

## **SALMONELLA**

### **SURVEY OF YEASTS FOR ANTAGONISTIC ACTIVITY AGAINST *SALMONELLA* POONA IN CANTALOUPE JUICE AND WOUNDS IN RINDS CO-INFECTED WITH PHYTOPATHOGENIC MOLDS (G. M. Richards, J. W. Buck, and L. R. Beuchat)**

Biological control is a process by which plant disease is minimized by application of a natural biological process and/or the product of a natural biological process, either preharvest or postharvest. Effective biological control of fungal pathogens by introduction of an antagonist has been reported on apple, apricot, cherry, citrus, grape, nectarine, peach, pear, pepper, persimmon, plum, potato, strawberry, and tomato. A yeast, *Candida oleophila* Montrocher, and a bacterium, *Pseudomonas syringae*, have been used to control postharvest diseases of pome and citrus fruits. Yeasts are the main group of microorganisms being investigated for biocontrol activity because they can colonize the surface of fruits and vegetables for long periods under reduced-moisture conditions, produce extracellular polysaccharides that enhance their survival, restrict colonization sites and flow of germination cues to fungal propagules, and use available nutrients to rapidly proliferate.

Metabiotic associations between phytopathogenic molds and foodborne bacteria on apples and tomatoes have been described. Some species of molds produce proteolytic enzymes that release alkaline by-products into the surrounding tissues that cause localized increase in pH. This may create a more favorable environment for enteric pathogens such as *Salmonella* to survive and grow. The objective of this study was to examine yeasts for their ability to impair survival and growth of *Salmonella* Poona in cantaloupe juice and in wounds on cantaloupe rind co-inoculated with phytopathogenic molds. Changes in the pH of cantaloupe juice and rind tissue, as well as the size of infected surface of and tissues surrounding wounds as affected by co-inoculation with yeasts, molds, and *S. Poona* were determined.

We examined ten yeasts for potential antagonistic activity against survival and growth of *S. Poona* in cantaloupe juice and decay by *Cladosporium cladosporioides* and *Geotrichum candidum* in wounds on cantaloupe rind. Cantaloupe juice was inoculated using five schemes: *S. Poona* only (1.10 log<sub>10</sub> cfu/ml), high (3.93 – 5.21 log<sub>10</sub> cfu/ml) or low populations (1.79 – 3.26 log<sub>10</sub> cfu/ml) of yeasts only, and *S. Poona* combined with high or low populations of yeasts. High initial populations of *Debaryomyces hansenii*, *Pichia guilliermondii*, and *Pseudozyma* sp. were antagonistic to *S. Poona* in cantaloupe juice stored at 20°C for 48 h. Wounds in cantaloupe rinds were inoculated with yeast and mold or yeast, mold, and *S. Poona* and cantaloupes were stored at 4°C for 14 days or 20°C for 7 days. The pH of rind tissue inoculated with *C. cladosporioides* and yeasts increased significantly ( $P \leq 0.05$ ) at 20°C. Wounds that were inoculated with *P. guilliermondii*, together with *C. cladosporioides* or *G. candidum*, did not show mold growth at 4 and 20°C. Populations of *S. Poona* (6.40, 7.26, and 7.98 log<sub>10</sub> cfu/sample) were lower in wounds co-inoculated with *G. candidum* and three of the test yeasts (*D. hansenii*, *P. guilliermondii*, and *Cryptococcus albidus*, respectively) compared to co-inoculation with *G. candidum* or the other seven yeasts. *Candida oleophila* and *Rhodotorula glutinis* showed the most promise in reducing the population of *S. Poona* in wounds in rinds of cantaloupes co-inoculated with *G. candidum* and stored at 4°C.

### **Evaluation of *Salmonella* Reduction in Broilers from Breeders Vaccinated with Live and Killed *Salmonella*: A Field Study (S.D. Young, O. Olusanya, K.H. Jones, T. Liu, K.A. Liljebjelke, and C.L. Hofacre)**

*Salmonella* reduction in broilers from commercial broiler breeders vaccinated with live and killed salmonella vaccines was evaluated. Broiler breeders were vaccinated with Poulvac ST (Fort Dodge, Overland Park, KS) live *Salmonella typhimurium* vaccine at day of age and then repeated at 2 and 6

weeks of age. The breeders were then administered a killed autogenous vaccine, containing *S. kentucky*, *S. heidelberg* and *S. hadar* (Merial, Gainesville, GA), at 10 and 18 weeks of age. Between the ages of 36-52 weeks of age, eggs from the breeder flocks were hatched and progeny were challenged at day of age by oral gavage with either  $1 \times 10^6$  cfu/chick in 4 separate experiments by either *S. kentucky*, *S. heidelberg*, *S. hadar*, or *S. enteritidis* each containing resistance to naladixic acid at 32 µg/ml. At 17-21 days of age, the broilers were sacrificed and one side of the cecum was cultured for *Salmonella* and the other side of the cecum was used for enumeration on positive samples. *Salmonella* was confirmed by O-antisera grouping. This study indicated a difference in *Salmonella* incidence and enumeration between the vaccinated and non-vaccinated breeder groups for certain species. When challenged with serotypes *S. kentucky*, *S. hadar* and *S. heidelberg*, protection was noted with a reduction of 28%, 17%, and 11%, respectively, when compared to the control groups. However, protection was not seen when challenged with *S. enteritidis*. Under the conditions of this study, live and killed vaccination of commercial broiler breeders with *Salmonella* contributes some protection to progeny when challenged at day of age.

#### **EFFICACY OF GASEOUS CHLORINE DIOXIDE AS A SANITIZER FOR KILLING *SALMONELLA*, YEASTS, AND MOLDS ON BLUEBERRIES, STRAWBERRIES, AND RASPBERRIES** (K. V. Sy, K. H. McWatters, and L. R. Beuchat)

Sanitizers such as gaseous chlorine dioxide ( $\text{ClO}_2$ ) have been explored as alternatives to aqueous chemicals for sanitizing fruits and vegetables eaten raw. Gaseous  $\text{ClO}_2$  has some advantages over chlorinated water in that it can break down phenolic compounds and remove phenolic tastes and odors from the water, does not react with ammonia, and has 2.5 times the oxidation capacity of chlorine. The bactericidal efficacy of gaseous  $\text{ClO}_2$  is also not markedly affected by pH as well as it has greater sporicidal activity. Several studies have shown gaseous  $\text{ClO}_2$  to be effective in killing enteric pathogens on several fruits and vegetables. However, the efficacy of gaseous  $\text{ClO}_2$  gas in killing or removing *Salmonella*, yeasts, and molds on small fruits has not been reported. The objective of this study was to evaluate gaseous  $\text{ClO}_2$  for its effectiveness in killing *Salmonella* inoculated onto the surface of blueberries, strawberries, and red raspberries. Inactivation of yeasts and molds naturally occurring on the fruits was also determined.

An inoculum (100 µl, 6.0 - 6.8  $\log_{10}$  cfu/g of fruit) containing five serotypes of *Salmonella enterica* was deposited on the skin, calyx tissue, or stem scar tissue of blueberries, skin or stem scar tissue of strawberries, and skin of red raspberries, dried for 2 h at 22°C, then held for 20 h at 4°C and 2 h at 22°C before treatment. Sachets containing reactant chemicals were formulated to release gaseous  $\text{ClO}_2$  at concentrations of 4.1, 6.2, and 8.0 mg/L of air within treatment times of 30, 60, and 120 min, respectively, at 23±1°C. Treatment with 8.0 mg of  $\text{ClO}_2$ /L significantly ( $\alpha = 0.05$ ) reduced the population of *Salmonella* on blueberries by 2.4 - 3.7  $\log_{10}$  cfu/g. Lethality was higher to cells in inoculum placed on the skin, compared to the stem scar tissue. Populations of *Salmonella* on strawberries treated with 8.0 mg of  $\text{ClO}_2$ /L were reduced by 3.8 - 4.4  $\log_{10}$  cfu/g. A significant reduction of 1.5  $\log_{10}$  cfu/g of raspberries was also achieved. Treatment with 4.1 - 8.0 mg of  $\text{ClO}_2$ /L caused reductions in populations of yeast and molds on blueberries, strawberries, and raspberries of 1.4 - 2.5, 1.4 - 4.2, and 2.6 - 3.0  $\log_{10}$  cfu/g, respectively. Lethality of  $\text{ClO}_2$  to *Salmonella*, yeasts, and molds was higher when fruits were treated at 75 - 90% relative humidity than at lower relative humidity. Treatment with 4.1 mg/L  $\text{ClO}_2$  did not markedly affect the sensory quality of fruits stored for up to 10 days at 8°C. Results indicate that gaseous  $\text{ClO}_2$  has promise as a sanitizer for small fruits.

#### **EFFECTIVENESS OF CLEANERS AND SANITIZERS IN KILLING *SALMONELLA* NEWPORT IN THE GUT OF A FREE-LIVING NEMATODE, *CAENORHABDITIS ELEGANS*** (S. J. Kenney, G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat)

Large microbial populations in the soil matrices, such as those amended with manure, have been reported to attract free-living nematodes. *Caenorhabditis elegans*, a free-living, microbivorous nematode found in the soil of temperate regions, has been reported to ingest *Escherichia coli* O157:H7, *Listeria monocytogenes*, several serotypes of *Salmonella enterica*, *Bacillus cereus*, and *Staphylococcus aureus*. Depending on environmental conditions, bacteria may persist within the gut of *C. elegans* for several days after consumption. Release of pathogens as a result of rupturing of the cuticle or defecation are ways that infected nematodes can contaminate the soil environment. Commercial cleaners and sanitizers used by the produce industry may contain surfactants to aid in the release of microorganisms, and perhaps also nematodes, from the surface of produce. Nematodes may subsequently become resident in or on produce contact areas such as water baths, belts, tables, and sorters in processing facilities. Pathogens from a single worm released onto processing equipment could theoretically release ingested cells of a pathogen and contaminate large amounts of produce. The plausibility of this series of events happening on a commercial level can be more easily assessed if the effectiveness of cleaners and sanitizers in killing pathogens ingested by nematodes is known. A study was undertaken to determine the effectiveness of two commercial cleaners and four sanitizers in killing *E. coli* OP50 and *S. Newport* in the gut of *C. elegans*. The effectiveness of these treatments in killing planktonic cells of *E. coli* OP50 and *Salmonella Newport* was also evaluated.

The efficacy of cleaners and sanitizers in killing *S. Newport* in the gut of *C. elegans* was studied. Adult worms were fed nalidixic acid-adapted cells of *E. coli* OP50 (control) or *S. Newport* for 24 h, washed, placed on paper discs, and incubated at 4 or 20°C and relative humidities of 33 or 98% for 24 h. Two commercial cleaners (Enforce<sup>®</sup> and K Foam Lo<sup>®</sup>) and four sanitizers (2% acetic acid, 2% lactic acid, Sanova<sup>®</sup>, and chlorine [50 and 200 µg/ml]) were applied to worms for 0, 2, or 10 min. Populations of *E. coli* and *S. Newport* (cfu/worm) in untreated and treated worms were determined by sonicating worms in 0.1% peptone and surface plating suspensions of released cells on tryptic soy agar containing nalidixic acid. Populations of *S. Newport* in worms exposed to 33 or 98% relative humidity at 4°C or 33% relative humidity at 20°C were significantly ( $P \leq 0.05$ ) lower than the number surviving exposure to 98% relative humidity at 20°C. In general, treatment of desiccated worms with cleaners and sanitizers was effective in significantly ( $P \leq 0.05$ ) reducing the number of ingested *S. Newport*. Results indicate that temperature and relative humidity influence the survival of *S. Newport* in the gut of *C. elegans*, and cleaners and sanitizers may not eliminate the pathogen.

#### **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 60.0°C. 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<sub>10</sub>, 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<sub>10</sub>, 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.

**MIGRATION OF *CAENORHABDITIS ELEGANS* TO MANURE AND MANURE COMPOST AND POTENTIAL VECTORING OF *SALMONELLA* NEWPORT TO FRUITS AND VEGETABLES** (S. J. Kenney, G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat)

It is not uncommon for animal manure and manure compost to be applied to cropland soil as fertilizers. The application of manure and manure compost to soil may attract nematodes that feed on bacteria. Free-living, microbivorous nematode populations have been reported to increase in soils to which cattle manure slurry has been applied. The extent to which various types of manure and manure composts are incorporated into the soil can influence populations of nematodes. Sand homogeneously amended with a humus-litter mixture has been reported to support higher populations of *Caenorhabditis elegans* compared to sand containing isolated patches of the humus-litter mixture. It is hypothesized that free-living nematodes such as *C. elegans* and possibly other genera may ingest human pathogens occasionally found in the soil and transport them through the soil matrix. As a worm migrates through soil it may come in contact with external tissues of plants, either by attraction mechanisms or by random chance. A study was undertaken to determine if *C. elegans* is attracted to bovine manure, turkey manure, composted bovine manure, composted turkey manure, and manure-amended soil inoculated with *Salmonella* Newport. Survival and reproduction of *C. elegans* in the same matrices not inoculated with *S. Newport* were investigated. Movement of *C. elegans* to lettuce, strawberries, and carrots on an agar medium and the ability of the nematode to transport *S. Newport* in soil to the surface of produce were also studied.

*C. elegans* moved most rapidly to turkey manure and strawberries, with 35% and 60% of worms, respectively, associating with samples within 30 min. Survival and reproduction of *C. elegans* in test materials was not affected by the presence of *S. Newport*. Bovine manure and bovine manure compost inoculated with *S. enterica* serotype Newport ( $8.6 \log_{10}$  cfu/g) were separately placed in the bottom of a glass jar and covered with a layer of soil (5 cm) inoculated (50 worms/g) or not inoculated with *C. elegans*. A piece of lettuce, strawberry, or carrot was placed on top of the soil before jars were sealed and held at 20°C for up to 10 days. In the system using soil inoculated with *C. elegans*, *S. Newport* initially in bovine manure was detected on the surface of lettuce, strawberry, and carrot samples within 3, 1, and 1 days, respectively. The pathogen was detected on lettuce, strawberry, and carrot within 1, 7, and 1 days, respectively, when initially present in bovine manure compost. With one exception, the pathogen was not detected on the produce over the 10-day incubation period when *C. elegans* was not present in the soil. Results indicate that *C. elegans* has the potential for transporting pathogens in soil to the surface of preharvest fruits and vegetables in contact with soil.

**INFECTION OF CANTALOUPE RIND WITH *CLADOSPORIUM CLADOSPORIODES* AND *PENICILLIUM EXPANSUM*, AND ASSOCIATED MIGRATION OF *SALMONELLA* POONA INTO EDIBLE TISSUES** (G. M. Richards and L. R. Beuchat)

Cantaloupe fruits are often in contact with the ground during their development, enhancing the potential for contamination by microorganisms capable of causing human diseases. They are susceptible to postharvest fungal rots, especially under warm, wet conditions. Complete loss of the commodity occurs when one or a few fungal pathogens invade and begin to breakdown the tissues. We undertook a study to determine if the growth of two molds known to cause decay of cantaloupes, *Cladosporium cladosporioides* and *Penicillium expansum*, in wounds on rinds facilitate migration of *Salmonella* Poona into sub-surface mesocarp tissues.

Two phytopathogens, *Cladosporium cladosporioides* and *Penicillium expansum*, in wounds on cantaloupe rinds, were studied to assess their potential to facilitate migration of *S. Poona* into sub-surface

mesocarp tissues. Wounded sites in cantaloupe rind were inoculated with *S. Poona* only, *S. Poona* and mold simultaneously, or mold followed by *S. Poona* 3 days later. A cylindrical plug (ca. 3 cm diameter and 4 cm deep) of inoculated tissue extending from the rind surface into edible tissues was removed and cut transversely into four segments (0 – 1, 1 – 2, 2 – 3, and 3 – 4 cm) representing distances from the rind surface. Regardless of the type of inoculum or the time of storage subsequent to inoculation, the pH of the tissues was significantly higher ( $P \leq 0.05$ ) as the distance from the rind surface increased. Test microorganisms and naturally-occurring microorganisms on the rind surface which were introduced into internal tissues during wounding, as well as physiological changes in cantaloupe tissue, contributed to these changes. *C. cladosporioides* and *P. expansum* were recovered from the inoculated rind and underlying tissues throughout storage at 20°C for 10 days. *S. Poona* persisted and grew in wounds on rinds on inoculated cantaloupe incubated at 20°C. Recovery of *S. Poona* from tissues 3 – 4 cm below the inoculated wound supports the hypothesis that it can migrate from the site of inoculation into adjacent mesocarp tissues. Survival and migration of *S. Poona* into the internal tissues of cantaloupes were enhanced by co-inoculation with *C. cladosporioides* and, to a lesser extent, *P. expansum*. Consumption of cantaloupes from which diseased tissue has been removed is not advisable because *S. Poona* and perhaps other enteric pathogens may still be present in remaining tissues.

#### **METABIOTIC ASSOCIATIONS OF MOLDS AND *SALMONELLA* POONA ON INTACT AND WOUNDED CANTALOUPE RIND (G. M. Richards and L. R. Beuchat)**

Several national and international outbreaks of salmonellosis have been epidemiologically linked to consumption of fresh cantaloupes. Cantaloupe fruits may be in direct contact with the ground during their development on long, running, non-climbing vines that are prostrate on the soil. The growth habit of cantaloupes enhances the potential for fruits to be contaminated by pathogens that may be present in the soil. Postharvest handling may also bring cantaloupes in direct contact with various sources of foodborne pathogens. Mesocarp tissues of fruits are particularly subject to contamination when rind surface integrity is compromised by disease, bruising, cutting, or peeling. Infection of cantaloupes by plant pathogenic fungi and contamination with foodborne pathogenic bacteria may occur before harvesting, at the time harvest, during handling, storage, transport, and marketing, or after purchase by the consumer. The behavior of foodborne pathogens such as *Salmonella* on or in cantaloupes as affected by metabiotic activities of plant pathogens has not been investigated. The objective of this study was to examine the association between selected molds pathogenic to cantaloupes and *Salmonella* Poona on the surface of intact rind and in wounds in the rind. Changes in pH caused by growth of molds were monitored, as were survival and growth of *S. Poona* in co-infected tissue as affected by temperature.

We tested proteolytic activity and measured changes in the pH of cantaloupe rind caused by growth of *Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Geotrichum candidum*, and *Penicillium expansum*. Survival and growth characteristics of *S. Poona* co-infected with each mold on the surface rind and in wounded rind tissue as affected by temperature were determined. *C. cladosporioides*, *G. candidum*, and *P. expansum*, but not *A. alternata* and *E. nigrum*, showed proteolytic activity on agar media containing gelatin and/or casein, with concurrent increases in pH, thus favoring survival and growth of salmonellae. Intact and mechanically wounded tissues of cantaloupe rinds were inoculated with a five-strain mixture of *S. Poona* and/or test mold. Five inoculation schemes were used: mold only, *S. Poona* only, mold and *S. Poona* simultaneously, mold then *S. Poona* 3 days later, and *S. Poona* then mold 3 days later. The pH of cantaloupe rinds inoculated with molds and stored at 20°C for 14 days was significantly higher ( $P \leq 0.05$ ) than on day 0. Only the pH of rinds inoculated with *C. cladosporioides* or *G. candidum* was significantly higher ( $P \leq 0.05$ ) on day 21 than on day 0, when cantaloupes were stored at 4°C. An initial population of *S. Poona* increased from  $3.3 \log_{10}$  cfu/sample (ca.  $7 \text{ cm}^2$ ) of cantaloupe rind to populations as high as  $9.5 \log_{10}$  cfu/sample during storage at 20°C for up to 14 days, regardless of co-inoculation with molds. Populations of *S. Poona* decreased or remained constant at 4°C for up to 21 days.

Results demonstrate that persistence and growth of *S. Poona* on intact, wounded, and decaying cantaloupe rind is not affected by the presence of molds.

**PERSISTENCE OF *ESCHERICHIA COLI* O157:H7, *SALMONELLA* NEWPORT, AND *SALMONELLA* POONA IN THE GUT OF A FREE-LIVING NEMATODE, *CAENORHABDITIS ELEGANS*, AND TRANSMISSION TO PROGENY AND UNINFECTED NEMATODES** (S. J. Kenney, G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat)

Free-living, bacterivorous nematodes are attracted to areas in soil in which large populations of bacteria are present, so their presence on produce grown in these soils would be likely. *Caenorhabditis elegans* has been reported to feed on human pathogenic bacteria such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Poona, *Salmonella* Typhimurium, *Bacillus cereus*, and *Staphylococcus aureus*, as well as on *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Ingestion of *S. Typhimurium*, *S. aureus*, and *P. aeruginosa* shortens the life span of *C. elegans*. We hypothesized that free-living nematodes may ingest human enteric pathogens present in soil matrices and harbor them in their gut. Ingested pathogens may then remain in the gut and be protected against environmental stresses imposed by desiccation or sanitizers used to decontaminate raw fruits and vegetables, even after the worm has died. A preliminary objective of this study was to confirm that *C. elegans* ingests *E. coli* O157:H7 and salmonellae. Major objectives were to determine persistence characteristics of the pathogens in the gut after ingestion, the effects of temperature and relative humidity on survival and growth of ingested cells, and transmission of ingested *Salmonella enterica* serotype Newport to adult progeny of *C. elegans* and to uninfected worms.

Worms were fed cells of a non-pathogenic strain of *E. coli* (OP50), *E. coli* O157:H7, *Sand s.* Newport, and *Salmonella* Poona, followed by incubating at 4, 20, or 37°C for up to 5 days. Initial populations of ingested pathogens significantly increased by up to 2.93 log<sub>10</sub> cfu/worm within 1 day at 20°C on K agar and remained constant for an additional 4 days. When worms were placed on Bacto agar, populations of ingested pathogens remained constant at 4°C, decreased significantly at 20°C, and increased significantly at 37°C within 3 days. Worms fed *E. coli* OP50 or *S. Newport* were incubated at 4 or 20°C at relative humidities of 33, 75, or 98% to determine survival characteristics of ingested bacteria. Fewer cells of the pathogens survived incubation at 33% relative humidity compared to higher relative humidities. Populations of ingested *E. coli* OP50 and *S. Newport* decreased by up to 1.65 and 3.44 log<sub>10</sub> cfu/worm, respectively, in worms incubated at 20°C and 33% relative humidity. Placement together on K agar of adult worms, labeled with green fluorescent protein (gfp) in the pharynx area, that had ingested gfp-labeled *S. Newport* and uninfected wild type worms resulted in transfer of the pathogen to gut of wild type worms. *S. Newport* was isolated from *C. elegans* two generations removed from exposure to the pathogen. Results of these studies show that *C. elegans* may serve as a temporary reservoir of foodborne pathogens, and could perhaps be a vector for contaminating preharvest fruits and vegetables, thus potentially increasing the risk of enteric infections associated with consumption of raw produce.

**FOLLOWING DRUG-RESISTANT *SALMONELLA* THRU THE FOOD CHAIN: A MOLECULAR ECOLOGY APPROACH.** (J.J. Maurer)

Antibiotic resistance has become an important food safety issue with the emergence of multi-drug resistant *Salmonella enterica* Typhimurium and Newport. There are several factors that impact on the emergence and maintenance of multi-drug resistance including: 1) antibiotic resistance gene reservoir; and 2) fitness cost. We collected poultry litter from commercial broiler chicken houses and isolated gram-negatives and *Salmonella*. Microbial DNA was isolated from poultry litter and integron associated antibiotic resistance genes were quantified. Poultry litter microflora was used to reconstitute the microflora of one day-old, commercial broiler chickens colonized with *S. ser* Typhimurium Nal<sup>r</sup>, Rif<sup>r</sup> T1 strain ± integron. *Salmonella* and antibiotic resistant gram-negatives were enumerated from poultry litter

by plating on MacConkey agar supplemented with various antibiotics. *Salmonella* isolated from bird pens were genetically typed by PFGE and screened for several antibiotic resistance genes. Antimicrobial susceptibilities were determined by standard, NCCLS approved methods. There was a significantly large reservoir of integron-associated antibiotic resistance genes in poultry litter, including gram-positive bacteria. Despite this large gene load, there was significant disparity in antibiotic resistance phenotype/genotype of litter *Enterobacteriaceae*. In birds experimentally colonized with *Salmonella*, a greater diversity of antibiotic resistance phenotypes/genotypes in *Salmonella* were associated with birds challenged with integron-positive *S. enterica* T1 strain. However, this *S. ser.* Typhimurium T1 strain lost its resident class 1 integron and was present in poultry litter at levels 2-3 logs less than integron-minus, T1 strain. There is a significantly large reservoir of integron-associated drug resistance genes in the poultry environment and ample opportunities for *Salmonella* to acquire these resistance genes. However, antibiotic resistance carriage may severely impact on *Salmonella*'s fitness in this environment.

