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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Phone: 352-294-5376/352-392-3889
E-mail: kcjeong@ufl.edu

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

ISSSC 2014 PROPOSAL: Techniques and Practices for Vibrio Reduction

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

PROJECT START DATE: September 1, 2014
PROJECT COMPETION 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*. Depite successful application of post-harvest processing (PHP) such as freezing, heating, high hydrostic 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 sustainabilty 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 oyters 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*.

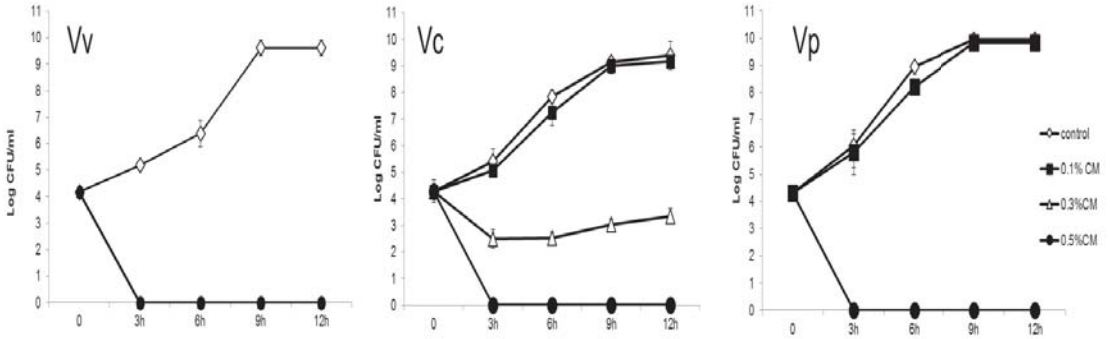

Figure 1. Effects of chitosan on *Vibrio* growth. *V. vulnificus* (Vv), *V. cholerae* (Vc), and *V. parahaemolyticus* (Vp) were cultured with chitosan (0, 0.1, 0.3, and 0.5%) at 37^{o}C in Luria Broth. Bacterial growth was evaluated by plate count and results were mean of 3 independent experiments with 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).

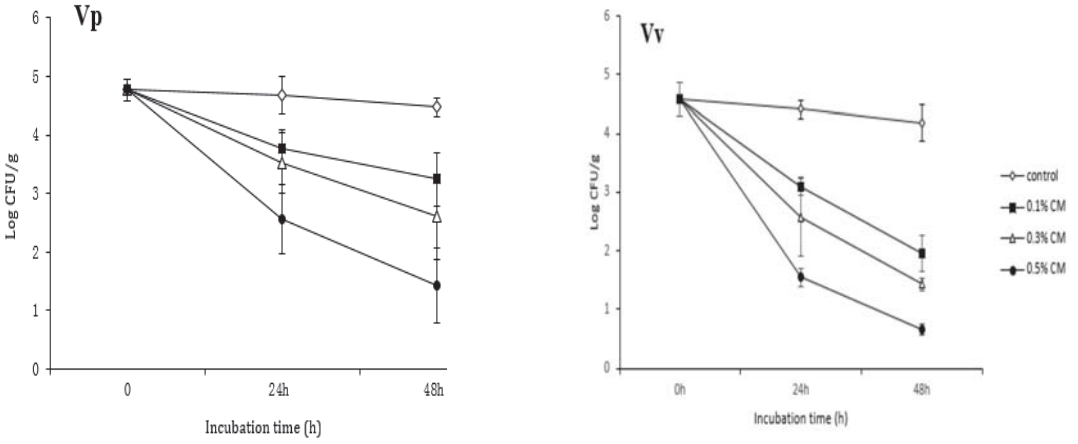

Figure 2. Effects of chitosan on *Vibrio* survival in live oysters. Effects of chitosan treatment (0, 0.1, 0.3, 0.5%) on levels of *V. vulnificus* (Vv) and *V. parahaemolyticus* (Vp) in inoculated oysters were determined by plate count on selective agars. The mean Log CFU/g ± standard deviation of triplicate 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. 10^6 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 tetraclycline 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

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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

Task:	Quarter			
	1	2	3	4
1) Optimized PHP				
In vitro studies	■			
Inoculated oyster studies		■	■	
Natural oyster studies			■	
2) Scale-up trials for oysters			■	■
3) Final report				■

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:**	
	Grant budget period: 9/1/14-8/31/15				
	Principal Investigator: Anita Wright			**Duration / Months:**	12
A.	**Salaries and Wages:**	**No. of People**	**Man Months**	**SEA GRANT FUNDS**	**MATCHING FUNDS**
1.	**Senior Personnel:**				9,623
a.	(Co) Principal Investigator				
b.	Associates (Faculty or Staff)				
	Sub total	**0**	**0.0**	**0**	**9,623**
2.	**Other Personnel:**				
a.	Professionals				
b.	Research Associates part time tech	1	6.0	10,000	
c.	Research Asst./Grad. Students				
d.	Prof. School Students				
e.	Pre-Bach Students				
f.	Secretarial-Clerical				
g.	Technical-Shop				
h.					
	Total Salaries and Wages	**1**	**6.0**	**10,000**	**9,623**
B.	Fringe Benefits (when charged a **(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**				
D.	**Expendable Supplies and Equipment**			**10,000**	
	Chitosan, micro, pcr				
E.	**Travel:**				
1.	Domestic - US and its Possessions (Inc. Puerto Rico)			4,000	0
2.	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				
8.					
9.					
	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**

Lori A. Howell, M.S., J.D.
27 Howell Drive
Eliot, Maine 03903
Plant and Sales: 207-439-2719
Fax: 207-439-7643
Email: lahowell@spinneycreek.com

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 (2004-2009). 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
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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@spinneycreek.com and lahowell@spinneycreek.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
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Fax: (352) 392-4400

UF UNIVERSITY of FLORIDA

DSR—1

Sponsored Projects
Approval Form

Principal Investigator: Anita Wright

Multiple PI Project: ☐ Yes ☑ No — For Multiple PI Projects one Contact PI must be identified in the signature block

Department: Food Science and Human Nutrition

College: IFAS

Current UPN#: (DSR Completes)

Project Title: Techniques and Practices for Vibrio Reduction

Funding Agency: Interstate Shellfish Sanitation Conference

If Known:

PeopleSoft Proposal #:

PeopleSoft Project #:

Type: ☑ New ☐ Renewal ☐ Continuation ☐ Supplemental ☐ Revised ☐ Change of PI ☐ Change Dept ID

Category: ☑ Research ☐ Training ☐ Extension ☐ Clinical Trial ☐ Other*

*(Fellowships, patient services, public service, conference, etc.)

UF/Dept Person to discuss Applicaton (name/phone/email):
Meri Nantz
(352) 392-1991 x 206
mnantz@ufl.edu

Application Deadline:
☐ Postmark ☑ Receipt ☐ None
Date: 07/31/14

Check all that apply:	Yes	No	Pending
*Human Subjects (IRB)	☐	☑	☐
*Animal Subjects (IACUC)	☐	☑	☐
Recombinant DNA/RNA	☐	☑	
Biohazards	☐	☑	

*(If yes, attach the IRB and/or the IACUC approval letter)

Application Mailing Instructions:
Mail Original and ____ Copies to:
mnantz@ufl.edu

☐ Grants.gov
☐ Other Electronic System
☐ FedEx
☐ Other Overnight
☐ First Class Mail
☐ Fax to:
☑ Email PDF
☐ Release back to PI
☐ Internal Only (no mailing)

(FedEx Account Number)

Cost Sharing: Yes ☑ No ☐

If yes, complete the following:
Mandatory: $ 12,299.00 Attach the required cost share letter and agency guidelines
Voluntary Committed: $ 0.00 Attach the "Dean's Approval" Letter

(DSR Use) DSR Staff: _____ Received _____ Action _____ Date _____

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 Endorsement: 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 Regulation: 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 Endorsement: 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 Distributions: 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: Check here if Contact PI ☑

7/25/14
DATE

NAME: Anita Wright
TITLE: Associate Professor
UFID #: 4980-4190 TELEPHONE #: (352) 392-1991 Ext. 311
DEPARTMENT: Food Science and Human Nutrition

Co-Principal Investigator:

NAME: K.C. Jeong DATE
TITLE: Assistant Professor
UFID #: 1067-6994 TELEPHONE #: 3523923889
DEPARTMENT: Animal Sciences

Department Chair:

7/25/14
DATE

NAME: Susan S. Percival
DEPARTMENT: Food Science and Human Nutrition

Other Endorsement (Where Needed):

NAME: Geoffery Dahl DATE
TITLE: Chair
ACADEMIC UNIT: Animal Sciences

College Dean:

7/31/14
DATE

NAME: Dr. Douglas L Archer or Dr. Mary L Duryea
COLLEGE: Dean for Research

Vice President for Research:

NAME: DATE
Division of Sponsored Research

DSR-1.PDF (September 3, 2009)

Please add additional signature sheets as needed.

Office of Research
Division of Sponsored Research
PO Box 115500 / 219 Grinter Hall
Gainesville, FL 32611-5500
Phone: (352) 392-1582
Fax: (352) 392-4400

UF UNIVERSITY of FLORIDA

DSR—1

Sponsored Projects
Approval Form

Principal Investigator: Anita Wright

Multiple PI Project: ☐ Yes ☑ No — For Multiple PI Projects one Contact PI must be identified in the signature block

Department: Food Science and Human Nutrition

College: IFAS

Current UPN#: (DSR Completes)

Project Title: Techniques and Practices for Vibrio Reduction

Funding Agency: Interstate Shellfish Sanitation Conference

If Known:
PeopleSoft Proposal #:
PeopleSoft Project #:

Type: ☑ New ☐ Renewal ☐ Continuation ☐ Supplemental ☐ Revised ☐ Change of PI ☐ Change Dept ID

Category: ☑ Research ☐ Training ☐ Extension ☐ Clinical Trial ☐ Other*
*(Fellowships, patient services, public service, conference, etc.)

UF/Dept Person to discuss Applicaton (name/phone/email):
Meri Nantz
(352) 392-1991 x 206
mnantz@ufl.edu

Application Deadline:
☐ Postmark ☑ Receipt ☐ None
Date: 07/31/14

Check all that apply:	Yes	No	Pending
*Human Subjects (IRB)	☐	☑	☐
*Animal Subjects (IACUC)	☐	☑	☐
Recombinant DNA/RNA	☐	☑	
Biohazards	☐	☑	

*(If yes, attach the IRB and/or the IACUC approval letter)

Application Mailing Instructions:
Mail Original and ____ Copies to:
mnantz@ufl.edu

☐ Grants.gov
☐ Other Electronic System
☐ FedEx
☐ Other Overnight
☐ First Class Mail
☐ Fax to:
☑ Email PDF
☐ Release back to PI
☐ Internal Only (no mailing)

Cost Sharing: ☑ Yes ☐ No

If yes, complete the following:
Mandatory: $ 12,299.00 **Attach the required cost share letter and agency guidelines**
Voluntary Committed: $ 0.00 **Attach the "Dean's Approval" Letter**

(DSR Use) DSR Staff: _____ **Received** _____ **Action** _____ **Date** _____

(FedEx Account Number)

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 Endorsement: 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 Regulation: 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 Endorsement: 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 Distributions: 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: Check here if Contact PI ☑

NAME: Anita Wright — DATE
TITLE: Associate Professor
UFID #: 4980-4190 — TELEPHONE #: (352) 392-1991 Ext. 311
DEPARTMENT: Food Science and Human Nutrition

Co-Principal Investigator: 7-25-14

NAME: K.C. Jeong — DATE
TITLE: Assistant Professor
UFID #: 1067-6994 — TELEPHONE #: 3523923889
DEPARTMENT: Animal Sciences

Department Chair:

NAME: Susan S. Percival — DATE
DEPARTMENT: Food Science and Human Nutrition

Other Endorsement (Where Needed): 7-25-14

NAME: Geoffery Dahl — DATE
TITLE: Chair
ACADEMIC UNIT: Animal Sciences

College Dean:

NAME: Dr. Douglas L Archer or Dr. Mary L Duryea — DATE
COLLEGE: Dean for Research

Vice President for Research:

NAME: — DATE
Division of Sponsored Research

DSR-1.PDF (September 3, 2009)

Please add additional signature sheets as needed.

UF UNIVERSITY of FLORIDA

Division of Sponsored Research
http://www.research.ufl.edu/research

219 Grinter Hall
PO Box 115500
Gainesville, FL 32611-5500

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.

Cost Sharing for Personnel	$12,298.81
Cost Sharing for Non Personnel	$0.00
Third Party Cost Sharing	$0.00
Total Commitment	$12,298.81

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

HONORS AND AWARDS: FDA Next Generation Sequencing Project –Director SE Regional Center (2012-present), Steering Committee for Vibrios (2011) and Vibrios in the Environment Conferences (2010) Conferences, ISSC Methods Committee (2005-2010), Advisory Board UF Emerging Infectious Disease Institute (2009-present), Governor's Task Force on Oyster Health in Apalachicola Bay, FL (2012), President SE Branch ASM (2011), Wall Street Journal Technology Innovation Award (2010)**,** UF Innovation Award (2009)**,** U MD "Best Poster" Award (1999)**,** Sigma Xi Graduate Research Award (1983)**,** NSF Undergraduate Research Fellowship (1974). Grant PI for USDA (2000-2004, 2004-2007, 2008-2012) and Fl Sea Grant (2003-2005, 2004-2006, 2006-2008, 2011-2013) Center for Produce Safety (2010-2012, 2013).

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):

1. Luo, Z., G. Gu, C. G. Mihai, P. Adams, G. Vellidis, A. H. C. van Bruggen, **A. C. Wright***. Development of a novel cross-streaking method for isolation, confirmation, and enumeration of *Salmonella* from irrigation ponds. *J. Microbiol. Methods*. In Press.
2. Li, B. Vellidis, G, H., Liu, M. Jay-Russell, S. Zhao, Z Hu, A.C Wright, and C. Elkins. Improved Detection and Isolation Scheme Reveals Diversity and Persistence of Salmonella enterica Subtypes in Surface Water in Southeastern U.S. (under review by Appl Environ.

Microbiol. The manuscript has been assigned the control number AEM00954-14.)
3. Gu, G., Luo, Z., Cevallos-Cevallos, J., Adams, P., Vellidis, G., Wright, A., and van Bruggen, A. 2013a. Factors affecting the occurrence of Escherichia coli O157 contamination in irrigation ponds on produce farms in the Suwannee River Watershed. *Canadian Journal of Microbiology*, 59(3): 175-82.
4. Gu, G., Luo, Z., Cevallos-Cevallos, J., Adams, P., Vellidis, G., Wright, A., and van Bruggen, A. 2013. Occurrence and population density of Campylobacter jejuni in irrigation ponds on produce farms in the Suwannee River Watershed. *Canadian Journal of Microbiology,* 59(5): 339-46.
5. Wright , A.C. and J. Harwood. (2013) "Vibrios" in Foodborne Infections and Intoxications, Fourth Edition. J. G. Morris, Jr, Editor. Academic Press. (ISBN-10: 0124160417; ISBN-13: 978-0124160415)
6. Tao, Z. A. Larsen, S.A. Bullard, A.C. Wright, and C. R. Arias. 2012. Prevalence and population structure of *Vibrio vulnificus* on recreational fishes from the northern Gulf of Mexico. *Appl. Environ. Microbiol.*
7. Staley, C., Jones, M.K., Wright, A. C., Harwood, V. J. 2011.Genetic and quantitative assessment of *V. vulnificus* populations in oyster (*Crassostrea virginica*) tissues. *Envirol Microbiol Reports*
8. Thiaville, P.C., Bourdage, K.L., Wright, A. C. et al. 2011. Genotype is correlated with but does not predict virulence of *V. vulnificus*. *Infect. Immun*. 9 (3): 1194-7.
9. Gulig, P. A., V. de Crecy-Lagard, V., A. C. Wright, et al., 2010. SOLiD sequencing of four *Vibrio vulnificus* genomes enables comparative genomic analysis. *BMC Genomics* 11, 512.
10. Gauthier, J. D., Jones, M. K., Thiaville, P., Joseph, J. L., Swain, R. A., Krediet, C. J., Gulig, P. A., Teplitski, M. A., Wright, A. C. 2010. Role of GacA in virulence of *V. vulnificus*. *Microbiology*.156:3722-3733.
11. Mahmud, Z. H., Wright, A.C. et al., 2010. Genetic characterization of *Vibrio vulnificus* strains from tilapia aquaculture in Bangladesh. *Appl Environ Microbiol* 76, 4890-4895.
12. Wright, A.C., Danyluk, M., Otwell, W.S. 2009. Pathogen analysis in raw foods: What the salad bar can learn from the raw bar. *Current Opin Biotech*. 20:172-177.
13. Srivastava, M., M. S. Tucker, P. A. Gulig, and A. C. Wright. 2009. Phase variation, capsular polysaccharide, pilin, and flagella contribute to uptake of the Eastern oyster (*Crassostrea virginica*) by *Vibrio vulnificus*. *Environ. Microbiol*. 11:1933-34.
14. Wright A.C., et al. 2007. Evaluation of post-harvest processed oysters by using PCR-based MPB enumeration of *Vibrio vulnificus* bacteria. *Appl Env Microbiol* 73:7477-7781

CURRENT AND PENDING SUPPORT: Anita C. Wright

NAME (List/PD #1 first)	SUPPORTING AGENCY AND AGENCY ACTIVE AWARD/PENDING PROPOSAL NUMBER	TOTAL $ AMOUNT	EFFECTIVE AND EXPIRATION DATES	% OF TIME COMMITTED	TITLE OF PROJECT
	Previous:				
Wright (PI), Teplitski, Gulig	USDA- AFRI	$372,000	7/08-12/12	5%	Post harvest treatment of Live Oysters and Investigation of Therapeutic Potential of Biological Controls
	Sea Grant			10%	
Wright (PI) Otwell Rodrick		$198,108	3/09-2/13		Implementation of *Vibrio* monitoring methods needed to sustain Florida coastal communities
	USDA- AFRI	$124,910	2/10-12/12	1%	
Jones (PI) Wright					Antimicrobial peptides for reduction of vibrios in oysters
Wright (PI), Van Bruggen, Danyluk, Adams	Center for Produce Safety	$333,000	10/11/-6/13	5%	Science-based evaluation of regional risks for *Salmonella* contamination of irrigation water at mixed produce farms in the Suwannee River watershed
		$99,000		10%	
Wright (PI), Jay-Russell	Center for Produce Safety		1/13-12/13		Science-based evaluation of risks associated with wildlife exposure for contamination of irrigation water by *Salmonella*
Wright (PI on subcontract) Blackmore (PI on project)	Subcontract to FL DOH		12/12-11/14	5%	**Next Generation Sequencing Project for Foodborne Pathogens**
	Pending:				
Wright (PI)	Center for Produce Safety	$162,260	1/15-12/17	1%	**Partnership for Next Generation Sequencing of Salmonella**
Wright (PI), Jeong, Salemi	Sea Grant	#339,751	9/1/14-8/31/16	20%	Sustaining Florida Aquaculture through Improved Process Technology and Monitoring of *Vibrio* species

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

EDECATION

- 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)

1. Jeon, S., W. Yeo, K. Galvao, and **K.C. Jeong**. 2014. Underlying Mechanism of antimicrobial activity of chitosan microparticles and implications for the treatment of infectious diseases (accepted by PLOS ONE)
2. Mir, R., T. A. Weppelmann, N.D. DiLorenzo, and **K.C. Jeong**. 2014. Age-specific prevalence of Shiga-toxin producing *Escherichia coli* in a cohort of beef cattle (in review, PLoS One).
3. Jeon, S., W. Yeo, K. Galvao, and **K.C. Jeong**. 2014. Underlying Mechanism of antimicrobial activity of chitosan microparticles and implications for the treatment of infectious diseases. PLoS One. 10.1371/journal.pone.0092723.
4. Aydin, M., G. Herzig, **K.C. Jeong**, S. Dunigan, P. Shah, and S. Ahn. 2014. Rapid and Sensitive Detection of *Escherichia coli* O157:H7 in Milk and Ground Beef Using Magnetic Bead-based Immunoassay Coupled with Tyramide Signal Amplification. J. Food Protection. 77:100-105.
5. Jeon, S., M. Elzo, N. DiLorenzo, C. Lamb, **K.C. Jeong**. 2013. Evaluation of animal genetic and physiological factors that affect the prevalence of *Escherichia coli* O157 in cattle. PLoS One. 10.1371/journal.pone.0055728.

6. **Jeong, K.C.**, O. Hiki, M.Y. Kang, D. Park, C.W. Kaspar. 2013. Prevalent and persistent *Escherichia coli* O157 strains on farms are selected by bovine passage. J. Vet. Microbiol. doi:10.1016/j.vetmic.2012.11.034.
7. Lim, M.S., J. Kim, J.G. Lim, B.S. Kim, **K.C. Jeong**, K.H. Lee, and S.H. Choi. 2011. Identification and characterization of a novel serine protease VvpS containing two functional domains and essential for autolysis of *Vibrio vulnificus*. J. Bacteriol. 193:3722-32
8. **Jeong, K.C**. and J. Yu. 2012. Investigation of *in vivo* protein interaction in Aspergillus spores, Methods Mol. Bio. 944:251-7.
9. **Jeong, K.C.,** M.Y. Kang, J.H. Kang, D.J. Baumler, and C.W. Kaspar. 2011. Reduction of *Escherichia coli* O157:H7 shedding in cattle by addition of chitosan microparticles to feed. Appl. Environ. Microbiol. 77:2611-2616.
10. Jeong, H.S., **K.C. Jeong**, H.K. Choi, K.-J. Park, K.-H. Lee, J.H. Rhee, and S.H. Choi. 2001. Differential expression of *Vibrio vulnificus* elastase gene in a growth phase-dependent manner by two different types of promoters. J. Biol. Chem. 276:13875-13880.
11. **Jeong, K.C.**, H.S. Jeong, S.E. Lee, S.S. Chung, J.H. Rhee, A.M. Starks, G.M. Escudero, P.A. Gulig, and S.H. Choi. 2000. Construction and phenotypic evaluation of a *Vibrio vulnificus vvpE* mutant for elastolytic protease. Infect. Immun. 68:5096-5106.
12. **Lee, S.E., Shin, S.Y. Kim, Y.R. Kim, D.H. Shin, S.S. Chung, Z.H. Lee, J.Y. Lee, K.C. Jeong, S.H. Choi, and J.H. Rhee.** 2000. *Vibrio vulnificus* has the transmembrane transcription activator ToxRS stimulating the expression of the hemolysin gene *vvh*. J. Bacteriol. 182:3405-3415.

B. Book chapters

1. **K.C. Jeong**, C.D. Vincent, E. Buford, and J.P. Vogel. Subcellular Localization of the Dot/Icm Type IV Secretion Proteins. *Legionella*: State of the art 30 years after its recognition. Nicholas P. Cianciotto [et al.]. Washington, D.C. ASM Press, 2006.
2. C.D. Vincent, **K.C. Jeong**, J. Sexton, E. Buford, and J.P. Vogel. The *Legionella pneumophila* Dot/Icm Type IV Secretion System. *Legionella*: State of the art 30 years after its recognition. Nicholas P. Cianciotto [et al.]. Washington, D.C. ASM Press, 2006.

CURRENT AND PENDING SUPPORT: K. C. Jeong

Externally funded

Start/ End Year	Amount funded	Funding Agency/Title	Role
2012/2013	$31,280	Milk Check Off, Effect of uterine administration of chitosan microparticles to lactating dairy cows with metritis on subsequent uterine bacterial microbiota	Co-PI Galvao (PI)
2012/2013	$12,000	AquaGen. Inc., Analysis of wastewater using nanomaterial-mediated biosensors	Co-PI McLamore (PI)
2012/2013	$3,837.50	AquaGen. Inc., Analysis of pathogens and protein content using nanomaterial-mediated biosensors	Co-PI McLamore (PI)

Nov. 2012-Oct. 2017	N/A	USDA/CRIS-Hatch, Prevalence, persistence, and transmission of Shiga Toxin-producing *Escherichia coli*	PI
Jul. 2013-Jun. 2014	$25,450	Milk Check Off, Use of chitosan microparticles to prevent metritis in lactating dairy cows	Co-PI Galvao (PI)
Jan. 2014-Dec. 2016	$481,320	USDA NIFA, Development of chitosan nanoparticles targeting pathogenic *Escherichia coli* in beef and dairy cattle	PI

Internally funded

Submission Year	Amount Requested	Funding Agency/Title	Role
2012	$10,000	Center for Veterinary Medicine, UF. Effect of uterine administration of chitosan microparticles to lactating dairy cows with metritis on subsequent uterine bacterial microbiota	Co-PI Galvao (PI)
Mar. 2013-Jun. 2014	$48,150	IFAS, UF. Underlying mechanisms of Antimicrobial resistance in cows with uterine diseases	PI
Mar. 2014	$50,000	IFAS, UF. Equipment grant	PI

Pending

Submission Year	Amount Requested	Funding Agency/Title	Role
2013 (Advanced to final round)	$99,000	UF-Opportunity Fund, Rising concern of antimicrobial resistance: Are food animal producers friends or foes?	PI
2014	$18,100	Milk Check Off, Bacterial diversity and succession in healthy cows and cows that develop uterine disease	Co-PI Galvao (PI)
2014	$35,320	Milk Check Off, Enhancing antimicrobial activity of chitosan microparticles to treat cows with uterine diseases	PI
2013	$357,050	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	Collaborator Adesogan (PI)
2014	$400,000 (This proposal)	Sea grant, Sustaining Florida Aquaculture through Monitoring Vibrio species and Improved Process Technology	Co-PI Wright (PI)
2014	$2.2 M	USDA NIFA, Food safety challenge, Effective mitigation strategies for antimicrobial resistance	PI

2014	$500,000	USDA NIFA, Animal health and disease, Using chitosan microparticles to prevent metritis in lactating dairy cows	Co-PI Galvao (PI)
2014	$500,000	USDA NIFA, Food Safety, Nutrition, and Health Cranberry application for prevention of inflammatory bowel disease by improving gastrointestinal health	Co-PI Park (PI)