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Microbiology Platform

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2009 PROJECT PROPOSALS

MICROBIOLOGY PLATFORM

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology

Title: Investigation of Norovirus Cross-contamination during Foodservice Procedures used in the Preparation of Fresh Produce

Project Leader(s): Alvin Lee, Carol Shieh, Stephen Grove

New or Continuing: Continuing **Start: October 2008** **End: September 2011**

Background and Justification:

Fruits and vegetables have increasingly been implicated as vehicles for norovirus (NoV) gastroenteritis. Such foods may become contaminated in the home or food service kitchen during preparation, or during serving by infected food handlers or patrons. The goal of this proposal is to examine cross-contamination of NoV during common procedures used in preparation of fresh produce, incorporate NoV transfer data into a quantitative risk assessment and evaluate the risk reduction after workers exposure to educational materials developed as a result of the research findings. Virus transfer data will be collected using human NoV (HNV) and murine NoV (MNV-1) between hands, fresh produce items, knives and cutting boards. The ability of handwashing and gloves to prevent or reduce virus transfer from hands during food preparation will also be examined.

A multi-institution team has been assembled for this project, consisting of researchers from NCFST and Rutgers University, and collaborators from FDA's Center for Food Safety and Applied Nutrition and Ecolab USA.

Project Objectives (and Milestones, with timeline, if a continuing project):

Milestones:

1. Develop efficient methods to recover and detect HNV and MNV-1 from food preparation surfaces by quantitative RT-PCR (qRT-PCR) techniques (12 mths).
2. Collect transfer data for MNV-1 between knives, cutting boards and produce items, and in some cases using co-inoculated HNV and MNV-1, such that MNV-1 data can be used as a surrogate for HNV in the risk assessment (18 mths).
3. Collect transfer data for MNV-1 between hands and surfaces common in fresh produce preparation, including an evaluation of the effectiveness of hand washing procedures to prevent or reduce MNV-1 transfer, and evaluation of the effect of gloved vs. ungloved hands on the transfer rate (20 mths).
4. Collect spread and transfer data for co-inoculated HNV and MNV-1 from a single produce item to others during a food-preparation process, e.g. salad preparation and mixing, such that MNV-1 data can be used as a surrogate for HNV in the risk assessment (24 mths).
5. Calculate appropriate virus transfer and reduction rates, and incorporate this information into a quantitative risk assessment (30 mths).
6. Develop educational materials based on research findings and evaluate material effectiveness through onsite food safety observational studies & risk modeling. Research findings to be disseminated via on-campus teaching, short courses, seminars, presentation at scientific meetings, etc (36 mths).
7. Evaluate the impact of outreach and training methods on behavioural change at the food service establishment level (36 mths).

Benefits to Stakeholders:

Cross-contamination data generated in this study will be incorporated into a quantitative risk assessment, which may be used to determine the risk management strategies that will have an impact on reducing the contamination of fresh produce food items in foodservice settings. Results will be communicated to food safety professionals and foodservice operators, with an evaluation of risk reduction based on behavioural change.

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology		
Title: Effects of Temperatures on HAV and MS2 Coliphage on Inoculated Produce		
Project Leader(s): Carol Shieh & David Laird		
New or Continuing: New	Start: October 2009	End: September 2011
Background and Justification: Large numbers of illnesses have been associated with viral contamination of fresh produce, which these viral contamination frequently occurs prior or during harvest. From postharvest to consumption, there are critical control points where research may provide control measures for future implementation of viral pathogen reduction. Shipping and storage conditions of fresh produce, particularly at optimal and abused temperatures, may affect the survival of viruses. In addition, our previous study showed that heat-dehydration at specific temperatures was found to effectively inactivate HAV in contaminated green onions. In contrast, freeze-drying is likely to preserve bioactivity of viruses. In this study, heat-dehydration will be applied to selected fresh fruits, in which their dehydrated forms are commonly found in the market. To better predict the survival of enteric viruses in produce, we propose to incorporate additional viral surrogate MS2 coliphage in this study for comparison. The bioassay of MS2 can be completed within hours or overnight.		
Project Objectives (and Milestones, with timeline, if a continuing project): <i>Milestones:</i> <ol style="list-style-type: none">1. Study the effects of shipping and storage temperatures at 4°C and 25°C on the survival of HAV and MS2 coliphage in produce, 7 months2. (a) Study the effects of various heat-dehydration temperatures on viruses that are inoculated on berries (b) Compare RT-PCR units to infectivity of viruses before and after the dehydration process, 10 months3. (a) Examine the cross-contamination pattern of HAV from contaminated onion samples to uncontaminated samples during chopping before dehydration (b) Examine the effect of low heat dehydration of HAV on onions when large numbers of samples are simultaneously being dehydrated, 7 months		
Benefits to Stakeholders: Results from the study will assist the prediction and analysis of the risks associated with viral contamination in produce.		

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology		
Title: Sample preparation for recovery and detection of viruses in fresh produce		
Project Leader(s): Diana Stewart		
New or Continuing: New	Start: July 1, 2009	End: June 30, 2011
Background and Justification: Outbreaks of viral foodborne gastrointestinal illness are frequently associated with consumption of contaminated fresh or minimally processed fruits and vegetables. Though detectable by time-consuming cell culture infectivity assays, molecular detection methods for viruses such as real-time RT-PCR are rapid and have increased sensitive. Unfortunately, sample preparation methods appropriate for recovery of the viruses from such a wide variety of foods matrices are not available. Sample preparation procedures must be developed to be relatively free of PCR inhibitors and maintain infectivity for post-assay recovery of the virus. Consequently, methods used for extraction or physical disassociation of the virus from the matrix and separation/concentration techniques will need to be compared to determine the combinations best suited for detection using both the infectivity assay and real-time RT-PCR.		
Project Objectives (and Milestones, with timeline, if a continuing project): <i>Milestones:</i> <ol style="list-style-type: none">1. Assess physical disruption techniques and extraction conditions for recovery of viruses from a variety of fresh produce items. 6/20102. Assess separation and concentration techniques by PCR efficiency and lack of inhibition. 6/20103. Compare optimized sample preparation method by real-time RT-PCR and tissue culture infectivity assay. 6/20114. Assess appropriate sample preparation methods for recovery of viruses from other foods or processed foods within a commodity. 6/2011		
Benefits to Stakeholders: The results from this project should provide efficient protocols for extracting viruses for input of food samples into a rapid real-time RT-PCR detection method. The optimized protocols will help to address the requirements of high-throughput needs in surveillance investigations as well as in emergency response in foodborne illness outbreaks.		

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology		
Title: Comparative Genomic Analysis of <i>Listeria monocytogenes</i> for Survival in Food Processing Environments		
Project Leader(s): Wei Zhang , Mary Lou Tortorello		
New or Continuing: Continuing	Start: July 1, 2006	End: December 2010
Background and Justification: <p><i>Listeria monocytogenes</i> causes deadly foodborne infectious disease and is a big food safety concern. <i>Listeria</i> contamination in RTE foods such as the dairy, meat and poultry products is very common. In the past 2 years, we have identified <i>Listeria monocytogenes</i> strains that are capable of surviving long-term starvation and persistent in colonizing food processing environments. In this study, we will determine the genetic basis (i.e. new genes) for its survival and persistence. Molecular comparative genome analysis will be conducted by using DNA microarrays and real-time quantitative PCR. Novel genetic factors identified from the above will then be characterized using mutagenesis in relevant models.</p>		
Project Objectives (and Milestones, with timeline, if a continuing project): <p><i>Objectives:</i> To determine the genetic basis that conveys repeated contamination of <i>Listeria monocytogenes</i> in food processing environments.</p> <p><i>Milestones:</i></p> <ol style="list-style-type: none">1. Design and fabricate <i>Listeria</i> whole-genome DNA microarrays-December 20092. Compare global expression profiles of resistant strains –May 20103. Statistically analyse and identify resistance-related genes in the <i>Listeria</i> genome-October 20104. Prepare final report and papers for peer-reviewed publication-December 2010		
Benefits to Stakeholders: <p>Knowledge gained about the genetic factors that enable <i>Listeria monocytogenes</i> to resist starvation stress will have a significant impact on understanding the physiology of <i>Listeria</i> survival and colonization in foods and food processing environments. Our results will lead to optimization of effective interventions (e.g. HACCP) to control <i>Listeria</i> contamination in foods and food processing environments.</p>		

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology		
Title: Validation of methods for detection of Salmonella in tomato processing water		
Project Leader(s): Mary Lou Tortorello, Tung-Jen Fu		
New or Continuing: New	Start: July 1, 2009	End: June 30, 2011
Background and Justification: <p>Fresh tomatoes have been the source of multiple outbreaks of salmonellosis, and faster pathogen detection tools may improve the efficiency of conducting outbreak investigations. In a previous study, we have noted some potential for using wash water for monitoring the contamination status of tomatoes in a washing tank. Testing of wash water allows for a larger sample to be assayed, and lessens the amount of background from the food matrix that can cause interferences in detection assays. A rapid PCR-based method recently has been developed for the FDA BAM for detecting the presence of Salmonella in tomatoes, but it is possible that this method may also be applied as a pathogen monitoring tool for tomato wash water. Other rapid methods, including other commercially-available PCR-based methods, may be validated as well. In this study, an examination of factors that may influence the sensitivity and specificity of the methods will be undertaken, including presence of background microflora, chlorine, organic load, and soil.</p>		
Project Objectives (and Milestones, with timeline, if a continuing project): <i>Objectives:</i> To evaluate the PCR-based BAM method and other rapid methods for detection of Salmonella in tomato process water. <i>Milestones:</i> <ol style="list-style-type: none">1. Evaluate sensitivity and specificity of the FDA BAM method for detecting Salmonella in tomato process water – Dec 20092. Select other rapid methods for detecting Salmonella in foods and evaluate them for tomato process water – Jun 20103. Test effect of chlorine or other sanitizers on FDA BAM method and selected rapid methods. - Dec 20104. Test effects of organic load and soil on FDA BAM and selected rapid methods - Jun 2011		
Benefits to Stakeholders: <p>Both industry and FDA will benefit from the evaluation and validation of rapid methods for their ability to detect Salmonella more efficiently in contaminated tomatoes.</p>		

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology		
Title: Salmonella desiccation resistance and survival in extremely low water activity foods		
Project Leader(s): Haiping Li, Sue Keller, Wei Zhang		
New or Continuing: New	Start: July 1, 2009	End: June 30, 2011
Background and Justification: <p><i>Salmonella</i> can survive long-term starvation and desiccation stresses and contaminate foods with extremely low water activities (such as peanut butter) where other enteric pathogens can not survive due to lack of free water. <i>Salmonella</i> has been implicated in numerous foodborne disease outbreaks associated with shelled eggs and more recently jalapeño peppers and peanut butters. In the past few decades, Typhimurium and Enteritidis have emerged to be the most common <i>Salmonella</i> serovars associated with foodborne gastroenteritis in the U. S. However, the stress physiology of <i>Salmonella</i> under starvation and desiccation and specific mechanisms of bacterial re-growth in low a_w foods has not been thoroughly investigated. In this project, we aim to use microbiological and molecular approaches to better understand the <i>Salmonella</i> stress physiology and to identify extrinsic and intrinsic factors that regulate <i>Salmonella</i> resistance and survival under long-term starvation and desiccation stresses.</p>		
Project Objectives (and Milestones, with timeline, if a continuing project): <p><i>Objectives:</i> To study the dry resistance, survival, and re-growth after rehydration of Salmonella in very low A_w food, e.g., peanut butter and derived products.</p> <p><i>Milestones:</i></p> <ol style="list-style-type: none">1. Develop methodology to study survival, thermal resistance, acid resistance of Salmonella strains after desiccation (exposure to very low A_w, e.g., peanut butter) - Dec 20092. Compare survival, re-growth, thermal resistance, acid resistance of Salmonella strains after rehydration of desiccated cells – Dec 20103. Study gene expressions in the Salmonella genomes under dry and rehydrated conditions – Dec 20104. Analyze data for thermal inactivation and identify relevant genes – Jun 2011		
Benefits to Stakeholders: <p>Knowledge gained about the extrinsic and intrinsic factors that regulate <i>Salmonella</i> survival and re-growth after desiccation will improve our basic understanding of the stress physiology of <i>Salmonella</i> in low a_w foods and may lead to developing more effective interventions (e.g. hurdle technologies) to minimize <i>Salmonella</i> contamination in low a_w foods.</p>		

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology

Title: Analysis of gene function in *E. coli* O157:H7 from outbreaks associated with fresh produce

Project Leader(s): Kaiping Deng, Mary Lou Tortorello, Wei Zhang

New or Continuing: New **Start: July 7, 2009** **End: December 30, 2011**

Background and Justification:

Fresh produce-related outbreaks associated with *E. coli* O157:H7 have been a growing public health problem. Compared to other outbreaks, the 2006 spinach-associated outbreak included more clinical cases of haemolytic uraemic syndrome, the most severe form of *E.coli* O157:H7 infection. A study of more than 500 *E. coli* O157 clinical strains indicated that the spinach outbreak strain TW14359 belongs to an *E. coli* O157:H7 clade exhibiting substantial genomic differences from the others, and it is of great interest to study this hyper-virulent strain at the genetic level. In our recent DNA microarray study of chlorine induced stress, we compared expression patterns in TW14359 and Sakai, a strain derived from the 1996 sprout outbreak. Of particular interest was significant up-regulation of gene *ycfR*, which has been shown to function in the regulation of acid resistance and biofilm formation, both of which are considered to be virulence traits; however, no information exists on the function of this gene in *E. coli* O157:H7. To better understand the molecular mechanism of the pathogenesis of these two outbreaks-related strains, especially the hyper-virulent strain TW14359, we will employ relevant bio-assays and gene mutagenesis to investigate the attachment and stress resistance of Sakai and TW14359 strains.

Project Objectives (and Milestones, with timeline, if a continuing project):

Objectives:
To elucidate the molecular mechanism of the attachment of outbreak-related *E. coli* strains on fresh produce.

Milestones:

1. Study cell surface hydrophobicity and aggregation using four *E. coli* strains: wild-type *E. coli* K-12, Sakai, TW14359 and Sakai *ycfR* mutant (Dec 2009).
2. Construct of an isogenic *ycfR* mutant in TW14359 strain. (May 2010).
3. Characterize the *ycfR* function using Sakai and TW14359 wild-type and *ycfR* mutants. (December 2010)
4. Clone the gene *ycfR* and complement the Sakai and TW14359 *ycfR* mutant (June 2011).

Benefits to Stakeholders:

The results obtained from this study will provide important information on the molecular mechanism of the pathogenesis of *E. coli* O157:H7 strains associated with fresh produce outbreaks. It will provide a scientific basis for produce industry to choose more effective interventions to reduce attachment of pathogenic strains during post-harvest process.

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology		
Title: A systems approach to minimize <i>E. coli</i> O157:H7 food safety hazards associated with fresh- and fresh-cut leafy greens.		
Project Leader(s): Tung-Jen Fu, Mary Lou Tortorello, Alvin Lee		
New or Continuing: Continuing	Start: September 1, 2007	End: August 31, 2011
Background and Justification: <p>In the past decade, fresh leafy greens have been the vehicle of more than 20 foodborne illness outbreaks. In this project NCFST is collaborating with three other universities in USDA-sponsored research toward improving the safety of leafy greens. Two aspects of the research will be pursued here: (1) use of process waters to assess the contamination status of leafy greens and (2) determination of pathogen behavior in packaged leafy greens during storage and distribution conditions. NCFST cooperative agreement funds are requested to fund student travel and publication costs.</p>		
Project Objectives (and Milestones, with timeline, if a continuing project): <p><i>Objectives:</i> Evaluate pre-concentration, separation and real-time PCR detection of surface- and internally-contaminated <i>E. coli</i> O157:H7 and generic <i>E. coli</i> from process waters. Assess effects of initial contamination level, temperature, time, indigenous microflora, sanitizing rinses and package environment on behavior of <i>E. coli</i> O157:H7.</p> <p><i>Milestones:</i></p> <ol style="list-style-type: none">Years 1 and 2: Evaluate pre-concentration, separation and real-time PCR detection of surface- and internally-contaminated <i>E. coli</i> O157:H7 and generic <i>E. coli</i> from process waters and assess pathogen detectability in fresh-cut processing waters Year 3: Assess pathogen detectability in fresh-cut processing waters and field irrigation waters using optimized concentration approach.Year 1: Assess effects of initial contamination level, temperature, time, and sanitizing rinses on behaviour of surface-inoculated <i>E. coli</i> O157:H7 and indigenous microflora in packaged leafy greens Year 2: Assess effects of modified atmosphere on surface-inoculated <i>E. coli</i> O157:H7 and indigenous microflora in packaged leafy greens. Assess effects of initial contamination level, temperature and time on behaviour of internalized <i>E. coli</i> O157:H7 in packaged leafy greens. Year 3: Assess effects of sanitizing rinses and modified atmosphere on behaviour of internalized <i>E. coli</i> O157:H7 in packaged leafy greens.		
Benefits to Stakeholders: <p>This research will enable industry and government stakeholders to identify risk mitigation strategies that may be influential in ensuring the safety of leafy greens.</p>		

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology		
Title: Genomic Analysis of <i>Clostridium botulinum</i>		
Project Leader(s): Wei Zhang , Guy Skinner		
New or Continuing: Continuing	Start: July 1, 2008	End: December 2010
Background and Justification: <p><i>Clostridium botulinum</i> can produce neurotoxins and cause severe foodborne illness and deaths. Botulism is responsible for approximately 24 foodborne outbreaks per year in the U.S. In 2006, <i>C. botulinum</i> was responsible for an outbreak associated with pasteurized carrot juice which resulted in 6 cases and one death. <i>C. botulinum</i> is generally grouped into two groups, proteolytic and nonproteolytic. These groups display very different minimum growth temperatures and heat resistances. However, the genetic factors that control such differences in growth, heat resistance and toxin production are poorly understood. In this project, we will use microarray-based approaches to study genes that regulate and control the thermal and/or high pressure resistance of proteolytic type <i>C. botulinum</i> spores.</p>		
Project Objectives (and Milestones, with timeline, if a continuing project): <p><i>Objectives:</i> To determine the genetic basis that conveys the thermal and high pressure resistance of <i>Clostridium botulinum</i> spores.</p> <p><i>Milestones:</i></p> <ol style="list-style-type: none">1. Compare genome expressions of selected <i>Clostridium botulinum</i> strains with enhanced thermal resistance (December 2009)2. Identify key genes in the <i>Clostridium botulinum</i> genomes (in both spores and vegetative cells) that control thermal resistance (May 2010)3. Fully sequence the genome of a <i>C. botulinum</i> strain that has strong thermal resistance (December 2010)		
Benefits to Stakeholders: <p>Fundamental mechanistic studies on the genetic factors that regulate heat resistance, of <i>C. botulinum</i> spores will have significant impact on understanding the physiology of <i>Clostridium</i> in foods and development of effective intervention strategies to control and prevent <i>Clostridium</i> growth and toxin production in low acid foods. In this project, we will begin to uncover the genetic and physiological basis by which <i>Clostridium</i> spores germinate in various food products (such as carrot juice). This project will also provide a scientific basis to food processors to optimize intervention, processing and storage conditions to effectively minimize <i>Clostridium</i> presence in finished food products. This project will be conducted in close collaboration with Professor Eric Johnson at University of Wisconsin Madison.</p>		

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology		
Title: Development of a procedure to diminish the clumping of <i>C. botulinum</i> spores to be used as indicator organisms.		
Project Leader(s): Wei Zhang		
New or Continuing: New	Start: July 1, 2009	End: June 30, 2010
Background and Justification: <p>Spores of <i>C. botulinum</i> used as indicator organisms in the high pressure sterilization field often show pronounced clumping. Clumping of these spores may easily lead to aberrant or misleading results because of the inherent error clumps induce in the enumeration of the survivors and controls: a clump leads to one colony forming unit (CFU) regardless of how many spores may form it.</p> <p>The exosporium of the spores may be expected to drive the clumping process and its removal is expected not to have any significant effect on the resistance of the spores to thermal processing and to high-pressure sterilization.</p> <p>Removal of the exosporium by mechanical means has been reported, and current status of the art may offer better alternatives. Therefore, the development of a modified process to prepare <i>C. botulinum</i> spores intended as biological indicators of high pressure sterilization processes is highly desirable. The resistance to high pressure sterilization of the spores produced using the alternative procedure must be verified experimentally.</p>		
Project Objectives (and Milestones, with timeline, if a continuing project): <p><i>Milestones:</i></p> <ol style="list-style-type: none">1. Literature Review – August 1st.2. Experimental plan – September 1st.3. Acquisition of materials and hiring student October 1st4. Creation of bioluminescent strains – March 1 20105. Verification of the resistance of the spores –April 1, 2010.6. Report – manuscript(s) for publication May 1, 2010.		
Benefits to Stakeholders: <p>The expected improvement in the reliability of experimental data corresponding to the survival of spores of <i>C. botulinum</i> to high-pressure sterilization processes will enhance the quality of our work related to cycle development, validation and also research and development in the area of high pressure sterilization.</p>		

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology

Title: Creation of bioluminescent strains of sporulated bacteria to be used as indicator organisms.

Project Leader(s): Wei Zhang, Alfredo Rodriguez

New or Continuing: New **Start: July 1, 2009** **End: June 30, 2010**

Background and Justification:
Bioluminescent strains of *B. subtilis v. niger* (now called *atropheus*) that had the same resistance to ethylene oxide sterilization processes as the original strains were produced as early as 1993. Bioluminescence enables enumeration of the survivors in approximately four hours, and it has the additional advantage that false positives are expected to be significantly diminished.
Current status of the art enables the production of such strains in a straightforward manner.
Strains of heat resistant *G. stearothermophilus*, *C. sporogenes*, and high-pressure resistant *C. botulinum* will be modified to add the bioluminescence gene to their chromosome and the resistance of the new strains verified experimentally.

Project Objectives (and Milestones, with timeline, if a continuing project):

Milestones:

1. Literature Review – August 1st.
2. Experimental plan – September 1st.
3. Acquisition of materials and hiring student October 1st
4. Creation of bioluminescent strains – March 1 2010
5. Verification of the resistance of the spores – April 1, 2010.
6. Report – manuscript(s) for publication May 1, 2010.

Benefits to Stakeholders:
The capability to get results of the survivors to lethal treatments in approximately 4 hours instead of the current process of incubation will accelerate our related work. Member companies that perform this type of studies will benefit accordingly. Finally, false positives are expected to diminish since these strains do not exist in nature.

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology	
Title: Effectiveness of ultrasound to de-soil complex food matrix embedded with <i>Bacillus sp</i> spores attached to various coupon surfaces	
Project Leader(s): Alvin Lee, Claudia Rodriguez	
New or Continuing: New	Start: October 1, 2009 End: December 30, 2009
Background and Justification: <p>Ultrasonic treatment can remove cells from various surfaces and is especially effective when used with a sanitizer (Seymour,2002). Increasing the time of ultrasound treatment and adding solids to a liquid can also increase the bactericidal effect (Scherba,1991). The cavitation created in a liquid by ultrasonic waves may have the mechanical effect of breaking up the bio-film architecture, thereby releasing attached cells into a planktonic state where they would be more susceptible to chemical sanitizers. The objective of the current study is to test the effectiveness of ultrasound as a de-soiling step prior to sanitization.</p>	
Project Objectives (and Milestones, with timeline, if a continuing project): <p><i>Objectives:</i> To evaluate the de-soiling of <i>Bacillus</i> spores embedded in complex food matrixes from various food contact surfaces based on the effect of ultrasound at various time, temperature and power intervals</p> <p><i>Milestones:</i></p> <ol style="list-style-type: none">1. Determine the calibration curve for ultrasound to analyze the relationship between power and amplitude. (1 month)2. Evaluate temperature profile at different power levels. (1 month)3. Determine the optimal temperature, time and power for the rate of removal of complex food matrix attached to coupons using various probes. (1 month)4. Determine the efficiency of ultrasound effective removal of the soil from various contact surfaces in presence of <i>Bacillus</i> spores. (1 month)	
Benefits to Stakeholders: <p>Previous data has shown that a wash step prior to sanitization of food contact surfaces commonly found in food processing plants (stainless steel, tile, buna-n etc..) will remove 3-4 log₁₀ of <i>Bacillus sp</i> spores. This project aims to analyse the potential practicality of ultrasound as a tool to remove soil from various food contact surfaces.</p>	

NEW (OR CONTINUING) PROJECT PROPOSAL (2009-2010)

Science Platform: Microbiology

Title: The Effect of Temperature History on the Ability of Proteolytic *Clostridium botulinum* to Produce Toxin in Extended Shelf-life Foods

Project Leader(s): Guy Skinner

New or Continuing: Continuing

Start: October 1, 2007

End: September 30, 2010

Background and Justification:

The risks associated with extended Shelf-life (ESL) foods have been frequently discussed. One hazard routinely discussed is the hazard of *Clostridium botulinum* neurotoxin formation by nonproteolytic strains which have the ability to grow and produce toxin at refrigerated temperatures. In ESL foods, refrigeration temperatures are relied upon to control the proteolytic types of *C. botulinum* which are inhibited at temperatures below 10-12C. Most botulism outbreaks related to ESL foods have been attributed to temperature abuse. It is accepted in the United States that the food distribution chain can not be counted upon to maintain refrigerated product temperatures to the consumer. Refrigeration inhibits proteolytic types of *C. botulinum* from growing and producing toxin. In addition to questions regarding the effectiveness of secondary barriers, many questions and concerns focus on the exposure of spores to the various temperature histories during storage and processing and what effect that exposure to elevated temperatures might have on spores germinating during storage or exposure to various levels of temperature abuse. It is believed that these time-temperature history scenarios may result in a traditionally safe product becoming a botulism risk when previous conditions had always resulted in safe products. Processors and food safety professionals will better understand how exposure of sensitive foods to certain conditions of time and temperature may result in botulism risk.

Project Objectives (and Milestones, with timeline, if a continuing project):

Objectives:

The objective of this project is to identify combinations of time and temperature that may stimulate spores of *C. botulinum* to germinate and form toxin where these spores would normally remain dormant. This may alert us to certain history effects that may occur during processing or storage which may increase the risk of botulism in certain products.

Milestones:

1. Identify the strains of *Clostridium botulinum* to be used in the project.
2. Identify the media or model food system.
3. Identify incubation temperatures and select level of factors and or combination of factors that result in inhibition against *C. botulinum*. Factors will be chosen from the following, aw, sodium chloride, pH (and acid), phosphate, etc.
4. Obtain or grow quantities of the *C. botulinum* strains identified for use in the project.
5. Perform inoculation studies using spores that have been exposed to various elevated temperature histories that may represent real abuse situations in foods. Subsequent to this preconditioning, inoculation studies will be performed at temperatures between 10 and 35C to test the effect on temperature exposure history on the ability of *C. botulinum* to form toxin in model systems.
6. Validate the results using selected food systems

Benefits to Stakeholders:

Although refrigeration temperatures can control the risk of toxin production from proteolytic strains of *Clostridium botulinum*, temperature abuse commonly occurs in such foods. Elevations in temperature can result in botulism from proteolytic strains. Botulism outbreaks have occurred in ESL foods from the growth and toxin production of proteolytic *C. botulinum*. Questions regarding toxin production in some products which have had a safe history for years exist. We plan to evaluate the effect of exposure to various time-temperature histories on the ability of *C. botulinum* spores to produce toxin under conditions that would be otherwise inhibitory. This may provide information as to why a food suddenly supports toxin formation when it has previously had a good safety record. Knowing the effect of various time-temperature exposures at the processor and consumer will result in avoidance of potentially hazardous situations and avoid botulism outbreaks.