LISTERIA MONOCYTOGENES

CHLORINE RESISTANCE OF *LISTERIA MONOCYTOGENES* BIOFILMS AND RELATIONSHIP TO SUBTYPE, CELL DENSITY AND PLANKTONIC CELL CHLORINE RESISTANCE

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

Strains of *Listeria monocytogenes* vary in their ability to produce biofilms. This research determined if cell density, planktonic chlorine resistance or subtype are associated with the resistance of *L. monocytogenes* biofilms to chlorine. Thirteen strains of *L. monocytogenes* were selected for this research based on biofilm accumulation on stainless steel and rep-PCR subtyping. These strains were challenged with chlorine to determine the resistance of individual strains of *L. monocytogenes*. Planktonic cells were exposed to 20 through 80 ppm sodium hypochlorite in 20 ppm increments for five minutes in triplicate per replication, and the experiment was replicated three times. The number of tubes with surviving *L. monocytogenes* was recorded for each isolate at each level of chlorine. Biofilms of each strain were grown on stainless steel coupons. The biofilms were exposed to 60 ppm of sodium hypochlorite. When in planktonic culture, four strains were able to survive exposure to 40 ppm of chlorine, while four strains were able to survive 80 ppm of chlorine in at least one of three tubes. The remaining five strains survived exposure to 60 ppm of chlorine. Biofilms of 11 strains survived exposure to 60 ppm of chlorine. No association of biofilm chlorine resistance and planktonic chlorine resistance was observed, however biofilm chorine resistance was similar for strains of the same subtype. Biofilm cell density was not associated with chlorine resistance. In addition, biofilms that survived chlorine treatment exhibited different biofilm morphologies. This data suggests that chlorine resistance mechanisms of planktonic cells and biofilms differ, with planktonic chlorine resistance being more affected by inducible traits, and biofilm chlorine resistance being more affected by traits not determined in this study.

FORMATION OF BIOFILM AT DIFFERENT NUTRIENT LEVELS BY VARIOUS GENOTYPES OF *LISTERIA MONOCYTOGENES*

(J.P. Folsom, G.R. Siragusa, and J.F. Frank)

Strains of *Listeria monocytogenes* exhibit a range of ability to form biofilms. The objectives of this study were to determine if genetically related strains exhibit similar biofilm-forming capacity, and the effect nutrient concentration has on the ability of different strains to produce biofilm. Biofilms of 30 strains of *L. monocytogenes,* obtained from a variety of sources, were grown on stainless steel in tryptic soy broth [TSB] or a 1:10 dilution of TSB [DTSB] for 24 hours at 32ºC. The amount of biofilm formed was determined using image analysis after staining the cells with bisBenzimide H 33258 (Hoechst 33258). The strains were genetically subtyped by repetitive element sequence-based PCR (rep-PCR) using the primer sets rep-PRO_{Dt} and rep-PRO_{G5}. Data were analyzed by using ANOVA and Duncan's multiple range test. Eleven strains produced the same amount of biofilm in the two media. Fourteen strains produced more biofilm in TSB than DTSB. Five strains produced more biofilm in DTSB than TSB. Serotype 4b strains produced more biofilm accumulation in TSB than serotype 1/2a strains, while serotype 1/2a strains produced more in DTSB than did serotype 4b strains. Growth in DTSB resulted in decreased biofilm accumulation for serotype 4b strains. There was no correlation between genetic subtype and the amount of biofilm accumulation. These results indicate that serotype 1/2a and serotype 4b strains differ in the regulation of their biofilm phenotype. The poor biofilm accumulation of serotype 4b isolates when grown in DTSB could be a factor in the predominance of serogroup 1/2 strains in food processing plants, where nutrients may be limited.

CHARACTERIZATION OF A CHLORINE TOLERANT MUTANT OF *LISTERIA MONOCYTOGENES*

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

Rough colony variants of *Listeria monocytogenes* ScottA (SA) were isolated from chlorine-treated cells that were tolerant to hypochlorous acid and produced increased amounts of biofilm. A derivative of one of these variants was smooth, produced even more biofilm and exhibited greater chlorine resistance biofilm when grown as a biofilm. The objective of this research was to compare the protein expression of the chlorine tolerance cultural variant to the wild type, to 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 S-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). The frequency at which chlorine tolerant variants of *L. monocytogenes* arise in food processing environments should be a topic for further investigation.

CROSS-CONTAMINATION OF *LISTERIA MONOCYTOGENES* BETWEEN PROCESSING EQUIPMENT AND DELI MEATS

(L. Ma, G. Zhang, C-M. Lin, and M.P. Doyle)

Contamination of ready-to-eat meats by *Listeria monocytogenes* has resulted in outbreaks of listeriosis and major product recalls. Food processing equipment such as slicers can serve as a potential contamination source. This study was conducted to determine (i) the dynamics of cross-contamination of *Listeria monocytogenes* from slicing equipment to two different types of turkey meat, cured and non-cured, (ii) the role of the conveyor belt in the transfer event, (iii) the fate of *L. monocytogenes* on contaminated samples during storage at 4°C for up to 90 days, and (iv) the efficacy of the BAX-PCR and USDA conventional enrichment culture assays in detecting *L. monocytogenes* on turkey meats. A five-strain mixture of *L. monocytogenes* was inoculated at ca. 500 CFU onto the blade of a commercial slicer. Five consecutive meat slices were packed per package, vacuum sealed, stored at 4°C, and sampled (entire package) at 1, 30, 60, and 90 days postslicing. Of the two types of deli meats, a larger number of *L. monocytogenes*-positive samples were obtained from non-cured turkey meat, 48 of 800 samples compared to 14 of 800 of cured turkey meat. Most of the *L. monocytogenes*-positive samples in cured turkey meat were detected at 30 days postslicing, whereas the largest number of *L. monocytogenes*-positive samples for non-cured turkey meat was recovered at 90 days postslicing. Slightly more (48 vs. 43 of 800 samples) *L. monocytogenes*-positive meat samples were obtained when the conveyor belt was used and the positive meat samples obtained at the middle or near the end of sliding were likely from contamination of the conveyor belt. For cured turkey meat, *L. monocytogenes* was detected in 5 meat samples by both the enrichment culture and BAX-PCR assays and in 9 samples only by enrichment culture assay. For non-cured turkey meat, *L. monocytogenes* was detected in 23 samples by both assays in 13 samples only by the enrichment assay, and in 10 samples only by the BAX-PCR assay. *L. monocytogenes* cell numbers were generally very low when detected on either type of turkey meat contaminated during processing. The results indicate that *L. monocytogenes* can be transferred from a contaminated slicer onto deli meats and survive storage at 4°C. The enrichment culture and BAX-PCR assays are complementary to each other in the detection of *L. monocytogenes*, especially for non-cured turkey meat.

INACTIVATION OF ZOONOTIC PATHOGENS DURING STATIC COMPOSTING OF CHICKEN LITTER AND PEANUT HULLS

(M.C. Erickson, C. Smith, J. Liao, G.E. Boyhan, M.P. Doyle, L. Ma, and X. Jiang)

During aerobic composting, the primary factor responsible for inactivation of fecal pathogens is heat generated from the metabolic activity of thermophilic microorganisms. Moreover, to ensure inactivation of pathogens at the surface of static compost piles, it is recommended that compost be turned periodically during the first weeks of composting. This safeguard practice, however, is not often implemented in situations where labor and resources are limited. To develop alternative management strategies for these situations, baseline data is needed to determine inactivation profiles of zoonotic pathogens at surface and interior sites of static piles. The fate of zoonotic pathogens [gfp-labeled *Escherichia coli* O157:H7 (Shiga toxin-negative) and *Listeria innocua* and rifampicin-resistant *Salmonella* Typhimurium (vaccine strain)] in the field was monitored at both interior and surface sites of static composting piles composed of chicken litter and peanut hulls. Zoonotic pathogen populations declined by 4-8 log CFU/g within 4 days of composting but were still detectable by enrichment culture. Despite exposures to

elevated temperatures, *Salmonella* continued to be detected in interior samples by enrichment for up to 14 days after composting was initiated. In surface samples, the fate of pathogens was dependent on the season and ambient temperature conditions in which composting was conducted. During the summer, *S.* Typhimurium, *E. coli* O157:H7 and *L. innocua* were detected by enrichment only in 3-day, 3-day, and 7-day compost surface samples, respectively. In contrast, 28, 56, and 56 days of composting in the late fall/early winter were required to reduce *S.* Typhimurium, *E. coli* O157:H7, and *L. innocua* populations, respectively, to levels detectable only by enrichment. In conclusion, zoonotic pathogens survived on the surface of unturned static composting piles containing chicken litter for up to 2 months.

INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* IN COW MANURE COMPOSTING SYSTEMS

(M.C. Erickson, J. Liao, L. Ma, M.P. Doyle, and X. Jiang)

Aerobic composting may be applied to manure whereby microbial metabolite degradation of organic matter generates heat for inactivation of pathogens. When equipment and manpower are not available to turn the compost mass and expose all the material to sufficient levels of heat, other management guidelines are needed to assure that pathogen inactivation of surface compost has been achieved. Towards that end, research has been addressing the potential for the initial carbon:nitrogen (C:N) ratio of the compost mixture to affect pathogen inactivation. Using a cow manure, straw, and cottonseed mixture in a laboratory-scale bioreactor, C:N ratio did not significantly affect the time to inactivation of *Listeria monocytogenes*. In contrast, *Escherichia coli* O157:H7 survived for significantly longer periods of time in 40:1 C:N systems than in 30:1 or 20:1 systems despite the fact that the cumulative heat exposure of the former system was much greater than the exposure encountered in the two latter systems. In addition, an escalation in pH to values between 8 and 9 occurred initially for 40:1 C:N systems whereas 20:1 and 30:1 systems experienced an initial decline in pH to values between 5.5 and 6 before climbing to alkaline values (8-9) after 2 days of composting. It is hypothesized that organic acids generated in the acidic stage of 20:1 and 30:1 systems may act in concert with heat to inactivate *E. coli* O157:H7. Such situations may be beneficial to the inactivation of pathogens on the surface of compost piles where temperatures are found to increase only slightly above ambient.

DOSE RESPONSE, INFECTIVITY, AND STILLBIRTHS IN PREGNANT GUINEA PIGS INOCULATED WITH *LISTERIA MONOCYTOGENES*

(D. Williams, E. A. Irvin, R. A. Chmielewski, J. F. Frank, and M. A. Smith)

Listeriosis is a severe disease that results from the foodborne pathogen, *Listeria monocytogenes* and is responsible for ~ 2500 cases and 500 deaths each year. Pregnant women are 20 times more likely to develop listeriosis than the general population with adverse pregnancy outcomes including low birth weight, spontaneous abortions, stillbirths, or neonatal meningitis. The objective of this study was to determine an infective dose, infectivity, and corresponding stillbirths in pregnant guinea pigs. Pregnant guinea pigs (n = 4-11/dose) were treated orally on gestation day (gd) 35 with 10^4 to 10^8 *L. monocytogenes* CFU in sterile whipping cream. Pregnancies were allowed to proceed normally until sacrifice on gd 56. *Listeria* species were determined by direct plating tissue samples on selective media, and *L. monocytogenes* was confirmed using a chromogenic substrate test. Among the treated dams, there was a dose dependent relationship in the colonization of *L. monocytogenes* within tissue samples. *L. monocytogenes* cells were recovered from 64, 73, 90, and 100 percent of liver samples from animals treated with 10^5, 10^6, 10^7, 10^8 CFU, respectively. Livers from each dose were examined microscopically after staining with H&E. No apparent lesions or chronic inflammation was seen in the control animals, but lesions were found in treated animals. At the lowest dose of 10^4 *L. monocytogenes* CFUs, some apoptotic hepatocytes and areas of minimal to mild acute inflammation were found. The lesions, in size and number, increased as the dose increased. In dams dosed with $\geq 10^6$ CFU, *L. monocytogenes* cells were cultured from 50% of the spleen and 33% of the gallbladder samples. Eleven of 34 dams treated with $\geq 10^5$ CFU delivered stillborn pups. *L. monocytogenes* cells were cultured from placenta, liver, and brain tissue from all stillbirths. However, the dams with nonviable fetuses shed *L. monocytogenes* for longer periods of time. Based on a log logistic model, the dose adversely affecting 50% of the pregnancies was approximately 10^7 *L. monocytogenes* CFU compared to that estimated from a human outbreak of 10^6 CFU. Listeriosis in pregnant guinea pigs can result in stillbirths, and the overall disease is similar to that described in non-human primates and in humans.


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