

## YEAST/MOLDS

### **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.

### **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 (ClO<sub>2</sub>) have been explored as alternatives to aqueous chemicals for sanitizing fruits and vegetables eaten raw. Gaseous ClO<sub>2</sub> 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 ClO<sub>2</sub> is also not markedly affected by pH as well as it has greater sporicidal activity. Several studies have shown gaseous ClO<sub>2</sub> to be effective in killing enteric pathogens on several fruits and vegetables. However, the efficacy of gaseous ClO<sub>2</sub> 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 ClO<sub>2</sub> 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<sub>10</sub> 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 ClO<sub>2</sub> 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 ClO<sub>2</sub>/L significantly ( $\alpha = 0.05$ ) reduced the population of *Salmonella* on blueberries by 2.4 - 3.7 log<sub>10</sub> 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 ClO<sub>2</sub>/L were reduced by 3.8 - 4.4 log<sub>10</sub> cfu/g. A significant reduction of 1.5 log<sub>10</sub> cfu/g of raspberries was also achieved. Treatment with 4.1 - 8.0 mg of ClO<sub>2</sub>/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<sub>10</sub> cfu/g, respectively. Lethality of ClO<sub>2</sub> 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 ClO<sub>2</sub> did not markedly affect the sensory quality of fruits stored for up to 10 days at 8°C. Results indicate that gaseous ClO<sub>2</sub> has promise as a sanitizer for small fruits.

#### **INFECTION OF CANTALOUPE RIND WITH *CLADOSPORIUM CLADOSPORIOIDES* 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<sub>10</sub> cfu/sample (ca. 7 cm<sup>2</sup>) of cantaloupe rind to populations as high as 9.5 log<sub>10</sub> 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.

#### **AN INTER-LABORATORY EVALUATION OF SELECTIVE MEDIA FOR THE DETECTION AND ENUMERATION OF YEASTS IN BLUE-VEINED CHEESE (B. C. Viljoen, A. Knox, L. R. Beuchat, T. Deak, M. Malfeito-Ferreira, T. K. Hansen, A. Hugo, M. Jakobsen, V. Loureiro, A. Lourens-Hattingh, and R. Vasdinnyei)**

Mold ripened blue-veined cheeses are produced from pasteurized cow milk to which lactic acid bacterial starters and a selected *Penicillium* species as a secondary starter culture have been added. Yeasts are not traditionally added as part of the starter culture or as adjunct starter cultures, but are often reported to develop as natural contaminants to high numbers (>10<sup>6</sup> cfu/g) in these cheeses. Since the survival of the yeast species and their interaction with other microorganisms are governed by unique environmental factors, it is imperative to select suitable media for surveying the yeast development during

processing and maturation, and for strain isolation purposes. We conducted an inter-laboratory study to determine the performance of mycological media for enumerating yeasts in blue-veined cheese.

Five laboratories in five countries evaluated 11 different selective media, designed to suppress mold and bacterial growth and support yeasts growth, for the recovery of yeast populations from blue veined cheeses. In addition, qualitative results were also incorporated. The yeast enumeration values were subjected to statistical analysis using analysis of variance (ANOVA) and the Tukey-Kramer multiple comparison test. With the exception of one laboratory, laboratories were unsuccessful in recovering yeasts on all the media. Six of the media proved inadequate for the enumeration of yeasts in a mold invested environment and were therefore omitted from statistical analysis. No significant differences in quantitative data obtained on Rose-Bengal Chloramphenicol Agar (RBCA), Dichloran Rose-Bengal Chloramphenicol Agar (DRBC), Dichloran 18% Glycerol Agar (DG18), and Malt extract agar supplemented with NaCl and oxytetracycline (MES) were detected by four of the collaborating laboratories whereas one laboratory found RBCA to be superior for yeast enumeration. DG18 and Malt Extract Agar with Biphenyl (MEB), however, were ranked superior based on qualitative results compared to the other media, attributed to distinctive individual yeast colonies and mold inhibition. RBCA, DRBC, DG18, and MES on the other hand, all proved to be adequate in supporting yeast colony development for quantitative analysis in samples obtained from blue veined cheeses.

