Optimization of Depuration-based Strategies using Chitosan Microparticles to Reduce Vibrio parahaemolyticus in the Eastern Oyster (Crassostrea virginica)

PRINCIPAL INVESTIGATOR: Dr. Anita Wright, Associate Professor
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CO-PRINCIPAL INVESTIGATOR: Dr. K. C. Jeong, Assistant Professor
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COLLABORATORS: Tom and Lori Howell, Owners, Spinney Creek Shellfish, Inc., Eliot ME; Dr.
Drs. Steve Jones and Cheryl Whistler, Associate Professors, University of New Hampshire, Durham, NH


PROPOSED BUDGET: Funds requested: $24,390
Matching Funds: $12,299

PROJECT START DATE: September 1, 2014
PROJECT COMPETITION DATE: August 31, 2014
EXECUTIVE SUMMARY

To sustain the U.S. shellfish industry, control measures are urgently needed for prevention of human disease associated with *Vibrio parahaemolyticus* and *V. vulnificus*. Despite successful application of post-harvest processing (PHP) such as freezing, heating, high hydrostatic pressure and irradiation for reduction of human exposure to these pathogens, disease incidence continues to increase (Newton et al., 2012). Moreover, most PHP protocols are lethal to molluscs, and the product is less suitable for the more lucrative “half-shell” market due to reduced shelf life and palatability. Our recent research focused on approaches that will support the sustainability of a live oyster product, using a practical, cost-effective methodology that is already approved (GRAS) for other food products and for drug delivery applications. Specifically, we examined the potential of chitosan microparticles as an effective PHP for reduction of *Vibrios* in live oysters. We found that application of 0.5% chitosan dramatically eliminated both *V. vulnificus* and *V. parahaemolyticus* in broth culture within 3 hours. A reduction of >3.52 log CFU/g was achieved for *Vibrios* in live oysters, thereby meeting the recommended ISSC validation criteria for oyster PHP (FDA, 2009). The results of these efforts provide the rationale for the requested funding. We propose to optimize chitosan PHP *in vitro* and in oysters at the University of Florida, with scale-up studies in a depuration-based platform to be conducted in collaboration with the Spinney Creek Shellfish, Inc. in Eliot, ME.

SCOPE, APPROACH AND METHODOLOGY

**Scope:** Although current PHP methods effectively lower *Vibrio* levels, they are generally expensive and detrimental to maintaining live oysters, as the moluscs are killed in the process. Options for *Vibrio* control in live oysters are the use of time-temperature controls, which impose serious restrictions on harvest practices, as well as management practices that include “relaying” or translocating oyster shellstock from areas with high numbers of potentially pathogenic *Vibrios* (usually lower salinity) to areas with reduced levels (higher salinity). Unfortunately, successful results from this strategy require extended exposure (up to a month), and little is known about possible consequences of this practice with regard to the spread of human and/or shellfish diseases. Thus, novel, rapid, and more economically feasible PHP strategies for successful treatment of live oysters are required. We propose the application of chitosan microparticles as a PHP that will greatly reduce or eliminate *Vibrio* spp. and is non-lethal to oysters (Fang et al., 2014, Abstracts of annual meeting of American Society for Microbiology and Fang et al., under review by Appl. Env. Microbiol.) Chitosan is a derivative of chitin, which is the second most abundant natural biopolymer on earth and is largely obtained from various marine organisms, such as the shells of crab, lobster and shrimp (Kurita, 2006). Because of low biodegradation of chitin, large amounts of crustacean exoskeleton wastes are accumulated after seafood processing, which accounts for 50-90% total solid waste landing in the US (Knorr, 1984). Chitosan is the soluble by-product of deacetylated chitin and has been shown to have a broad range of antimicrobial activity (Prashanth and Tharanathan, 2007). Microparticles are produced with minor modifications and have increased efficacy, especially under conditions of high salinity and pH (Jeong et al., 2011). In this respect, commercial production and application of chitosan from inexpensive seafood refuse is not only economically acceptable for the use of an oceanic resource, but also provides a solution for waste disposal.
**Supporting research:** Our research demonstrated the anti-*Vibrio* activity of 0.5% (wt:vol) chitosan microparticles when added to nutrient medium, resulting in growth cessation by 3 hours post treatment, and in fact, all *Vibrio* spp. examined were reduced to non-detectable levels (Figure 1). At lower chitosan concentrations efficacy of the treatment varied among species, with activity against *V. vulnificus* > *V. parahaemolyticus* > *V. cholerae*.

**Figure 1. Effects of chitosan on *Vibrio* growth.** *V. vulnificus* (*Vv*), *V. cholerae* (*Vc*), and *V. parahaemolyticus* (*Vp*) were cultured with chitosan (0, 0.1, 0.3, and 0.5%) at 37°C in Luria Broth. Bacterial growth was evaluated by plate count and results were mean of 3 independent experiments with three biological replicates each (standard deviations are shown by error bars).

The crucial test for the feasibility of chitosan PHP is assessing its activity in live oyster. Oysters were artificially inoculated with *Vibrios* by adding bacteria to seawater in holding tanks, as described below in methodologies. Significant (p<0.05) reductions were observed after 24h exposure, and >4 log CFU/g reduction was seen for both species by 48 hours using 0.5% chitosan (Figure 2).

**Figure 2. Effects of chitosan on *Vibrio* survival in live oysters.** Effects of chitosan treatment (0, 0.1, 0.3, 0.5%) on levels of *V. vulnificus* (*Vv*) and *V. parahaemolyticus* (*Vp*) in inoculated oysters were determined by plate count on selective agars. The mean Log CFU/g ± standard deviation of triplicate experiments was determined three independent experiments with three biological replicates each (standard deviations are shown by error bars).
As artificial inoculation of *Vibrios* in oysters may not reflect the response of natural populations of *Vibrios*, oysters were obtained during summer months when levels of *Vibrios* are elevated and subjected to chitosan treatment in two independent experiments. Results were consistent with artificial inoculations, and significant reductions (p<0.05) in *V. parahaemolyticus* and *V. vulnificus* levels were observed for all chitosan-treated oysters compared to control samples after 24 hours. Furthermore, treatments using 0.5% chitosan achieved the criteria of the NSSP guidelines for validation of live oyster PHP by **showing a >3.52 log CFU/g reduction** in trials where pre-treatment concentrations exceeded 10,000 bacteria/g. All treated samples met the Canadian Food Inspection Agency (CFIA) end-product guidelines for raw oysters, limiting *V. parahaemolyticus* counts to no more than 1 sample in 5 exceeding 100 total *V. parahaemolyticus* /gram and no single sample exceeding 10,000 total *V. parahaemolyticus* /gm.

**Approach and Methodology:** Our approach will be to optimize the chitosan PHP for applications to live oysters. Experiments described above showed greater efficacy for chitosan activity against *V. vulnificus* compared to *V. parahaemolyticus*. Thus, initial studies will focus on optimizing anti-*V. parahaemolyticus* activity in live oysters, as this pathogen poses the primary threat to the oyster industry due to large number of cases that have emerged in recent years. In vitro studies will examine time/temperature/salinity gradients that may function to enhance activity against fully virulent strains of this pathogen. Once optimum in vitro activity is achieved, small-scale oyster experiments, using both artificial and natural populations of bacteria, will be conducted to optimize the biological activity in live oysters. Scale-up of the chitosan treatment will be examined at the Spinney Creek Shellfish, Inc. facility using large holding tanks with recirculating seawater to achieve the desired reductions. Shelf life issues will be addressed by examining microbial populations at 1 and 2 weeks post-treatment.

Thus, objectives for proposed research include the following:

1. **Optimization of chitosan microparticle treatment as a novel intervention strategy for reduction of Vibrios in oysters** (to be conducted at the University of Florida);
2. **Scale-up of chitosan PHP for Vibrios in live oysters in saltwater holding tanks** (to be conducted at the Spinney Creek Shellfish, Inc, Eliot ME).

**Chitosan microparticles (chitosan) preparation:** The chitosan preparation and quality control will be performed by Dr. Jeong’s laboratory and follows a previously described protocol (Jeong et al, 2011). Briefly, a 1% (w/v) chitosan solution is prepared as a mixture of 2% (v/v) acetic acid and 1% (w/v) Tween®80. After addition of 2 ml of sodium sulfate (10% [wt/vol]), the chitosan solution is stirred and sonicated to increase cross-linking and then centrifuged, washed and dried.

**In vitro optimization of chitosan treatment:** Dr. Wright’s lab will perform optimization studies of chitosan PHP by examining bacterial survival in artificial seawater using various parameters of temperature, pH, and salinity. Experiments will be conducted in triplicate with 6 biological replicates each. Clinical strains of *Vibrio* spp. will be used for optimization of chitosan treatment: *V. vulnificus* (Vv) CMCP6 and MO6-24-O; *V. parahaemolyticus* TX2106 (Vp), and additional pathogenic strains of *V. parahaemolyticus* from recent disease outbreaks will also be obtained from researchers at the University of New Hampshire. Stock cultures are frozen at -80°C in Luria-Bertani with NaCl broth prepared with 1.0% tryptone, 0.5% yeast extract, and 1.0% NaCl (LBN) in deionized water with 50% glycerol, pH 8.4.
Chitosan PHP optimization in live oysters. Using optimized conditions based on in vitro results, optimization of chitosan PHP for elimination of Vp will be conducted on artificially inoculated live oysters (C. virginica) obtained from a local seafood market or obtained from Spinney Creek Shellfish, Inc. Oysters will be transported on ice packs and acclimated in dry storage at room temperature for 30 minutes in order to avoid temperature shock. Live oysters are then cleaned under tap water to remove any shells, dirt or debris, and up to 30 oysters are placed in 30 gallon Nalgene tanks containing 20 L ASW (Salinity, pH and temperature to be determined by in vitro studies) for 24 hrs at room temperature (25±1ºC) using two pumps for charcoal filtration. Following acclimation in ASW, chitosan PHP will be evaluated using either artificially inoculated or naturally infected oysters. For artificial inoculations, tetracycline treatment is performed to reduce the background Vibrio levels prior to infection, as previously described (Srivastava et al., 2009). Basically, oysters (n=6) are transferred to smaller tanks, containing 6 L ASW with tetracycline (10 µg/ml) and incubated without filtration for 24 hours in order to eliminate Vibrios prior to inoculation. Antibiotics are subsequently removed by incubation of oysters in fresh ASW for 24 hrs with charcoal filtration. Oysters are artificially inoculated by addition of V. vulnificus or V. parahaemolyticus (ca. 10⁶ CFU/ml) to the fresh ASW and are incubated without filtration for 24 h. Inoculated oysters are then transferred to new ASW containing various concentrations (0, 0.1, 0.3, and 0.5%) of chitosan for 24-48h. Oysters are shucked under sterile conditions using a shucking knife rinsed with ethanol (70%) and flamed. Individual oyster meats are aseptically collected in a 50 ml sterile conical tube, weighed, and homogenized with an equal volume of PBS for 30 seconds using a sterile mini blender (Seward, Stomacher® 80 Biomaster, Lab System) to prepare a 1:2 dilution sample suspension. Subsequently, serial 10-fold dilutions in PBS are spread plated in duplicate for presumptive identification of Vv on mCPC (Yellow colonies, Warner and Oliver, 2007) or on Vibrio CHROMagar™ (mauve colonies) and reported as log CFU/g. Experiments using chitosan on natural populations of Vibrios in oysters will be conducted as above but without tetracycline and by using a PCR-based MPN (Wright et al., 2007) and the DuPont Vibrio multiplex QPCR assay for detection of Vibrios. All experiments will be conducted in triplicate with 6 biological replicates each.

Scale-up of chitosan treatment in live oysters. Scale-up of chitosan PHP for live oyster application research will be conducted in collaboration with Tom and Lori Howell at Spinney Creek Shellfish Inc. They have a batch depuration facility that is not available in Florida and are equipped with a laboratory that conforms to requirements of the National Shellfish Sanitation Program. These studies are proposed in a separate proposal entitled “Depuration-based Strategies adding Chitosan Micro-particles to Reduce Vibrio parahaemolyticus in American Oysters using the Multi-Phasic Media Method for Process Verification”. (See attached letter of support).

Statistical analysis. Results of microbiological tests will be transformed to log values for statistical analysis. Significant differences among CFU/g levels of bacterial populations among treated and untreated samples will be tested using a T-test: Paired Two Samples for Means (Excel, Microsoft, Redmond, WA). Significant differences between means of treatments will be established at p<0.05. Analyses of variance (ANOVA) will be performed to test the null hypotheses that there were no effects of chemical and physical stressors on CFU/g levels of bacterial population collected from samples. If a null hypothesis is rejected, a Tukey test will be
used to identify differences. Tests will be performed at the 0.05 level of significance using Statistical Analysis Systems Software (SAS, version 8).

REFERENCES


Fang, L., K. C. Jeong, A. C. Wright. 2014. Chitosan Microparticles are effective for the reduction of *Vibrio* Species in live oysters. Abstracts of the Annual Meeting of the American Society for Microbiology, Boston, MA.


**PROJECT DELIVERABLES:** Measurable outcomes include a much-needed and optimized PHP for application to live oysters. Efforts will subsequently scale-up this PHP at a depuration facility in New England for *V. parahaemolytics* control in collaboration with Spinney Creek Shellfish, Inc.

**Timeline of major tasks in proposed research**

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<th>Task:</th>
<th>Quarter</th>
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<tr>
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<tr>
<td>1) Optimized PHP</td>
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<td>In vitro studies</td>
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<td>Inoculated oyster studies</td>
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<td>Natural oyster studies</td>
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<td>2) Scale-up trials for oysters</td>
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<td>3) Final report</td>
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**PROJECT MANAGEMENT APPROACH:**

Dr. Wright will provide overall supervision of all aspects of the project and be responsible for data management and reporting. Dr. Wright’s lab will conduct PHP optimization studies. A state-funded PhD student (Lei Fang) in her lab will assist in these studies. Dr. K.C. Jeong will supervise the technician to provide the chitosan preparation and quality control. Collaborators include Spinney Creek Shellfish, Inc. (Eliot, ME), who will provide facilities for chitosan PHP scale-up and validation using on-site tanks. Results will also be communicated through presentations at national (American Society for Microbiology) and international (International Molluscan Shellfish Safety Conference) meetings and the GOMA website. Data will be published in peer-reviewed journals and presented at national and international meetings. Dr. Wright has conducted *Vibrio* research for the last 35 years and served on the Methods Committee for ISSC and contributed to methods that are currently approved by ISSC.
DETAILED AND ITEMIZED BUDGET

Salaries: Salary is requested for part-time technician (33% effort=$10,000) with fringe calculated at 3.9% of salary ($390). This person will work with Dr. Jeong to prepare chitosan and assist in optimization studies. No salary is requested for PIs. Total Salary and fringe =$10,390.

Expendable supplies and equipment: Microbiological reagents, including selective media ($2000) and various expendables ($1000) are requested for presumptive evaluation of *Vibrio* survival in chitosan-treated samples. Molecular PCR reagents, Dupon Qualicon Vibrio Kits ($1000 each x 2) and other reagents ($1000) are needed for confirmation of *Vibrios* ($3,000 total) are requested for Dr. Wright's Lab. Chitosan and other reagents ($4,000) are requested for generation of chitosan microparticles are requested for Dr. Jeong. Total supplies = $10,000.

Travel: Funds are requested for travel for Dr. Wright and her student to participate in scale-up studies, including 2 trips to Maine. Each trip will be for 5 days for 2 people and include airfare ($1000) hotel ($500), food ($200) car rental ($300) or for a total of approximately $2000/trip. Total travel=$4000.

Indirect costs: Not allowable.

Total costs: $24,390.

Matching funds: Dr. Wright will contribute 10% effort in salary ($9624) and fringe ($2675) as matching funds. Total = $12,299.
### 2014 ISSC BUDGET

**Grantee Institution:** University of Florida  
**Grant/Project Number:**  
**Principal Investigator:** Anita Wright  
**Duration / Months:** 12

#### A. Salaries and Wages:

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<th>Months</th>
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<th>MATCHING FUNDS</th>
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<td>1. Senior Personnel:</td>
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<td>a. (Co) Principal Investigator</td>
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<td>b. Associates (Faculty or Staff)</td>
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<td>Sub total</td>
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<td>2. Other Personnel:</td>
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<td>Total Salaries and Wages</td>
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#### B. Fringe Benefits (when charged @ (OPS= 3.9%))

| | | 390 | 2,675 |

**Total Salaries, Wages, and Fringe Benefits (A and B)** | 10,390 | 12,299 |

#### C. Permanent Equipment

| Diversilab microcappilary unit | | 10,000 |

#### E. Travel:

| Domestic - US and its Possessions (Inc. Puerto Rico) | | 4,000 | 0 |
| International | | 0 |
| Travel - Total | | 4,000 | 0 |

#### F. Publications and Documentation Cost

| | | |

#### G. Other Costs:

| 1. Computer Costs | | |
| 2. Consultants | | |
| 3. Copying, Library, and Communication | | |
| 4. Analytical and Shop Services | | |
| 5. Fuel, Boat time, Vehicle Usage, Space Rental | | |
| 6. Tuition / Stipend | | |
| 7. Subcontract - Other Institutions | | |

| Total Other Costs | | 0 | 0 |

**Total Direct Costs (A through G)** | 24,390 | 12,299 |

**Modified Total Direct Cost** | 24,390 | 12,299 |

**Indirect Costs:** Not allowed

**Other IDC, Explain in budget justification** | 0 | 0 |

**Total Indirect Costs** | 0 | 0 |

**Total Costs** | 24,390 | 12,299 |
July 29, 2014

To whom it may concern,

I am writing to express the support of Spinney Creek Shellfish, Inc. for the project entitled, Optimization of Depuration-based Strategies using Chitosan Microparticles to Reduce Vibrio parahaemolyticus in the Eastern Oyster (Crassostrea virginica), which will be submitted in response to the July 2014 ISSC funding opportunity.

Spinney Creek Shellfish owns and operates a multi species depuration plant in Maine. We process and market several of our own brands of value-added, quality-assured molluscan shellfish. A substantial portion of our income is derived from oyster and hard clam sales. The east coast Vibrio parahaemolyticus illnesses of the past few summers threatens not just our own business, but also those of over a thousand oyster and clam farms on the east coast. We have participated in Vibrio research for more than 20 years, and the proposal submitted by Drs. Wright and Joeng shows the most promise that we have seen over this period. The preliminary data offered by the principal investigators is extremely promising and is worthy of your immediate support.

Spinney Creek Shellfish has committed to collaborate on this project and will provide process system design and use of our facilities, including installation of an experimental system, as appropriate. This proposed technology, if fully vetted, promises to be the secret ingredient to reducing both Vibrio vulnificus, and Vibrio parahaemolyticus levels in shellfish. This would provide an additional tool for shellfish growers and processors to reducing risk of vibrio illness, strengthening consumer confidence and eliminating one of the major obstacles in the shellfish business. We strongly urge your support for this project.

Sincerely,

Lori A. Howell
Vice President
Appendix 1- References

Dr. Jody Harwood, Professor and Interim Chair, Department of integrative Research, University of South Florida, Tampa, Florida. Email: vharwood@usf.edu; Phone: 813 974-1524

Dr. Glenn Morris, Director Emerging Pathogens Institute, University of Florida, Gainesville, Florida 32611; Email: jgmorris@epi.ufl.edu; Phone: (352) 273-7526

Dr. Cova Arias, Professor, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, Auburn, Alabama; Email: ariascr@auburn.edu; Phone: 334-844-9215

Appendix 2- Project Team Staffing

Principal Investigator: Dr. Anita Wright, Associate Professor

Address: Food Science and Human Nutrition Department, University of Florida, PO Box 110370, Bldg 475 Newell Dr., Gainesville, FL 32611-0370
Phone: 352-392-1991 ext311
E-mail: acw@ufl.edu

Dr. Wright has been involved in Vibrio research for over 30 years. She has courtesy appointments in the Microbiology and Cell Science Department and the Emerging Pathogens Institute at the University of Florida. Her research efforts include the investigation of virulence factors for V. vulnificus, development of rapid diagnostics for Vibrios and other pathogens, and the validation of oyster PHP in collaboration with the Seafood Extension program at the University of Florida. Besides an active research program, she teaches both undergraduate and graduate courses in Food Microbiology and has chaired 5 PhD and 10 MS committees and served as advisor for numerous others. She was past president of the Southeastern Branch of the American Association for Microbiology and was a recipient of an Innovation Award from the University of Florida. Since her appointment she has been continuously funded with awards from USDA, Sea Grant, and the Center for Produce Safety. Currently, Dr. Wright is the director of the SE Regional Center for the FDA Next Generation Sequencing Project. She served on the Governor’s Task Force on Oyster Health in Apalachicola Bay, FL (2012-present). She was a member of steering committees for Vibrios 2011 Conference, Santiago DeCompastella, Spain (2011); Vibrios in the Environment Conference (2010); Florida Marine Biotechnology Summit (2002-2007). She organized a Vibrio session for the 2014 National Shellfisheries Association in Jacksonville, FL. She is a past member of the ISSC Methods Committee (2005-2009) and for Advisory Boards for University of Florida Emerging Infectious Disease Institute (2009-2013) and for BioFlorida (2004-2009). She chaired the Graduate Committee Food Science and Human Nutrition Department (2004-2010) and was a member University of Florida Curriculum Committee (2004-2010). She currently serves on the UF Graduate Scholarship and the Faculty Enhancement Committees. She also served on review panels for USDA Food Safety NRI (2002, 2004, 2010). She was past president of the Southeastern Branch of the American Society for Microbiology (2010)
Co-Principal Investigator: Dr. K. C. Jeong, Assistant Professor

Address: Animal Science, University of Florida, Emerging Pathogens Institute, P.O. Box 103633, Gainesville, FL 32610-3633
Phone: 352-294-5376/352-392-3889
E-mail: kcjeong@ufl.edu

Dr. Jeong has pioneered veterinary application of chitosan treatments in cattle. His ultimate research goal is to develop interventions for pathogens in animals for the benefit of animals and humans. To achieve this goal, his research areas are not only in basic sciences but also in applied sciences. The primary goal of basic science research is to understand molecular mechanisms of antimicrobial resistance, colonization, host-microbe interactions, and survival of pathogens in hosts. Identification of genetic traits responsible for the survival of pathogens in hosts and characterization of genes and proteins will provide insights for the development of intervention technologies. Dr. Jeong’s repertoire of knowledge in molecular biology, biochemistry, cell biology, genomics, metagenomics, and genetic techniques will be applicable to various aspects of researches. Developing intervention technologies to fight against pathogens using micro and nanoparticles is another key part of his research program. Chitosan microparticles have been developed as an alternative antimicrobial agent, and his research has focused on the increment of efficacy in chitosan microparticles targeting a broad spectrum of pathogens, including antimicrobial resistant microorganisms. Furthermore, development of nanoparticles with high specificity against pathogens has been funded by the USDA.

Additional Staff:

Lei Fang, PhD Student Food Science and Human Nutrition Department, University of Florida, PO Box 110370, Bldg 475 Newell Dr., Gainesville, FL 32611-0370
Lei is a fourth year PhD student with anticipated graduation in Fall, 2015. She is a recipient of the University of Florida Alumni Award and is fully funded through 2015. The supporting data described in the proposal on the application of chitosan for elimination of Vibrios in oysters is a component of her PhD dissertation.

Collaborators:

Tom and Lori Howell: Owners, Spinney Creek Shellfish, Inc., 27 Howell Drive, Eliot, Maine 03903; Phone: 207-439-2719, Ext. 1; E-mail: tlhowell@spinney creek.com and lahowell@spinney creek.com. Tom and Lori have owned their shellfish depuration facility in Maine for nearly 30 years and will provide the expertise and facilities for scaling up depuration system for the chitosan PHP.

Drs. Steve Jones and Cheryl Whistler, Jackson Estuarine Laboratory and the University of New Hampshire, Durham, NH Durham, NH 03824; E-mail: shj@unh.edu and cac36@unh.edu. The University of New Hampshire team will provide expertise in the evaluation of pathogenic V. parahaemolyticus in oysters.

Project team members are not bonded. We are unaware of any requirement for bonding of employees for this project. Please advise if we have overlooked this. We affirm that
no employees working on this project have ever been convicted of a felony.

Appendix 2- Company overview

Official Name: University of Florida, Gainesville, Florida (UF Duns Number: 969663814), Division of Sponsored Research. PO Box 115500, 219 Grinter Hall, Gainesville, FL 32611; Phone 352-392-1582; Fax 395-392-4400.

Key Contact Name: Anita Wright Food Science and Human Nutrition Department, University of Florida, PO Box 110370, Bldg 475 Newell Dr., Gainesville, FL 32611-0370; Phone: 352-392-1991 ext311; Fax 352-392-9467

Person authorized to contractually bind the organization for any proposal: Brian Prindle, Associate Director, Division of Sponsored Research, University of Florida, Gainesville, Florida.

University of Florida is one of the oldest land grant universities and was established in 1853.

The submitters have no conflict of interest for the proposed research.
Office of Research  
Division of Sponsored Research 
PO Box 115500 / 219 Grinter Hall 
Gainesville, FL 32611-5500 
Phone: (352) 392-1583 
Fax: (352) 392-4600

UNIVERSITY OF FLORIDA

DFR—1 
Sponsored Projects 
Approval Form

Principal Investigator: Anita Wright  
Multiple PI Project: [ ] Yes [ ] No  
Department: Food Science and Human Nutrition  
College: IFAS 
Current UPRM: [ ] DSR Completes

Project Title: Techniques and Practices for Vibrio Reduction

Funding Agency: Interstate Shellfish Sanitation Conference

Type: [ ] New [ ] Renewal [ ] Collaboration [ ] Supplemental [ ] Revised
Change of PI [ ] Yes [ ] No  
Change Dept ID [ ] Yes [ ] No  
UF/Dept Person to discuss Application [ ] Yes [ ] No  
Other* [ ] Yes [ ] No

Human Subjects [ ] Yes [ ] No  
Animal Subjects [ ] Yes [ ] No  
Turnover DNA/RNA [ ] Yes [ ] No  
Biobanks [ ] Yes [ ] No  
[ ] (If, you, attach the IRB and/or the IACUC approval letter)

Application Mailing Instructions:  
Mail Original and ___ copies to: rmrntz@ufl.edu

Check all that apply:  
[ ] Grants.gov  
[ ] Other Electronic System  
[ ] Fax  
[ ] Other Oversight  
[ ] First Class Mail  
[ ] Fax to:  
[ ] Email PDF  
[ ] Release back to P1  
[ ] Internal Only (no mailing)

Cost Sharing:  
[ ] Mandatory: $12,299.00 (Attach the required cost share letter and agency guidelines)  
[ ] Voluntary Committed: $0.00 (Attach the “Grant’s Approval” Letter)

(DSF Used) DSR Staff:  
Received  
Action  
Date

(PEDES Account Number)

Multiple Principal Investigators: For those projects designated as a Multiple PI Project the named PIs share the responsibility for directing and managing the project in accordance with University and Sponsor policies and procedures. The Contract PI will be responsible for relaying communications between all of the PIs, University Officials and the Sponsor.

Principal Investigator Assurance Statement: By signing below you agree to perform the work and manage the project in accordance with University and Sponsor policies and procedures.

Investigator(s) Assurance Statement as Required by Federal Regulations: Investigator(s) by signing this DSR-1 form, further certify that: (1) the information submitted within the application is true, complete and accurate to the best of their knowledge; (2) that any false, fictitious, or fraudulent statements or claims may subject the Investigator(s) to criminal, civil, or administrative penalties; and (3) that the Principal Investigator(s) agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports and the final report if a grant is awarded as a result of the application.

University Assurance: This project has been reviewed by the officials whose signatures appear below as they relate to their areas and are satisfied that all faculty involved in the project have agreed to participate and that all obligations and commitments described herein are acceptable.

Indirect Cost Distribution: Upon receipt of DSR’s Notice of Award, Principal Investigator(s) are instructed to use the Office of Research web-based F&A Manager to declare how the indirect costs collected under the award shall be distributed. The return of indirect costs generally occurs in the Fall of each year and is based upon the indirect costs collected from grants and contracts during the preceding fiscal year (July 1 - June 30).

Principal Investigator:  
NAME: Anita Wright  
TITLE: Associate Professor  
DATE: 7/25/14

DIRECTIONS: Print all names.  
NAME: K.C. Jeong  
TITLE: Assistant Professor  
DATE: 7/25/14

DEPARTMENT: Food Science and Human Nutrition

NAME: Dr. Douglas L Archer or Dr. Mary L Duriea  
COLLEGE: Food Science and Human Nutrition

NAME: K.C. Jeong  
TITLE: Assistant Professor  
DATE: 7/25/14

DEPARTMENT: Food Science and Human Nutrition

NAME: Dr. Douglas L Archer or Dr. Mary L Duriea  
COLLEGE: Food Science and Human Nutrition

NAME: DSR-1 PDF (September 3, 2009)
Multiple Principal Investigator Projects: For those projects designated as a Multiple PI Project the listed PIs share the responsibility for directing and managing the project in accordance with University and Sponsor policies and procedures. The Contact PI will be responsible for relaying communications between all of the PIs, University Officials and the Sponsor.

Principal Investigator: Anita Wright

Multiple PI Project: Yes

Department: Food Science and Human Nutrition

DSS-1 #: 390-4100

Phone: (352) 392-1991 ext. 211

Date: 7-25-14

Please add additional signature sheets as needed.

NAME: Anita Wright

TITLE: Associate Professor

PHONE: (352) 392-1991 ext. 211

DEPARTMENT: Food Science and Human Nutrition

Department Chair: Susan E. Perzival

DEPARTMENT: Food Science and Human Nutrition

College Dean:

Dr. Douglas L. Archer or Dr. Mary L. Dunye

COLLEGE: Dean for Research

Date: September 1, 2009

Please add additional signature sheets as needed.
Cost Sharing Commitment

TO: Division of Sponsored Research
FROM: PI: Anita Wright
UNIT: Food Science and Human Nutrition
SUBJECT: Proposal Title: Techniques and Practices for Vibrio Reduction
Sponsor: Interstate Shellfish Sanitation Conference

This proposal involves Voluntary Cost Sharing. The justification is given below.

Justification: Preference will be given to submitters offering to match ISSC funding support. The ISSC is encouraging one to one matching funds.

As the Unit Leader, I have received and concur with the justification provided for the voluntary cost sharing amount that we are now obligating for this proposal. I understand that the Third Party Cost Sharing (if any) is committed independently from my Unit's resources by the letters provided and attached to this document.

<table>
<thead>
<tr>
<th></th>
<th>Amount</th>
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<tbody>
<tr>
<td>Cost Sharing for Personnel</td>
<td>$12,298.81</td>
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<td>Cost Sharing for Non Personnel</td>
<td>$0.00</td>
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<td>Third Party Cost Sharing</td>
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<td>Total Commitment</td>
<td>$12,298.81</td>
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This Commitment is acknowledged and agreed to on July 29, 2014.

Unit Head Signature: ____________________________
Susan S. Percival

Dean's Signature: ____________________________
Dr. Douglas L. Archer or Dr. Mary L. Duryea
ANITA C. WRIGHT

ADDRESS: University of Florida
Food Science and Human Nutrition Dept.
PO Box 110370 Gainesville, FL 32611

PHONE: 352-392-1991 x 311
EMAIL: acw@ufl.edu

EDUCATION
B.S. Florida State University, Tallahassee, FL 1974 Experimental Psychology
M.S. University of North Carolina at Charlotte, NC 1983 Biology
Ph.D. University of Maryland, Baltimore, MD 1997 Molecular Microbiology

POSITIONS
2005-present Associate Professor, University of Florida, Gainesville, Florida.
1999-2005 Assistant Professor, University of Florida, Gainesville, Florida
1997-1999 Post Doctoral Associate, Center for Marine Biotechnology, Baltimore, MD
1984-1997 Research Associate, University of MD Medical School, Baltimore, MD


RESEARCH INTERESTS:
Dr. Wight is a food microbiologist who focuses on the ecology and evolution of foodborne pathogens in environmental reservoirs. Her research experience includes investigations on the virulence, survival, and environmental distribution of Vibrio and Salmonella species. Outcomes have resulted in the development of rapid methods for the enumeration and molecular characterization of these pathogens in aquatic reservoirs and food products. Collaborations with the Emerging Pathogens Institute explored the role of aquaculture in the evolution of virulence of V. vulnificus in Bangladesh. She directs the Southeastern component of the U.S. FDA Next Generation Sequencing Project and will access these resources for phylodynamic evaluation of pathogens. She has over 40 peer-reviewed papers and has received more than $2 million in grants support. She has supervised five PhD and 8 MS students during her tenure at UF.

SELECTED PUBLICATIONS (Out of 46 total):
Microbiol. The manuscript has been assigned the control number AEM00954-14.)


## CURRENT AND PENDING SUPPORT: Anita C. Wright

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<tr>
<th>NAME (List/PD #1 first)</th>
<th>SUPPORTING AGENCY AND AGENCY ACTIVE AWARD/PENDING PROPOSAL NUMBER</th>
<th>TOTAL $ AMOUNT</th>
<th>EFFECTIVE AND EXPIRATION DATES</th>
<th>% OF TIME COMMITTED</th>
<th>TITLE OF PROJECT</th>
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<td>Wright (PI), Teplitski, Gulig</td>
<td>Previous: USDA- AFRI</td>
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<td>7/08-12/12</td>
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<td>Post harvest treatment of Live Oysters and Investigation of Therapeutic Potential of Biological Controls</td>
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<td>Wright (PI)</td>
<td>Sea Grant</td>
<td>$198,108</td>
<td>3/09-2/13</td>
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<td>Implementation of Vibrio monitoring methods needed to sustain Florida coastal communities</td>
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<td>Otwell Rodrick</td>
<td>USDA- AFRI</td>
<td>$124,910</td>
<td>2/10-12/12</td>
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<td>Antimicrobial peptides for reduction of vibrios in oysters</td>
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<td>Jones (PI) Wright</td>
<td>Center for Produce Safety</td>
<td>$333,000</td>
<td>10/11/-6/13</td>
<td>5%</td>
<td>Science-based evaluation of regional risks for Salmonella contamination of irrigation water at mixed produce farms in the Suwannee River watershed</td>
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<td>Wright (PI), Van Bruggen, Danyluk, Adams</td>
<td>Center for Produce Safety</td>
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<td>1/13-12/13</td>
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<td>Science-based evaluation of risks associated with wildlife exposure for contamination of irrigation water by Salmonella</td>
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<td>Wright (PI), Jay-Russell</td>
<td>Subcontract to FL DOH</td>
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<td>12/12-11/14</td>
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<td>Next Generation Sequencing Project for Foodborne Pathogens</td>
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<td>Wright (PI on subcontract) Blackmore (PI on project)</td>
<td>Pending: Center for Produce Safety</td>
<td>$162,260</td>
<td>1/15-12/17</td>
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<td>Partnership for Next Generation Sequencing of Salmonella</td>
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<td>Wright (PI), Jeong, Salemi</td>
<td>Sea Grant</td>
<td>#339,751</td>
<td>9/1/14- 8/31/16</td>
<td>20%</td>
<td>Sustaining Florida Aquaculture through Improved Process Technology and Monitoring of Vibrio species</td>
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</table>
Kwang Cheol (K.C.) Jeong, Ph.D.

Assistant Professor of Microbiology, Department of Animal Sciences; and Emerging Pathogens Institute, University of Florida. 2055 Mowry Rd, PO Box 10009, Gainesville, FL 32611
Phone: 352-294-5376, E-mail: kcjeong@ufl.edu

EDUCATION
• Ph. D. University of Wisconsin-Madison, Food Microbiology and Toxicology; and Department of Bacteriology, Madison, Wisconsin (2004)
• M. S. Chonnam National University, Food Science and Technology, Korea (1998)
• B. S. Chonnam National University, Food Science and Technology, Korea (1996)

APPOINTMENTS
• Assistant professor, University of Florida (2011 – present)
• Faculty of Animal Molecular and Cellular Biology, University of Florida (2011 – present)
• Postdoctoral Research Associate, Department of Molecular Microbiology, Washington University in St. Louis, School of Medicine (2005 – 2010)

RESEARCH INTERESTS
Dr. K. C. Jeong’s ultimate research goal is to intervene pathogens for the benefit of animals and humans. Developing intervention technologies to fight against pathogens using micro and nanoparticles is another key part of his research program.

PROFESSIONAL MEMBERSHIPS AND HONORS
• Associate Faculty Member in Faculty of 1000 (2010 – present), member of American Society for Microbiology, IAFP, FAFP, IFT, Sigma Xi, and R&D planning/evaluation board of Ministry of Trade, Industry and Energy (MOTIE) in Korea (2013 – present), Member of
• First place, Poster competition, Annual meeting of Food Research Institute, University of Wisconsin, Madison, WI (2003). Berg/Morse Fellowship Award, Washington University in St. Louis, School of Medicine (2007). The labarotorian of the year-2012, Florida Association of Food Protection (2013). Career development award, IFAS, University of Florida (2013).

PUBLICATIONS (Most relevant to the current application-selected from 26 publications)


**B. Book chapters**


**CURRENT AND PENDING SUPPORT: K. C. Jeong**

<table>
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<th>Externally funded</th>
<th>Start/End Year</th>
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<td>chitosan microparticles to lactating dairy cows with</td>
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<tr>
<td>2012</td>
<td>$10,000</td>
<td>Center for Veterinary Medicine, UF. Effect of uterine administration of chitosan microparticles to lactating dairy cows with metritis on subsequent uterine bacterial microbiota</td>
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<td>2013</td>
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<td>IFAS, UF. Underlying mechanisms of Antimicrobial resistance in cows with uterine diseases</td>
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<td>UF-Opportunity Fund, Rising concern of antimicrobial resistance: Are food animal producers friends or foes?</td>
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<td>Israel, The effect of selected lactic acid bacteria on the microbial composition and on the survival of pathogens in the rumen in context with their probiotic effects on ruminants</td>
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<td>USDA NIFA, Food Safety, Nutrition, and Health Cranberry application for prevention of inflammatory bowel disease by improving gastrointestinal health</td>
<td>Co-PI Park (PI)</td>
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