Project Title

Development of Quantifiable Pocket-Size Detection Kits for Foodborne Pathogens in Raw and Post-Processed Aquaculture Food Products

PI and Co-PI

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Year funded
2013- $35,000

Participants (FTE's for the reporting year)

M.S. Demarcus Carter: 1.0

Accomplishments

Development of rapid single tube assays for identification and enumeration of pathogenic vibrio using classical microbiological approach

Objectives

The objectives of this research were to develop a quantifiable pocket-size detection kit which (1) will give the results within a maximum of 24 h, (2) optimize recovery of pathogenic *Vibrio spp*. (Vibrio vulnificus in PHP samples), (3) be validated by comparison with FDA/USDA methods for ≥97% sensitivity and specificity, (4) will be designed for on-site testing without the need for expensive equipment.

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)

Vibrio vulnificus (Vv) is the deadliest pathogen in the US food supply and is predominantly associated with consumption of raw oysters grown in warm estuarine waters by individuals with chronic underlying illnesses or the immunocompromised. Environmental monitoring, market surveys and research efforts are hindered by the lack of efficient and economical assays for Vv. We developed and evaluated an assay based on classical microbiological characteristics of Vv including esculin hydrolysis, resistance to colistin and polymyxin B and motility. A bi-phasic medium composed of two agar layers overlaid with alkaline peptone water (APW), which allowed enrichment, selectivity and differentiation in a single step. A clear black visual signal was produced from esculin hydrolysis indicative of Vv presence was observed after overnight incubations of pure cultures and shellfish homogenates without any additional steps or manipulations. The assay was adapted to a 96-well format to simplify medium preparation and inoculation of samples into a most probable number (MPN) format that is typically applied for enumeration of Vv in shellfish samples. Testing with 47 diverse Vv strains and 26 neighbor vibrio strains demonstrated complete agreement with DNA probe colony hybridization and PCR results. During the summer of 2013, oysters and clams were collected from AL, CT and NY and shipped overnight under refrigeration to the FDA Gulf Coast Seafood Laboratory for analysis. Shellfish homogenates from each sample were prepared and inoculated into our bi-phasic medium as well as standard APW enrichment medium and incubated overnight at 35C. MPN estimates based on esculin hydrolysis in the bi-phasic medium agreed well with those obtained by real-time PCR of APW enrichments. The simplicity and economy of this assay could greatly

increase Vv analytical capacity in many laboratories that currently lack resources, expensive equipment and molecular expertise needed for real-time PCR and other available methods.

Project Results/Outcomes

The detection kit will be applicable to all current testing needs for determining levels of total *V. vulnificus* 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

The high cost of current *V. vulnificus* methods deters PHP progress for all but the largest processors and threatens the viability of the Gulf oyster industry. A simple and economical in-house testing program in the oyster processing facilities provides a smarter business model to advance PHP technology and sustain the Gulf oyster industry.

Project Impacts/Benefits

The impact of a new testing technology on in-house *V. vulnificus* testing can be seen in several different ways which benefit U.S. seafood industry. We expect practical approaches in the oyster processing plants which will demonstrate immediate impacts on U.S. oyster industry and regulatory agencies. First, PHP oyster processors will increase use of the one-tube *V. vulnificus* detection kit as a *V. vulnificus* control tool. The regulatory agencies will utilize the detection kit for early *V. vulnificus* control strategy and place immediate modification PHP upon high number of incidence and have a power to decrease *V. vulnificus* in PHP oyster.

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.