

# *Platform: Microbiology*

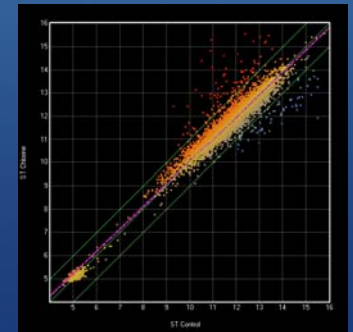
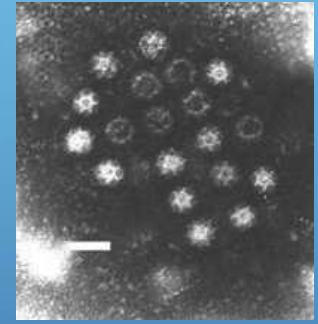
Mary Lou Tortorello

NCFST Technical Advisory Committee  
Meeting

May 14, 2009

# Program Areas

- *Recovery, survival, inactivation of viruses*
- *Post-harvest factors to enhance safety of fresh produce*
- *Pathogen stress responses using new molecular tools to understand survival in conditions relevant to food manufacturing*
- *Characteristics and behavior of bacterial endospores*



# Comparison: Current and Proposed Projects

	Current FY 08-09	Proposed FY 09-10		
Program Area	Number	Number	Status	
			Continuing	New
<b>Viruses</b>	3	3	1	2
<b>Produce</b>	2	3	1	2
<b>Stress</b>	2	2	1	1
<b>Spores</b>	3	5	3	2
<i>Totals</i>	<i>10</i>	<i>13</i>	<i>6</i>	<i>7</i>

## *Continuing Project:*

# **Norovirus Cross-Contamination during Foodservice Procedures used in Preparation of Fresh Produce**

- Program: Viruses
- Objective: Examine cross-contamination during common procedures used in preparation of fresh produce, incorporate transfer data into quantitative risk assessment and evaluate risk reduction after worker education
- Leaders: Lee, Grove, Shieh
- Collaborators: Don Schaffner, Rutgers; Katie Swanson, Ecolab
- Duration: 2008 - 2011
- Score: 5.48



## *Continuing Project:*

# **Norovirus Cross-Contamination during Foodservice Procedures used in Preparation of Fresh Produce**

- Milestones:

- Methods development for norovirus quantitation
- Assess transfer:
  - between food contact surfaces
  - between surfaces and foods
  - between hands and foods
- Model and develop risk assessment
- Conduct and assess training

- Benefits: Improved risk reduction strategies



## *New Project:*

# **Effects of Temperature on HAV and MS2 Coliphage on Inoculated Produce**

- Programs: Viruses, Produce
- Objective: Study temperature effects on survival of viruses in dehydration of fruits
- Leaders: Shieh, Laird
- Duration: 2009 - 2011
- Score: 4.87

## *New Project:*

# **Effects of Temperature on HAV and MS2 Coliphage on Inoculated Produce**

- Milestones:
  - Evaluate 4 and 25°C: HAV and MS2
  - Assess survival during heat dehydration: berries
  - Estimate cross-contamination during chopping: onions
- Benefits: Provide data for virus risk assessments in produce

*New Project:*  
**Sample Preparation for Recovery and Detection of  
Viruses in Fresh Produce**

- Programs: Viruses, Produce
- Objective: Develop sample preparation and recovery procedures for extraction of viruses from various food matrices
- Leaders: Stewart, Shieh, Tortorello
- Collaborator: FDA ORA-CFSAN Virus Initiative Group
- Duration: 2009 - 2011
- Score: 6.63



## *New Project:*

# **Sample Preparation for Recovery and Detection of Viruses in Fresh Produce**

- Milestones:

- Assess physical disruption and extraction methods from various fresh produce items
- Evaluate separation and concentration techniques for PCR application
- Compare optimized methods in PCR and infectivity assay
- Extend to other foods within commodity group

- Benefits: Improved virus detection methods



***Continuing Project:***  
**Comparative Genomic Analysis of**  
***Listeria monocytogenes* for Survival in Food**  
**Processing Environments**

- Program: Stresses
- Objective: Identify genes that function in survival in foods and processing environments
- Leader: Zhang, Tortorello
- Collaborators: Steve Salzberg, UMD-CP
- Duration: 2006 - 2010
- Score: 6.58

*Continuing Project:*  
**Comparative Genomic Analysis of**  
*Listeria monocytogenes*

- Milestones:
  - Design and fabricate *Listeria* genome microarrays
  - Compare profiles of lineages I, II and III
  - Identify and analyze genes relevant to survival in foods and processing environments
- Benefits: Leading to better understanding of survival and colonization in foods and processing environments

## *New Project:*

# **Validation of Methods for Detection of *Salmonella* in Tomato Processing Water**

- Program: Produce
- Objective: Evaluate PCR-based FDA BAM method for detecting *Salmonella* in processing waters
- Leaders: Fu, Tortorello
- Collaborator: Eric Brown, FDA-CFSAN
- Duration: 2009 - 2011
- Score: 5.91

## *New Project:*

# **Validation of Methods for Detection of *Salmonella* in Tomato Processing Water**

- Milestones:
  - Evaluate sensitivity and specificity of BAM method for detection in tomato process water
  - Compare to other rapid and conventional methods
  - Test effects of sanitizers
  - Test effects of organic load and soil
- Benefits: Improved methods for detection of *Salmonella*

## *New Project:*

# **Salmonella Desiccation Resistance and Survival in Extremely Low Aw Foods**

- Program: Stresses
- Objective: Study dry resistance, survival and re-growth after rehydration of cells in very low Aw food
- Leaders: Li, Zhang, Keller, Tortorello, Cole
- Collaborators: TBD, external funding?
- Duration: 2009 - 2011
- Score: 7.04

## *New Project:*

# **Salmonella Desiccation Resistance and Survival in Extremely Low Aw Foods**

- Milestones:
  - Develop methodology to study survival, thermal resistance, and acid resistance of strains after desiccation
  - Compare survival and resistance after rehydration
  - Study gene expression in dry and rehydrated conditions
  - Analyze data for thermal inactivation and identify relevant genes
- Benefits: Improved understanding of basis for *Salmonella* resistance to desiccation

## *New Project:*

# **Analysis of Gene Function in *E. coli* O157:H7 from Outbreaks Associated with Fresh Produce**

- Programs: Produce, Stresses
- Objective: Elucidate molecular mechanism of attachment of *E. coli* strains on fresh produce
- Leaders: Deng, Zhang, Tortorello
- Duration: 2009 - 2011
- Score: 5.54

## *New Project:*

# **Analysis of Gene Function in *E. coli* O157:H7 from Outbreaks Associated with Fresh Produce**

- Milestones:
  - Study cell surface hydrophobicity and aggregation in wild type K12, Sakai, TW14359 and Sakai *ycfR* mutant
  - Construct TW14359 *ycfR* mutant
  - Characterize *ycfR* function in isogenic sets
  - Cloning and complementation
- Benefits: Understand molecular basis for *E. coli* attachment to design better interventions

## *Continuing Project:*

# **A Systems Approach to Minimize *E. coli* O157:H7 Food Safety Hazards in Fresh- and Fresh-Cut Leafy Greens**

- Program: Produce
- Objectives: Provide data for farm-to-table risk reduction measures
- Leaders: Fu, Lee, Tortorello, Zhang, Nnoka, Cole
- Collaborators:
  - Mike Doyle and Marilyn Erickson, UGA
  - Elliott Ryser and Ewen Todd, MSU
  - Xiuping Jiang, Clemson
- Duration: 2007 - 2011
- Score: 6.67



## *Continuing Project:*

# **A Systems Approach to Minimize *E. coli* O157:H7 Food Safety Hazards in Fresh- and Fresh-Cut Leafy Greens**

- Milestones:
  - Evaluate concentration, separation and PCR detection of *E. coli* from process waters
  - Assess packaging and storage / distribution conditions on pathogen behavior
  - Assess ultrasonics/sanitizers
  - Communicate findings via outreach
- Benefits: Risk mitigation strategies for ensuring safety



## ***Continuing Project:*** ***Genomic Analysis of Clostridium botulinum***

- Program: Spores
- Objective: Compare selected *Clostridium botulinum* strains genetically and determine the genetic basis that conveys enhanced resistance to food processing stresses.
- Leaders: Zhang, Skinner
- Collaborator: Eric Johnson, UW-FRI
- Duration: 2008 - 2010
- Score: 5.83

# *New Project:*

## *Genomic Analysis of Clostridium botulinum*

- Milestones:
  - Compare genome expression of selected *C. botulinum* strains with enhanced thermal resistance
  - Identify key genes
  - Fully sequence the genome of a highly resistant strain
- Benefits: uncover basic mechanisms of heat resistance and spore germination; provide a scientific basis to optimize interventions to minimize presence in foods

## *New Project:*

# **Development of a Procedure to Diminish Clumping of *Clostridium botulinum* Spores**

- Programs: Spores
- Objective: Develop procedures for removal of exosporium from *C. botulinum* spores
- Leaders: A. Rodriguez, Zhang
- Duration: 2009 - 2010
- Score: 4.95

## *New Project:*

# **Development of a Procedure to Diminish Clumping of *Clostridium botulinum* Spores**

- Milestones:
  - Develop procedures to remove exosporium from sporulated cells
  - Verify resistances to HPP, heat
- Benefits: Improved methodology for conducting processing studies

## *New Project:*

# **Creation of Bioluminescent Strains of Sporeforming Bacteria to Be Used as Indicators**

- Programs: Spores
- Objective: Develop bioluminescent strains of sporeformers for using in processing studies
- Leaders: A. Rodriguez, Zhang
- Duration: 2009 - 2010
- Score: 6.38

## *New Project:*

# **Creation of Bioluminescent Strains of Sporeforming Bacteria to Be Used as Indicators**

- Milestones:
  - Develop bioluminescent strains, e.g. *C. sporogenes*, *C. botulinum*, *G. stearothermophilus*
  - Verify resistances to HPP, heat
- Benefits: Improved methodology for conducting processing studies

## *Continuing Project:*

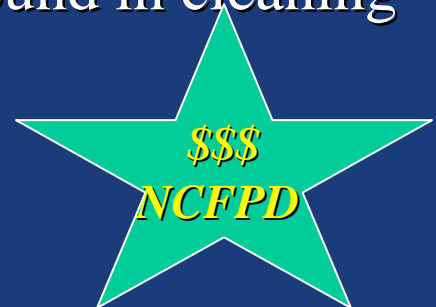
# **Effectiveness of Ultrasound to De-Soil Complex Food Matrix Embedded with Bacillus sp. Spores Attached to Various Coupons**

- Program: Spores
- Objective: Evaluate ultrasound for de-soiling of Bacillus spores in foods and food contact surfaces
- Leaders: Lee, C. Rodriguez
- Duration: Oct 2009 - Dec 2009
- Score: 6.25



# *Continuing Project:* **Effectiveness of Ultrasound to De-Soil Complex Food Matrix Embedded with Bacillus sp. Spores Attached to Various Coupons**

- Milestones:
  - Determine calibration curve for ultrasound to analyze power and amplitude relationship
  - Evaluate temperature profile at different power levels
  - Determine optimal temperature, time, and power for rate of removal of food matrix attached to coupons
  - Determine efficiency of ultrasound for removal of spores
- Benefits: Determination of practicality of ultrasound in cleaning food contact surfaces



## *Continuing Project:*

# **Effect of Temperature History on Ability of Proteolytic *Clostridium botulinum* to Produce Toxin in Extended Shelf-Life foods**

- Program: Spores
- Objective: Evaluate how time-temperature history of spores influences toxin production and efficacy of secondary barriers
- Leader: Skinner
- Duration: 2007 - 2010
- Score: 7.13

## *Continuing Project:*

# **Effect of Temperature History on Ability of Proteolytic *Clostridium botulinum* to Produce Toxin in Extended Shelf-Life foods**

- Milestones:
  - Identify strains, media, temperatures and factors resulting in inhibition
  - Precondition spores to elevated temperature histories and test effect on toxin formation including secondary barriers
  - Validate results in selected food systems
- Benefits: Enhance safety by identifying secondary barriers that may be useful in ESL foods

# Project Scoring

