Center for Food Safety, University of Georgia, Griffin, GA (http://www.griffin.uga.edu/cfs)
2004 Highlights

LISTERIA MONOCYTOGENES

CONTROL OF *LISTERIA MONOCYTOGENES* BY COMPETITIVE EXCLUSION BACTERIA IN FLOOR DRAINS OF A POULTRY PROCESSING PLANT (T. Zhao, M. P. Doyle, T. C. Podtburg, P. Zhao, B. E. Schmidt, D. A. Baker, B. Cords, and R. Howell)

Recent outbreak investigations revealed that contamination of the environment of food processing facilities is a primary source of *L. monocytogenes* in many commercially prepared ready-to-eat (RTE) processed foods. Studies have revealed that certain strains of *L. monocytogenes* can become well established in a food processing facility in locations such as floor drains and remain members of the resident microbial flora for months or years. Although significant improvements in plant layout and equipment design, and procedures for cleaning and sanitizing have been made, it is believed that *L. monocytogenes* will continue to be introduced into the environment in which RTE foods are exposed for further processing and packaging.

Controlling the widely distributed psychrotrophic *L. monocytogenes* in food processing facilities has been a formidable challenge for the entire food industry, from the smallest to the largest food processor. Besides the pathogen's widespread occurrence in nature, it is nonfastidious, grows at refrigeration temperature, forms protective biofilms, and thrives in moist environments. Floor drains in food processing facilities are a particularly important niche for its existence and can be a control point of contamination for the processing plant environment and food products.

Decontaminating floor drains of *Listeria* is especially challenging because when entrapped in a biofilm, listeriae are afforded unusual protection against disinfectants and treatment available to control pathogens on environmental surfaces. Our goal was to characterize microorganisms that would thrive in combination with *Listeria* within its biofilm at a wide range of temperatures that occur in food processing facilities (especially at refrigeration conditions) and would compete to control listerial growth and possibly eliminate the pathogen.

Based on previous studies, two competitive exclusion (CE) bacteria, strains C-1-92 (*Lactococcus lactis* subsp. *lactis)* and 152 (*Enterococcus durans*) were selected for use to treat floor drains in a raw meat poultry processing facility to reduce/eliminate *Listeria monocytogenes*. In cooperation with industry partners, Ecolab and Gold Kist, Inc., a poultry processing plant located in Athens, Georgia was chosen for the field trial. Before treatment, the floor drains were tested every two weeks for five times plus one time after sanitation of the plant for *Listeria*. Samples were collected from five locations in each of five floor drains. The sampling locations included (a) bottom of drain, (b) right side of drain, (c) left side of drain, (d) under metal support of drain, and (e) surface of the floor within 1 foot of the drain.

The average number of *Listeria* in floor drains sampled at six different times (at 2-week intervals) ranged from 3.3 to 4.0 $\log_{10}$ cfu/cm^2 for drain #1, from 4.2 to 5.4 $\log_{10}$ cfu/cm^2 for drain #3, from 3.4 to 4.5 $\log_{10}$ cfu/cm^2 for drain #4, from 3.2 to 4.2 $\log_{10}$ cfu/cm^2 for drain #6, and from 6.1 to 8.2 $\log_{10}$ cfu/cm^2 for drain #8. Following these samplings, 10^7 CE bacteria/ml in foam-based medium developed by Ecolab (St. Paul, MN) were applied to the floor drains daily for four times during the first week (Monday through Thursday). Then the treatment was applied twice a week (Tuesday and Thursday) for the next three weeks. Samples were collected for *Listeria* count determinations once a week for the five weeks following application. The average number of *Listeria* ($\log_{10}$ cfu/cm^2) in samples collected one week after treatments were applied were <1.7 (positive only by selective enrichment) for drain #1, 2.0 to 3.7 for drain #3, 0 (negative by selective enrichment) to <1.7 for drain #4, 0 (all negative by selective enrichment) for drain #6 and 2.2 to 4.6 $\log_{10}$/cm^2 for drain #8. Results indicate that application of these two CE bacteria can greatly reduce *Listeria* cell numbers in floor drains at 2 to 30ºC in a poultry processing facility.

SURVIVAL, GROWTH, AND THERMAL RESISTANCE OF *LISTERIA MONOCYTOGENES* IN PRODUCTS CONTAINING PEANUT AND CHOCOLATE (S. J. Kenney and L. R. Beuchat)

Outbreaks of listeriosis linked to the consumption of ready-to-eat (RTE) foods have raised interest in better understanding the survival and growth characteristics of *Listeria monocytogenes* in these products. Among RTE products of interest are those containing chocolate. Outbreaks of listeriosis have been epidemiologically linked to the consumption of butter, chocolate milk and whole-fat and reduced-fat (2%) milk. To date, there have been no outbreaks of listeriosis associated with the consumption of peanut products. However, peanut butter has been epidemiologically linked to an outbreak of salmonellosis in Australia caused by *Salmonella* Mbandaka. *Salmonella* can survive in peanut butter for at least 24 weeks at 5 or 21°C. The wide-spread presence of *L. monocytogenes* in nature, its ability to grow at refrigeration temperatures, and the severity of infections it is capable of causing raises interest in defining its survival and growth characteristics in conventional and newly developed RTE foods. The objective of this study was to evaluate the behavioral characteristics of *L. monocytogenes* in two newly developed food products, a peanut beverage, and a chocolate-peanut spread, as affected by temperature and a_w. Survival and growth of *L. monocytogenes* in these products subjected to various environmental conditions was determined. Behavior of the pathogen in newly developed products was compared to its behavior in similar products available in retail markets.

Tolerance of *L. monocytogenes* was evaluated in a peanut-based beverage (3.1% fat), whole-fat (3.5%) milk, whole-fat (4.0%) and reduced-fat (1.0%) chocolate milk, a chocolate-peanut spread (39% fat), and peanut butter (53% fat). The $D_{60°C}$ value in peanut beverage (3.2 min) was not significantly different ($P > 0.05$) than the $D_{60°C}$ value in whole-fat milk (3.3 min) or whole-fat chocolate milk (4.5 min) but significantly lower ($P \leq 0.05$) than the $D_{60°C}$ value in reduced-fat chocolate milk (5.9 min). The pathogen was significantly more resistant to heat when enmeshed in chocolate-peanut spread (a_w 0.46) ($D_{60°C}$ = 37.5 min) and peanut butter (a_w 0.32) ($D_{60°C}$ = 26.0 min) than in liquid products. At 10°C, the pathogen grew most rapidly in whole-fat chocolate milk and slowest in peanut beverage. At 22°C, populations increased significantly within 12 and 16 h in whole-fat milk and reduced-fat chocolate milk, respectively, and within 8 h in whole-fat chocolate milk and peanut beverage. Initial populations (3.37 – 4.42 log cfu/g) of *L. monocytogenes* in chocolate-peanut spread and peanut butter adjusted to a_w 0.33 and 0.65 declined but the pathogen was not eliminated over a 24-week period at 20°C. Survival was enhanced at reduced a_w. Results indicate that a pasteurization process similar to that used for full-fat milk would be adequate to ensure the destruction of *L. monocytogenes* in peanut beverage. The pathogen survives for at least 24 weeks in chocolate-peanut spread and peanut butter at an a_w range encompassing that found in these products.

THE ROLE OF AEROSOL IN TRANSMISSION OF MICROORGANISMS (INCLUDING *LISTERIA*) TO READY-TO-EAT MEAT/POULTRY PRODUCTS. (L. Ma, C. M. Lin, Z. Yan, J. Kornacki, O. Oyarzabal, and M.P. Doyle)

Airborne contamination of *Listeria monocytogenes* in food processing facilities may or may not be an important contributing factor in disseminating *L. monocytogenes* in such facilities. However, aerosol studies in food processing plants have been limited by lack of a suitable surrogate microorganism for *L. monocytogenes*. The objectives of this study were to investigate the potential of using *Jonesia denitrificans* as a surrogate for aerosol studies of *L. monocytogenes* and to study the role of aerosols in the transmission of microorganisms (including *L. monocytogenes*) to ready-to-eat meat/poultry products.

The settling rates of aerosol-borne *J. denitrificans* released into a bioaerosol chamber were determined. Studies revealed that settling rates depend on particle size and relative humidity of the environment. Larger particles settled from the air more rapidly than smaller particles, with 5-µm particles completely settled out of the air within a few minutes of releasing and 0.3-µm particles remaining airborne (<1 $\log_{10}$ reduction) for 4 hours. In most instances, relative humidity (RH) at 40 or 75% had minimal effect on settling rates, although settling rates of *J. denitrificans* were slightly greater at 75% RH than at 40% RH. Overall, *J. denitrificans* had similar settling rates as *L. monocytogenes* (previous studies).

The contamination level of *J. denitrificans* on turkey meat following its aerosolization in the bioaerosol chamber was similar to that of *L. monocytogenes* which was dependent on initial inoculum cell numbers, exposure time, and relative humidity. The greater the number of cells in the aerosol, the greater the number of contaminated turkey samples and the less exposure time for contamination to occur. No turkey samples were *J. denitrificans* or *L. monocytogenes* positive within 4 hours exposure time when the initial cell number was $\leq 2.5x10^2$ or $\leq 1.5x10^2$ cfu/L air, respectively, and all samples were positive within 5 to 30 minutes of exposure when cell inoculum populations were $\geq 3.5x10^5$ cfu/L air. More samples were positive in the 75% RH environment when the inoculum was 10^3 cfu/L air but relative humidity had little influence on the number of contaminated samples for higher or lower levels of inoculum. Both the detectable cell numbers of *J. denitrificans* and *L. monocytogenes* on positive samples of non-cured turkey meat were generally low, ranging from 1 to 12 cfu per three slices. These results suggest that even when relatively large cell numbers are in the aerosols in a room, relatively small numbers contaminate the surface of products during a short exposure.

Releasing *J. denitrificans* at 10^3 cfu/L as an aerosol into a deboning room of a poultry processing pilot facility revealed that the distance from the air conditioning units from which the bacteria were aerosolized influenced the level of *J. denitrificans* contamination that occurred. The greatest degree of contamination occurred at 100 to 150 cm from the air conditioners, and least at 50 and 250 cm from the units. For samples obtained at 100 cm, the greatest average number of *J. denitrificans* on agar media was $2.4x10^2$ cfu/plate, and greatest percentage of meat samples positive at a sampling distance was 40%. Results indicate that releasing as an aerosol at a high population (10^3 cfu/L), *J. denitrificans* can contaminate agar plate and meat surfaces at a range of 250 cm from air conditioning units with the greatest degree of contamination occurring within 100 to 150 cm of an air conditioning unit. Interestingly, swab sampling of environmental surfaces of the deboning room immediately after aerosolizing *J. denitrificans* yielded negative results; indicating *J. denitrificans* is not a good environmental survivor.

FATE OF ACID-ADAPTED AND NONADAPTED *ESCHERICHIA COLI*, *LISTERIA MONOCYTOGENES*, AND *SALMONELLA* ON GROUND OR WHOLE BEEF JERKY (R. A. Morrow, M. A. Harrison, and J. A. Harrison)

The objective of this study was to determine the fate of acid-adapted and nonadapted *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on ground and whole beef jerky strips during the home-style jerky process. Each organism and meat type was compared separately and analyzed using a split-plot experimental design. To achieve acid-adapted and nonadapted cultures, each pathogen was grown in tryptic soy broth with and without dextrose, respectively. After incubation, the pH of the acid-adapted culture was 4.88 and the nonadapted was 6.97. Inoculated strips were dried in a vertical dehydrator with an air temperature of 60.0ºC. For ground beef strips, samples were taken at time 0, 2, 4, 6, and 10 h. After 10 h, population reductions of acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* were 5.86 and 5.30, 4.73 and 3.96, and 4.28, and 4.51 log_{10}, respectively. When population reductions were compared for the same organism, there was no significant difference ($p>0.05$) between acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on ground beef strips. Whole beef strips were sampled after inoculation, after marination, and at 4, 8, 12, and 14 h. Population reductions after 14 h for acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* were 5.25 and 5.13, 4.85 and 4.82, and 4.81 and 4.87 log_{10}, respectively. When population reductions were compared for the same organism, there was no significant difference ($p>0.05$) between acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* on whole beef strips.

LISTERIA MONOCYTOGENES SURVIVAL IN REFRIGERATOR DILL PICKLES (J. Kim, E. M. D'Sa, M. A. Harrison, J. A. Harrison, and E. L. Andress)

Listeria monocytogenes can survive and grow in refrigerated foods with pH levels of approx. 4.0-5.0 and salt concentrations of 3-4%. Refrigerator dill pickles fit this description. Contamination of this product with *L. monocytogenes* could cause serious problems since these items are not heated prior to consumption. This study determined whether *L. monocytogenes* survives and grows in refrigerator dill pickles at three salt levels (1.3, 3.8, and 7.6%). Cucumbers were inoculated with *L. monocytogenes*. Brine mixtures were poured over the cucumbers and they were held at room temperature for one week and then stored under refrigeration for up to 3 months. The pH and percent NaCl and total aerobic, psychrotrophic, lactic acid bacteria, and *Listeria* counts were measured following the addition of brine, at 2, 4, and 7 days, during storage at room temperature, and then later at weekly intervals during refrigerated storage. There was a rapid decrease in pickle pH after four days at room temperature (from 6.2-6.3 to 4.4-4.8) followed by a gradual decrease. The percent NaCl in the pickles increased only slightly while held at room temperature from 0 to 0.101, 0.234, and 0.448% in 1.3, 3.8, and 7.6% salt mixtures, respectively. The initial *Listeria* population was 6-7 log cfu/in^2 on the surface and 4-5 $\log_{10}$ cfu/g internally. There was approximately 1 log increase during fermentation at room temperature followed by a population decline during refrigerated storage, with a greater decrease in the pickles with the highest NaCl content. Populations of total aerobes and lactic acid bacteria increased. Based on old recommendations consumption of refrigerator dill pickles could typically be anytime after 3 days of refrigerated storage. Since *L. monocytogenes* may still be viable well after this point, there is a food safety risk and no recommendations to prepare this product in the home should be distributed.

DEVELOPMENT OF ANIMAL MODELS FOR *LISTERIA MONOCYTOGENES* (N. Mytle, D. Williams, E.A. Irvin, G. A. Anderson, and M. A. Smith)

Many foodborne illnesses are more serious for certain segments of the population such as immunocompromised individuals. At-risk populations include people taking immunosuppressive drugs or people who have illnesses that suppress the immune system. The very young and very old are also more susceptible to certain foodborne pathogens although the mechanisms of their decreased ability to respond to pathogens are poorly understood. *Listeria monocytogenes* is a foodborne pathogen that has serious and oftentimes fatal effects in at-risk populations. Using *L. monocytogenes* as an example, animal models are being examined for their similarities and differences in susceptibility to listeriosis.

Listeriosis during pregnancy frequently results in stillbirths or neonatal illness and death. Using pregnant rhesus monkeys, pregnant guinea pigs or adult mice, dose response information was collected for *L. monocytogenes* using endpoints of stillbirth and fecal shedding for primates and guinea pigs, and mortality for mice. Pregnant rhesus monkeys were sedated and administered *L. monocytogenes* by nasogastric intubation at doses ranging from 10^3 - 10^8 cfu at the beginning of the third trimester. Pregnancies were allowed to continue normally until parturition. Seven out of 33 animals had adverse pregnancy outcomes.

Experiments conducted in our lab using pregnant guinea pigs confirm they are susceptible to *Listeria*-induced stillbirths. Pregnant guinea pigs were fed orally with 10^7 cfu *L. monocytogenes* in whipping cream at about mid-gestation and sacrificed 21 days later. Twenty-eight percent of fecal samples collected from treated guinea pigs were positive for *L. monocytogenes* while samples from control animals were negative. In control animals, 95% of the fetuses were viable compared to 68.4% at 10^6 cfu and 25% in 10^7 cfu treated animals. In animals treated with 10^7 cfu *L. monocytogenes*, 75% of placentas and 67% fetal livers were positive for *L. monocytogenes*.

Normal or immunocompromised ICR female mice were administered *L. monocytogenes* by intraperitoneal injection. LD_{50}s were determined for 3-and 5-days post-treatment. The mouse LD_{50} is 10^5 cfu. Preliminary results from guinea pigs indicate a fetal LD_{50} at approximately 10^6-10^7 cfu. Dose response curves for nonhuman primates indicates 50% fetal mortality at a dose of approximately 10^7 cfu which is similar to FDA's estimated LD_{50} from the human Mexican style cheese outbreak. Thus, when comparing the LD_{50}s of humans, primates and mice after exposure to *L. monocytogenes*, primates and guinea pigs more closely predict the human LD_{50} than mice. Choosing an appropriate animal model is essential to understanding the biological basis for at-risk populations.