

ECOLOGY OF PATHOGENS

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.

SURFACE AND INTERNALIZED *ESCHERICHIA COLI* O157:H7 ON FIELD GROWN SPINACH TREATED WITH SPRAY CONTAMINATED IRRIGATION WATER

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Shiga toxin-producing *Escherichia coli* O157:H7 (STEC) strains have been identified as a cause of serious human gastrointestinal disease accompanied by severe complications, such as bloody diarrhea, hemorrhagic colitis, and the life-threatening hemolytic-uremic syndrome. Contamination of fresh produce by *E. coli* O157:H7 continues to be a problem facing the produce industry. For example, in the 2006 spinach outbreak, STEC contamination led to numerous reports of symptomatic enteritis and three deaths. In this and other outbreaks, several sources of contamination have been identified and include human cross-contamination events during pre- and post-harvest activities, improper composting of plant and animal waste, and direct or indirect fecal contamination from livestock or feral animals. In particular, run-off from contaminated pastures or animal facilities may reach surface waters used for irrigation. In turn, pathogens within the irrigation water may be transferred to produce in the field. Although numerous field studies have demonstrated surface contamination of produce plants in the field, it is unclear whether internalization of the pathogens within the plant tissue occurs. To date, evidence to support this contamination route is based on growth chamber and hydroponic systems. Therefore, this study sought to differentiate the site of *E. coli* O157:H7 contamination when the pathogen was applied to field grown spinach through spray irrigation water.

Four Shiga toxin-negative strains of *E. coli* O157:H7, tagged with a Green fluorescent protein plasmid, were mixed in equal proportions and applied to spinach fields during the mid- (7-weeks post-transplantation) and late-growing season (10-weeks post-transplantation) to give concentrations in irrigation water of either 10² (low-dose), 10⁴ (mid-dose), or 10⁶ (high-dose) CFU/ml. Spray irrigation involved the use of a hand held sprayer held approximately 6 inches from the top of the plant. To five plots containing 64 plants each, 4 L of the appropriately diluted inoculum was applied to the leaf surfaces. To differentiate internalized and surface populations, leaves were treated with a HgCl₂/ethanol disinfectant wash prior to grinding the tissue samples for analysis of the former group. *E. coli* O157:H7 were quantified by direct plate counts or detected through enrichment culture.

Immediately following spray inoculation to the leaf surface, *E. coli* O157:H7 could not be detected through enrichment culture either internally or on the leaf surface of low-dose treated plants. In the case of mid-dose treated plants, *E. coli* O157:H7 was only detected on the surface (4 of 20 samples) whereas for high-dose treated

plants, the pathogen was detected on both the surface (17 of 20) and internally (5 of 20). Seven days post-spraying, all spinach leaves tested negative for surface or internal contamination. Good agricultural practices with regard to irrigation water and the abstention of overhead spray irrigation one week prior to harvest will limit contamination of produce through irrigation water.

EVALUATION OF PATHOGEN INTERNALIZATION WITHIN FIELD GROWN SPINACH EXPOSED TO *ESCHERICHIA COLI* O157:H7 CONTAMINATED DRIP IRRIGATION WATER

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Between 1990 and 2005, produce-associated outbreaks accounted for approximately 13% of all reported foodborne outbreaks with a known food item. Although traceback to the farm has occurred for a number of these produce outbreaks, definitive identification for the mode of contamination remains uncertain, however, both direct and indirect contamination routes are possible. Suspected pre-harvest sources include contaminated manure, manure compost, sewage sludge, irrigation water runoff, water from livestock operations, exposure to waste products from wild and domestic animals, as well as trophic interactions between plants and plant foragers like birds, mammals, and insects. With any of these sources, once introduced into the field, soil may act as a pathogen reservoir and transfer of pathogen to plants may occur through direct contact of aerial tissue with the ground or through rain or irrigated water splashes of soil onto the aerial tissue. Growth chamber and hydroponic system studies, however, have also demonstrated that pathogens in the soil are internalized into the roots of vegetable plants and in some cases translocated to aerial tissues. To investigate whether internalization occurs in field-grown spinach, this study applied *E. coli* O157:H7-contaminated drip irrigation water to soil and evaluated the fate of this pathogen.

Four Shiga toxin-negative strains, tagged with a Green fluorescent protein plasmid, were mixed in equal proportions and applied to spinach fields during the early-, mid-, and late-growing season to give concentrations in irrigation water of either 10^2 (low-dose), 10^4 (mid-dose), or 10^6 (high-dose) CFU/ml. Up to 3-weeks after application of 50 ml of one of the doses to the soil at the base of each plant, soil, leaf, and root samples were collected and *E. coli* O157:H7 quantified by direct plate counts or detected through enrichment culture. To differentiate internalized and surface populations, leaves and roots were treated with an HgCl₂/ethanol disinfectant wash prior to grinding the tissue samples for analysis of the former group.

E. coli O157:H7 persisted in the soil 21, 22, and 7 days following application of contaminated irrigation water to soil in the early-, mid-, and late-growing seasons. Despite persistence in the soil, no internalized *E. coli* O157:H7 was detected in any of the spinach leaves. Internalized *E. coli* O157:H7 was also not detected in root samples collected up to 3-weeks following the early- or late-season exposure. Internalized pathogen was detected in root samples collected 7-days after the mid-season soil exposure. At this sampling time, enrichment of root samples yielded 3 of 10, 1 of 10, and 1 of 10 for the low-, mid-, and high-dose treatments, respectively. One week later when the plants were evaluated on Day 14, the pathogen was no longer detected nor was it detected on Day 22 which was considered the harvest point. These results indicate that internalization events are rare but when they occur, the pathogen does not persist.

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 *Enterobacter 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.

TRANSFER OF *ESCHERICHIA COLI* O157:H7 TO ICEBERG LETTUCE VIA SIMULATED FIELD CORING

(P. J. Taormina, L. R. Beuchat, M. C. Erickson, L. Ma, G. Zhang, and M. P. Doyle)

Between 1995 and 2006, nine outbreaks of *E. coli* O157 infections were attributed to consumption of lettuce or spinach grown in or near the Salinas Valley in California. Investigation of this region led to the conclusion that *E. coli* O157 contamination of growing fields and produce is a dynamic and interrelated process involving transport and distribution of the pathogen via the watershed or possibly other non-water mechanisms. Epidemiologic evidence and traceback investigations indicated that STEC within the Salinas Valley growing region may be transferred in some way to leafy greens during cultivation and/or harvesting. Questions remain as to the mechanism(s) whereby cells of STEC become associated with lettuce in ways that they survive processing and distribution and reach the consumer. A major portion of commercially grown iceberg lettuce is cut and cored in the field. It is thought that soil might be transferred to edible tissues during this practice. Referred to in the industry as "field-coring" or "cut and core," the process involves field workers using hand-held devices consisting of a stainless steel blade, shaft, and cylindrical coring ring to sever lettuce heads from roots and remove the core. Initial stem cuts are made near the soil surface using a wedge-shaped metal blade. The coring ring is then inserted around the stem of the lettuce head to remove the core. Depending on worker accuracy with each event, as well as soil conditions, blades of field-coring devices may contact the contaminated soil and transfer it to lettuce tissues, resulting in cross contamination. Transfer of foodborne pathogens from stainless steel to lettuce can occur to various extents, depending on the amount of water on the leaf surface. The potential for cut tissues of lettuce to become contaminated with the pathogen as a result of physical damage incurred during harvesting merits further study. Specifically, the potential and degree to which *E. coli* O157:H7 can become transferred to cored lettuce during field-coring needs to be determined. We undertook a study to determine the extent of contamination of iceberg lettuce via soil inoculated with *E. coli* O157:H7 by simulating mechanical damage of lettuce tissue resulting from cut-and-core practices used in commercial harvesting operations. The efficacy of chlorinated water treatment for removal or inactivation of *E. coli* O157:H7 on coring blades and cored lettuce was determined. The goal of the study was to provide information identifying various factors associated with contamination and elimination of *E. coli* O157:H7 on field-cored iceberg lettuce. Chlorinated water treatment was evaluated for its efficacy in removing or inactivating *E. coli* O157:H7 on the blade portion of the field-coring device (FCD) and on cored lettuce. FCD inoculated by immersing blades in soil containing *E. coli* O157:H7 at 3.74 or 6.57 log CFU/g contained 3.13 and 4.97 log CFU/blade, respectively. Treatment of inoculated FCD blades by immersing in chlorinated water (200 µg/ml, total chlorine) for 10 s resulted in a reduction of 1.56 log CFU/blade, which was 1.42 log CFU/blade greater than achieved using water, but insufficient to eliminate the pathogen on blades. FCD inoculated by contacting soil containing *E. coli* O157:H7 at 2.72 and 1.67 log CFU/g, then repeatedly used to cut and core ten lettuce heads, transferred the pathogen to ten and five consecutively processed heads, respectively. Lettuce cores remained positive for the pathogen after spraying with 100 µg/ml free chlorine for 120 s at 2.81 kg/cm² (40 psi), regardless of the inoculum level. The number of *E. coli* O157:H7 recovered from inoculated lettuce cores treated for 10 s with chlorine was significantly ($P \leq 0.05$) different than the number recovered from tissues treated with water. Dipping contaminated FCD in chlorinated water may not be effective in killing the pathogen and controlling cross-contamination from head to head. Spraying contaminated lettuce with chlorinated or untreated water reduces but does not eliminate *E. coli* O157:H7.

FATE OF *ESCHERICHIA COLI* O157:H7 ON FRESH AND FRESH-CUT ICEBERG LETTUCE AND SPINACH IN THE PRESENCE OF NORMAL BACKGROUND MICROFLORA

(M. Harrison, W. Hurst, and W. Kerr)

Produce such as lettuce and spinach can become contaminated with foodborne pathogens at numerous points from the field to the retail market. UGA food scientists used the systems approach to determine the fate of *E. coli* O157:H7 in the presence of normal background microorganisms on iceberg lettuce and baby spinach under conditions that mimic actual practices between production and retail sale. While *E. coli* O157:H7 levels decreased on products handled and stored under recommended conditions, survivors persisted. Factors in the system significantly affecting *E. coli* O157:H7 populations from the time iceberg lettuce or baby spinach was harvested to the time products were put into retail bags were field temperature, time before cooling, and wash treatment. Time after cooling until lettuce was bagged was significant. However, for spinach this step was highly insignificant. *E. coli* O157:H7 contamination level on lettuce was not significantly different after vacuum cooling compared to before cooling. On greens packaged and stored at 4°C, *E. coli* O157:H7 contamination was detected, although populations decreased in many cases by at least 1.5 logs.

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.