Request for Report for Projects Awarded in 2013 and 2014 by
Mississippi Center for Food Safety and Post-Harvest Technology

Title: High-risk *Listeria monocytogenes* specific collagen binding protein and its role in catfish fillet attachment

Award year: 2013

PI: Attila Karsi

Co-PI: Mark Lawrence

Collaborator: Bhaskar Das

1. Objectives.
   - Design and synthesize small molecule inhibitors against high-risk *L. monocytogenes* specific Lmof2365_2117 protein.

   - Evaluate the efficacy of small molecule inhibitors in preventing catfish fillet attachment of high-risk *L. monocytogenes*.

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

   All objectives of our project were completed. Briefly, during the extension period of our project, effects of designed small molecules on the growth of high-risk wild-type *L. monocytogenes* (Lmof2365) were determined. We found that none of the small molecules affected the growth kinetics of Lmof2365. After this, we tested the effect of small molecules on catfish fillet attachment of Lmof2365. We found that four small molecules changed the attachment properties of Lmof2365. SM01, SM03, and SM07 caused a decrease on the fillet attachment of Lmof2365, while an increase on the fillet attachment of Lmof2365 was observed when SM08 was used.

3. Objectives not accomplished and impediments to meeting objectives.

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

5. Students supported

   a. PhDs (% FTE and name)

   Attila Karsi: 10%
   Mark Lawrence: 1%
   Hossam Abdelhamed: 10%
   Ali Akgul: 25%
   Nawar Al-Janabi: 25%

b. M.S. (% FTE and name)
c. Undergraduate (number of students)

6. Leveraged Funds: External Competitive Funding Applied and Awarded based on findings from this project.
    a. Applied for:
        i. Funding agency
        ii. Program
        iii. Funding request ($$)
    b. Awarded:
        i. Funding agency
        ii. Program
        iii. Funding awarded ($$)

7. Outputs – In addition to the above, please populate the following sections to be included in a report to be compiled in a FSI Research Accomplishment Booklet. The project report will also be posted in a FSI website to be developed.

Please submit reports in Microsoft Word Document (except the published journal articles in pdf format) to Ms. Kaila Peggs by May 15.

Project Summary (Issue/Response)

Listeria monocytogenes is an important food borne pathogen in ready-to-eat aquaculture products. We determined that *L. monocytogenes 2117* gene (LmF2365_2117) encoding a cell wall surface anchor protein is present only in high-risk serovars 4b, 1/2a, 1/2b, 1/2c. However, role of LmF2365_2117 protein in high-risk *L. monocytogenes* attachment to catfish fillet is still unexplored. The goal of this research was to design and synthesize small molecule inhibitors against high-risk *L. monocytogenes* specific Lmof2365_2117 protein, and to evaluate the efficacy of small molecule inhibitors in preventing catfish fillet attachment of high-risk *L. monocytogenes*.

Project Results/Outcomes

Our fillet attachment experiments showed that there was a significant ($p < 0.01$) difference in attachment of LmF2365 and LmF2365Δ2117 strains. (Figure 1). Wash procedures (short vs long) did not have any effect on the outcome and wash and bacteria did not show any interaction.

Ten compounds with different pharmacophore groups were synthesized. Compounds 1-4 are 2,4-disubstituted-phthalazin-1(2H)-one derivatives with boron and without boronic acid and potassium salts of trifluoroborate. Compounds 5-8 are 3,7 disubstituted-2H-benzo[b][1,4] oxazine derivatives, and compound 9-10 are pyridine substituted boronic acid derivatives to mimic the dipeptides. All compounds are characterized by 1H proton NMR, 13C carbon NMR and HRMS to identify the exact structure and purity (above 98% purity).

Effects of designed small molecules on the growth of high-risk wild-type *L. monocytogenes* (Lmof2365) were determined. We found that none of the small molecules affected the growth of Lmof2365. Then, we tested the effect of small molecules on catfish fillet attachment of Lmof2365. We found that four small molecules changed the attachment properties of Lmof2365. SM01, SM03, and SM07 caused a significant ($p < 0.05$) decrease on the fillet attachment of Lmof2365, while a significant ($p < 0.05$) increase on the fillet attachment of Lmof2365 was observed when SM08 was used (Figure 2).

Project Impacts/Benefits

L. monocytogenes is an important food born pathogen causing death in at-risk people. Ready-to-eat aquaculture

products may get contaminated with *L. monocytogenes* and attachment of *Listeria* to fish fillet is an important step for pathogen proliferation. Our goal was to block attachment of *Listeria* to catfish fillet by using small molecules that were designed using the high-risk *L. monocytogenes* specific Lmof2365_2117 protein. In this project, ten compounds with different pharmacophore groups were synthesized. We found that three small molecules (SM01, SM03, and SM07) decreased the attachment properties of Lmof2365. After further evaluation of these small molecules' safety in food, they could be used to block listeria attachment in fish fillets and other food products.

Project Deliverables

-Peer reviewed articles

- Reddy S, Akgul A, **Karsi A**, Wills RW, Lawrence ML. 2014. The role of *Listeria monocytogenes* cell wall surface anchor protein LapB in virulence, adherence, and intracellular replication. Microbial Pathogenesis. Submitted.

- Abdelhamed H, Lawrence ML, **Karsi A**. 2015. A novel suicide plasmid for efficient gene mutation in *Listeria monocytogenes*. Plasmid. Submitted.

-Meeting Abstracts

- Akgul A, Lawrence ML, **Karsi A**. 2014. High-risk *Listeria monocytogenes* specific cell wall surface anchor protein and its role in catfish fillet attachment. American Society for Microbiology South Central Branch Meeting, Fayetville, AR, USA.

- Abdelhamed H, Lawrence ML, **Karsi A**. 2014. A novel suicide plasmid for efficient mutation of genes in Gram-positive bacteria. American Society for Microbiology South Central Branch Meeting, Fayetville, AR, USA.

Graphics

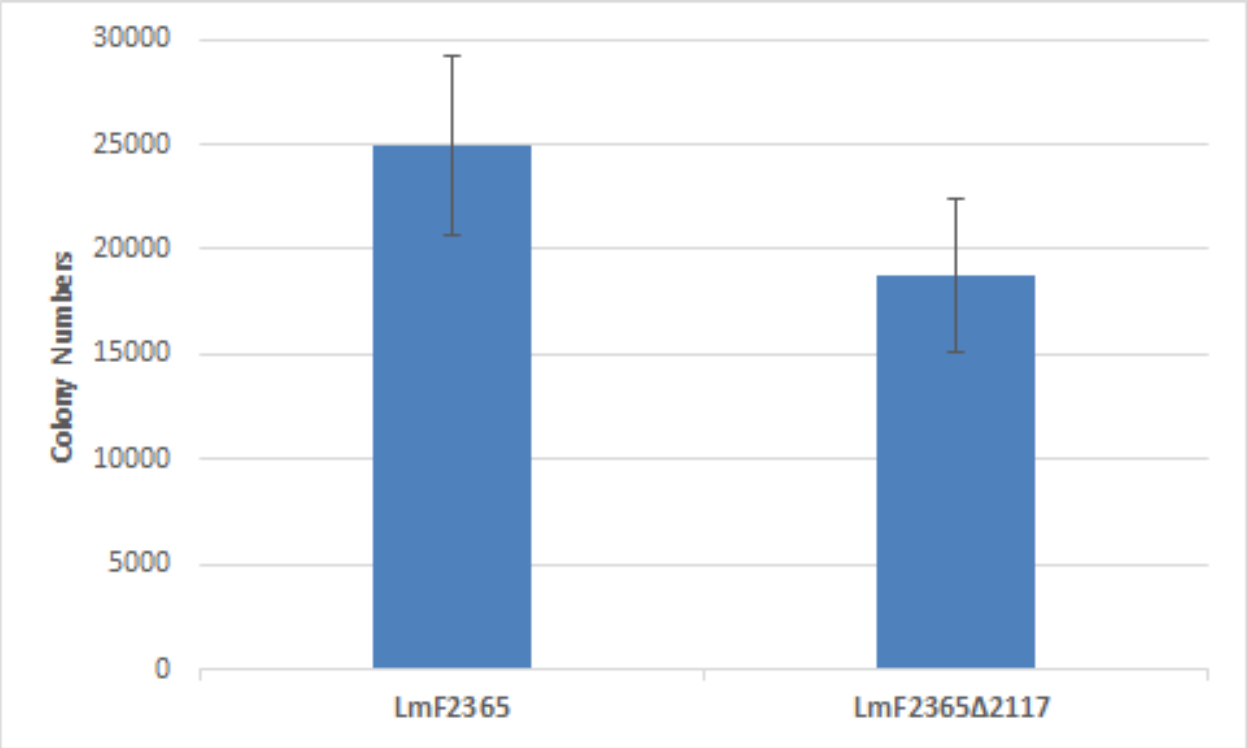

Figure 1. Catfish fillet attachment of high-risk wild-type *L. monocytogenes* (Lmof2365) and *2117* mutant (LmF2365Δ2117). There was a significant ($P < 0.01$) difference in attachment of LmF2365 and LmF2365Δ2117.

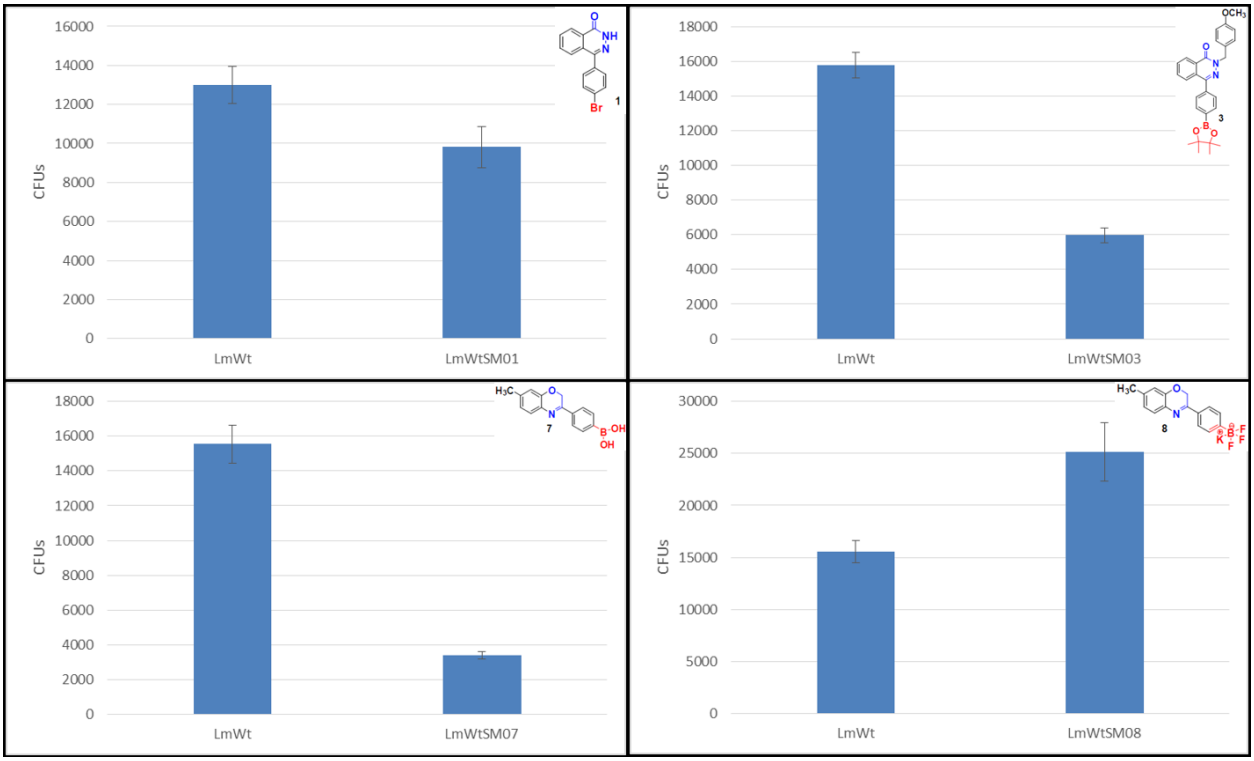

Figure 2. Catfish fillet attachment of high-risk wild-type *L. monocytogenes* (Lmof2365) in presence of small molecules. There was a significant ($P < 0.05$) difference in attachment of LmF2365 when treated with small molecules. SM01, SM03, and SM07 caused a decrease on catfish fillet attachment, while an increase was observed when SM08 was used.

Attached Refereed Journal Publications in Separate Files

- Reddy et al submitted to Molecular Pathogenesis. The decision was major revision and further experiments are being made.

- Abdelhamed et al submitted to Plasmid. The decision was minor revision and manuscript is being revised and the revised version to be submitted next week.