Title: Validation of Gold Nanoparticle DNA-based Biosensors for the Detection of Non-PCR Amplified Bacterial Food Borne Pathogens in Food Matrices

Principal Investigator: Sylvia Vetrone, Whittier College
Center of Excellence: Food Protection and Defense Institute (FPDI)
COE Lead/Co-Lead Institution: University of Minnesota

Project Start Date: 07/2014

Project Completion Date: 06/2015

Project Status: Complete

Research Theme: Agent Behavior

Participating State(s): California, Michigan, Minnesota

Amount Awarded to Date: $119,380

Award Number: 2010-ST-061-FD0001

Abstract: Our DNA-based biosensor, which utilizes gold nanoparticles for signal amplification and magnetic nanoparticles for easy and clean separation from samples, can detect non-PCR amplified genomic DNA of the bacterial foodborne pathogen Salmonella Enteritidis within basic, and acidic liquid food matrices (2% milk and 100% orange juice) --something that had not been demonstrated before. Consequently, in this project we set forth to improve upon the DNA extraction method, DNA-biosensor sensitivity and full validation of the detection capabilities of the biosensor, to achieve a system that can establish high sensitive detection within a matter of hours. In particular, we shortened the DNA extraction method by 65 % down to 30 minutes, along with the hybridization of samples to the biosensor components down to three hours, for an overall reduction of prep and run time at about 4 hours. In addition, our findings indicate that while the DNA-biosensor cannot reliably detect non-PCR amplified genomic pathogenic DNA targets within high viscosity food matrices (egg yolk and dog biscuits), it is able to detect pathogenic DNA targets at concentrations as low as 15 ng/ml from liquid and low viscosity food matrices (chicken and spinach), which are within the range (7-50 ng/mL) similar to those achieved with a PCR method. We believe these results to be very promising as we move towards transitioning this detection method towards a prototype and commercialization. Future efforts will focus on improving sample preparation of food matrices with high viscosity for DNA extraction.

Project Type: Research

End User Engagement:

- DHS Science and Technology Directorate
- Food and Agriculture Industries
- Food and Drug Administration
- State and Local Food and Agriculture Labs
- U.S. Department of Agriculture

Executive Summary (2015): Since the 2001 anthrax distribution through the United States Postal Service, the prospect of bioterrorism in food, water, and agriculture has identified critical needs in prevention, protection, and mitigation for homeland security. Rapid, onsite, point-of-care diagnosis is one such need. Although, a wide range of detection methods for bacterial food pathogens are commercially available, they can take 2-7 days for confirmation and require special reagents, large equipment, and facilities. Consequently, there has been much attention towards the development of rapid, sensitive, low cost, portable biosensors for the biological detection of pathogens that incorporate the use of nanoparticles for DNA detection. While these biosensors have been shown to detect specific DNA from various pathogens, many of them only detect purified and polymerase chain reaction (PCR)

amplified DNA targets (DNAt), and not from genomic DNAt extracted from pure bacterial samples or contaminated food matrices. Likewise, PCR is often criticized for its complex, expensive, time-consuming, and labor-intensive procedure requirements. Therefore, the primary objective of this project was to validate the ability of a gold nanoparticle DNA-based biosensor detection system to recognize non-PCR amplified Salmonella Enteritidis genomic DNAt extracted from solid food matrices. In is project, we improved upon the DNA extraction method and DNA detection scheme to reduce the overall time from preparation of test samples to detection without lowering DNAt yield or detection sensitivity. In particular, we shortened the DNA extraction method by 65 % down to 30 minutes, along with the hybridization of DNAt samples to the biosensor components down to three hours, for an overall reduction of prep and run time at about four hours. Additionally, by running test samples in triplicate, we were able to establish the sensitivity limits of detection by the biosensor. These results indicate that genomic DNAt extracted from low-viscosity food matrices (chicken and spinach) could be detected down to 15 ng/mL, a level of detection comparable to that achieved with PCR-amplification, between 7-50 ng/mL. Lastly, our findings also suggest that while the DNA-biosensor it is able to detect non-PCR amplified genomic DNAt extracted from liquid and low viscosity food matrices, it was unable to reliably detect non-PCR amplified genomic DNAt extracted from high viscosity food matrices (egg yolk and dog biscuits). Consequently, future efforts will focus on improving sample preparation of high viscosity food matrices for DNAt extraction. Taken together, these results are very promising as we move towards transitioning this detection system towards a prototype and commercialization. The validation of this gold nanoparticle DNA-based biosensor detection system demonstrates that it can provide recognition of DNAt within hours- not days, is inexpensive to fabricate, environmental-friendly, only requires an inexpensive multimeter for signal read-out, and can be highly portable for field applications. We believe these combined parameters make this gold nanoparticle DNA-based biosensor detection system an attractive method for bioterrorism countermeasure for prevention, protection, and mitigation.

Contact Information:

Food Protection and Defense Institute
University of Minnesota - Twin Cities
R285 LES Bldg
St. Paul, MN 55108
612-624-2458
fpdi@umn.edu