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As the Authorized Departmental Officer’s Designated Representative (ADOOR), I am the official ARS contact person for the collaboration agreement with the CFSE at Purdue University. Trained as a bio-physical chemist, I am also the Research Leader of the Microbial and Residue Chemistry Research Unit at the Eastern Regional Research Center (ERRC) of the Agricultural Research Service (ARS), United States Department of Agriculture (USDA).

One of the missions of my Research Unit is to develop rapid, sensitive and specific food pathogen detection methods by biosensor processes for possible adoptions by regulatory agencies and the food industry. To date, we have developed a few processes that can sensitively detect pathogens such as *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes* in meats and produces within eight hours. Examples are pathogen detection processes suitable for ground meats and produces involving the use of a combination of immunomagnetic capture and time-resolved fluorescence measurements of lanthanides. To broaden the applicability of developed biosensor processes, we have utilized phage-display technique to generate proper immuno-reagents for other pathogens. One of the long term goals of our research is to develop microarray detection platform that can perform multiple pathogen/toxin detection and identification. The collaborations with CFSE on the common research goals have reached a new level in the past year. Under the new partnership arrangement, Dr. Tao Geng, a Purdue postdoctor, expanded his fiber optical waveguide biosensor research at ERRC. His excellent research progress was included in a joint Purdue-ARS manuscript for journal. This productive personnel arrangement is expected to continue. I strongly believe this partnership will synergistically enable us to reach our common goals.
The Center for Food Safety Engineering (CFSE) at Purdue University in collaboration with the USDA-ARS Eastern Regional Research Laboratory, celebrates another successful year. We continue to build upon multi-disciplinary team approaches to solve problems related to detection and control of foodborne pathogens. Our strength lies in our ability to combine talents of Engineering scientists with researchers from Agriculture, Consumer and Family Sciences, Science, and Veterinary Sciences.

Research teams are now working on emerging and exciting technologies to improve microbial and chemical detection. Detection systems are being developed for bacterial foodborne pathogens including *Listeria monocytogenes*, *E. coli O157:H7*, *Campylobacter* spp., *Salmonella* spp., and for chemical toxins such as organic pesticides (e.g., paraquat), toxic metals (e.g., arsenic), and aromatic compounds. We are researching a wide variety of detection technologies including enzyme linked immunosorbant assays, polymerase chain reactions, impedance-based microbiology, infrared spectroscopy, atomic force microscopy, bioluminescence, and DNA/RNA probes. We are also working on separation technologies that help bring a higher concentration of targeted pathogens to the detection device. For example, the cell concentration recovery (CCR) kit, developed by our *Listeria* biochip team, uses different separation technologies, including microfiltration, to concentrate *Listeria* cells from complex food systems (i.e., hot dogs). A biosensor has also been developed for food samples that separate target pathogens using dielectrophoresis, followed by target capture using immunobiology reactions. Then, the cell actually grows on the chip and impedance is measured over time to relate back to initial cell number. The CCR kit coupled with the *Listeria* biosensor has potential to be a very powerful detection tool.

The following research report highlights projects funded in 2004-2005. This year, our collaboration with USDA-ARS has lead to 17 peer reviewed research publications, over 25 presentations at national meetings, and graduation of 7 Masters and Ph.D. students. This past summer, we were honored by an invitation to speak at the 25th Annual Rapid Methods Workshop held at Kansas State University. CFSE researchers conducted a 1/2 day program on development of biosensors for detection of foodborne pathogens.

These are certainly very exciting times for CFSE and Purdue University. I am proud of our combined efforts and look forward to our growth in the coming years. To learn more about our center, please visit our Web site at www.cfse.purdue.edu or feel free to contact me directly.
**Project Rationale**

*L. monocytogenes* has emerged as one of the most important food pathogens with a “zero tolerance” policy in ready-to-eat processed meats and dairy foods. This bacterium not only causes serious illness but also is lethal in infants, people over 60, and immune-compromised individuals.

Current methods of detecting this bacterium rely upon enrichment in the numbers of bacteria present in a sample. The food or food extract is incubated in special growth media for 12 to 24 h and the resulting culture is tested for *L. monocytogenes* using procedures that require an additional 3 to 24 h. The elapsed time, referred to as time to result or TTR, is problematic since some foods are consumed before test results would be available.

Rapid and affordable technologies to detect low numbers of *L. monocytogenes* cells directly from food, and which distinguish living from dead cells, are needed. This multi-disciplinary, multi-departmental research project is addressing the fundamental engineering and science required for development of microchip, bio-based assays that are transportable to the field, useable in a manufacturing plant environment and capable of rapidly detecting *L. monocytogenes* at the point of use. This research has the goal of microscale detection of *Listeria monocytogenes* on a real-time or near-real-time basis within 4 hours, and reducing the time of culture steps with rapid cell concentration and recovery based on membrane technology.

Our multidisciplinary research team is addressing the development, engineering and validation of such a microchip system that combines bioseparations technology for rapid concentration and recovery of microbial cells and bionanotechnology to construct systems capable of interrogating fluids for pathogens. Our approach is resulting in a technology platform capable of detecting other types of foodborne and medically relevant pathogens although the focus of the research is on rapid detection of *L. monocytogenes* by a combination of technologies that will ultimately give a time to result in hours.

**Project Objectives**

- Develop rapid concentration and recovery of microorganisms from food samples for subsequent interrogation of pathogens.
- Study the mechanisms and approaches for rapid filtration to better understand the probable role of lipids and fats in clogging the membranes, and the identification of problems in the rapid filtration of fluids extracted from vegetables, as well as meats.
- Establish a method of microfiltration for 250 mL samples of hotdog broth from massaged hotdogs.
- Study the interfacing of biological molecules with electronic components as part of our integral approach to derivatizing surfaces of membranes, derivatizing and immobilizing antibodies on surfaces of fiber optic probes, treating surfaces of microfabricated devices to minimize non-specific retention, and using electrical means to capture, collect, and concentrate microorganisms, on-chip.
- Establish methods for microfiltration, selective and non-selective capture of microorganisms, transport of microbes from one component to the next in a micro-fabricated system, and detection of their viability against a background of other non-target constituents based on macromolecular properties of the cells or surfaces.
- Design a buffer that not only enhances growth but also maximizes difference in electric conductivity between target and background signals, where the background due to the inherent conductivity of the fluid itself is minimized.
- Improve biospecificity of target capture by applying known blocking agents (BSA, Tween 20) to surfaces, and using polylysine di-functional reagent to immobilize ligands, to which rabbit anti-Listeria, or monoclonal anti-Listeria antibodies may be attached in an active conformation.
Interface biochip systems with electronic reading devices for sample monitoring and data collection.

**Project Highlights**

The first integration of sampling, cell concentration and recovery, and detection into a single protocol, with a time to result of 4 hours, is the single most significant accomplishment this year. While an integrated system is not ready for testing, it provides a first important step towards a conceptual prototype for testing in industry. Cell concentration and recovery was carried out using the Concentration Cell Recovery (CCR) kit consisting of membranes, syringes, pipette tips, special buffers, and a step-by-step protocol (on a CD) for carrying out cell concentration coupled to detection using a fiber optic probe to which antibodies had been fixed. The experiment was carried out by inoculating *L. monocytogenes* into hotdog, massaging the hotdog in buffer, and then concentrating, recovering, and detecting the cells. For an amount of 106 cells/50 g hotdog, a recovery of 25 to 30% of viable cells in a concentrated form was achieved with a fiber optic probe. The probe was derivatized with C11E9 antibody for selective capture of the *L. monocytogenes*. While the number of cells in the sample were between 105 to 106, which is significantly higher than the threshold we will ultimately achieve, this experiment highlighted scientific issues that still need to be addressed as we begin integrating the various technologies that make-up the system.

On-chip concentration of particles was demonstrated using dielectrophoresis. In addition, the capture of cells by dielectrophoresis, and by antibodies immobilized onto the chip was demonstrated in a dramatic manner, using time-lapse optical imaging of fluorescently labeled microorganisms. This result addresses an important facet of on-chip processing for detecting target cells: capture of a specific target cell in a microfluidic system. The microfabrication of the chip, the placement of antibodies on chip, and microorganism capture are all key components toward development of an integrated biosystem.

The rapid concentration and recovery of cells from foods has been shown to be feasible using the CCR kit, a flat membrane-based system. This has now evolved into a system where concentration and recovery may be carried out in a continuous mode. Computer control of the CCR process, and a software interface to monitor the process has resulted in a reproducible, as well as rapid method to concentrate up to 250 mL of massaged hotdog extract into a sample volume of between 100 to 500 mL, in 30 to 60 minutes. This holds promise for obtaining small volumes of concentrated microbial populations from large volumes of samples with a low microbial populations for applications to a number of different types of pathogen testing methods.
**Project Rationale**

Inadvertent contamination of foods with harmful microorganisms can result in multiple concerns including loss in productivity, expenses related to healthcare, investigation, litigation, destruction of vast quantities of agricultural products, and loss of human life. In order to streamline efforts in the circumvention of food poisoning related incidents, the food industry and regulatory agencies would value a fully automated testing system for the rapid throughput analysis of foods for contaminant pathogens (E. coli O157:H7, Salmonella, Listeria spp., etc.). Ideally, the testing platform would be a technician-operated instrument that can simultaneously screen food samples for the presumptive presence of multiple bacteria and, if desired, confirm their presence and characterize the pathogens through identification of virulence-related or other genes. This approach would ultimately eliminate the need for the time-consuming conventional cell culture/isolation/confirmation procedures currently used. The specific aim of this project was to develop a nucleic acid microarray for the detection of multiple PCR products for the identification of E. coli O157:H7, Salmonella spp., and L. monocytogenes based on molecular beacon technology. This project leverages capabilities in DNA microarray technology to develop a gene-specific assay that does not require costly labeling and purification methods to detect the presence of the target gene.

**Project Objectives**

- Construct an initial prototype gene array consisting of four marker genes for E. coli O157:H7 utilizing molecular beacon probes immobilized on a glass slide.
- Construct a microarray containing 4 targets from Salmonella spp. and L. monocytogenes from previously constructed target probe and amplicon sequences along with microarrays including all 3 sets of probes for the simultaneous detection of E. coli O157:H7, Salmonella spp., and L. monocytogenes and internal controls.
- Evaluate previously developed multiplex PCR reactions for simultaneous amplification of the multiple pathogen targets utilizing the developed microarrays.

**Project Highlights**

The development of the prototype microarray was the single most significant accomplishment of 2005. The initial assay developed uses 4 different genes associated with the virulence and identification of E. coli O157:H7 providing a set of marker genes to reduce false negative and false positives, yet the microarray platform is scalable genes representing many different organisms could easily be added. Another important attribute of the system is the potential for reuse. The use of a wavelength shift as a detection format allows the reset of the array for subsequent rehybridization to verify results or use with another sample. Initial results show the lower detection limit to be approximately 1.0-1.5 ng/µL of DNA, however optimization of this parameter has not been evaluated.

Furthermore, the assay protocol has been developed to quickly provide results (5 min. hybridization). Initial results also indicate that the assay system is not only qualitative but quantitative.

“In order to streamline efforts in the circumvention of food poisoning related incidents, the food industry and regulatory agencies would value a fully automated testing system for the rapid throughput analysis of foods for contaminant pathogens.”
**Project Rationale**

Listeria monocytogenes and enterohemorrhagic *Escherichia coli* are important foodborne pathogens of concern in the United States because of their low infective dose and prevalence in foods. For the detection and evaluation of foods contaminated with these pathogens, USDA/FSIS recommends initial enrichment and subsequent plating on a selective agar media, which follows further identification by biochemical, serological or genetic means. However, these latter steps are time consuming and may take more than 2-3 days.

This multidisciplinary project focuses on improving the key subsystems of a light scattering instrument (previously funded project), known as BARDOT (Bacteria Rapid Detection using Optical Scattering Technology), as well as adding to the bacteria colony scattering signature database. The targets include *Listeria*, *Salmonella*, *E. coli*, *Bacillus* and other food related bacteria. The anticipated detection time would be less than 10-15 minutes after the colonies are grown on the agar plate. We foresee this non-destructive/noninvasive light scattering label-free detection technology would be simple and easy to perform with minimum of training for rapid identification without additional bacterial sample processing steps.

Potentially, this technology could be used for the rapid detection/identification tool for viable bacteria from food, clinical, or environmental sources.

**Project Objectives**

- To improve the BARDOT design, including supporting physics-based models, for more repeatability and maximum discrimination of forward scattering signatures of colonies.
- To acquire scatter images of colonies of select foodborne pathogens.
- To analyze the bacterial colonies of different foodborne bacteria on non-selective and selective agar media and cellular properties by electron microscopy and GC-MS.
- To validate the technology by using naturally or deliberately contaminated food samples.
- To analyze the scatter signal images using ‘Standard feature extraction’, ‘Moments of shape analysis’ and ‘Neural network based classification’ methods.

**Project Highlights**

Ability to differentiate colonies of *Listeria* species on solid agar surface by a non-contact, noninvasive scatterometer device would dramatically simplify the detection and identification of pathogens from food. Light scattering images of different *Listeria* species was analyzed using an improved image analysis software program that utilizes computational image analysis and pattern recognition tools such as Zernike moments and principle component analysis (PCA). 157 images representing different *Listeria* species were classified and most *Listeria* species were clustered separately with the exception of some strains of *Listeria innocua* and *L. monocytogenes* which had shared overlapping patterns. This was not surprising since these two species share a high degree of similarities in their genome sequence. We foresee this technology would have a widespread application in monitoring bacterial pathogens in food, environment, water, and air samples added deliberately or present naturally.
MULTI-PATHOGEN SCREENING AND/OR CONFIRMATION VIA MICROARRAY DETECTION.

Project Rationale
Foodborne disease is one of the most common causes of morbidity and mortality in the world, and more than 200 known diseases are transmitted through food. In the United States there are about 76 million cases and of which 325,000 require hospitalization and 5,000 die each year. The foodborne pathogens of greatest concern due to incidence rate and/or severe disease implications are: E. coli O157:H7, Salmonella, Listeria monocytogenes, Toxoplasma and Campylobacter. Detection of these pathogenic bacteria during food processing and storage is crucial for the microbiological safety and prevention of possible outbreaks.

Antibody-based detection methods are regarded as rapid and efficient and are widely used in conventional ELISA and dipstick methods. In recent years, antibodies have been successfully used in the development of biosensor tools for rapid detection. Therefore, specificity and avidity of a given antibody for the target bacteria is extremely important, specifically those originating from stressful environments of food. Stress can affect antigen expression on bacterial cells thus affecting antibody-based detection. This project uses antibody-based methods to simultaneously detect Listeria monocytogenes, Salmonella enterica and E. coli O157:H7.

Once completed this detection approach would allow development of a detection kit for multiple pathogens during a single test. This breakthrough would save time and money for product testing and also would aid regulatory agencies when evaluating food products for key food pathogens.

Project Objectives
Three major objectives were pursued in this project and included:
- Development of antibody specific for L. monocytogenes, Salmonella enterica and E. coli O157:H7.
- Determination of the effect of environmental and physiological stresses on antigen expression.
- Development of a sandwich ELISA for each pathogen.

Project Highlights
Two polyclonal antibodies PAb Lm404 and LmC369 were demonstrated to be specific for Internalin B (InlB) and actin polymerization protein (ActA) of L. monocytogenes, respectively. These antibodies could be potentially used for specific detection of this bacterium from products or test samples in immunosensor applications. Therefore, ability of these antibodies to detect bacteria obtained from different selective and nonselective culture media and from meat samples were analyzed. Differences in antigen expression were observed in different strains.

Selective media – like BLEB, UVM and FB suppressed PAb Lm 404 reactive InlB expression whereas only FB suppressed Lm C369 reactive ActA expression. Three L. monocytogenes serotypes commonly associated with outbreaks were tested for expression in hotdogs at 37°C or 4°C. At 37°C all strains expressed InlB and ActA; however, at 4°C no strains showed expression of either InlB or ActA. These data indicate that PAb Lm404 could be used only when bacteria cultured in non selective media while PAb Lm C369 could be used in an immunosensor with bacteria directly taken from selective enrichment broth.
**Project Rationale**

Most *Fusarium* species produce mycotoxins in cereal grains and foods and three mycotoxins, fumonisins, trichothecenes and zearalenone, have been linked to potential human health problems. These mycotoxins are produced worldwide in grains during growth in the fields, storage in grain elevators and during processing operations for some foods. Once formed, these mycotoxins are difficult to destroy in grains and foods because they resist most food processing operations, including heat. Therefore, there is a need to detect *Fusarium* species in grains and foods before they can grow and produce mycotoxins. Currently, there are no rapid methods to detect *Fusarium* species. Our previous work focused on developing rapid methods to detect *Fusarium* species in grains and foods by using techniques based on enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). ELISA and PCR assays were developed that could detect *Fusarium* species as a general group and also a multiplex PCR was developed to detect the major species of *Fusarium* that produce three major mycotoxins: fumonisin-producing *Fusarium verticillioides* and *Fusarium proliferatum*, and trichothecene- and zearalenone-producing *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium sporotrichioides*. Continued work is being done by using the antibodies for immunocapture of the *Fusarium* species that then can be detected by multiplex real-time PCR.

**Project Objectives**

The overall goal of our current work is to develop a biosensor protocol, using antibodies that were produced to capture antigens of mycotoxin-producing *Fusarium* species, and the combination of these antibodies with the real-time PCR assay to rapidly identify *Fusarium* species. The specific objectives for the research are to:

- Develop a method to capture the antigens of mycotoxin-producing *Fusarium* species using the antibodies produced against *Fusarium graminearum* and *F. verticillioides*.
- Develop real-time PCR to primers that are specific for the *Tri6* gene involved in trichothecene biosynthesis and to *Fum1* the gene involved in fumonisin biosynthesis.
- Use this immunocapture-real-time PCR to detect *Fusarium* species in foods and grains.

**Project Highlights**

FastPrep™ kit was used to disrupt conidia of *Fusarium verticillioides* and *Fusarium graminearum* for easier ability to do real-time PCR. The conidia of *Fusarium* species have been difficult to lyse and release DNA for real-time PCR, and the sonication techniques used were labor and time intensive making the method more difficult and expensive. Research was done using a Stomacher™, microwave/vortex combination, bal mill and FastPrep™ kit to develop a procedure to disrupt the conidia for *Fusarium* species. The use of the FastPrep™ kit can be done in a few minutes and the protocol is easy for an untrained person to do; therefore, we are hopeful that the method can be developed for routine industry use.
Project Rationale

Foodborne pathogens in meat and poultry products continue to be a serious health concern with an estimated 5 million illnesses and 4000 deaths each year. Salmonella, Campylobacter jejuni, and E. coli O157:H7 are among the top foodborne pathogens with an incidence rate of 15.1, 13.8 and 1.6 cases per 100,000 persons each year in the U.S., respectively. To reduce microbial contamination in meat products and carcasses, aqueous chemical treatments, such as: chlorinated water, chlorine dioxide solution, organic acids, ozone, trisodium phosphate, and others have been widely used and investigated. However, these treatments are limited to a 1-2 log reduction of pathogens on meat surfaces. Highly effective sanitizers need to be developed for meat and poultry products. ClO2 gas may be an alternative antimicrobial agent for the elimination of pathogens on meat products. Results from this research will hopefully help improve the safety of meat and poultry products. Furthermore, it will enhance our understanding of the potential benefits and feasibility of using ClO2 gas as a new antimicrobial treatment for meat industry.

Project Objectives

The overall goal of this project was to improve meat product safety by developing and applying novel disinfection technologies. The specific objectives for the research were to:

- Determine the efficacy of ClO2 gas treatments for control of Salmonella spp. and generic E. coli on different tissue surfaces of beef (lean, fat), pork (skin, lean, fat), and broilers (skin, lean).
- Identify the most resistant tissue surfaces (from objective 1) and optimize ClO2 gas treatments (concentration and contact time) for the most efficient reduction of Salmonella and generic E. coli.
- Determine pathogen reduction of C. jejuni (beef, pork, and boilers) and E. coli O157:H7 (beef) using optimized ClO2 gas treatment parameters (from Objective 2) for its application in meat processing.
- Determine the effects of ClO2 gas treatments on the chemical and physical qualities of the meats (residual ClO2, chlorite, free available chlorine, and chloramines).

Project Highlights

A range of 1-4 log reductions of generic E. coli K12, Salmonella, C. jejuni, and E. coli O157:H7 were achieved on different tissue types from beef (fat and lean), pork (skin, fat and lean) and broiler (skin and lean) after 500-4500ppm gaseous ClO2 treatments for 10-20min using a continuous flow system. Pork surfaces (skin, fat and lean) and chicken skin showed the highest log reductions (3-4). ClO2 treatment negatively impacted fresh lean color, but only had a minimal effect on the color of fat and skin. Lipid oxidation was increased in treated products. Residues of free available chlorine, chloramines, ClO2, and chlorite in treated products were detected at levels of less than 0.05 mg/kg, which is lower than residue limit (0.1 mg/l) in drinking water. ClO2 can be used as an alternative sanitizer to reduce microbial contamination on whole animal carcasses or as one of a combination of treatments including trisodium phosphate wash, air cooling/chilling, and organic acid treatments.
**Project Rationale**
Conventional detection methods for identification of foodborne pathogens can take 24 to 48 hours or much longer. In order to facilitate timely intervention measures, the food industry needs more rapid detection methods and a sensor able to accurately and rapidly identify low levels of microbial foodborne contaminants within food systems or cultural media. We are investigating the efficacy of infrared (IR) technology as a means of rapid detection of select bacterial pathogens. There are many literature reports on the ability of FTIR methods to accurately and rapidly identify, classify, quantify, and differentiate between many types of bacteria. FTIR is a physico-chemical method that allows the chemically-based discrimination of intact cells without their destruction and produces complex whole-organism biochemical fingerprints (spectra) which are reproducible and distinct for different bacteria. We are combining sample handling procedures with FTIR approaches to improve the usefulness and effectiveness of FTIR for detection and identification of foodborne pathogens.

**Project Objectives**
We have 3 main research goals that include:
- Creation of a library of Fourier-transform infrared (FTIR) spectra of bacterial cell wall components and whole cells needed for pathogen identification and differentiation.
- Development of FT-IR methods for identification and quantification of these pathogens from water, cultural media, and select foods. This will include standardizing sampling procedures, quantification methods, spectral analysis procedures, and developing an overall chemometric approach for the analysis of FTIR data.
- Design of an IR sensor based on the most promising few-wavelength algorithms developed using FTIR data generated from research activities in the first two objectives.

**Project Highlights**
We successfully developed an approach for sample preparation, FTIR spectral collection, and data analysis that is able to both quantify and identify *Salmonella* and *E. coli* O157:H7 from mixtures of bacteria in culture media. This approach will be used as the format for development of the portable IR sensor. To develop this approach, we evaluated a variety of sample preparation methods, FTIR data collection methods, and analytical approaches for raw spectra. Further development of this approach will enable the design of a sensor that can be used in a production or retail facility to characterize a food sample as contaminated or free of select pathogenic bacteria in less time than current methods for detection.

“We successfully developed an approach...to both quantify and identify *Salmonella* and *E. coli* O157:H7...”

**INVESTIGATORS**
- Lisa Mauer (Principal), Maribeth Cousin, Jay Gore, Jean Guard-Petter, Brad Reuhs, Sivakumar Santhanakrishnan (College of Agriculture, School of Engineering)
**Project Rationale**
To minimize the risk of contaminants on our food supply, food production, processing, and retail establishments must be able to quickly and accurately identify and remove microbial foodborne contaminants such as pathogenic Salmonella, and Escherichia coli O157:H7. Conventional detection methods take at least 24 to 48 hours to differentiate and identify microorganisms; therefore, measures taken to counteract food contamination can be delayed until completion of the detection test. A popular category of commercially available rapid detection kits include immunological methods which are based on antigen-antibody reactions and DNA based methods, which include Polymerase Chain Reaction (PCR), Nucleic Acid Sequence Based Amplification (NASBA), hybridization, and microarrays techniques that measure residual DNA and mRNA that are specific for the target organisms. These reactions and measurements are independent of the physiological state of the cell and can measure all cells for up to 30 h post-thermal inactivation, therefore, a clear discrimination of the cell viability cannot be made. Detection of viable vs. non-viable cells is an important characteristic of a successful detection technology. This project studies the application of rapid FTIR techniques to detect viable cells in water, cultural media, and foods.

**Project Objectives**
- Develop FTIR methods and analytical approaches to differentiate between viable and non-viable cells of Salmonella spp. and E. coli O157:H7 in water, cultural media, and selected foods.
- Validate a miniature IR sensor for detecting the live bacteria.

**Project Highlights**
Our most significant finding confirmed that the FTIR analytical approach could successfully differentiate viable vs. non-viable bacteria as well as the specific type of processing treatment applied (heat, UV, salt, alcohol) leading to cell inactivation. Spectra from viable and non-viable cells were collected after using selected treatments commonly applied in the food industry and in the laboratory and then the spectra was analyzed using common chemometric methods. Results showed that the FTIR technique clearly differentiated viable vs. non-viable cells with 100% accuracy. In many cases, the spectra from FTIR were capable of categorizing non-viable cells based on the method used to destroy them.

“...the FTIR analytical approach could successfully differentiate viable vs. non-viable bacteria...”
**Project Rationale**

Our nation must protect against environmental sources of pollution that can contaminate the food supply, and guard against deliberate acts of terrorism intended to impact human health and/or weaken our economic base. The overall goal for our interdisciplinary research is the development of a core bioreporter-based chemical biosensor (BCB) platform for the eventual production of inexpensive biosensors to detect chemical agents that threaten our environment and food supply. The BCB platform consists of an enclosure/microenvironment system that contains minimal nutrients, genetically modified bioreporters, and in some applications an analytical transducer. The technology exploits the abilities of living microorganisms to sense and react to chemical stimuli; to be genetically manipulated to contain reporter genes, and to form biofilms which promote survival. Enclosures/microenvironment systems will be developed to facilitate resuscitation of microorganisms from storage and sustain them within biofilms in an optimal sensing state for the detection of chemical agents. Our research will utilize novel bioluminescent and pigmented bioreporters for biosensors that are genetically programmed to detect organic pesticides (e.g., paraquat), toxic metals (e.g., arsenic), and aromatic compounds. This research is expected to enable the development of BCB technology for rapid, inexpensive, selective (minimizing false-positive) and sensitive detection of many chemical threat agents. After core technology is developed, inexpensive application specific devices will be designed for use by agricultural personnel and/or scientists to identify environmental contamination or product tampering in both point-of-use and long-term monitoring applications.

**Project Objectives**

- Develop a prototype enclosure(s) containing a micro-environment that supports bio-reporting biofilms and in some applications contains a transducer(s) to facilitate rapid detection and long-term monitoring of biological responses to toxic compounds.
- Develop bioreporters for use in the enclosure/micro-environment for the detection of arsenic, paraquat, and aromatic solvents.
- Develop ancillary methodologies for determination of variables required for modeling.
- Combine constructed bioreporters with a prototype enclosure/micro-environment and obtain concentration dependent bioreporter response data for chemical agent detection.
- Use ancillary and empirical data from BCB and ancillary testing to develop models to help understand the bacterial response, and improve the analytical performance of the biosensors.

**Project Highlights**

This project has just recently been funded, therefore, research has just begun. At this point, we have assembled our multi-disciplinary team, hired graduate students, purchased required supplies, and begun work toward our research objectives.

“**This research is expected to enable the development of BCB technology for rapid, inexpensive, selective and sensitive detection of many chemical threat agents.**”
A biosensor can be defined as a device that is used to monitor or analyze a biological system or a particular biological or chemical entity. We live amidst a sea of biosensors around us and this technology has many applications, ranging from medical diagnostics, food safety, environmental monitoring, and homeland security. There has been considerable progress in the development of the biosensors. Glucose monitors for example, which are used by over 15 million diabetics in the U.S. Commercially available PCR technology can detect just a few strands of DNA. Antibodies against membrane-bound proteins or from lysates is the other most commonly used approach. Typically no more than 10% capture efficiency can be achieved with antibodies, with sensitivities in the nM-pM range for proteins. Aptamers are nucleic acid-based molecules that could potentially substitute antibodies, with improved properties. A critical aspect of the biosensor system is that of the sample preparation and acquisition, and means to get the target analytes close to the sensing element. This problem is especially problematic for food samples, blood or body fluids, etc., Blood and serum contain immunoglobulins, which are present at many orders of magnitude higher concentrations than the target proteins. Proteins cannot be amplified, and are especially difficult to detect in low concentration and means to rapidly separate and concentrate such proteins are very much needed. For the case of food safety, the goal is to detect as few as 10 live cells/100ml of fluid (Listeria Monocytogenes, for which FDA mandates a zero tolerance policy). This is very much equivalent to looking for a needle in a haystack. Lucky for us, bacteria can multiply in most cases, unless it is injured and stressed to not be able to grow. And here lies one of the main limitations of current techniques; it takes a long time for the bacteria to reach a concentration, which can be detected by current techniques. Many commercial systems now provide PCR result from a sample of 5-20ul solution, with less than 10 copies of the DNA in it, within 30-45 minutes. However, it must be noted that a cell amplification step precedes the PCR step, to bring the concentration to, let’s say, ~5 copies of the DNA in 20ul, or a concentration of ~250cells/ml.

Biosensors based on surface-plasmon resonance, quartz crystal micro-balance, etc. are increasing used to perform label free detection. Microfluidics and nanotechnology also promise to bring to reality, novel sensor arrays for real-time and label free detection of biological entities with high sensitivity and selectivity. Cantilevers with mechanical output, silicon nano-wires and carbon nanotubes with electrical output, and quantum dots and nano-shells with optical output are all very promising label-free candidates; some in the process of being commercialized now.

There is still a clear need for better ways to devise and develop sample preparation, extraction, and concentration techniques. Techniques to provide signal amplification, which are not limited by the time of cellular processes and multiplication, and especially for targets that cannot be currently amplified, are very much needed. Someday, in the not so distant future, the goals of instant pathogen detection from food and instant disease diagnostics for personalized medicine will become a reality.

"Glucose monitors are used by over 15 million diabetics in the U.S."
This past June, a group of Purdue University and USDA-ARS researchers representing the Center for Food Safety Engineering were invited to speak at the 25th International Workshop on Rapid Methods and Automation in Microbiology, held at Kansas State University. Our group presented a 1/2 day program focusing on the development and utilization of biosensors to detect biological foodborne pathogens. The symposium was part of “Molecular Detection Day”.

We were honored to be part of such a great workshop that Dr. Daniel Fung, from Kansas State, has provided leadership now for 25 years. The program content that was presented included information about CFSE research on bioseparation techniques and biosensor development, focusing on our work with *Listeria monocytogenes*. The presentations that were given are provided below. Our group hopes to continue and even expand our participation in next year’s program.

<table>
<thead>
<tr>
<th>PRESENTATION TOPIC</th>
<th>PRESENTER</th>
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<tbody>
<tr>
<td>Center for Food Safety Engineering at Purdue</td>
<td>Dr. Richard H. Linton, Director Center for Food Safety Engineering</td>
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<tr>
<td>Micro-scale bioseparations for pathogenic cell concentration and recovery</td>
<td>Dr. Michael Ladisch, Professor Agricultural and Biological Engineering</td>
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<tr>
<td>Optical immunosensors and cell-based detection for <em>Listeria monocytogenes</em></td>
<td>Dr. Arun Bhunia, Professor Department of Food Science</td>
</tr>
<tr>
<td>Nanotechnology and microfluidics for pathogen detection</td>
<td>Dr. Rashid Bashir, Associate Professor Electrical and Computer Engineering</td>
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<tr>
<td>Novel detection using bioluminescence techniques</td>
<td>Dr. Bruce Applegate, Associate Professor Department of Food Science</td>
</tr>
<tr>
<td>Phage-display method for antibody production and new sandwich methods for pathogen detection</td>
<td>George Paoli, Research Scientist USDA/ARS, Wyndmoor, PA</td>
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</tbody>
</table>


Abstracts for major papers/posters presented (2004-2005):


Book Chapters (2004-2005):


Invited Lectures and Seminars (2004-2005):


Bhunia, A.K. Biosensor – the next frontier in pathogen detection Auburn University, Auburn, AL. (March, 2004).

Huang, T., Chen, W., Geng, T., Gomez, R., Bashir, R., Bhunia, A., Ladisch, M.R., Fundamentals of Nanotechnology: Relationship to Food Science and Technology, Invited Lecture, Paper 45-1, at the Annual Meeting of Institute of Food Technologists, Symposium: Nanoscale Science, Engineering and Technology for Food Safety and Engineering, Las Vegas, NV (July 14, 2004).


Ladisch, M.R., Rapid Prototyping of Purification Platforms, University of Arizona (March 1, 2005).

Ladisch, M., Biotechnology Industry Organization, Nanotechnology and Press-fit Microdevices, The 2nd Annual World Congress on Industrial Biotechnology and Bioprocessing, Orlando, FL (April 20, 2005).


Patents Granted (2004-2005):

The logo of Purdue University’s Center for Food Safety Engineering (CFSE) represents the center in many more ways than one. Understanding the iconography of its images is one way to understand and appreciate CFSE.

**The Gear — Engineering**
One of the primary components of the CFSE logo is a gear, symbolizing the engineering component of the center. CFSE engages many of the different branches of engineering, and the gear calls to mind the basics of that discipline.

The teeth of the gear bear the insignia of the five Purdue schools that contribute to the center, organized alphabetically in a counterclockwise arc starting from the upper right.
- The first insignia represents the School of Agriculture;
- The second insignia represents the School of Consumer and Family Sciences;
- The third insignia represents the Schools of Engineering;
- The fourth insignia represents the School of Science; and
- The fifth insignia represents the School of Veterinary Medicine.

**The Circuit Board — Progress**
The circuit board in the center is actually part of the revolutionary biochip developed by Purdue researchers. In the CFSE logo, the biochip symbolizes the combination of engineering and food safety for technological progress.

**The Petri Dish — Food Safety**
The petri dish in the lower portion represents food safety, as epitomized by traditional microbial identification methods. One of the center’s goals is to develop more rapid methods of identifying microbes.

**The Circle — Synthesis**
The circle bounding the petri dish and the biochip inside the gear represents the synthesis of old and new technologies for improved food safety.