

SALMONELLA SPP.

DETECTION OF SALMONELLA SPP. BY PCR IN POULTRY LITTER

(R. Sanchez-Ingunza, M.A. Harrison, R.A. Morrow, and C. Reilly)

Salmonella spp. are important foodborne pathogens in the United States and can be disseminated through the food chain via poultry products, by-products and poultry litter. Poultry litter can be used as a soil amendment. Prompt identification of this microorganism in poultry litter by a reliable and rapid method is important to establish an effective program for controlling *Salmonella*. This study determined the optimal pre-enrichment and enrichment incubation conditions and detection limits for PCR to detect *Salmonella* in poultry litter. The detection capability of PCR was compared to culture methods using 15 *Salmonella enterica* serotypes. As PCR targets, *sdia* and *hila* genes were used. The results were compared by calculating the kappa indexes at 95% CI. The shortest combination times for the pre-enrichment and enrichment steps which led to positive results by PCR were 6 h in 0.1% peptone water and 18 h in Rappaport-Vassiliadis broth, respectively. The calculated detection limit for the PCR tests was 10 *Salmonella* CFU/100 g of litter. The aforementioned time schedule and calculated detection limit were selected for further analysis with the 15 *Salmonella* strains. There was no significant difference in the PCR identification of *Salmonella* when either *sdia* or *hila* were used as targets ($p < 0.0001$). When PCR and the conventional methods were compared, they disagreed and this disagreement was statistically significant ($p < 0.05$). PCR was not able to detect *S. Agona* while *S. Montevideo* and *S. Typhimurium* were not re-isolated by the conventional microbiological method. The results of these experiments suggest that shorter incubation times might lead to false negative results in PCR tests. The analysis of multiple samples at longer incubation times for the enrichment step might increase PCR sensitivity and reduce the disagreement between tests.

SURVIVAL AND GROWTH OF SALMONELLA IN SALSA AND RELATED INGREDIENTS

(L. Ma, G. Zhang, P. Gerner-Smidt, R.V. Tauxe, and M.P. Doyle)

A large outbreak of *Salmonella* Saintpaul infection associated with raw jalapeno peppers, Serrano peppers, and possibly tomatoes was reported in the United States in 2008. During the outbreak, two clusters of illness were significantly associated with eating salsa. Experiments were done to determine the survival and growth characteristics of *Salmonella* in salsa and related major ingredients, i.e., tomatoes, jalapeno peppers, and cilantro. Intact and chopped vegetables and different formulations of salsas were inoculated with a five-strain mixture of *Salmonella* spp. and then stored at 4, 12, and 21°C for up to 7 days. *Salmonella* populations and total aerobic counts were monitored during storage. *Salmonella* did not grow but survived on intact tomatoes and jalapeno peppers, whereas significant growth at 12 and 21°C was observed on intact cilantro. In general, growth of *Salmonella* occurred in all chopped vegetables when stored at 12 and 21°C, with chopped jalapeno peppers being the most supportive of growth. Regardless of differences in salsa formulation, no growth of *Salmonella* (initial inoculation ca. 3 log CFU/g) was observed in salsa when stored at 4°C; however, rapid or gradual decreases in *Salmonella* population was only observed in formulations that contained both fresh garlic and lime juice. *Salmonella* grew at 12 and 21°C in salsas except those formulations that contained both fresh garlic and lime juice, in which salmonellae rapidly or gradually were inactivated depending on formulation. These results highlight the importance of preharvest pathogen contamination control of fresh produce and proper formulation and storage of salsa.

MICROBIOLOGICAL EXAMINATION OF VEGETABLE SEED SPROUTS IN KOREA

(H. Kim, Y. Lee, L. R. Beuchat, and J.-H. Ryu)

Consumption of sprouted vegetable seeds in Korea has been increasing due in part to their nutritional benefits. Since sprouts are often eaten without being heated or cooked, they have occasionally been implicated in foodborne diseases such as in *Escherichia coli* O157:H7 infections and salmonellosis. Sprouts caused at least 37 outbreaks of foodborne disease in several countries between 1995 and 2005. Growth of *Salmonella* and *E. coli* O157:H7 that may be present on seeds or introduced from the environment during sprouting and subsequent

handling may occur. *Enterobacter sakazakii* (*Cronobacter*) is known to grow on several types of fresh-cut fruits and vegetables but its incidence and behavior on seed sprouts have not been described. Studies on the general microbiological quality of soybean sprouts commonly consumed in Korea have been investigated. However, there is limited information on the microbiological quality of other types of seed sprouts. The objective of this study was to determine the general microbial quality and the prevalence of *Salmonella*, *E. coli* O157:H7, and *E. sakazakii* in different types of sprouts and seeds commercially available for the consumers in Korea. We profiled the microbiological quality of sprouts and seeds sold at retail shops in Seoul, Korea. Ninety samples of radish sprouts and mixed sprouts purchased at department stores, supermarkets, and traditional markets, and 96 samples of radish, alfalfa, and turnip seeds purchased from on-line stores were analyzed to determine the number of total aerobic bacteria (TAB) and molds or yeasts (MY) and the incidence of *Salmonella*, *E. coli* O157:H7, and *E. sakazakii*. Significantly higher numbers of TAB (7.52 log CFU/g) and MY (7.36 log CFU/g) were present on mixed sprouts compared to radish sprouts (6.97 and 6.50 CFU/g, respectively). Populations of TAB and MY on the sprouts were not significantly affected by location of purchase. Radish seeds contained TAB and MY populations of 4.08 log CFU/g and 2.42 log CFU/g, respectively, whereas populations of TAB were only 2.54 - 2.84 log CFU/g and MY were 0.82 - 1.69 log CFU/g on alfalfa and turnip seeds, respectively. *Salmonella* and *E. coli* O157:H7 were not detected on any of the sprout and seed samples tested. *E. sakazakii* was not found on seeds but 13.3% of the mixed sprout samples contained this potentially pathogenic bacterium.

REDUCTION OF SALMONELLAE ON PRODUCE AND POULTRY BY ORGANIC ACID PLUS DETERGENT (M.P. Doyle)

A group of organic acids, including lactic acid, acetic acid, and levulinic acid, and sodium dodecyl sulfate (SDS), were evaluated individually or in combination for their ability to inactivate *Salmonella* and *E. coli* O157:H7. Results from pure culture assays in water with the treatment chemical reveal that 0.5% organic acid and 0.05-1% SDS, when used individually, reduced pathogen cell numbers by <2 log CFU/ml within 20 min at 21°C. Combining any of these organic acids at 0.5% with 0.05% SDS resulted in >7 log CFU/ml inactivation of *Salmonella* and *E. coli* O157:H7 within 10 sec at 21°C. A combination of levulinic acid and SDS was evaluated at different concentrations for pathogen reduction on lettuce at 21°C, on poultry (wings and skin) at 8°C, and in water containing chicken feces or feathers at 21°C. Results revealed that treatment of lettuce with a combination of 3% levulinic acid plus 1% SDS, for < 20 sec reduced both *Salmonella* and *E. coli* O157:H7 populations by > 6.7 log CFU/g on lettuce. *Salmonella* and aerobic bacteria populations on chicken wings were reduced by > 5 log CFU/g by treatment with 3% levulinic acid plus 2% SDS, for 1 min. Treating water heavily contaminated with chicken feces with 3% levulinic acid plus 2% SDS, reduced *Salmonella* populations by >7 log CFU/ml within 20 sec. The application of levulinic acid plus SDS as a wash solution may have practical application for killing foodborne enteric pathogens on fresh produce and uncooked poultry.

INACTIVATION OF SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI O157:H7 IN ALFALFA SEEDS BY LEVULINIC ACID AND SODIUM DODECYL SULFATE WASHING TREATMENT

(T. Zhao, P. Zhao, and M.P. Doyle)

Since 1995, raw sprouts have been increasingly implicated as the vehicles of outbreaks of *E. coli* O157:H7 and *Salmonella* nationally and internationally. Most involved alfalfa sprouts, but cress, mung bean, and clover sprouts were also implicated. Thirteen *Salmonella* serotypes were isolated from the clinical cases. Various intervention approaches, including heating and chemical treatments (NaOCl, Ca(OCl)₂, acidified NaClO₂, acidified ClO₂, Na₃PO₄, Vegi-Clean, Tsunami, Vortexx, and H₂O₂) have been evaluated for their efficacy for reduction of *E. coli* O157:H7 contamination in alfalfa seeds. Results revealed that none of the above chemical treatments was able to eliminate or satisfactorily reduce *E. coli* O157:H7 on alfalfa seeds and sprouts. The focus of this research project was to determine the optimum concentration and exposure time of our newly developed chemical solution (levulinic acids plus sodium dodecyl sulfate, SDS) for treatment of alfalfa seeds for reduction of *E. coli* O157:H7 and *Salmonella* and to determine whether this treatment would affect seed germination characteristics. A 5-strain mixture of *E. coli* O157:H7 or *S. Typhimurium* at 10⁸ CFU/g was inoculated onto the alfalfa seeds. The seeds were dried at 21°C in a laminar flow hood for up to 72 h. *E. coli* O157:H7 counts at 4, 24, 48, and 72 h were 8.1, 4.8, 4.0 and 4.0 log CFU/g, respectively; and of *S. Typhimurium* at 4, 24, 48, and 72 h were 6.6, 4.4, 4.3, and 4.1 log CFU/g, respectively. The 0.5% levulinic acid and 0.05% SDS treatment for 5 min

at 21°C reduced *E. coli* O157:H7 and *S. Typhimurium* populations by 5.6, >3.3, >2.4, >2.3; and 6.4, >2.7, 4.3, and 2.4 log CFU/g on seeds contaminated and dried for 4, 24, 48, and 72 h, respectively. However, some samples treated were positive by selective enrichment only. Seeds contaminated with 10⁴ *E. coli* O157:H7 cells/g and dried for 2 h at 21°C in a laminar flow hood were tested with 0.5% levulinic acid plus 0.05% SDS at 40°C for up to 5 min. *E. coli* O157:H7 in samples (25 g) tested at 1, 2, 3, and 5 min of exposure were negative by a direct plating method, but were positive by selective enrichment (<0.7 log CFU/g). All the levulinic acid + SDS used for treatment at 1, 2, 3, and 5 min were negative for *E. coli* O157:H7 by selective enrichment culture.

INACTIVATION OF *SALMONELLA* SPP. AND *E. COLI* O157:H7 ON TOMATOES BY ALLYL ISOTHIOCYANATE, CARVACROL AND CINNAMALDEHYDE IN VAPOR-STATE
(M.M. Obaidat and J.F. Frank)

The activity of various volatile antimicrobials in vapor state was determined. *Salmonella* spp. and *E. coli* O157:H7 on sliced and whole tomatoes were treated in a 120 ml sealed container with various concentrations of allyl isothiocyanate (AIT), cinnamaldehyde and carvacrol in vapor-state, with incubation at 4 and 10°C for up to 10 days and at 25°C for up to 10 h. AIT exhibited the greatest inactivation against the pathogens on sliced and whole tomatoes followed by cinnamaldehyde. The lowest level of AIT (1µl/120 ml volume) inactivated *Salmonellae* on sliced tomatoes by 1.0 and 3.5 log CFU at 4 and 10°C, respectively, in 10 days and by 2.8 log CFU at 25°C in 10 h. This level of AIT inactivated *Salmonellae* on whole tomatoes by 1.5 and 2.2 log CFU at 4 and 10°C, respectively, in 10 days and by 2.8 log CFU at 25°C in 10 h. AIT also inactivated *E. coli* O157:H7 on sliced tomatoes by 4.0 and 3.0 log at 4 and 10°C, respectively, in 10 days with no inactivation at 25°C in 10 h. AIT reduced *E. coli* O157:H7 on whole tomatoes surface by 3.0 and 1.0 log CFU at 4 and 10°C, respectively, in 10 days and by 2.0 log CFU at 25°C in 10 h. Greater inactivation occurred for all treatments at 10 than at 4°C. Pathogens on sliced tomato were not inactivated at 25°C. Antimicrobials in vapor form may be useful for controlling pathogens on fresh tomatoes marketed in packages containing head space.

INACTIVATION OF PATHOGENS IN CHICKEN LITTER COMPOST MIXTURES
(M. C. Erickson, J. Liao, and X. Jiang)

Initial C:N ratios of cow manure compost formulations were found to have a significant effect on survival of *Salmonella* spp. but not *Listeria monocytogenes*. A study was done to determine how C:N ratios influence pathogen survival when chicken litter was used as the manure source. Laboratory-scale bioreactors were used for composting manure mixtures formulated to initial C:N ratios of 20:1, 30:1, and 40:1. The initial C:N ratios had a significant effect on survival of both *Salmonella* spp. and *L. monocytogenes*, with greatest survival in formulations of 40:1 compared to 20:1 or 30:1. Heat was not the contributing factor to differences in pathogen survival as pathogens received slightly less heat in the 20:1 or 30:1 formulations than in 40:1 formulations. More ammonia was produced in the 20:1 and 30:1 formulations than the 40:1 formulations and likely contributed to pathogen inactivation.

INACTIVATION OF PATHOGENS IN ANIMAL MANURE COMPOST SYSTEMS SIMULATING SURFACE CONDITIONS
(M. C. Erickson, C. Smith, and X. Jiang)

Compost mixtures were formulated with wheat straw, cottonseed meal, and different manures (hog, chicken, or cow) to have initial C:N ratios of 20:1, 30:1, or 40:1. The mixtures were placed in trays to simulate responses at surface sites of compost piles and then held at 20, 30, or 40°C. On a weekly basis, moisture levels in samples were adjusted to initial values (30% or 60%). At both 20 and 30°C, *Salmonella* spp. was inactivated more rapidly in compost formulated with hog manure, followed by those formulated with chicken manure and then cow manure. Inactivation of *Salmonella* was greater in chicken compost formulations of 20:1 compared to formulations of 40:1 when held at either 20 or 30°C. The pH of all compost mixtures increased more when moisture contents were initially formulated to 60% compared to 30%. Weekly additions of water to reconstitute the compost samples to initial moisture contents also resulted in higher pH values than samples not reconstituted. Adjustment of moisture to initial levels on a weekly basis had no significant effect on survival of *Salmonella* spp. in compost mixtures. Initial pH of cow manure did influence the subsequent survival of *Salmonella* and *E. coli*

O157:H7 in compost mixtures held at 40°C. Reductions in populations of both pathogens after 4 weeks of storage were only ~2-3-log CFU/g in compost formulations containing cow manure with an initial pH of ~8.5-9.0. In contrast, a 6-log CFU/g reduction occurred within one week for both pathogens when compost formulations contained cow manure with an initial pH of ~7. In these systems, volatile acid concentrations were much higher than in the pH 9 cow manure compost and suggests that these acids may act in concert with heat to inactivate pathogens more rapidly than heat alone.

