affect the physiological state of *Salmonella* and EHEC on the surface of lettuce plants.

Objective 3: Determine if the physiological state of *Salmonella* and EHEC on lettuce plants influences subsequent survival during decontamination washes as well as during gastric passage.

The research methods and results of the project are presented, following the same three original objectives.

Objective 1 and 2

Methods and results for these two objectives are combined for this report as they both used similar methodological approaches, Obj. 1 focused on determining the physiological state of *Salmonella* and EHEC on lettuce plants over time, while Obj.2 focused on determining the physiological state of *Salmonella* and EHEC on lettuce plants grown under different environmental conditions.

Strain selection. There are two major genetic lineages of EHEC; EHEC 1 consists of strains of serotype O157:H7 and O55:H7 and EHEC 2 consists of serotypes O26:H11 and O111:H8. We thus originally planned to test one strain of EHEC O157:H7 and one strain of EHEC O26:H11 as well as one *Salmonella* Newport and one *Salmonella* Typhimurium strain. Initial experiments with multiple strains of each serotype highlighted that considerable variation in survival on lettuce plants can occur for strains of the same serotype (Figure 1). Based on these data, we modified our plan to include two strains of each serotype (for a total of 8 strains) and focus on environmental conditions representing two harvest seasons (rather than conditions representing 4 harvest seasons). This modification allowed us to provide a more in-depth set of data for each season tested, which can be used to determine which factor may be more important for pre-harvest survival, the strain or the harvest season. The final strains used are listed in Table 1.

Lettuce plant cultivation and inoculation. Romaine lettuce seeds (*Lactuca sativa*) purchased from Living Whole Foods (Springville, UT) were seeded into sterile soil (Sungro Sunshine LC1 consisting of coarse perlite, dolomitic limestone, gypsum and Canadian sphagnum peat moss) in 4.5 inch plastic pots. Lettuce was grown in the North Dakota Agricultural Research Experiment Station greenhouse facility at 13°C-15°C during the night and 18°C-20°C during the day with a photoperiod of 14.5 hours. Plants were watered as needed.

All bacterial isolates were stored at -80°C in Brain-Heart infusion (BHI) broth with glycerol. Each isolate was freshly streaked to Luria-Bertani (LB) agar from frozen stock and incubated for 24 h at 37°C. A single colony was transferred to 5 ml of LB followed by incubation at 37°C for 15 h. After 15 h, 100 µl LB culture was transferred to 100 ml LB broth with incubation at 37°C, and shaking at 215 rpm, for 15 h. Following growth in LB for 15 h, cells were collected by centrifugation at 8000 rpm for 5 min (Avanti J-25 Centrifuge, Beckman Coulter). Supernatant was discarded and inoculum was prepared by suspending the cell pellet in

50 ml Phosphate Buffered Saline (PBS) for a final concentration of approx. 10^9 cells/ml. After 28-35 days of growth, 8 pots of lettuce were inoculated with each isolate via spray inoculation in a biosafety cabinet. A hand-held TLC sprayer (model 422530-0050, Kontes Glass Company, Vineland, N.J) was used to deliver inoculum by spraying for 5 s (approx. 1 ml) onto the lettuce leaves of each pot (Lang M et al, 2004). The carrier gas was nitrogen at approximately 10 Psi. Inoculated plants were placed in a plastic tray filled with 2 cm water and kept in a greenhouse growth chamber (Conviron PGW40, Winnipeg, Manitoba, Canada).

Environmental conditions and their effect on pathogen survival. Survival of the 8 pathogen strains (4 EHEC and 4 *Salmonella)* on lettuce was evaluated under conditions representing the two harvest seasons that showed the largest differences in climatological conditions, the March and June harvest seasons. Climate data for the major lettuce harvest seasons in Salinas, California (March, June, September, and November; USDA National Agricultural Statistics Service) were obtained from the Salinas Municipal Airport weather station for 2009-2011 from the National Climatic Data Center (www.ncdc.noaa.gov). The March harvest season conditions were 12 h photoperiod, max temp 17. 2°C, min temp 6.7°C; the June harvest season conditions were 14.8 h photoperiod, max temp 20°C, min temp 12.2°C. For both harvest conditions, experiment were conducted under two different humidity levels, 45% RH (relative humidity) and 75% RH.

Our data showed that the effects of RH on EHEC survival on lettuce is dependent on the harvest season conditions. For the June harvest season, higher RH leads to significantly lower survival of EHEC, while lower RH leads to significantly greater survival (Figure 1). For the March harvest season, lower RH leads to significantly lower survival, while higher RH leads to greater survival one day post-inoculation. Over the 5 day period, the total impact of RH under March harvest season conditions is minimal; survival is similar for 45% RH and 75% RH.

For *Salmonella*, we observed no differences in survival on lettuce among the different conditions one day post-inoculation (Figure 2). Significant differences in *Salmonella* survival were observed on lettuce 5 days post-inoculation, where the effect of RH on survival is again dependent on harvest season conditions. For the June harvest season, higher RH leads to lower survival, while lower RH leads to higher survival on day 5. For the March harvest season, RH has no effect on survival – the average log decrease in CFU/g lettuce after 5 days is 1.68 for March 75% RH and is 1.62 for March 45% RH.

Characterization of physiological state by RNA sequencing. RNA sequencing ("RNA-seq") was used to characterize the levels of mRNA in the different pathogen strains grown on lettuce; analysis of RNA-seq data provides an in-depth evaluation of the physiological state of the bacteria. To extract RNA from the EHEC and Salmonella grown on the lettuce under different conditions, leaves were added to plant extraction buffer as described previously (Fink et al. 2012). Bacteria were subsequently washed from the leaf surface and centrifuged; bacterial pellets stored at -80°C. RNA was be extracted from cell pellets using the hot phenol method as described previously (Bergholz et al. 2007). rRNA was removed from samples with the Microbe Express kit (Ambion), and quality of the RNA assessed on a Bioanalyzer (Agilent). RNA was prepared for sequencing using the ScriptSeq kit (Epicentre), followed by RNA-seq on the Illumina HiSeq at the Cornell University Core Life Science Laboratory (CLSL).

For EHEC O157:H7, the majority of significant changes in gene expression during incubation on lettuce were observed 3 days post-inoculation. Genes encoding proteins known to

contribute to the osmotic stress response, the cell envelope stress response, and the oxidative stress response had higher levels of expression on day 3 compared to days 1 and 5. These genes included *osmB, osmC,* and *osmE,* all known to be induced by osmotic changes and to contribute to resisting osmotic stress in *E. coli* (Jung et al. 1990, Gutierrez et al. 1991). Activation of the cell envelope stress response was indicated by increased expression levels of *cpxP* and *degP*, encoding proteins involved in responding to misfolded proteins in the periplasm, and *pspABCDE*, encoding proteins involved in responding to damage to the cell envelope (Danese et al. 1998). Genes encoding proteins that mitigate the effects of oxidative damage were also induced, including *sodC*, which encodes superoxide dismutase, and is known to contribute to resistance to oxidative stresses, including chlorine stress (Wang et al. 2009). Increased expression of *sodC* was also observed for EHEC O26. EHEC O26 also had increased expression levels of genes encoding proteins involved in attachment of the pathogen to surfaces, including *yadM*. Not as many changes in gene expression were observed for *Salmonella*, though genes encoding proteins located on the cell surface, as well as those involved in attachment, were upregulated, including *cmeC* and *yiaD*.

Objective 3

Survival studies in chlorine wash. We have measured the ability of EHEC and *Salmonella* strains to survive a chlorine wash (2 minutes, 50 ppm XY-12 [EcoLab]) following 1, 3, and 5 days on lettuce plants held at 75% humidity under June harvest conditions. Inoculated lettuce was also washed for 2 minutes in sterile water as a control. The log CFU/g lettuce recovered from lettuce washed with chlorine was compared to that recovered from lettuce washed with water. The data presented in Figures 3 and 4 are the difference in recovery between lettuce washed with water and with chlorine. For *Salmonella* strains inoculated on lettuce for one day, the average log decrease due to chlorine was ~ 1.5 for all strains. Significant changes in chlorine resistance was not observed as the length of time on pre-harvest lettuce increased, the average log decrease due to chlorine remained ~ 1.5 on days 3 and 5. For EHEC strains inoculated on lettuce for one day, the average log decrease due to chlorine was ~ 1 for all strains. Over time, differences in chlorine survival among strains were observed for 3 and 5 days post-inoculation, where the O157:H7 strain from the 2006 spinach outbreak (TB65) had significantly greater survival compared to the other EHEC strains. It appears that longer incubation on lettuce led to greater chlorine survival for that strain, where the difference between cells recovered from water and from chlorine was much smaller for cells that had been on lettuce for 3 and for 5 days. These data are important as they suggest that validation studies on chlorine washes may over-estimate the effectiveness of these washes if challenge strains have not been adapted to lettuce for at least 3 days.

Effect of physiological state of *Salmonella* and EHEC on lettuce plants on subsequent survival during gastric passage. In order to assess the effect of the *Salmonella* and EHEC physiological state on pathogen survival during gastric passage, we analyzed the RNA-seq data to specifically determine whether genes that are important of acid stress survival are up- or down regulated on bacteria grown on lettuce and exposed to different conditions. Proteins known to contribute to acid resistance at pH < 3.0 in *E. coli* include the glutamate decarboxylase system (Lin et al. 1996), the arginine decarboxylase system (Lin et al. 1996), the general stress response sigma factor RpoS (Small et al. 1994), and the acid shock response protein (Seputiene et al. 2003). With the exception of the glutamate decarboxylase system, the same set of proteins

contributes to acid resistance in *Salmonella* (Lee et al. 1994, Lee et al. 1995, Spector et al. 1999). We did not observe any of these acid resistance genes either up- or down-regulated in *Salmonella* on pre-harvest lettuce. For EHEC, we did observe upregulation of *asr*, encoding the acid shock response protein. None of the other acid resistance genes were up- or down-regulated in EHEC. Together, these data suggest that incubation on pre-harvest lettuce is unlikely to increase subsequent acid resistance of either pathogen.

Outcomes and Accomplishments

Overall, this project provided considerable new insights into the physiological state of two key pathogens of concern in the leafy green industry (*Salmonella* and Enterohemorrhagic *E. coli* [EHEC]). Specific key scientific accomplishments include (i) successful development and implementation of methods to isolate complete RNA from bacteria grown on leafy greens; (ii) the description of the complete transcriptome of four *Salmonella* and four EHEC strains; and (iii) initial data on the effects of strain diversity and environmental conditions on the transcriptome and physiology of *Salmonella* and EHEC growing on leafy greens. The description of the complete transcriptome of four *Salmonella* and four EHEC strains grown on leafy greens did not only allow us to describe specific adaptation of these pathogens to leafy greens, but also provides a tremendous community resource that can be leveraged for future research and discoveries. For example, these data identify specific genes that produce high level of mRNA when these pathogens are grown on leafy greens, which will facilitate development of future detection methods that target these mRNA species. For example, if a pathogen produces about a 500 mRNA copies (versus having only one copy of the DNA for this gene), targeting this genes should allow for considerably more rapid detection as a shorter or no enrichment can be used.

In addition to the scientific outcomes, this project also continued to the training of future food safety researchers and professionals with expertise in produce food safety. For example, Dr. Teresa Bergholz who contributed to the preparation of the original proposal when she was still at Cornell, has moved to North Dakota State University as an Assistant Professor, where she continued to do produce safety related research. A Masters student in Dr. Bergholz's lab, Deepti Tyagi, collected the majority of data associated with this project, and successfully defended her Masters' thesis "Impact of pre-harvest environmental factors on the survival of enterohemorrhagic *E. coli* and *Salmonella* on lettuce" in December 2014.

Summary of Findings and Recommendations

Key findings

- Different strains within the same EHEC or *Salmonella* serotype can show considerable variation in survival on lettuce plants
- Our data suggest that validation studies on post-harvest interventions and specifically chlorine washes may not correctly estimate the effectiveness of these washes, unless challenge strains have not been adapted to lettuce for at least 3 days.
- EHEC and *Salmonella* associated with pre-harvest lettuce are experiencing osmotic and oxidative stresses, as indicated by the changes in gene expression during incubation on lettuce. This may suggest changes in the susceptibility of these pathogens to osmotic and oxidative stresses after adaptation to lettuce surfaces in the pre-harvest environment.

Recommendations

- Evaluation and validation of post-harvest pathogen interventions for leafy greens should use pathogen strains that have been adapted to the leafy green environment for at least 3 days
- Our data show that both strain and environment can have a significant impact on physiological state, suggesting that further work is needed to determine how strains and pre-growth conditions should be selected for challenges studies and evaluation of interventions
- Genes that show consistent high transcript levels on lettuce should be further pursued as potential targets for mRNA-based detection systems, which may provide significant advantages over traditional DNA-based methods as (i) mRNA is found at a higher level than DNA in a given cell, thus allowing for more sensitive detection in a shorter time frame and (ii) as mRNA is rapidly degraded in a dead bacterial cell. Our data suggest that *osmB, osmC, sodC,* and outer membrane protein encoding genes such as *yiaD, yadM,* and *cmeC* are likely candidates for mRNA-based detection, and should be evaluated further.

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APPENDICES

Publications and Presentations (required)

Tyagi, D., J. Sherwood, K. Sanders, M. Wiedmann, and T. M. Bergholz. Differences in survival among Enterohemorrhagic *E. coli* and *Salmonella* on pre-harvest lettuce. American Society for Microbiology General Meeting, Boston, Massachusetts. 2014.

Tyagi, D., J. Sherwood, and T. M. Bergholz. Effect of humidity on survival of foodborne pathogens on pre-harvest lettuce. North Dakota Academy of Science Annual Meeting, Valley City, North Dakota. 2014

Tyagi, D., J. Sherwood, K. Sanders, and T. M. Bergholz. Influence of pre-harvest environmental factors on stress resistance of *E. coli* and *Salmonella* on lettuce. North Central Branch ASM Meeting, Brookings, South Dakota. 2013.

Budget Summary (required)

Funds utilized as of: 2/16/15

Cornell University	
Salaries	$148,943.94
Fringe benefits	$53,007.36
Supplies	$34,180.63
Services	$13,251.02
Communications	$210.77
Indirect Costs	$9,199.46
North Dakota State University	
Sub-award	$18,830.85 (amount invoiced by NDSU to date)
Total Expenses	$277,624.03

We expect to use all funds awarded to our project. While the award is not yet fully spent, remaining invoices from North Dakota State University and expenses for travel to the June 2015 CPS meeting will fully expand the funds.

Tables and Figures (optional)

Table 1. *E. coli* and *Salmonella* strains used in this study

Isolate	Pathogen	Serotype	Source	Year of Isolation
FSL R8-2543	*Salmonella*	Newport	Human sporadic	2008
FSL R8-4110	*Salmonella*	Newport	Bovine feces	2009
FSL P3-1552	*Salmonella*	Typhimurium	Soil	2012
FSL R6-0207	*Salmonella*	Typhimurium	Human sporadic	2006
TW08264	EHEC	O157:H7	Japan sprouts outbreak (Sakai)	1996
TW014359	EHEC	O157:H7	US Spinach outbreak	2006
TW09184	EHEC	O26:H11	Human sporadic	2003
TW016501	EHEC	O26:H11	US Sprouts outbreak	2012

Figure 1. Survival of EHEC strains under each RH and harvest season condition

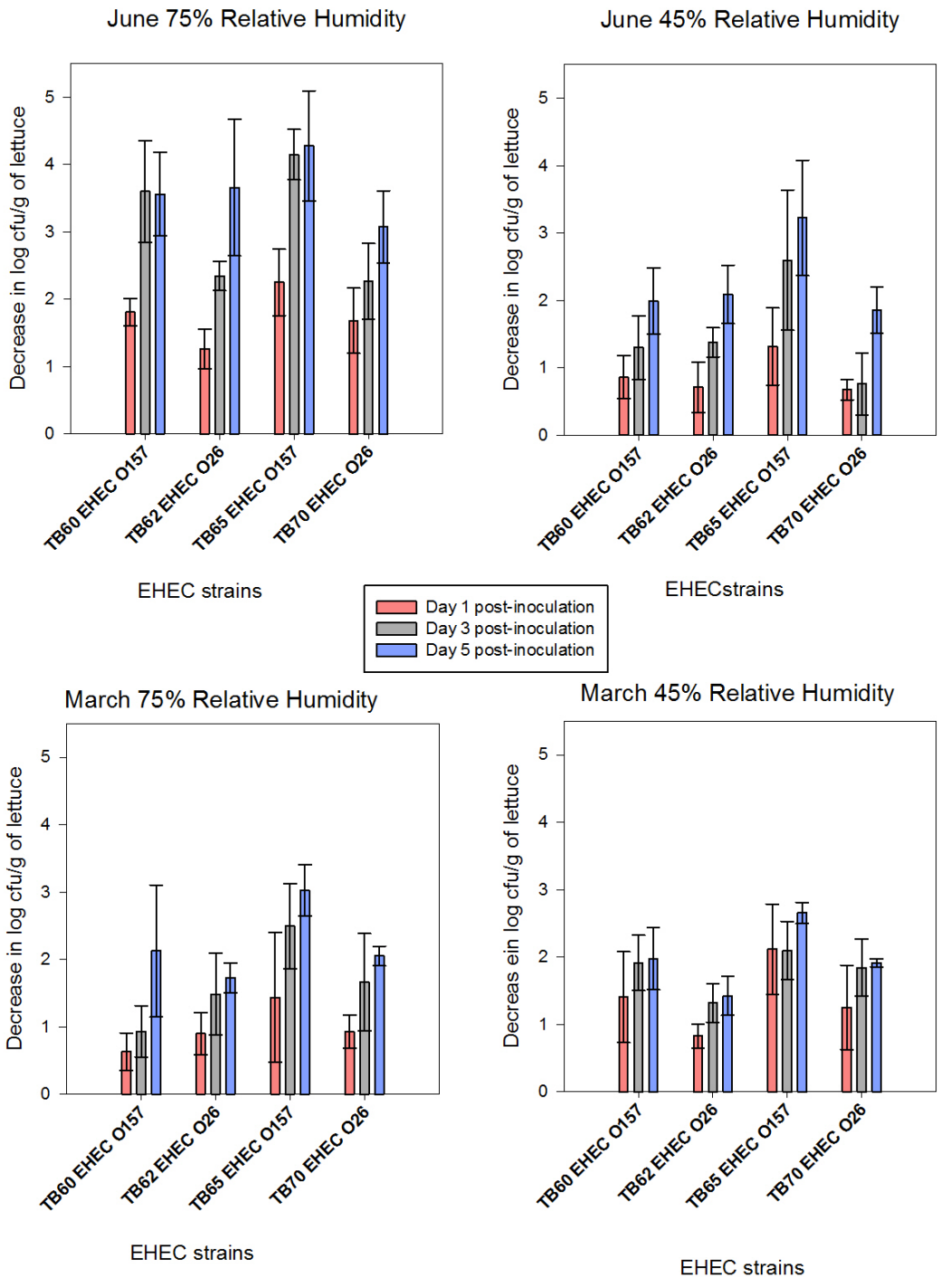


Figure 2. Survival of *Salmonella* strains under each RH and harvest season condition

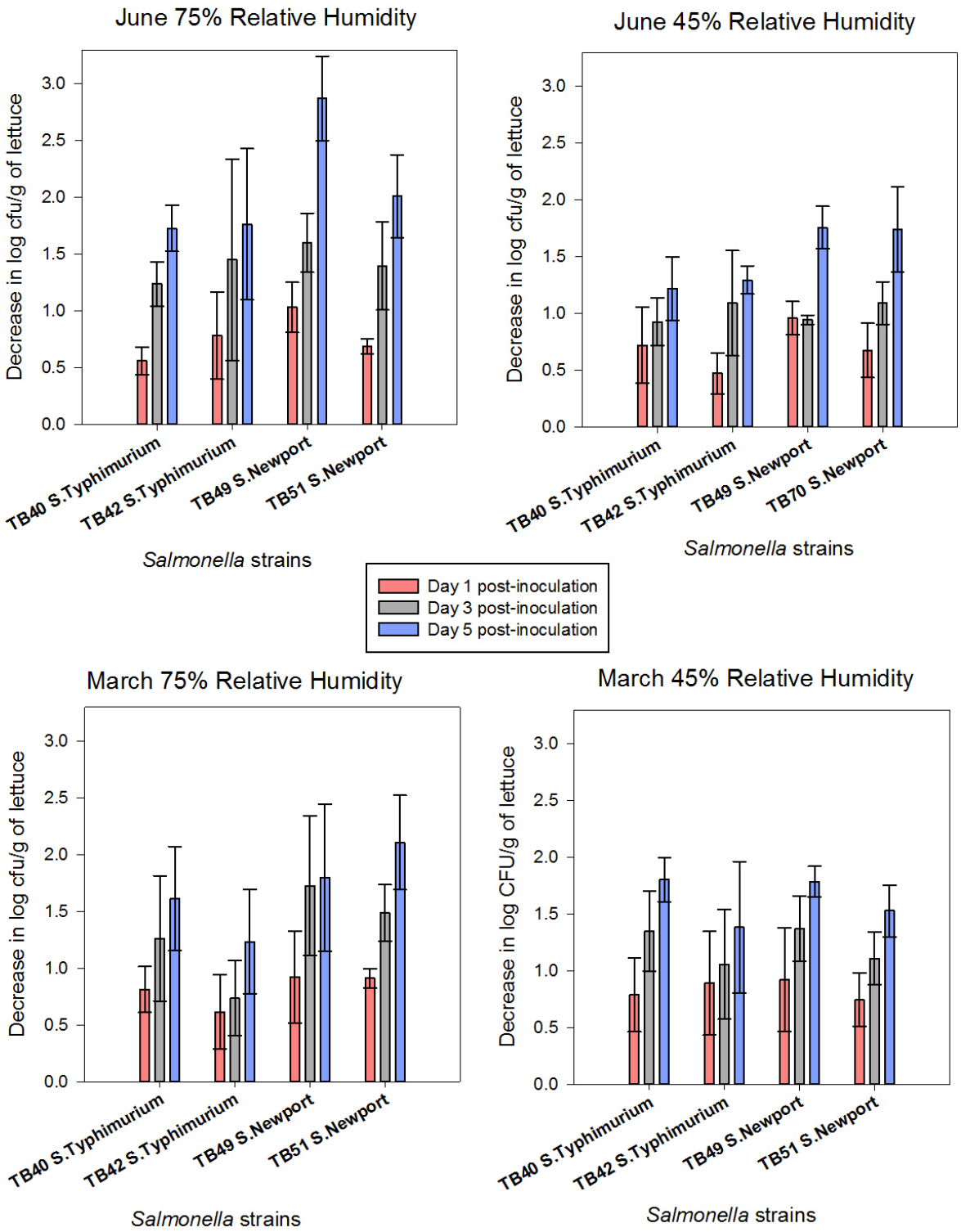


Figure 3. Chlorine resistance of *Salmonella* strains after incubation on pre-harvest lettuce. Inoculated lettuce plants were incubated in a growth chamber under June harvest conditions and 75% RH. After 1, 3, and 5 days of incubation in the growth chamber, inoculated lettuce was collected, subdivided, and a portion washed in sterile water and a portion washed in 50 ppm chlorine for 2 minutes. The difference in the number of bacteria present after water wash and chlorine wash are presented for 2 independent replicates with 2 technical replicates each. Different letters above a bar indicate a significant difference.

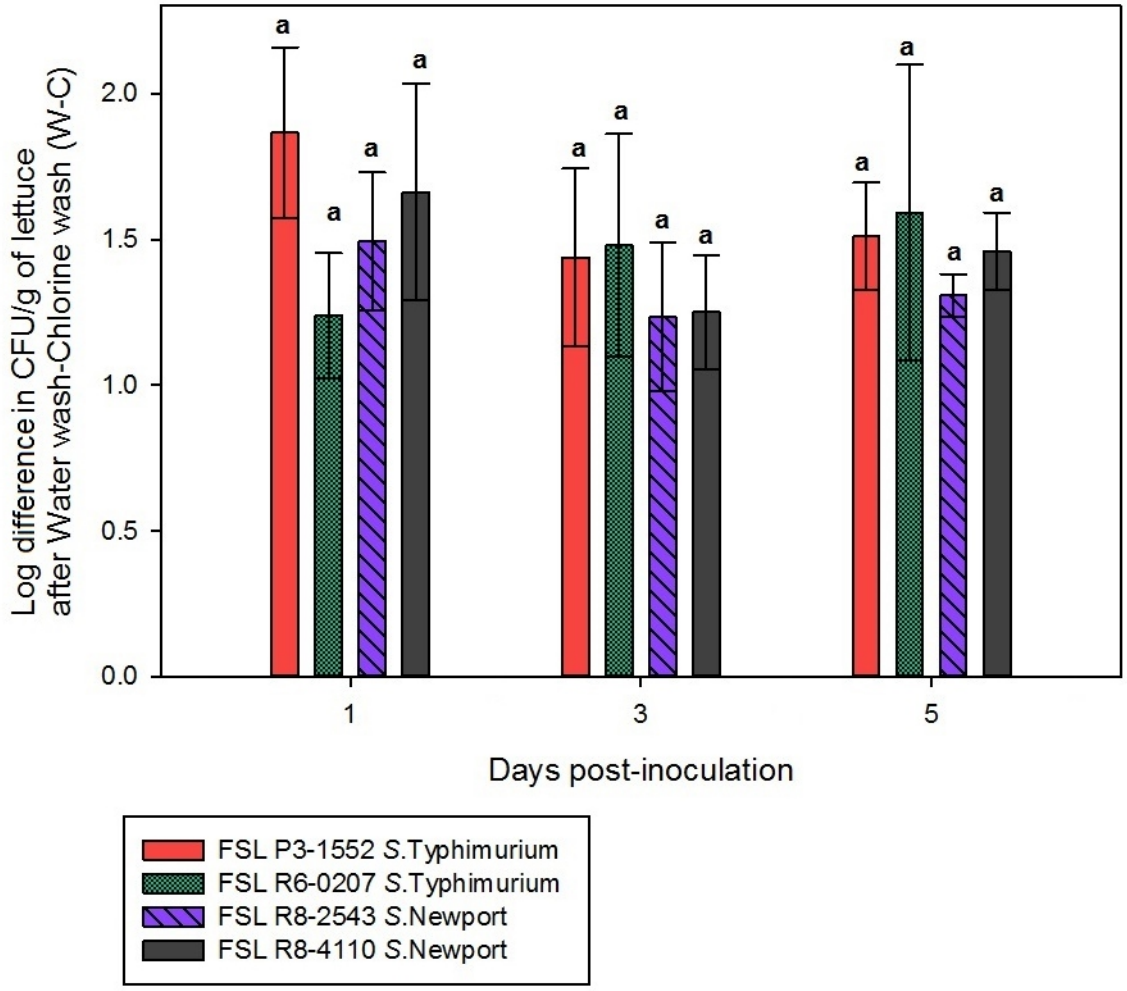
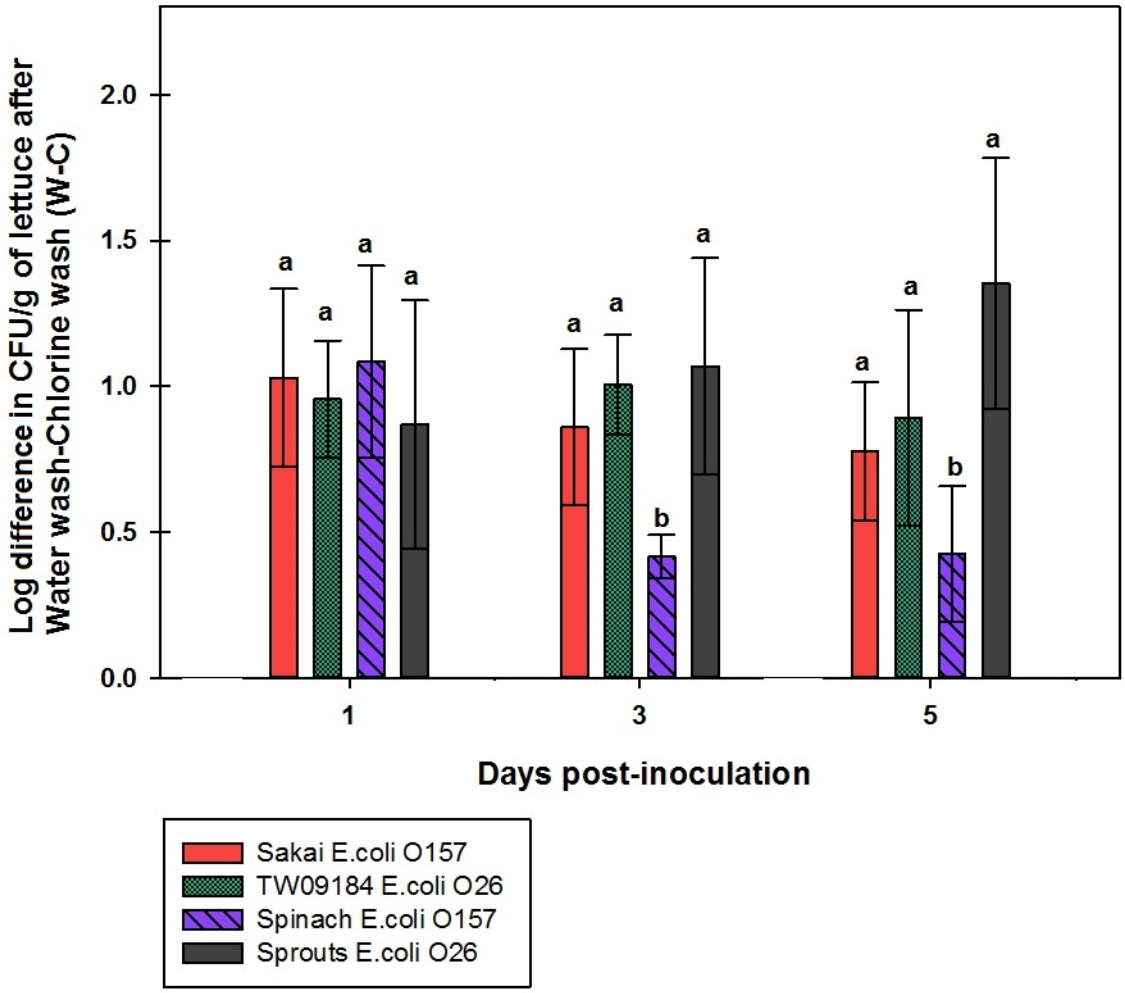


Figure 4. Chlorine resistance of EHEC strains after incubation on pre-harvest lettuce. Inoculated lettuce plants were incubated in a growth chamber under June harvest conditions and 75% RH. After 1, 3, and 5 days of incubation in the growth chamber, inoculated lettuce was collected, subdivided, and a portion washed in sterile water and a portion washed in 50 ppm chlorine for 2 minutes. The difference in the number of bacteria present after water wash and chlorine wash are presented for 2 independent replicates with 2 technical replicates each. Different letters above a bar indicate a significant difference.



Suggestions to CPS (optional)

This work here highlights the importance of using standard sets of bacterial isolates and strains for different studies; it may be worth for CPS to consider developing a standard collection of produce associated pathogen strain that are made available to differ researchers, as well as companies, for projects.