

Request for Report for Projects Awarded in 2013 and 2014 by

Mississippi Center for Food Safety and Post-Harvest Technology

Title: Persistence, adherence, and survival of acid-, alkali-, heat-, oxidative-, and salt-stress adapted cells of high-risk *Listeria monocytogenes* serotypes under normal catfish processing conditions and their prevention/destruction.

Award year: 2013

PI: Ramakrishna Nannapaneni

Co-PI:

Collaborator:

1. Objectives.

- (1) Detect highly-stable stress-adaptive responses of high-risk *Lm* serotypes when pre-exposed to mild acid-, alkali-, heat-, oxidative- and salt-stress.
- (2) Determine which of the acid-, alkali-, heat-, oxidative- or salt-stress adapted cells of high-risk *Lm* serotypes exhibit most adherence to simulated stainless steel food-contact surfaces found in catfish processing plants.
- (3) Determine which of the approved disinfectants (or their sequential application) can prevent/destroy the biofilms of most adherent stress-adapted cells of high-risk *Lm* serotypes

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

We have completed Objectives 1 and 3 and Objective 2 biofilms work is in progress. We have identified a high diversity of heat tolerance within strains of *L. monocytogenes* serotypes. Differences in survival of heat stress adapted cells of *L. monocytogenes* serotype 1/2a in various disinfectants/sanitizers and essential oils was examined. The survival of heat stress adapted cells of *L. monocytogenes* was decreased by 2.2 - 2.7 logs in lethal acid, alkali, chlorine and quaternary ammonium compounds. However, the survival of heat stress adapted cells of *L. monocytogenes* cells was increased by 3.5 - 4.0 logs in two essential oils, carvacrol and bay oil.

3. Objectives not accomplished and impediments to meeting objectives.

None

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

Continuing work on *Listeria monocytogenes* biofilms with different catfish processing surfaces will be reported in the next year annual meeting.

5. Students supported

- a. PhDs (% FTE and name)

Piumi Abeyesundara (100% FTE)
Nitin Dhowlaghar (100% FTE)
 - b. M.S. (% FTE and name)

Sulagna Saha (20% FTE)
 - c. Undergraduate (number of students): None
6. Leveraged Funds: External Competitive Funding Applied and Awarded based on findings from this project.
- a. Applied for:
 - i. Funding agency: AFRI 2014 Food Safety Challenge
 - ii. Program: Submitted two LOI for A4131 and A4171.
 - iii. Funding request (\$\$): None
 - b. Awarded: None
 - 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)

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

Listeria monocytogenes exhibits sophisticated adaptive mechanisms to counteract higher levels of lethal acid, heat, salt or oxidative stresses after pre-exposure to sublethal concentrations of homogenous stress. Our findings show that temperature plays a significant role in the induction of acid-stress adaptation in *Listeria monocytogenes* and two distinct patterns were observed: (I) Presence of sublethal acid at 37°C or 22°C significantly induced acid-stress adaptation; and (II) Presence of sublethal acid at 4°C did not induce any acid-stress adaptation. Both patterns were confirmed by two experimental models: (1) *L. monocytogenes* cells were first grown at 37°C and then exposed to sublethal acid at 37°C, 22°C and 4°C prior to lethal acid challenge; (2) Alternatively, *L. monocytogenes* cells were first grown at 4°C for 20 days before pre-exposure to sublethal acid and then challenged with lethal acid. Regardless of whether *L. monocytogenes* cells were simultaneously exposed with both cold stress and sublethal acid stress, or subjected to cold growth first before exposure to sublethal acid, no acid-stress adaptation was induced at 4°C. Bead beating treatment prior to mild acid pre-exposure at 4°C partially induced acid adaptation in *L. monocytogenes*. Our findings suggest that cold temperature can prevent the risk of acid-stress adaptation in *L. monocytogenes*.

Project Results/Outcomes

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

Several factors play critical roles in controlling the induction of acid-stress adaptation in *L. monocytogenes*. Two distinct patterns of acid adaptation were observed: (I) conditions where acid adaptation occurred; and (II) conditions where acid adaptation did not occur. For both strains, cells pre-exposed to mild acid stress at 22°C or 37°C for 1 h exhibited about 2 to 5 log CFU/ml better survival than non-stressed cells. In contrast, no difference in survival between mild acid-stressed and non-stressed cells was observed if pre-exposure was performed at 4°C. For Scott A, 4°C mild acid-stressed had 2 to 3 log CFU/ml lower survival compared to 22°C or 37°C mild acid-stressed cells after 90 min in pH 3.5 TSB-YE. For Bug600, this difference was about 2 log CFU/ml. This conceptual finding was also true if the lethal acid challenge was performed at 4°C or 22°C. Lack of mild acid adaptation at 4°C was also observed in *L. monocytogenes* cells grown at 4°C. For both strains, no difference existed between survival of mild acid-stressed and non-stressed cells if mild acid pre-exposure was performed at 4°C. However, at 37°C, mild acid-stressed cells had about 2 log CFU/ml higher survival compared to non-stressed cells. Addition of sodium chloride during pre-exposure did not induce acid adaptation at 4°C as survival of non-acid-stressed cells was similar to that of mild acid-stressed cells. In contrast, pronounced acid adaptation was observed when the cells were pre-exposed to pH 5.0 supplemented with sodium chloride at 37°C. In the presence of low concentrations (0.5% to 6%) of sodium chloride, mild acid-stressed cells exhibited at least 3 log CFU/ml greater survival than non-acid-stressed cells. Being exposed to low acid at 4°C, *L. monocytogenes* encounters two different types of physiological stress, namely acid stress and cold stress. *L. monocytogenes* can adapt to cold environment by expressing different protein patterns which contribute to its modulated metabolism pathway, nutrient uptake, protein folding and lipid

biosynthesis. Hence, it is likely that between acid and cold adaptation, cold adaptation becomes the priority task for the bacterium to deal with. We hypothesized that *L. monocytogenes* could start to respond to acid stress after cells were fully cold adapted. So we tested whether cold grown log phase cells which are fully cold acclimatized can show acid adaptation at 4°C. However, cold grown log cells still failed to activate acid adaptation at 4°C. Therefore, the actual cold adaptation event may not be the sole factor for repressed acid adaptation at 4°C.

A group of 37 strains representing all 13 serotypes of *Listeria monocytogenes* with initial cell density of 10^7 CFU/ml were analyzed for their heat tolerance at 60°C for 10 min. These *L. monocytogenes* strains were categorized into three heat tolerance groups: low (strains with < 2 log survival), medium (2 to 4 log survival) and high (4 to 6 log survival) heat tolerance. Serotype 1/2a strains exhibited relatively lower heat tolerance since 7 out of 8 tested strains were classified as low heat tolerant. Of the two 1/2b serotypes tested, one was very heat sensitive (non-detectable) and the other very heat resistant (5.4 log CFU/ml survival). Among the 16 serotype 4b strains, survival varied from non-detectable to 4 log CFU/ml. When one *L. monocytogenes* strain from each representing group was subjected to sublethal heat stress at 48°C for 30 or 60 min, the survival of heat stressed cells at 60°C for 10 min increased by 5 log CFU/ml (or $D_{60^\circ\text{C}}$ values nearly doubled) compared to the non-stressed control cells. Sublethal heat stress at 48°C for 60 or 90 min increased lag phase of *L. monocytogenes* in tryptic soy broth supplemented with 0.6% yeast extracts (TSB-YE) at room temperature by 3 to 5 hours compared to non-stressed control cells. The heat stress adaptation in *L. monocytogenes* was reversed within 2 h at room temperature but well maintained up to 24 h at 4°C. Our results show that there is a high diversity in heat tolerance within strains of *L. monocytogenes* serotypes and their heat stress adaptation once acquired is still preserved after cooling step which should be taken into account while conducting risk analysis for this pathogen.

Project Impacts/Benefits

In this box type 250—300 words project Impacts/Benefits statement.

Listeriosis outbreaks may potentially occur when a small number of *L. monocytogenes* cells multiply to reach approximately 1000 CFU in contaminated ready-to-eat food products. Hence, multiplication capacity of this bacterium in ready-to-eat food products is closely related to its infectious dose and virulence potential. Heat stress induced cell injury impairs cell growth capability which may increase the resuscitation time and these cells may still be living and infective. Our study revealed an extensive diversity of heat tolerance response in serotypes of *L. monocytogenes* which led to a sub-classification of low, medium and high heat tolerant strains. As a result of sublethal heat stress adaptation, $D_{60^{\circ}\text{C}}$ values of low, medium and high heat tolerant strains of *L. monocytogenes* increased by maximum 2.5 times and lag phase was prolonged by 3 to 7 h. We have also observed that acid-stress adaptation that typically occurs in *L. monocytogenes* at 22°C or 37°C was not induced when cells were pre-exposed to sublethal acid at 4°C. Our findings suggest that cold processing or cold storage temperature can prevent the risk of creating acid-stress resistant phenotypes of *L. monocytogenes*.

Project Deliverables

In this box list complete citations for all publications, presentations, workshops, field days, and other deliverables that came out of this project. Please use the following style (J. of Food Sci):

Shen Q, Soni KA, Nannapaneni R. 2014. Influence of temperature on acid-stress adaptation in *Listeria monocytogenes*. *Foodborne Pathog Disease* 11(1):43-49.

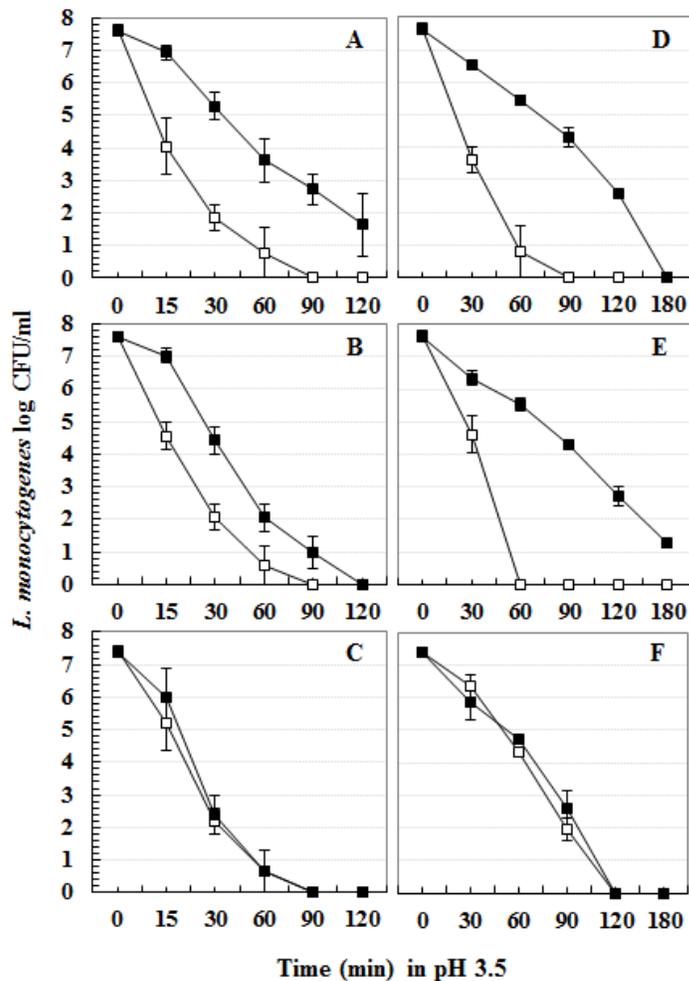
Shen Q, Jangam P, Soni KA, and Nannapaneni R. 2014. Low, Medium and High Heat Tolerant Strains of *Listeria monocytogenes* and Increased Heat Stress Resistance after exposure to Sublethal Heat. *Journal of Food Protection* 77:1298-1307.

Saha, S. 2014. Transmission electron microscopy study of *Listeria monocytogenes* serotype 1/2a cells exposed to sublethal heat stress and carvacrol [MS Thesis], Mississippi State Department of Food Science, Nutrition and Health Promotion, 77p.

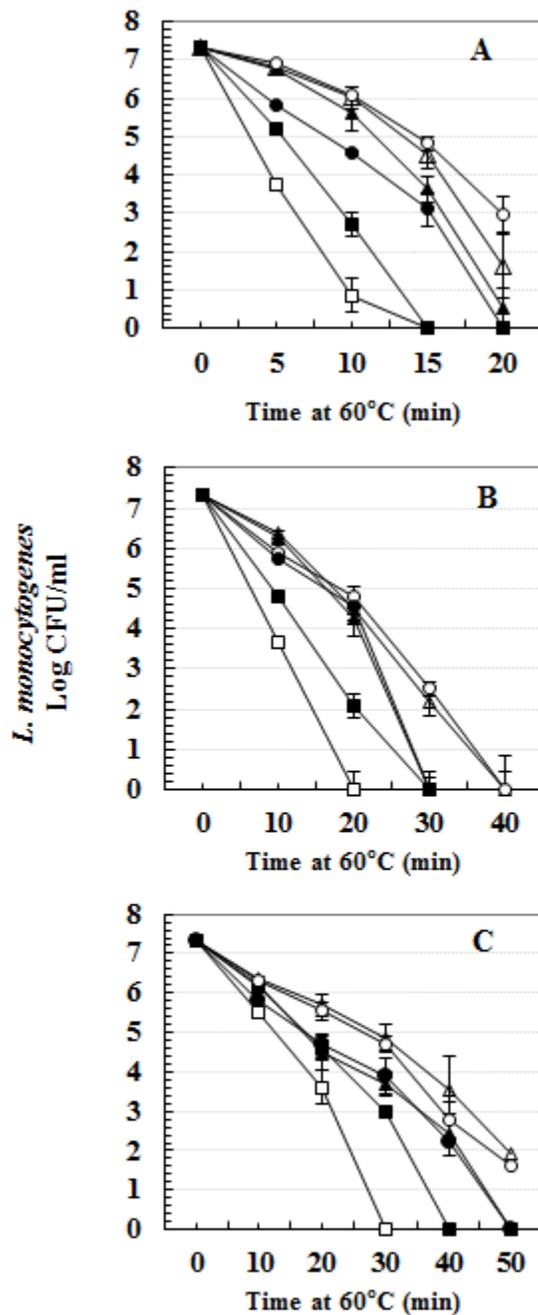
Saha, S, Dhowlaghar N, Lawrence A, Nannapaneni R, Sharma, S. 2014. Transmission electron microscopy study of *Listeria monocytogenes* serotype 1/2a cells exposed to sublethal heat stress and carvacrol. *Mississippi Academy of Science* (Submitted).

Graphics

Include one or two graphics (picture or figure, colored preferred) that illustrate project outcomes. Please make sure you provide labels and appropriate units for all dimensions, and a a brief explanation for each figure/graph.



Survival of *L. monocytogenes* Scott A (serotype 4b) (A,B,C) and Bug600 (serotype 1/2a) (D,E,F) in lethal pH 3.5 TSB-YE at 37°C after 1 h pre-exposure to sublethal pH 5.0 (■) or pH 7.2 (□) at three temperatures: (A,D) 37°C; (B,E) 22°C; and (C,F) 4°C.



Effect of sublethal heat stress at 48°C for 0 (□), 5 (■), 15 (▲), 30 (△), 60 (○) and 90 (●) min on survival at 60°C in three *L. monocytogenes* serotypes: (A) BUG 600 (serotype 1/2a); (B) NRRL B-33157 (serotype 4b); and (C) F4260 (serotype 1/2b).

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.

The following two journal articles are attached as PDF files:

Shen Q, Jangam P, Soni KA, and Nannapaneni R. 2014. Low, Medium and High Heat Tolerant Strains of *Listeria monocytogenes* and Increased Heat Stress Resistance after exposure to Sublethal Heat. *Journal of Food Protection* 77:1298-1307.

Shen Q, Soni KA, Nannapaneni R. 2014. Influence of temperature on acid-stress adaptation in *Listeria monocytogenes*. *Foodborne Pathog Disease* 11(1):43-49.