

## YEASTS/MOLDS

### PROTEOLYTIC YEASTS ISOLATED FROM RAW, RIPE TOMATOES AND METABIOTIC ASSOCIATION OF *GEOTRICHUM CANDIDUM* WITH *SALMONELLA* (W. N. Wade, R. Vasdinnyi, T. Deak, and L. R. Beuchat)

Post-harvest decay of tomatoes can be caused by several molds, but the *Alternaria*, *Botrytis*, *Cladosporium*, *Fusarium*, *Rhizoctonia*, and *Rhizopus* species are most commonly involved. Yeasts and yeast-like organisms such as *Geotrichum candidum*, which causes sour rot of tomatoes, also contribute to post-harvest losses. Metabiotic associations between molds and bacteria capable of causing human diseases are a public health concern. Growth of *Alternaria* and *Fusarium* in fresh tomatoes has been reported to increase the pH of tissues to values as high as 8, allowing *Clostridium botulinum* to grow and produce toxin. The pH of tomato juice has been shown to increase from 4.1 to greater than 9.0 when inoculated with molds. *Aspergillus gracilis* and species of *Penicillium* and *Cladosporium* have been reported to grow in tomato juice and increase the pH to levels supporting toxin production by *C. botulinum*. We have observed that *Alternaria alternata* and *Cladosporium* species co-inoculated with *Salmonella* into raw ripe tomatoes increase the pH of pulp, resulting in enhancement of the rate of growth of the pathogen. Food spoilage yeasts are infrequently examined for proteolytic activity, although some species known to grow in a wide range of foods can cause significant proteolysis. Several genera, including *Aureobasidium*, *Candida*, *Endomycopsis*, *Kluyveromyces*, and numerous sporobolomycetes exhibit proteolytic activity. Highly proteolytic *Candida* species have been isolated from ripe amapa fruit and yeasts known to have proteolytic activity grow well in guava and tomato fruits. In results from investigations of the extracellular enzymatic activity profiles of yeast and yeast-like strains isolated from tropical environments, 7 of 196 (3.6%) strains of ascomycetes and 48 of 155 (31%) strains of basidiomycetes exhibited protease activity.

Several outbreaks of salmonellosis associated with consuming raw tomatoes have been documented. Environmental and ecological factors that may affect the survival and growth of *Salmonella* in uncooked tomatoes have not been clearly defined, although pre- and post-harvest infection with yeasts and molds may play a role. Metabiotic associations between yeasts and foodborne bacterial pathogens that may occasionally occur as incidental contaminants on raw tomatoes have not been described. We did a survey of raw, ripe, decayed, or damaged tomatoes to determine the presence of proteolytic yeasts. We then studied the survival and growth of *Salmonella* co-inoculated with a proteolytic strain of *G. candidum* into raw ripe tomatoes. Sixty-two of the 371 (16.7%) fungi isolated from 215 decayed or damaged tomatoes and 12 of the 62 (19.4%) yeasts showed proteolytic activity on gelatin agar and/or standard methods caseinate agar. The pH of tomato pericarp (pulp) tissue from which 9 or the 12 yeasts were isolated ranged from 4.3 to 7.5 (mean = 5.3) compared to 4.2 - 5.1 (mean 4.8) for sound pulp tissue in the same tomatoes. The 12 proteolytic yeasts consisted of four strains of *Cryptococcus albidus*, two strains each of *Debaryomyces hansenii* and *Trichosporon pullulans*, and one strain each of *Cryptococcus humicolus*, *Cryptococcus laurentii*, *Geotrichum candidum*, and *Sporidiobolus pararoseus*. Survival and growth characteristics of a five-serotype mixture of *Salmonella* co-inoculated with *G. candidum* into sound (not chill injured) and chill-injured tomatoes were studied. Storage of sound tomatoes at 15°C for 10 days resulted in an increase in population of 7.6 log<sub>10</sub> cfu of *Salmonella*/g of a 2-g sample of co-infected pulp tissue. Increases were less in tissue inoculated with *Salmonella* only, *Salmonella* on day 0 followed by *G. candidum* on day 3, or *G. candidum* on day 3, or *G. candidum* on day 0 followed by *Salmonella* on day 3. Trends were similar in sound inoculated tomatoes stored at 25°C. Growth of *Salmonella* was enhanced in chill-injured tomatoes compared to sound tomatoes; a population of 10 log<sub>10</sub> cfu/g of chill-injured pulp tissue was reached within 10 days at 25°C. Results clearly show that growth of a proteolytic, alkalinizing yeast such as *G. candidum* in raw tomatoes enhances conditions for growth of *Salmonella*. The removal of tomatoes infected with proteolytic yeasts and other fungi from lots intended for minimally processed tomato products is an essential step in reducing the risk of human diseases caused by pathogenic bacteria favored by increased pH of decayed pulp tissue.