Project report submitted in Microsoft Word version. Do not provide a pdf version.

Project Leader (PI): Dr. Mark L. Lawrence

Co-PI(s): Dr. Attila Karsi

Collaborator(s): Dr. Todd Ward

Objective(s):

1) Evaluate the use of MALDI-TOF mass spectra for differentiation of *L. monocytogenes* high-risk serogroups and epidemic clones. In our previous FSI-funded work, we identified candidate protein mass spectra that can distinguish high-risk serogroups. We will evaluate additional listerial isolates used to validate a DNA probe method. This will allow comparison of the protein and DNA-based methods, and it will allow evaluation of the protein detection method for detection of epidemic clones.

2) Identify proteins that are unique to high-risk *L. monoyctogenes* serogroups and epidemic clones. Biomarkers identified in Aim 1 have potential for development of simple antibody-based methods for differentiating high-risk isterial serogroups or epidemic clones. To enable development of these methods, we will use SDS PAGE followed by MALDI TOF/TOF for peptide identification.

Milestones for FY 2016-17:.

Title: Detection of protein biomarkers for high-risk *Listeria monocytogenes*

Objective 1. Partially met. We redirected our strategy for this objective to using electrospray ionization tandem mass spectrometry (ESI MS/MS). This was done for two reasons: 1) consultation with Dr. Tibor Pechan at MSU Institution for Genomics, Biocomputing, and Biotechnology (IGBB) indicated that this strategy was better for exploratory research to identify unique peptides, and 2) due to changes in circumstances, a Bruker MALDI-TOF Biotyper was not available. New methods for protein isolation from *L. monocytogenes* for ESI MS/MS were optimized, and mass spectrometry was conducted on Lineage III strain ST33077.

Objective 2. Partially met. We redirected our strategy for this objective to using 2D gel electrophoresis instead of one dimensional SDS PAGE. This was based on consultation with Dr. Pechan; two dimensional gels have more discriminatory power than one dimensional gels. Analysis of Lineage III strain ST33077 was completed.

Progress Report:

In our 2016-2017 FSI project, we began a new approach to identify protein biomarkers for separation of the major *L. monocytogenes* genetic lineages. We successfully optimized sample preparation methods and liquid chromatography (LC) conditions prior to electrospray ionization tandem mass spectrometry (ESI MS/MS) analysis using *L. monocytogenes* lineage III strain ST33077. Mass spectrometry data was matched against the ORF database from the ST33077 genome sequence. A total of 10 distinct proteins

with no homologs in representative strains from *L. monocytogenes* genetic Lineages I and II were detected in Lineage III strain ST33077. In addition to analysis by LC ESI MS/MS, we also used two-dimensional gel electrophoresis (2-DE) to analyze the proteome of strain ST33077. Our findings were similar to published results showing that the pI of most listerial proteins are in the acidic range.

Accomplishments

Results showed that grinding *L. monocytogenes* after freezing in liquid nitrogen improved protein yield versus traditional sonication methods. Results also showed that increasing LC gradient length and fractionation by molecular weight prior to LC dramatically increased the proteome coverage. BLAST analysis of the ten Lineage III-unique proteins against the NCBI *Listeria* RefSeq proteome database provided best hits to a teichoic acid biosynthesis protein, GNAT family N-acetyltransferase, lactoylglutathione lyase, endonuclease, type IV secretion protein Rhs, a membrane protein, and four hypothetical proteins Further analysis of these proteins by BLAST analysis against the NCBI non-redundant protein database revealed that several of them do not have significant similarity to *L. monocytogenes* proteins from any sequenced genomes from Lineages I and II. Two-dimensional gel electrophoresis analysis of *L. monocytogenes* allowed visualization of approximately 250 proteins in the 4-7 pI range using Coomassie Blue.

Significant Activities that Support Special Target Populations: (100 words or less)

Listeria monocytogenes is estimated by the CDC to be the third leading cause of death from foodborne illness with an approximate 20% case fatality rate. The goal of this project is to improve detection and characterization of *L. monocytogenes* and enable implementation of improved food safety measures in food industries. In particular, a protein-based detection method for differentiating high-risk *L. monocytogenes* serogroups and epidemic clones would facilitate identification of listerial subgroups in diagnostic microbiology laboratories and in food processing facilities. This increased discriminatory capability would assist in assessment of risk from *L. monocytogenes* isolates and accelerate epidemiological investigations.

Technology Transfer:

None

International Cooperation / Collaboration

None

Publications: Please use the following format, examples

Reddy, S., Akgul, A., Karsi, A., Abdelhamed, H., Wills, R. W., and Lawrence, M. L. (2016). The role of *Listeria monocytogenes* cell wall surface anchor protein LapB in virulence, adherence, and intracellular replication. *Microbial Pathogenesis*, 92: 19-25.

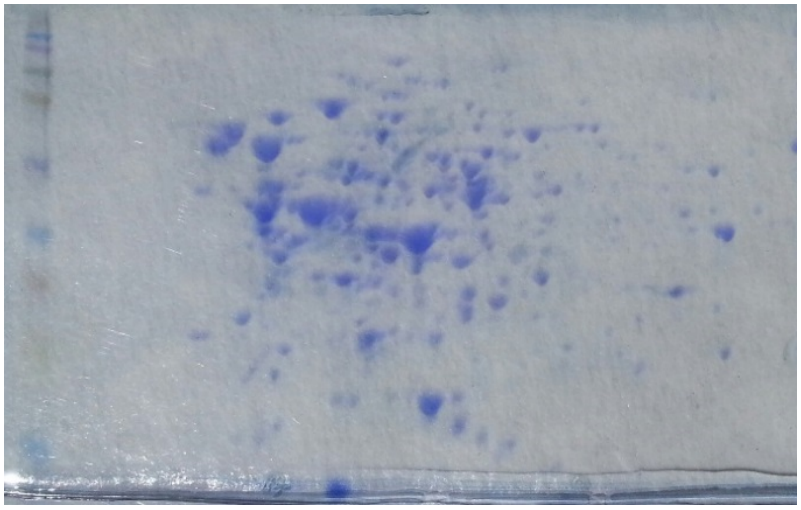
Reddy, S., Turaga, G., Abdelhamed, H., Banes, M. M., Wills, R. W., and Lawrence, M. L. (Submitted). *Listeria monocytogenes* PdeE, a phosphodiesterase that contributes to virulence and has hydrolytic activity against cyclic mononucleotides and cyclic dinucleotides. *Microbial Pathogenesis*.

Presentations: Please use the following format

None to report.

Please attach a photo or figure with a brief explanation to showcase the achievement of your project that will be placed on our food safety research website

Thank you very much.



Two dimensional gel electrophoresis of *L. monocytogenes* strain ST33077. We visualized approximately 250 proteins in the 4-7 pI range using Coomassie Blue.