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# Reports on Food Safety Projects

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#### Pathogenic detection using phage display

**Salmonella spp bacteriophage:** To facilitate the construction of a modified P22 bacteriophage for *Salmonella spp.* it was necessary to construct a recombination vector for insertion of a modified tail spike protein. The recombination vector was constructed by removing approximately 1 kb of the P22 genome in the region of orf 201 and orf 80 to allow the insertion of DNA for recombination. A multicloning site and a TA cloning site were inserted to facilitate rapid insertion of modified DNA to construct the appropriate epitope. Primers were utilized to amplify the tailspike protein from P22 with the appropriate His modifications. The preparative host strain repressor gene cassette was constructed for insertion into the preparative host strain genome. Work is continuing on inserting the modified tailspike gene in the previously constructed P22 recombination vector. Appropriate promoter and terminator configurations were added to the modified tailspike protein to allow repression of expression of the His modified tailspike protein in the preparative host strain. Preparative host strain containing the *lacI* repressor gene cassette was constructed.

### E. coli O157:H7 bacteriophage:

The apparent host specificity of  $\phi$ V10, its ability to lysogenically convert without transducing host genes, and its lack of identifiable virulence determinants suggest that  $\phi$ V10 should be useful in biotechnology and that its use in biotechnology should not constitute any undue hazard, making it a good candidate for detection assays.

*Listeria monocytogenes* bacteriophage: An initial screening of a *Listeria monocytogenes* library to isolate a lysogenic bacteriophage producing preliminary results that suggest success and we are currently further evaluating the isolated bacteriophage and continuing to screen the library.

#### Packaging:

The initial proposed product would contain a lateral flow assay for the multiplexed determination of multiple pathogens. We have begun collaboration with Embedded Concepts, which has patented technology which allows the embedding of antibodies into plastics. This innovation would greatly enhance the potential for integration of the phage based assay into food packaging material without the need foe lateral flow simplifying development of stand alone kits involving sample preparation and detection all in one.

# FOOD PROCESSING AND PACKAGING

Research efforts for 2005-2006 focused on: 1) characterizing the effects of space-relevant radiation doses of gamma-radiation on select foods, food ingredients, and dietary supplements and identifying threshold radiation levels for quality changes, and 2) characterizing the impact plant cultivars and growth conditions have on nutrient profiles, antioxidant capacity, functionality, and acceptability of foods.

#### Radiation Effects on Oils

Radiation can initiate a process known as autoxidation, where an initiator species reacts with the lipid, removes a hydrogen, and produces a lipid free radical. This lipid free radical can then react with other fatty acids and produce an accelerating chain reaction of similar events during propagation. Products of this oxidation of fatty acids include peroxides, alcohols, aldehydes, and carbonyls. Further reactions with these products lead to off odors and flavors accustomed to rancid fats. Studies using soybean and peanut oils (with and without added antioxidant, irradiated at 0, 3, 10, 100, 200, 400, 600, 800, and 1000Gy, and stored at 65°C) found that addition of 0.02% TBHQ (antioxidant) reduces total oxidation and delays oxidation maxima from 7 months to 42 months for soybean oil and from 21 to 49 months for peanut oil. *Radiation Effects on Antioxidants* 

Radiation doses during transit and on the surface of Mars are expected to be as high as 3 sieverts (approx. equivalent to 3 Gy), excluding solar events. Studies have shown that antioxidants may provide long term health protection from oxidative stress caused by radiation exposure; therefore, to counteract the impact of elevated radiation exposure on astronaut health, consumption of antioxidants will be important. In addition to vitamin supplements, antioxidants within foods will also provide protection. While radiation dose significantly affected the stability of vitamin C in solution, stability of vitamin C in dry formulations

(vitamin C supplements, multivitamins, and ascorbic acid powder) was stable following radiation doses of up to 1000 Gy.

# Radiation Effects on Fruits and Vegetables

Determination of appropriate cultivars for not only growth but also food use are important to the success of a sustainable human habitat. Preliminary studies have shown that cultivar selection and growth conditions impact the composition and acceptability of crops such as sweet potato and carrot. While moisture, ash, protein, total sugar, water activity, calcium, magnesium, sodium, and potassium were relatively constant upon exposure to all radiation doses, total extractable fat, vitamin C, carotenoids, and lycopene were significantly affected by radiation doses. The finding that radiation exposure may increase the extractability of fats from plant tissues could be utilized for further food processing development and commercial applications.

#### Radiation Effects on Wheat

Wheat is the most studied candidate crop and has the highest priority. Apogee and Perigee cultivars of wheat were developed at Utah State University to yield high amounts of wheat berries with a minimum amount of inedible crop waste. While growth conditions have been characterized, further analysis of these cultivars must be conducted to characterize the protein, lipid, and starch functionalities related to food quality, as well as antioxidant capacities, and to compare the food-functionality of these cultivars to common cultivars used in the food industry. Because wheat can be shipped as a bulk ingredient and/or grown in space, additional studies investigated the effects of radiation exposure on the quality and functionality of wheat. For a Mars mission, results indicate that Apogee is a more suitable candidate cultivar than Perigee due to its overall stability and nutritional properties.

# OPTIMAL FOOD SAFETY IN ADVANCED LIFE SUPPORT

A. *Inactivation of Salmonella by pulsed light sterilization and sanitizers*. The 1% peracetic acid solution was the most effective treatment while the 2% commercial sanitizer was the least effective sanitizer over 8 days of storage. Overall, 1% peracetic acid was the most effective in reducing the populations Salmonella spp. on the surface of whole tomatoes.

*Pulsed Light and Sanitizer Treatment.* Several preliminary studies conducted in our laboratory with tomatoes aided in the determination of a suitable distance from the light source and the number of pulses that could be used without a significant temperature increase. After 2 and 8 days of storage, 2.02 to 4.54, 0.80 to 2.10 and 5.76 to 4.86 log CFU/ml of *Salmonella* was recovered from the surface of the tomatoes treated with a combination of pulsed-uv light and 1% hydrogen peroxide, 1% peracetic acid and 2% commercial sanitizer, demonstrating the use of pulsed-uv light in combination with sanitizers is highly effective in inactivating *Salmonella* on surface of whole tomatoes. Results also indicated the use of pulsed-uv light alone wasn't as effective in significantly inactivating Salmonella on the surface of whole tomatoes compared to the combination and control treatments.

*B. Microbial ecology of water from hydroponic distribution system.* The development of bacterial communities in hydroponic water distribution systems leads to a food chain which supports the growth of microorganisms incompatible with water quality requirements and esthetics. In this study, our hypothesis was that most plant or waterborne microorganisms can thrive in a hydroponic system during growth of salad crops, due to the abundant of nutrients present in the water and generally created by the plants. There was a significant decline ( $p \le 0.05$ ) in the numbers of mesophilic microorganisms in the hydroponic system over 12 weeks of the experiment. The reduction was sustained after 7 weeks. After seven weeks, Pseudomonads were the only bacteria detected by traditional plating methods.

*C. Pre-harvest intervention for L. monocytogenes on carrot seeds.* No *Listeria monocytogenes* was detected in uninoculated carrot seeds. Some cells that adhered to the seeds during dipping process may have been killed during drying and storage before chlorine, hydrogen peroxide and heat treatment. Researchers have suggested the process of drying seeds after inoculation could, in effect, protect bacteria that entered the seeds through cracks and crevices against inactivation by chlorine. It's suggested that when

active chlorine comes into contact with an environment with high levels of organic matter, such as carrot seeds, the potency is quickly diminished.

This very likely could have happen in this experiment, resulting in cells surviving chlorine concentrations as high as 0.1% in dip solutions to inactivate *L. monocytogenes* that infiltrated the seeds. With a scanning electron microscope, *L. monocytogenes* biofilms, an adhesive and threadlike substance surrounding the bacterial cells, was observed on the inoculated carrot seeds after 7 days of storage at 8 °C. There was no reduction in the *Listeria* populations on the carrot seeds soaked at 21°C (control) for 5 or 10 min. During the 7 and 21-day storage, *L. monocytogenes* within the liquid contiguous to the surface of the carrot seeds developed biofilms, making *L. monocytogenes* more resistant to the disinfectants and heat treatments. The increase in CFU per milliliter of dip solution and CFU per gram of seed appearing in 10 min and 2 min treatment for chlorine and hydrogen peroxide, when compared with the control dip (water) suggested that longer exposure in peptone solution also facilitates release of *L. monocytogenes* from the seeds.