

SESSION IIIB: ORAL PRESENTATIONS

A Ribosomal RNA-based System to Monitor Microbial Contaminants in the Space Environment

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The risk of microbial contaminants in space environments is realistic and important for a variety of reasons. Concerns about disease during human space missions are particularly important considering the clinically significant changes the immune system incurs during spaceflight. Humans shed microorganisms and viruses into the environment, and during spaceflight some normally benign microbes may become pathogenic. Additionally, our responsibility as explorers of the cosmos includes ensuring planetary protection by avoiding the introduction of anthropogenic contamination into new environments we might visit. A sensitive, highly specific system to detect and monitor these contaminants is crucial. We are currently developing a monitoring system based on 16S ribosomal RNA probes to identify bacterial contaminants and are adapting it to a variety of assays that exploit the exciting technologies of molecular beacons and microarrays.

Microbial Ecology and Molecular Characterization of Pathogens in Feedyard Playa Air and Water Samples in the High Plains of Texas

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Concentrated animal feeding operations (CAFO) significantly impact the environment due to nutrient inputs present in the manure, and the sheer volume of bulk materials that must be processed and/or disposed of. Consequently, management issues such as odor, presence of human pathogens, endotoxins (ET), and dust emissions are introduced. The impact of these nutrient inputs and human pathogens on the microbial ecology of feedyard playa air and water is under investigation. Airborne microbial concentrations of CAFO were determined by using 2- and 6-stage Andersen biological cascade impactors and SKC air samplers positioned upwind, on-site, and downwind of each feedyard playa while water samples were obtained from feedyard playas. All samples collected were cultured on a variety of nonselective and selective media. Water samples were extracted to obtain bulk nucleic acids using MO BIO water kits and selectively enriched for *E. coli* O157:H7 or *Salmonella* sp. A key objective of this project is to develop the tools and necessary database information to: 1) rapidly detect environmental *E. coli* O157:H7 isolates, 2) assess the biodiversity of environmental *Escherichia* sp. inhabiting feedyard playas, and 3) to monitor the fate and transport of *E. coli* O157:H7 in the environment. Molecular methods (AP-PCR) to detect and discriminate between various *E. coli* sp were developed by taking advantage of the fact that the complete genomes of K12-MG1655, O157:H7-EDL933 and O157:H7B-Sakai have been sequenced. A global alignment of all three genomes (approximately the 1st 0.5 megabases in 100 kb segments) was conducted using the Clustal W program. Genomic regions of high homology interspersed with insertions/deletions or regions of low homology were selected for the design of primers to be used in AP-PCR assays. Seven PCR primer pairs (A-G) were selected from several hundred possible pairs that generate a single diagnostic amplicon (250 to 1800 bps) per MG1655, EDL-933, or Sakai genomes. These primer pairs were validated in PCR reactions using genomic DNA obtained from the strains MG1655, EDL-933, and Sakai that were sequenced. The usefulness of these primer pairs was evaluated employing the DEC A set of strains (n=72) obtained from the National Food Safety and Toxicology Center, Michigan State University and suspected *E. coli* O157:H7 isolates obtained from enrichments of feedyard playa samples. In addition RAPD-PCR studies using 10-mer primers from Operon were conducted to assess their potential in generating useful DNA fingerprints of environmental isolates of *E. coli* O157:H7 in order to assess the biodiversity of *E. coli* strains inhabiting feedyard playas.

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