Report for Projects Awarded in 2013 and 2014 by

Mississippi Center for Food Safety and Post-Harvest Technology

Title: Biofilm Formation and Transferability of *Listeria monocytogenes* on the Surfaces of Food and Processing Equipment

Award year: April 1, 2013 to March 31, 2015 (a six-month no cost extension granted)

PI: Chinling Wang, D.V.M., M.S., Ph.D., Associate Professor, Department of Basic Science, College of Veterinary Medicine

Collaborator:

1. Objectives.

   The objective of this project is to identify factors of *L. monocytogenes* that are required for biofilm formation on processing equipment and RTE surfaces, and understand the transferability of the pathogen to food.

   Objective 1. Identify factors of *L. monocytogenes* that are required for biofilm formation on processing equipment (completed)

   Objective 2. Understand the transferability of biofilm-contaminated equipment to food and the persistence of biofilms (on-going)

2. New Accomplishments toward objectives. Please indicate if all objectives listed were completed.

   Objective 1 (completed)

   A) We have identify a novel attachment factor Lcp of L. monocytogenes that adhere to lettuce, spinach and cantaloupe skin. This Lcp factor does not contribute to biofilm formation. These results is published in Appl Envrion Microbiol. 70:6855-61, 2013.

   B) We also successfully constructed an *adlp* mutant by deleting an *adlp* encoding for alkylbase DNA glycosylase-like protein. The deletion of *adlp L. monocytogenes* enhanced the flagellar motility and decreased biofilm formation. The *adlp* mutant strain also impaired the virulence in mice.

   Objective 2 (on-going)

   To determine the transferability of contaminated equipment to food, the slicer blade was immersed in 100 ml 10^5 or 10^7 CFU/ml *L. monocytogenes* for 15 min. When the contaminated blade was used to slice RTE meat, we found *L. monocytogenes* contamination from slice 1 to 3. When we stored the contaminated RTE in refrigeration temperature, we were able to isolate *L. monocytogenes* from slice 1 to 10 but not slice number 15 after day 3 and day 7 at 4C storage. This objective is on-going).

3. Objectives not accomplished and impediments to meeting objectives.
   We continued to work on Objective 2 is expected to complete by September, 2015.

4. If continuing project, when will new and/or long term objectives be completed?

   We did not apply for 2015 FSI grant but we will apply for the next cycle.

5. Students supported

   a. PhDs (% FTE and name)- Yue-Jia Lee 50% FTE

6. Leveraged Funds:

   Awarded:

   i. Funding agency: Elanco Pharmaceutical Company
   ii. Funding awarded $68,283 (FY 2014)

7. Outputs –

Project Summary (Issue/Response)

In this box type 300—400 word project summary in 10 pt font.

Listeria monocytogenes is well known to possess a strong attachment and biofilm formation abilities. Due to the fact that the bacterium has unique ability to attach to various ready-to-eat (RTE) surfaces and processing equipment, it makes difficult to prevent biofilm formation and cross contamination in food processing environments. The objective of this project is to identify factors of *L. monocytogenes* that are required for biofilm formation on processing equipment and RTE surfaces, and understand the transferability of the pathogen to food.

A study to determine the attachment of *L. monocytogenes* serotype 4b strain F2365 on vegetables and fruits was conducted. In initial study, we screened 32 genes encoding surface proteins and lipases of the strain to find highly expressed genes on lettuce leaves. Results showed that transcription levels of LMOf2365_0413, LMOf2365_0498, LMOf2365_0859, LMOf2365_2052, and LMOf2365_2812 were significantly up-regulated on lettuce leaves. *In silico* analysis showed that LMOf2365_0859 contains a putative cellulose binding domain. Thus, we hypothesized that this gene may be involved in an attachment to vegetables and named *Listeria* cellulose-binding protein (*lcp*). *lcp* mutant (Δ*lcp*) and *lcp* complement (F2365::pMAD:*cat*:*lcp*) strains were generated by the homologous recombination. The attachment ability of a wild type (WT), Δ*lcp*, and a complemented strain to lettuce leaves was evaluated, indicating that the attachment of the Δ*lcp* to lettuce was significantly less than the WT and the complemented strain. Similar results were observed in baby spinach and cantaloupe. Fluorescence microscopy and field emission scanning microscopy analysis further support these findings. Binding ability of *L. monocytogenes* to cellulose was determined using cellulose acetate-coated plate. Results showed that a binding ability of Δ*lcp* was significantly lower than that of wild type. Combined, these results strongly suggest that LCP plays an important role in an

attachment to vegetables and fruits.

Temperature-dependent alteration of flagellar motility genes expression is essential for food-borne pathogen *Listeria monocytogenes* to respond to a changing environment. In this study, a genetic determinant (*lmof2365_0220*) encoding a putative protein that is structurally similar to *Bacillus cereu*s alkylbase DNA glycosylase AlkD was identified involving in transcriptional repression of flagellar motility genes, and was named *adlp* (AlkD-like Protein, Adlp). Deletion of *aldp* gene activated the expression of flagellar motility genes at 37°C and disrupted temperature-dependent inhibition of motility of *L. monocytogenes*. The deletion of *adlp* significantly decreased biofilm formation and impaired survivability of bacteria under several stress conditions.

Project Results/Outcomes

In this box type 500—750 word summary of project results/outcomes.

We have identify a novel attachment factor Lcp of *L. monocytogenes* that adheres to lettuce, spinach and cantaloupe skin. This Lcp factor did not facilitate biofilm formation. These results is published in Appl Envrion Microbiol. 70:6855-61, 2013. We also successfully constructed an *adlp* mutant by deleting an *adlp* gene encoding for alkylbase DNA glycosylase-like protein. The deletion of *adlp L. monocytogenes* enhanced the flagellar motility and decreased biofilm formation. The *adlp* mutant strain also impaired the virulence in mice. To determine the transferability of contaminated equipment to food, the slicer blade was immersed in 100 ml 10^5 or 10^7 CFU/ml *L. monocytogenes* for 5 min and air dried for 15 min. When the contaminated blade was used to slice RTE meat, we found *L. monocytogenes* contamination from slices 1 to 3. When we stored the contaminated RTE in refrigeration temperature, we were able to isolate *L. monocytogenes* from slice 1 to 10 but not slice number 15 after day 3 and day 7 at 4C storage. The results suggest that initial cultural negative samples collected from contaminated slicers still pose a risk of contamination. This objective is on-going.

Project Impacts/Benefits

We have published one manuscript and three submitted under this funding period. The information is useful for developing new strategies for preventing or controlling this important food-borne pathogen by eliminating factors that may foster the biofilm formation on processing equipment and prevent further transferring the bacterium to ready-to-eat products. This project also enhances our competitiveness for extramural funding such as private industry and federal funding.

Project Deliverables

Zhang, T., D. Bae and C. Wang. Deletion of an alkD-like Genetic Determinant in *Listeria monocytogenes* affects biofilm formation, virulence, and stress responses at the 114th ASM General Meeting, Boston, MA. (Poster presentation)

Graphics

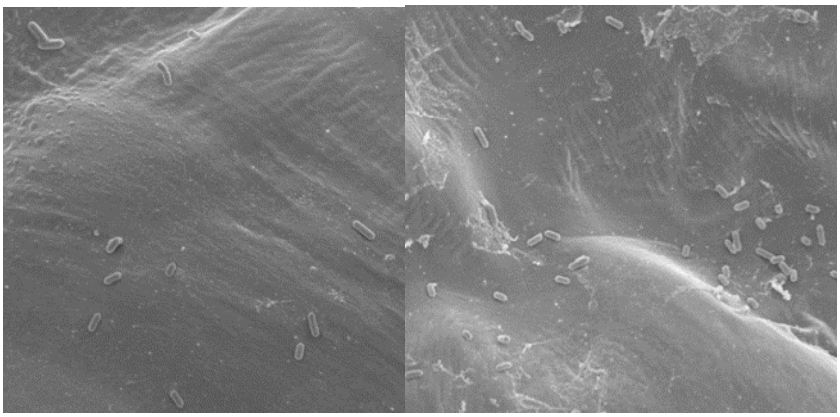

Figure showed that *lcp* mutant *L. monocytogenes* lost its ability to attach on lettuce (left) and the wild type strain attached to lettuce surface efficiently (right).

Attached Refereed Journal Publications in Separate Files

Please attached published journal articles (in pdf format if available) for this project. Manuscripts accepted or in review process may be submitted in word files. Thank you very much for your cooperation. See attached.