Bioamplification using phage display for the detection of Salmonella spp. and its evaluation as a technology platform for the simultaneous detection of multiple pathogens in the same sample

Investigators: Bruce Applegate and Michael R. Ladisch

Initial construction of the recombinant M13 to be utilized in the assay was unsuccessful. Therefore, an alternate strategy was pursued. We obtained an M13 GIII phage display library and isolated phage containing a streptavidin binding epitope. These findings allowed for the development of purification protocols and proof in principle assays for the ultimate success of the project. The P22 recombination vector was obtained and modified to allow insertion of tail spike protein. We constructed a P22 sensitive Salmonella strain which overexpresses the Lac repressor to facilitate the production of the 6xHis tag modification P22 phage. The modified P22 successfully propagated the 6xHis tag modified phage without expressing the modified tailspike protein which will then only be expressed by infecting wild type strains. This was accomplished by inserting a constitutively expressed 6xHis into the chromosome of the Salmonella strain. This was crucial to the specific application but also for validation of the overall concept of suppressing the displayed epitopes prior to infection of a wild type host.

Multiplexed Detection of Pathogens using Fluorescence Resonance Energy Transfer in a Spatial Detection Format

Investigators: Bruce Applegate and Sergei Savikhin

We determined the sequences for the amplicons and molecular beacon probes for the simultaneous PCR of 15 different toxin genes associated with the pathogens in the study. The primer and probe sets were synthesized for evaluation in the multiplex PCR reaction. The amplicon primer sets were tested for cross reactivity with individual strain DNA and mixtures in a multiplex PCR format and no cross reactivity was determined. This was a critical accomplishment for the project as it provides DNA to be analyzed in the proposed spatial format detection platform. We also determined the minimal primer concentrations to amplify the target amplicons to equivalent detectable levels (in the multiplex format) using fluorescence analyzed by capillary electrophoresis. Subsequent detection limits were also determined (102 copies of target). Primers were synthesized with fluorescence tags to allow identification of peaks and used in the multiplex PCR reaction using capillary electrophoresis as opposed to electrophoresis since the fragments are similar sizes and can not be resolved by electrophoresis. The optimization allows the amplification of target amplicons in varying initial concentrations without loss of detection of lower concentration amplicons which is observed when primers are utilized in excess concentrations. This is an important development in the overall goal of the project but could also be utilized in current amplification detection methods currently in use.

Development of Immunocapture Real-time PCR to Detect Fusarium Species in Grains and Foods

Investigators: Maribeth A. Cousin and Charles P. Woloshuk

Research was done to develop an immunocapture method for Fusarium species by using different solid support systems: microcentrifuge tubes, magnetic and non-magnetic beads, and microtiter plates. It is important to develop a simple method to take the mold away from the food particles before going to PCR. A modified ELISA using microtiter plates gave the best capture and was used before going to PCR. If a method could be developed to easily and quickly detect species, then these molds could be prevented from producing mycotoxins in agricultural commodities and foods.

Project Objectives
1. Develop real-time PCR to detect Fusarium species
2. Develop real-time PCR to specifically detect Fusarium species that produce fumonisins and trichothecenes
3. Capture Fusarium species with antibodies
4. Combine immunoassay with PCR to specifically detect Fusarium verticillioides that produce fumonisins and Fusarium graminearum that produce trichothecenes

Healthcare and Public Safety Systems for the Detection of Listeria monocytogenes in foods

Investigators: Michael R. Ladisch, Rashid Bashir, Arun Bhunia, and J. Paul Robinson

A second generation silicon-based microfluidic biochip was fabricated that enables collection and concentration of cells accompanied by impedance-based detection of their presence on the chip. The chip was designed, fabricated, and tested in a manner that integrates off-chip processing with on-chip microfluidic handling and interrogation of a small sample volume. This is an important step forward towards achieving the goal of systems integration for a laboratory prototype for L. monocytogenes detection in samples derived from ready-to-eat meat products. Research also demonstrates how environmental stress reduces binding of L. monocytogenes to its bioreceptor and consequently reduces sensitivity for detection of the pathogen. This work showed how enrichment media might be formulated to optimize the physiological status of the microorganism and to enhance its binding to a pathogen specific antibody while minimizing “clustering,” thereby reducing probability of a false negative result. The outcome of this work will ultimately enhance reliability and sensitivity of biochip-based detection of L. monocytogenes by formulating special media or buffers for this purpose.

Multi-pathogen screening and/or confirmation via microarray detection

Investigator: Arun Bhunia

After genomics/proteomics analysis, nine rabbit polyclonal antibodies (PAb) were developed against different target peptide antigens of L. monocytogenes. All but one showed a specific reaction with the target peptide. Of which, only PAD LmH404 (internalin B) and LmCB39 (ActA) (actin polymerization protein) showed specific reactions for the proteins from L. monocytogenes and not from any other Listeria species tested. However, the remaining PAbs reacted with multiple protein bands and showed cross-reactions with other Listeria species. The lack of specific binding could be due to the denaturation and altered folding of the antigen on the cell surface. InB and ActA peptides appeared to be the most promising targets for L. monocytogenes specific antibody production. The major drawback for these antibodies involved high background reactions with E. coli and Salmonella. We believe this may be due to high titer levels (for antibodies against several common microorganisms in the serum) in rabbits. Specific pathogen-free rabbits are currently being considered to improve antibody development for these antigens.