Project Title

Validation of Vibrio parahaemolyticus Detection Kit in Raw and Post-Processed Aquaculture Food Products

PI and Co-PI

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Reporting period: October 2013 to September 2014

Year funded: $30,000

New accomplishments

Vibrio analytical capability and capacity in the U.S. is extremely limited due to the high cost of equipment, supplies and highly skilled analysts needed to test oysters or other seafood using currently available and accepted methods. Adequate capability exists at a few state and federal shellfish control authorities and a handful of academic laboratories but not at any commercial laboratories in the Gulf region. In general this capability is directed at research and PHP validation/verification mainly for large firms handling high product volumes. Laboratories with vibrio analytical capability generally lack resources for monitoring at needed spatial and temporal granularity to identify high risk products or for surge capacity during outbreaks. Validation of the proposed two-phase testing approach for *V. parahaemolyticus* would provide an alternative method that would eliminate the need for expensive equipment and highly trained analysts and reduce the cost of expendable supplies by a factor of 10-fold relative to currently used methods such as real-time PCR. The above reductions in analytical resources and technical complexity would allow virtually all state shellfish control authorities to quickly achieve sufficient vibrio analytical capabilities and capacity to meet all monitoring needs and the surge capacity to manage outbreak response. Affordable testing could be provided by commercial laboratories to assist with validation and verification of PHP oysters by industry. Field testing capability could potentially expand testing to processing plants located in remote oyster growing areas. Taken together this expansion of capability and capacity would be transformational for risk management based on vibrio analysis, as MASGC is already funding a similar project for *V. vulnificus*.

Objectives

The objectives of this project are (1) to validate the arabinose and urea test kits for the detection of total and pathogenic *V*. *parahaemolyticus* in raw shellfish (live and processed) using procedures currently accepted by ISSC as the reference method; (2) to introduce the innovative key technologies and their detection/quantification concepts to educate extension

personnel; and (3) to transfer technology to the oyster industry and institutions that conduct post-harvest process (PHP) validation and verification testing.

Student supported

M.S. 100 % FET Demarcus Carter

Leveraged funds

Applied for

Funding agent: NOAA
Program: National Oceanic and Atmospheric Administration Sea Grant Aquaculture Research Program
Funding requested: $89,039.

Awarded

Funding agent: NOAA
Program: National Oceanic and Atmospheric Administration Sea Grant Aquaculture Research Program
Funding requested: $89,039.

Kim, T. DePaola, A., Jones J., Silva J.L., Koo, J. $89,039. National Oceanic and Atmospheric Administration Sea Grant Aquaculture Research Program. 2012-2014. Innovative Application of Classic Microbiology for Detecting *Vibrio vulnificus* in Raw and Post-Harvest Processed Oysters.

Outputs

Since we have developed the Vibrio assay kit, target audiences are food industry and their regulatory agents which needs rapid, easy and cost-effective Vibrio test kit. Oyster processing plants, university and national seafood labs have interest to adapt our technology to screen their products and environmental samples. Their feedback will be corrected to improve the kits.

Project Summary (Issue/Response)

The proposed project will address the FY2014 Topical Priority "*Research to inform pending, regulatory decisions on the local, state, or federal level leading to an information product-- such as a tool, technology, template, or model-- needed to make final decisions on a specific question regarding impacts of aquaculture"*. Some commonly used aquaculture practices, such as desiccation for anti-biofouling, allow pathogenic *Vibrio spp*. to proliferate in shellfish. Due to the potential public health risk of this anti-biofouling practice, the regulatory authorities required Alabama farmers to re-submerge oysters for 30 days prior to final harvest in 2013. The minimum amount of time the oysters need to be re-submerged after desiccation to allow *Vibrio* levels to return to background levels is currently being investigated. Validation of rapid tests kits for enumeration of total and pathogenic *V. parahaemolyticus* in oysters will provide investigators a rapid and cost-effective tool to evaluate not only the practice of re-submersion following anti-biofouling, but also other aquaculture practices that state and federal regulators may find likely to increase the risk of vibrio illness.

We have previously optimized overnight detection assays based on biomarker expression that provide exquisite selectivity and sensitivity for total and pathogenic *V. parahaemolyticus* using pure cultures, naturally contaminated, and inoculated oysters. The key features of these test kits is a novel two phase culture approach that allows simultaneous enrichment, separation and confirmation of *V. parahaemolyticus* which requires multiple steps and several days to complete using currently accepted regulatory methods. The liquid phase enhances recovery of stressed cells during enrichment while the solid phase provides selectivity by chemical suppression of background microflora and differentiation through two biomarkers, arabinose fermentation (total *V. parahaemolyticus*; Figure 1A) and urease expression (pathogenic *V. parahaemolyticus*; Figure1B). These two biomarkers were determined based on biochemical properties of 144 clinical and oyster strains of *V. parahaemolyticus* isolates provided by CDC and FDA, respectively. Arabinose was fermented by 92 and 93% of clinical and oyster *V. parahaemolyticus* isolates, respectively and all arabinose negative strains were urease positive (Jones et al., 2012). Additionally, DePaola et al. (2003) observed that 95% of pathogenic *V. parahaemolyticus* strains isolated from Gulf oysters produced urease. This novel detection kit is adaptable to standard test tubes or 96-well plates for overnight quantification of *V. parahaemolyticus*. Of even greater relevance is the Pacific NW invader strain that is responsible

for a 300% increase in Atlantic *V. parahaemolyticus* illnesses since 2012 produces urease positive. Illness outbreaks caused by this strain in 2013 lead to multiple closures and recalls that greatly disrupted the oyster aquaculture industry in several states. The ISSC has requested that FDA reassess the *V. parahaemolyticus* risk calculator that states use to evaluate their monthly risk levels. The availability of a validated method that is simple, fast and economical will greatly enhance the accuracy of the risk models that underpin the *V. parahaemolyticus* risk calculator. These tests provide a simple, rapid (18h) result for total and potentially pathogenic *V. parahaemolyticus* levels in oysters. Initial testing demonstrated 100% specificity against 48 *V. parahaemolyticus* and 26 non-*V. parahaemolyticus* and sensitivity of <10 cells/test. Using the 96-well plate format, comparability testing demonstrated excellent reliability of these test kits, with 183 naturally-incurred oyster samples from the Gulf, Atlantic, and Pacific Coasts tested and good agreement ($P < 0.05$) was observed between the test kit for total *V. parahaemolyticus* and real-time PCR.

Project Results/Outcomes

The detection kit will be applicable to all current testing needs for determining levels of total *V. parahaemolyticus* in oysters will have the following performance characteristics.

1) Detect a single *Vibrio parahaemolyticus* cell in a 0.1 g sample portion
2) Recover stressed *Vibrio parahaemolyticus* cells in raw (temperature abused) and PHP oysters.
3) Provide results within 20 h
4) Perform equivalently as standard FDA BAM method with regard to sensitivity (97%) and specificity (97%)
5) Require minimal training for analyst
6) Cost < $1/sample

Project Impacts/Benefits

The success of this project leaded the Vp test kits to a patent product available in US and international commercial market. Additionally, the method can be submitted to the ISSC as an approved method for use in validation and verification of PHP oyster processes.

Project Deliverables

Patent Disclosure and US Patent Application, Vibrio Assay Methods and Kits, Tech ID #2013.0883

Graphics

Not available

Attached Refereed Journal Publications in Separate Files

Please attached published journal articles (in pdf format if available) for this project. Manuscripts accepted or in review process may be submitted in word files. Thank you very much for your cooperation.