

## ***LISTERIA***

### **BIOMARKERS OF *LISTERIA MONOCYTOGENES* INFECTION AND TREATMENT WITH A SYNTHETIC ANTI-INTERNALIN PEPTIDE IN PREGNANT GUINEA PIGS**

(E. Irvin, D. Williams, A. Puckett, and M.A. Smith)

*Listeria monocytogenes* is a bacterial pathogen known to cause spontaneous abortions and stillbirths. The mechanisms of *Listeria*-induced miscarriages and stillbirths are still largely unknown. Previously, pregnant primates and pregnant guinea pigs were used to develop a dose-response model for infection with *L. monocytogenes*. Our recent studies have focused on the maternal and fetal effects during the course of infection and biomarkers that might predict induction of a stillbirth. We hypothesize that after infection with *L. monocytogenes*, changes in the placental immunological status will occur. Additionally, we hypothesize that treatment with a synthetic peptide designed to bind to internalin A (anti-In1a) will decrease the invasiveness of *L. monocytogenes* in maternal and fetal tissues. Pregnant guinea pigs were treated on gd 35 with doses of  $10^4$  to  $10^8$  CFUs *L. monocytogenes* and sacrificed at post-treatment days 2, 6, 9, and 20 (gd 37, 41, 44, and 55). *Listeria* species were determined by plating enriched maternal and fetal tissues as well as maternal fecal samples on selective media. *L. monocytogenes* was confirmed using a chromogenic substrate test. The immunological response of the placenta was determined by qRT-PCR analysis of specific Th1 (IFN- $\lambda$ , TNF- $\alpha$ , IL-2) and Th2 (IL-5, IL-10) cytokines. Expression levels were determined using the comparative relative quantity method and are expressed as a fold-change compared to control levels. For the peptide study, three experimental groups were used and all treatments were administered orally. (A) Pregnant guinea pigs were treated with the peptide only, (B) 100 $\mu$ g/ml anti-In1a peptide was administered followed by an administration of  $10^8$  *L. monocytogenes* CFU/ml one hour later, or C) 100 $\mu$ g/ml anti-In1a peptide and  $10^8$  *L. monocytogenes* CFU/ml were pre-incubated for one hour prior to administration. Tissue infectivity was not affected by the duration of infection as *L. monocytogenes* was isolated from tissues samples at the earliest post-treatment day 2. However, cytokine expression levels were affected by the duration of infection with no changes occurring in placental cytokine expression levels from treated dams at post-treatment day 2. Th1 placental cytokine expression levels were significantly altered in treated dams at post-treatment days 6, 9, and 20. Th2 placental cytokine expression levels were not altered after maternal treatment with *L. monocytogenes*. For the anti-In1a study, of the guinea pigs that were exposed to *L. monocytogenes* 1 hour following the peptide (B), *L. monocytogenes* was isolated from maternal liver and spleen. Interestingly, *L. monocytogenes* could not be cultured from the placentas or fetuses of this group. Additionally, pre-incubation of the peptide with *L. monocytogenes* did not reduce infection of maternal tissues. Current data suggest that when administered to pregnant guinea pigs prior to *L. monocytogenes* exposure, anti-In1a peptide may reduce the occurrence of fetal infection and stillbirths.

### **PROTEOMIC ANALYSIS OF A HYPOCHLOROUS ACID TOLERANT *LISTERIA MONOCYTOGENES* CULTURAL VARIANT EXHIBITING ENHANCED BIOFILM PRODUCTION**

(J.P. Folsom and J.F. Frank)

Following exposure of *Listeria monocytogenes* ScottA (SA) to hypochlorous acid, rough colony variants were identified that were tolerant of hypochlorous acid and produced increased amounts of biofilm. A derivative of one of these variants was smooth, produced even more biofilm and exhibited greater biofilm chlorine resistance. The objective of this research was to compare the protein expression of a cultural variant to SA, and identify proteins that may be associated with biofilm production and chlorine tolerance. Suspension chlorine tolerance for several cultural variants (SAR, SAR5, and SBS) was determined by exposure to 60-120 ppm hypochlorous acid for five minutes. Hypochlorous acid tolerance of biofilms was determined after growing biofilms on stainless steel followed by exposure to 200 ppm hypochlorous acid for 5 min. All cultural variants were able to survive 120 ppm of hypochlorous acid in suspension. There was little difference in the hypochlorous acid tolerance of the cultural variant planktonic cells. The cultural variants produced greater amounts of biofilm than the SA form, and were more hypochlorous acid tolerant. The SBS variant was selected for proteomic comparison because it was the variant that produced the most biofilm and was most tolerant of hypochlorous acid when grown as a biofilm. Protein expression of planktonic and biofilm cells of SBS was compared to SA by using two dimensional difference gel electrophoresis. The 50s ribosomal protein, L10 was down regulated in biofilm SBS. Other

proteins down regulated in planktonic SBS were the peroxide resistance protein (Dpr) and a sugar binding protein (LMO0181). This sugar binding protein was also up regulated in biofilm SBS. One protein spot down regulated in planktonic SBS contained both 50s ribosomal protein L7/L12 and an unknown protein (LMO1888).

### **INACTIVATION OF PATHOGENS IN COMPOST MIXTURES AS INFLUENCED BY TYPE OF MANURE**

(M.C. Erickson, C. Smith, X. Jiang, and M.P. Doyle)

During aerobic composting, heat is generated from the metabolic activity of thermophilic microorganisms and may contribute to inactivation of contaminant pathogens at internal sites of static piles. At the surface of compost piles, however, heat dissipation contributes to reduced temperatures and in turn reduced pathogen inactivation. It was the objective of this study to investigate whether pathogen inactivation at the surface would be affected by the compost composition and in particular the type of manure.

Chicken, cow, and hog manures served as the source of nitrogen in compost mixtures while straw and cottonseed meal were used as carbon amendments. Mixtures varied in the C:N ratio, having initial values of 20:1, 30:1, or 40:1 and were inoculated with both gfp-labeled *Salmonella* spp. and gfp-labeled *Listeria monocytogenes*. Mixtures were placed in trays (simulating surface sites of static compost piles) and held in environmental controlled chambers at 20° or 30°C and under different levels of light exposure. On a weekly basis, moisture levels in samples were adjusted to initial values (30% or 60%). Samples were periodically taken for enumeration of pathogens and measurement of moisture and pH.

At both 20° and 30°C, pathogen survival was greatest in compost mixtures formulated with cow manure followed by mixtures formulated with chicken manure and then hog manure. Regardless of the manure used in the compost mixture formulation, however, *L. monocytogenes* populations decreased faster than *Salmonella* spp. populations. Exposure to conditions simulating bright sunlight accelerated pathogen inactivation.

### **COMPETITIVE INHIBITION MICROORGANISMS FOR THE CONTROL OF ZOO NOTIC PATHOGENS IN COMPOST**

(L. Ma, G. Zhang, V. Mantripragada, M. C. Erickson, and M. P. Doyle)

Indigenous microflora may play a significant role in suppression of zoonotic pathogens during static composting. The objective of this project was to isolate competitive inhibition (CI) microorganisms from static compost piles for the control of zoonotic pathogens. Compost samples from the surface of static compost piles were collected during the study of the fate of zoonotic pathogens (*E. coli* O157:H7, *Listeria innocua*, and *Salmonella* Typhimurium) in static composting of chicken litter and peanut hulls. Only samples that exhibited a large decline in inoculated pathogen populations in two consecutive sampling times were used for the isolation of CI microorganisms. Two methods were used to screen for potential CI bacteria against target pathogens (*E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella*): a deferred antagonism test and a co-culture test. A total of 20 potential CI isolates against either one or all of three target pathogens were selected from 16 compost samples. Cross inhibitory activity among these isolates revealed that nine of the isolates were compatible. Characterization of these isolates by DNA sequencing of the 16S rRNA gene is currently in progress. Future studies will incorporate these isolates into cured compost materials and evaluate their potential to inhibit the growth of *Salmonella*.