

PREVENTATIVE TREATMENTS

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 log₁₀ cfu/ml), high (3.93 – 5.21 log₁₀ cfu/ml) or low populations (1.79 – 3.26 log₁₀ 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 log₁₀ 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 1×10^6 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 $\mu\text{g/ml}$. 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.

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/cm² for drain #1, from 4.2 to 5.4 \log_{10} cfu/cm² for drain #3, from 3.4 to 4.5 \log_{10} cfu/cm² for drain #4, from 3.2 to 4.2 \log_{10} cfu/cm² for drain #6, and from 6.1 to 8.2 \log_{10} cfu/cm² 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/cm²) 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} /cm² for drain #8. Results indicate that application of these two CE bacteria can greatly reduce *Listeria* cell numbers in floor drains at 2 to 30°C in a poultry processing facility.

TREATMENTS FOR CONTROL OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* IN DRINKING WATER CONTAMINATED WITH RUMEN CONTENT OR FECES (P. Zhao, M. P. Doyle, T. Zhao, J. W. West, J. Bernard, and H. Cross)

E. coli O157:H7 has emerged in the last 10 years as an important foodborne pathogen with an estimated 73,000 cases annually in the U.S. Cattle are the major reservoir and studies revealed that when present in cattle drinking water, *E. coli* O157:H7 was disseminated to other cattle using the contaminated water source. Hence, drinking water for cattle is an important vehicle of *E. coli* O157:H7 transmission. Studies indicate that once contaminated in the drinking water of a cattle farm, *E. coli* O157:H7 can survive for many months.

A variety of treatments have been evaluated for their efficacy in killing *E. coli* O157:H7 in drinking water contaminated with rumen content or cattle feces. Results revealed that most had minimal effect on killing *E. coli* O157:H7 because these treatments were neutralized by organic materials present in the rumen content or feces. The objective of this study was to identify practical treatments to eliminate or control *E. coli* O157:H7 in drinking water simulating on-farm conditions.

Survival of *E. coli* O157:H7 in water containing rumen content at different water:rumen content, *E. coli* O157:H7 cell numbers, and temperatures was determined. At 21°C, *E. coli* O157:H7 inoculated at a high inoculum ($10^{5.8}$ cfu/ml) survived for 8, 15, 23, >56 and 24 weeks and at a low inoculum ($10^{2.9}$ cfu/ml) survived for 8, 11, 10, 11 and 10 weeks at a water:rumen content ratio of 5:1, 10:1, 25:1, 50:1 and 100:1, respectively.

Different treatments, including lactic acid, acidic calcium sulfate, chlorine, chlorine dioxide, hydrogen peroxide, caprylic acid, ozone, butyric acid, sodium benzoate and competitive inhibition *E. coli* were tested individually or in combination for inactivation of *E. coli* O157:H7 in the presence of rumen content. Chlorine (5 ppm) and ozone treatment (22-24 ppm at 5°C or 8-12 ppm at 21°C) of water had minimal effect on killing *E. coli* O157:H7 in the presence of rumen content at ratios of 50:1 and higher. Treatment by competitive inhibition *E. coli* in water with rumen content also had minimal effect on *E. coli* O157:H7 counts compared with untreated controls. Four chemical treatment combinations including: (a) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.05% caprylic acid (Treatment A); b) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.1% sodium benzoate (Treatment B); (c) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.5% butyric acid (Treatment C); (d) 0.1% lactic acid, 0.9% acidic calcium sulfate and 100 ppm chlorine dioxide (Treatment D) were highly effective at 21°C in killing *E. coli* O157:H7, O26:H11 and O111:NM/ml in water heavily contaminated with rumen content (ratio of 10:1 water:rumen content, v/w) or feces (ratio of 20:1, water:feces, v/w). Among them, Treatments A, B and C killed >5 \log_{10} *E. coli* O157:H7, O26:H11 and O111:NM/ml within 30 min in water containing rumen content. For Treatment D, *E. coli* O157:H7, O26:H11, and O111:NM were reduced within 30 min by 2.8, 4.3, and 3.2 \log cfu/ml in water containing rumen content, respectively, and by 3.5, 4.9, and 4.6 \log cfu/ml in water with feces, respectively.

Cattle fed ad libitum water containing Treatment A, C, or control (untreated water) for two treatment periods at 7-day increments drank an average of 15.2, 13.8, and 30.3 L/day, respectively. Cattle provided

water containing 0.1% lactic acid plus 0.9% acidic calcium sulfate (pH 2.1) drank 18.6 L/day. The amount of water consumed for all water treatments was significantly different from the control, and there were no significant differences among water treatments. The covariant was significant, but there were no differences among cow groups or between the two treatment periods. This implies that the covariant effectively removed variation among animals from the statistical analysis, that the randomly assigned groups were similar, and that the treatment effect was consistent between the two experimental periods. To ensure that treatment effects on water intake were not due to differences in cow body size, cow body weight (BW) was converted to MBW ($BW^{0.75}$), and intake of water per MBW was calculated. Treatment effects for water intake/MBW were similar to those for total water intake. Because water intake was substantially reduced when treated with the chemicals described above, optimal on-farm use of such treatments would be periodic, rather than continuous. In addition, application of chemicals to drinking water systems followed by flushing to remove or dilute the chemicals after 30 minutes of exposure is recommended.

