

ECOLOGY OF PATHOGENS

SURFACE MATERIAL, TEMPERATURE AND SOIL EFFECTS ON THE SURVIVAL OF SELECTED FOODBORNE PATHOGENS IN THE PRESENCE OF CONDENSATE

(J. Allan, J. L. Kornacki, and Z. Yan)

Survival of foodborne pathogens in food processing facilities is affected by factors including surface materials, nutrients, moisture and temperatures. The effects of surface-type [stainless steel, Delrin[®] (DuPont) acetal resin, and fiberglass reinforced plastic wall paneling (FRP), and mortar surfaces], soil, and temperature on the survival of *Listeria monocytogenes*, *Salmonella* spp., and *Yersinia enterocolitica* were evaluated in the presence of condensation. Surface coupons soiled and non-soiled with sterile porcine serum were exposed to cell suspensions made from five-strain cocktails of each organism (10^7 cfu/ml) in Butterfield's phosphate buffer (BPB) and incubated for 2 h at 25°C allowing attachment of cells. Three surfaces, stainless steel, Delrin and FRP, were included in the same experiment. The coupons were rinsed to remove unattached cells, incubated at either 4°C or 10°C under condensate-forming conditions, and sampled at six time intervals over a 15-day period. For enumeration, cells were removed from the coupons by vigorous shaking in 100 ml BPB with 3 g of glass beads and the BPB was plated on tryptic soy agar with 0.6 % yeast extract. The results showed that stainless steel did not support the survival of *Listeria* as well as acetal resin or FRP. Acetal resin and stainless steel were less supportive of *Salmonella* than FRP. All three surfaces supported the survival of *Yersinia* over the 15-day trial equally at 10°C. Temperature had little effect on survival of *Listeria* or *Salmonella* across all three surfaces. However, *Yersinia* displayed growth on FRP at 10°C, but death at 4°C. Serum had a protective effect on *L. monocytogenes* on all surfaces, but did not affect survival of *Salmonella* or *Yersinia* on stainless steel, acetal resin, or FRP.

Since mortar surface is very different from the three surfaces described above, it was tested separately. The method to enumerate bacterial cells on the mortar surface involved applying sonication to remove bacterial cells and determining the cfu/coupon at 9 to 10 sampling periods over a total of 120 h. In general, the mortar surface had a significant inhibitory effect against all the bacteria tested compared to the three surfaces described above because of alkaline pH (increased to pH 11 within 6 h) when submerged in BPB. *Listeria* and *Salmonella* survived better on mortar than *Yersinia* throughout the 120-h incubation period, partially due to the alkaline resistance of *L. monocytogenes* and *Salmonella* spp. Serum had a protective effect on the survival of all three organisms. Differences in temperature did not affect the survival of *Salmonella* or *Yersinia*, whereas populations of *L. monocytogenes* declined more rapidly at 10°C than at 4°C after 24 h.

FACTORS AFFECTING PRODUCTION OF EXTRACELLULAR CARBOHYDRATE COMPLEXES

BY *ESCHERICHIA COLI* O157:H7

(J.-H. Ryu and L. R. Beuchat)

Numerous environmental factors have been reported to promote exopolysaccharide (EPS) production. These include high levels of oxygen, limited availability of nitrogen, desiccation, low temperature, and nutrient deprivation. The influence of waxes on colonization of microorganisms on plants and implication of biofilms in the ecology and management of epiphytic bacteria on plant leaves have been described. However, environmental factors specifically related to EPS production and biofilm formation by foodborne pathogens on the surface of raw fruits and vegetables have been given only meager research attention. The rate and amount of EPS produced by *Escherichia coli* O157:H7 and other pathogens, as affected by environmental conditions to which whole and fresh-cut produce is commonly exposed, are unknown.

In this study, we studied the effects of atmospheric gas composition, pH, nutrient source, and temperature on production of extracellular carbohydrates complexes (ECC) by *E. coli* O157:H7 and investigated the production of ECC on media formulated from juice of raw fruits and vegetables. ECC is defined in this study as those carbohydrates secreted from cells or loosely attached to the cell surface which can be detached by heat treatment. The reason for measuring the amount of ECC rather than the amount of purified EPS produced was that it is difficult to achieve a complete physical separation of EPS from other polysaccharides such as lipopolysaccharides.

EPS is probably present with other carbohydrates, including mono- or oligosaccharides secreted by cells or cleaved from the EPS physically or enzymatically, and polysaccharides derived from cell surface components in the form of carbohydrate complexes.

The influence of environmental conditions on cell growth, the total amount of ECC produced, and the amount of ECC produced on a per cell basis by *E. coli* O157:H7 strains ATCC 43895 (wild type) and 43895-EPS (natural mutant, extensive EPS producer) was studied. To determine the effects of pH on the production of ECC on a per cell basis, *E. coli* O157:H7 was grown aerobically at 12°C and 22°C on tryptic soy agar (TSA) acidified at pH 7.0, 6.5, 6.0, 5.5, 5.0, 4.5, and 4.0. Lettuce, alfalfa sprout, cantaloupe, tomato, and apple juice agars (pH 4.46 to 6.50) were also evaluated for their support of the ECC production. Conditions generally favorable for growth of *E. coli* O157:H7 were rich nutrient medium (TSA) versus heated lettuce juice agar (HLJA) or minimal salts medium (MSM), 22°C and an aerobic atmosphere compared to modified atmosphere (1% O₂, 10% CO₂, and 89% N₂). Conditions favorable for production of ECC on a per cell basis were HLJA, 12°C, and an aerobic atmosphere. There was a negative relationship between cell growth and production of ECC on a per cell basis, and environmental conditions that affected the total amount of ECC produced based on initial population reflected a combination of environmental conditions influencing both cell growth and ECC production on a per cell basis. A relative growth index factor (RGIF) was calculated to better understand ECC production as affected by various environmental conditions simultaneously. The production of ECC on a per cell basis by strain 43895-EPS showed a negative linear relationship with pH of TSA at both 12°C and 22°C. This strain generally produced a greater amount of ECC on fresh juice agar than on TSA at the same pH but production of ECC on alfalfa sprout juice agar (FJA, pH 6.45) at 22°C was significantly less than on TSA (pH 6.50). This indicates that nutrient limitation is not based only on nutrient availability. There may be other factors that repress the production of ECC on FJA and the effects of those factors may be temperature dependent. Further studies will be required to better understand the relationship between nutrient availability and other factors on the production of ECC by *E. coli* O157:H7 on raw produce.

**ETHANOL-MEDIATED VARIATIONS IN CELLULAR FATTY ACID COMPOSITION AND PROTEIN PROFILES
OF TWO GENOTYPICALLY DIFFERENT STRAINS OF *ESCHERICHIA COLI* O157:H7
(R. Y.-Y. Chiou, R. D. Phillips, P. Zhao, M. P. Doyle, and L. R. Beuchat)**

Ethanol can be found in foods, particularly in fruits and fruit products and in fermented foods and beverages, and in food processing environments as a result of fermentation of sugars by naturally occurring microorganisms. Ethanol and cleaners containing ethanol and other alcohols are also used in some areas of food processing plants to reduce or remove microorganisms on equipment and for promoting good worker hygiene. Occasionally, low concentrations of residual ethanol may be present on treated surfaces of equipment and in environmental niches not properly cleaned and sanitized. This provides an opportunity for pathogens to adapt and grow in environments with sublethal concentrations of ethanol.

The use of ethanol in the food processing industry is largely for the purpose of killing microorganisms rather than as a preservative, although low concentrations of ethanol have been examined for controlling the growth of spoilage and pathogenic species. The efficacy of ethanol as a preservative in a wide range of foods was studied. Growth inhibition of *Staphylococcus aureus* by ethanol has been shown to be caused by factors other than reduced a_w. Treatment of a high-moisture bakery product with ethanol vapor delays the growth of and toxin production by *Clostridium botulinum*. Little is known about physiological changes in *Escherichia coli* O157:H7 and other foodborne pathogens exposed to sublethal concentrations of ethanol. However, the prospect of using ethanol as a preservative in foods raises the need for more information on the behavior of spoilage and pathogenic microorganisms that may adapt to otherwise lethal concentrations of ethanol and subsequently exhibit altered survival or growth behavior and increased resistance to other environmental stresses imposed by traditional preservation technologies.

It is important to gain a better understanding of the mechanisms *E. coli* O157:H7 may possess to adapt and grow in ethanol-containing substrates in order to more accurately assess the level of safety hazard they may represent. In this study, two strains of *E. coli* O157:H7 isolated from unpasteurized apple juice and salami were grown in ethanol-supplemented tryptic soy broth (TSB) as a model food system. Characterization of growth patterns and changes in pH and glucose content in TSB as affected by ethanol content were determined. Early stationary-growth phase cells grown in TSB supplemented with 5.0% ethanol were analyzed for fatty acid

composition, protein content, and SDS-PAGE protein patterns. Cells grown in TSB and in TSB supplemented with ethanol were subjected to pulsed-field gel electrophoresis (PFGE) analysis to compare genomic DNA fingerprints.

Two strains of *E. coli* O157:H7 were grown in tryptic soy broth (TSB, pH 7.1) supplemented with 0, 2.5, 5.0, 7.5, and 10% ethanol at 30°C for up to 54 h. Growth rates in TSB supplemented with 0, 2.5, and 5.0% ethanol decreased with an increase of ethanol concentration. Growth was not observed in TSB supplemented with 7.5 or 10% ethanol. The pH of TSB containing 5.0% ethanol decreased to 5.8 within 12 h, then increased to 7.0 at 54 h. The ethanol content in TSB supplemented with 2.5 or 5.0% ethanol did not change substantially during the first 36 h of incubation but decreased slightly thereafter, indicating utilization or degradation of ethanol by both strains. Glucose was depleted in TSB supplemented with 0, 2.5, or 5.0% ethanol within 12 h. Cells grown under ethanol stress contained a higher amount of fatty acids. With the exceptions of *cis*-oleic acid and nonadecanoic acid, higher amounts of fatty acid were present in stationary-phase cells of the two strains grown in TSB supplemented with 5.0% ethanol for 30 h compared to cells grown in TSB without ethanol for 22 h. The *trans*-oleic acid content was 10-fold higher in the cells grown in TSB with 5.0% ethanol than in TSB without ethanol. In contrast, *cis*-oleic acid was not detected in ethanol-stressed cells but was present at concentrations of 0.32 and 0.36 mg/g of cells of the two strains grown in TSB without ethanol. Protein content was higher in ethanol-stressed cells than in non-stressed cells. SDS-PAGE protein profiles varied qualitatively as affected by strain and the presence of ethanol in TSB. An ethanol-mediated protein (MW 28 kDa) was observed in the ethanol-stressed cells but not in control cells. It is concluded that the two test strains of *E. coli* O157:H7 underwent phenotypic modifications in cellular fatty acid composition and protein profiles in response to ethanol stress. The potential for cross protection against subsequent stresses applied in food preservation technologies as a result of these changes is under investigation.

**ATTACHMENT OF *ESCHERICHIA COLI* O157:H7 GROWN IN TRYPTIC SOY BROTH
AND NUTRIENT BROTH TO APPLE AND LETTUCE AS RELATED
TO CELL HYDROPHOBICITY, SURFACE CHARGE, AND CAPSULE PRODUCTION
(A. N. Hassan and J. F. Frank)**

This study investigated the effect of growth in tryptic soy broth (TSB) and nutrient broth (NB) on the ability of *E. coli* O157:H7 to attach to lettuce and apple. In addition, surface hydrophobicity, charge and capsule production were determined by cells grown in these media. Cells grown in NB attached less to lettuce and apple surfaces than did those grown in TSB. TSB, but not NB, supported capsule production by *E. coli* O157:H7. Cells grown in TSB were more hydrophilic than those grown in NB. No difference was found in the electrokinetic properties of cells grown in these media. Electrostatic and hydrophobic interactions and surface proteins did not appear to play an important role in the attachment of *E. coli* O157:H7 to these surfaces. Of the factors studied, only capsule production was associated with attachment ability.

**INTERACTION OF A FREE-LIVING SOIL NEMATODE, *CANENORHABDITIS ELEGANS*,
WITH SURROGATES OF FOODBORNE PATHOGENIC BACTERIA
(G. L. Anderson, K. N. Caldwell, L. R. Beuchat, and P. L. Williams)**

The agricultural impacts of plant and animal parasitic nematodes have long been recognized and, by virtue of their effects on fruit and vegetable production, have been extensively studied. Comparatively little is known regarding the impact of free-living microbiovorous nematodes on produce production and safety, although they are the most abundant and wide spread soil mesofauna. The association of free-living nematodes and various genera of bacteria has been studied. While it is recognized that free-living nematodes avoid certain bacteria, it is clear that they do not uniformly avoid foodborne pathogens. Two human enteric pathogens, *Salmonella* and *Shigella*, are reportedly ingested and defecated by free-living saprozoic nematodes and *Salmonella* Typhimurium is known to infect the free-living soil nematode *Caenorhabditis elegans*. From these reports, it appears that free-living nematodes may be important as vectors of pathogenic bacteria, including some forms capable of causing human disease.

Soil is a source of microbial contamination of fruit and vegetables, as evidenced by the isolation of soil-residing pathogenic bacteria from produce. In a survey of vegetables for the presence of amoebae and *Salmonella*, nematode eggs and larvae have been recovered using a naccional-ether method. The recovery of nematodes from uncooked vegetables indicates that agronomic conditions and marketing practices may be conducive to the survival of nematodes on fresh produce. This also indicates that if free-living nematodes are present on raw produce, they

may serve as vehicles for contamination with pathogenic bacteria, either by contact with their surface or via eggs or voided material from their gastrointestinal tract.

We undertook a study to evaluate the interaction of *C. elegans* with bacterial surrogates for foodborne pathogens occasionally occurring or persisting in soil. Nematode/bacterial interactions were characterized to determine the propensity of young adult worms to be attracted to bacterial colonies, to compare the feeding and development of young adult worms cultured on this diverse group of bacteria, and to examine the dispersal of bacteria by *C. elegans* following feeding on monoxenic cultures. We evaluated the association between a free-living soil nematode, *C. elegans*, with *Escherichia coli*, an avirulent strain of *S. Typhimurium*, *Listeria welshimeri*, and *Bacillus cereus*. On an agar medium, young adult worms quickly moved toward colonies of all four bacteria; over 90% of 3-day adults entered colonies within 16 min after inoculation. After 48 h, worms moved in and out of colonies of *L. welshimeri* and *B. cereus*, but remained associated with *E. coli* and *S. Typhimurium* colonies for at least 96 h. Young adult worms fed on cells of the four bacteria suspended in K medium. Worms survived and reproduced using nutrients derived from all test bacteria, as evidenced by eggs laid by second generation worms after culturing for 96 h. Development was slightly slower in worms fed on Gram-positive bacteria compared to Gram-negative bacteria. Worms fed for 24 h on bacterial lawns formed on tryptic soy agar dispersed bacteria over a 3-h period when transferred to a bacteria-free agar surface. Results suggest that *C. elegans* and, perhaps, other free-living nematodes are potential vectors for both Gram-positive and Gram-negative bacteria, including foodborne pathogens in soil.

**PROTEOLYTIC YEASTS ISOLATED FROM RAW, RIPE TOMATOES
AND METABIOTIC ASSOCIATION OF *GEOTRICHUM CANDIDUM* WITH *SALMONELLA*
(W. N. Wade, R. Vasdinyei, 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 log₁₀ 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 log₁₀ 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.

**ATTRACTION OF A FREE-LIVING NEMATODE, *CAENORHABDITIS ELEGANS*,
TO FOODBORNE PATHOGENIC BACTERIA, AND ITS POTENTIAL AS A VECTOR OF *SALMONELLA* POONA
FOR PREHARVEST CONTAMINATION OF CANTALOUPE
(K. N. Caldwell, G. L. Anderson, P. L. Williams, and L. R. Beuchat)**

Soil is a source of microbial contamination of fruits and vegetables, as evidenced by the isolation of soil-residing pathogenic bacteria from produce. Free-living, microbivorous nematodes are among the primary grazers of bacteria in soils and also have potential to serve as vectors of microorganisms, including enteric pathogens, to the surface of fruits and vegetables. Most nematologists do not attach particular importance to free-living nematodes as vectors of plant pathogens. However, a critical examination of the role nematodes may play in plant and perhaps human diseases has been suggested. *Caenorhabditis elegans*, a microbivorous, free-living nematode, has been used extensively in biological studies. Feeding primarily on bacteria, the adult worm lives approximately 2 weeks under optimal environmental conditions. The worm is routinely cultured in the laboratory on *Escherichia coli* OP50, a uracil-deficient non-pathogenic strain that grows slowly on K agar but serves as a nutrient source for multiplication and reproduction. The objectives of this study were to determine the propensity of *C. elegans* to migrate toward three human enteric pathogens and cantaloupe juice, as well as its survival and reproductive behavior in the presence of these pathogens. The potential of *C. elegans* as a vector to transport *Salmonella* in soil to the surface of cantaloupe rind was also investigated.

The propensity of *C. elegans* to be attracted to seven strains of *Escherichia coli* O157:H7, eight serotypes of *Salmonella*, six strains of *Listeria monocytogenes*, and cantaloupe juice was investigated. Adult worms (20 - 30) were placed on the surface of K agar midway between a 24-h bacterial colony and 10 µl of uninoculated tryptic soy broth (TSB) or cantaloupe juice positioned 1.5 cm apart. The number of nematodes that migrated to the colony, TSB, or cantaloupe juice within 5, 10, 15, and 20 min at 21°C was determined, followed by incubating plates at 37°C for up to 7 days to determine the ability of *C. elegans* to survive and reproduce in bacterial colonies. The nematode was attracted to colonies of all test pathogens, and survived and reproduced within colonies for up to 7 days. *C. elegans* was not attracted to cantaloupe juice. The potential of *C. elegans* to serve as a vector to transport *Salmonella* Poona to cantaloupe rind was investigated. Adult worms that had been immersed in a suspension of *S. Poona* were deposited 1 or 3 cm below the surface of soil on which a piece of cantaloupe rind was placed. The rind was analyzed for the presence of *S. Poona* after 1, 3, 7, and 10 days at 21°C. The presence of *S. Poona* was evident more quickly on rind positioned on soil beneath which *C. elegans* inoculated with *S. Poona* was initially deposited compared to rind on soil beneath which *S. Poona* alone was deposited. The time required to detect *S. Poona* on rind was longer when the rind was placed 3 cm above the inoculum, compared to 1 cm. Free-living nematodes may play a role in the preharvest dispersal of incidental human pathogens in soil to the surface of raw fruits and vegetables in contact with soil during development and maturation, as evidenced by the behavior of *C. elegans* as a test model.

**INTESTINAL CARRIAGE OF *CAMPYLOBACTER* AND *SALMONELLA* IN TURKEYS
IN RESPONSE TO SUB-THERAPEUTIC LEVELS OF ANTIMICROBIALS IN FEED
(N. A. Cox, S. E. Craven, M. T. Musgrove, M. E. Berrang, and N. J. Stern)**

Since the 1950s, antimicrobials have been added to poultry feed at sub-therapeutic levels to minimize illness and promote growth. Despite the benefits to the agricultural industry and domestic animals, there are fierce debates worldwide on whether or not this practice carries a consequence in terms of human health. Turkeys and broilers

provided these additives have increased weight gain, muscle yield, and feed conversion, in part due to decreases in diseases such as coccidiosis and necrotic enteritis. Benefits achieved by adding these compounds to animal feeds are attributed in part to a shift in the gut microflora. However, studies have been published in which it was determined that competitive exclusion cultures, administered to birds to control colonization by human pathogens such as *Salmonella*, can be negatively affected by antimicrobials commonly used in poultry rations. Other published studies have reported an increase in *Salmonella* levels when experimentally challenged birds were fed diets containing low levels of antimicrobials. This study demonstrated that although naturally occurring populations of *Campylobacter* were virtually unaffected by antimicrobial feed additives, *Salmonella* populations were significantly decreased when commercial turkeys were fed rations containing flavomycin, virginiamycin, or monensin.

