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US Ireland Partnership Program: BEACONS: Biosafety for Environmental Contaminants Using Novel Sensors

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Federal Agency and Organization Element to Which Report is Submitted:	4900
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Project Title:	US-Ireland Partnership Program: BEACONS: Biosafety for EnvironmentAl COntaminants using Novel Sensors
PD/PI Name:	Laurie B Connell, Principal Investigator Gregory J Doucette, Co-Principal Investigator Rosemary L Smith, Co-Principal Investigator
Recipient Organization:	University of Maine
Project/Grant Period:	05/01/2009 - 04/30/2013
Reporting Period:	05/01/2012 - 04/30/2013
Submitting Official (if other than PD\PI):	Laurie B Connell Principal Investigator
Submission Date:	05/31/2013
Signature of Submitting Official (signature shall be submitted in accordance with agency specific instructions)	Laurie B Connell

Accomplishments

*** What are the major goals of the project?**

We had three fronts of activities for the US portion of our project.

1) Prepare saxiphilin from toad serum to be used as a probe molecule for STX.

2) Surface plasmon resonance (SPR)
detection of STX on the SPR platform using antibodies developed by our Irish partners.
comparison of platforms
detection of total RNA extracted from Alexandrium cell cultures

3) Colorimetric detection
applying to total RNA extracted from Alexandrium cell cultures
testing a handheld, low-cost colorimeter designed to be used with this assay.

*** What was accomplished under these goals (you must provide information for at least one of the 4 categories below)?**

Major Activities:

Specific Objectives:

Significant Results:

Significant Findings and Accomplishments:

1) Our overall aim for this project was to develop a saxiphilin-based assay that would have represented a significant advancement in our ability to detect the major PSTs on field-portable platforms. Unfortunately, due to technical and personnel issues of collaborating partner A. Roberson (USFDA), the final purification of saxiphilin protein could not be completed prior to termination of the project. Nonetheless, as an alternative approach, we adopted an antibody-based strategy using immunoreagents produced by our QUB partners and have made progress towards developing a PST detection assay formatted for use on a field-portable SPR instrument. Considerable work still remains to be done in order to accommodate limitations specific to this field-portable instrument (*i.e.* use of miniature SPR chips and development of surface chemistries) and to achieve acceptable sensitivity for detection of multiple PSTs. This effort will be expanded upon and taken up during a follow-on project.

2) The SPR assay compared excellently with the laboratory based SPR from BiaCore. However, high variability was encountered with the small SPR instrument. Work was focused on reduction of this variability. Flow rate and sample dilution reduced variability as well as an additional calibration step resolved many of these issues and brought the variability into an acceptable range.

3) Because development of probes for the MBio based assay to be used in our intial project proposal was very difficult because of collaboration issues with MBio we designed colorimetric probes for another device that can be moved onto additional platforms at a later date. Recently (spring 2013) our collaborators in Belfast UK (QUB) has obtained the ability to print devices that will work in the MBio assay, therefore we will be able to move forward in the future without the complication of the MBio collaboration. However, we prepared for this opportunity by development of additional probes as discussed in section 3.

A small handheld version of a detection instrument was developed based on PNA probes. The instrument is very inexpensive (~$150 USD) and easy to use. Assay

development was significantly enhanced by optimizing the detection limit, improving the signal-to-noise ratio, determination of optimal probe design, hybridization temperature as well as sample preparation.

Work still needs to be done to move the PNA probes to a fluorescence-based system compatible with the current MBio assay used by the US Ireland partners. This effort will be moved forward in follow-up projects.

Key outcomes or Other achievements:

1) a handheld inexpensive dye-based detction paltform was developed and tested with cultured material for detection of target organismAlexandrium.

2) the dye-based chemistry was develped to detect RNA with hig specificity and low background

3) PNA binding and regeneration parameters have been developed for SPR based platforms for gold surfaces

4) PNA probes have been developed for the SPR platform

5) PST antibodies have been applied to the SPR platform and the assay was developed

*** What opportunities for training and professional development has the project provided?**

Over the project 5 undergradates from the US have been active in the evelopment of the project. Two graduate students have been active in this project and one Post-doc. In addition one gradyuate student from the US spent a summer in Belfast Northern Ireland at the QUB laboratories w=orking with our Northern Irish partner to develop the binding parameteres for PNA to the SPR chip surfaces. In exchange an Irish graduate student spent 6 weeks in Maine working on develpment of antibody attachment to our portable SPR instrument.

*** How have the results been disseminated to communities of interest?**
Nothing to report.

Supporting Files

Filename	Description	Uploaded By	Uploaded On
For this reporting period describe.pdf	more complete description of major findings as well as figures	Laurie Connell	05/31/2013

Products

Books
Bratcher, A R (2011). *Portable biosensor detection of the harmful dinoflagellate Alexandrium using surface plasmon resonance and peptide nucleic acid probes. Oceans '11.* Status = PUBLISHED; Acknowledgment of Federal Support = Yes ; Peer Reviewed = Yes

Book Chapters
Bratcher, A R and L B Connell (2012). The use of peptide nucleic acids in surface plasmon resonance for detection of red tide algae. *Molecular Biological Technologies for Ocean Sensing* S. M. Tiquia. Springer Verlag:. 135. Status = PUBLISHED; Acknowledgement of Federal Support = Yes ; Peer Reviewed = Yes

Conference Papers and Presentations
Bratcher, A R, L B Connell and P Millard (2011). *An SPR biosensor for detection of the harmful dinoflagellate*

Alexandrium. 14th International Meeting on Harmful Algae. Hersonissos, Greece. Status = OTHER; Acknowledgement of Federal Support = Yes

Duy, J, R L Smith, S D Collins and L B Connell (2011). *Field-aplicable colorimetric detection of Alexandrium tamarense species complex using peptide nucleic acid probes and a symetrical cynanine dye.*. 14th International Meeting on Harmful Algae. Hersonissos, Greece. Status = OTHER; Acknowledgement of Federal Support = Yes

Duy, J, R L Smith, S D Collins and L B Connell (2011). *Low-cost colorimeter development for the field-based detection of harmful algal blooms.*. OCEANS '11 MTS/IEEE Kona,. Kona, HI. Status = PUBLISHED; Acknowledgement of Federal Support = Yes

Marshall, N.M., Campbell, K., Elliott, C.T., Doucette, G.J. (2011). *Multiplexed detection of paralytic shellfish toxins and domoic acid on a portable surface plasmon resonance platform.*. 6th Symposium on Harmful Marine Algae in the U.S.. Austin, TX. Status = OTHER; Acknowledgement of Federal Support = Yes

Doucette, G.J., Asong, J., Marshall, N., Smith, J.L., Campbell, K., Elliott, C.T., Anderson, D.M. 1 (2012). *PSP toxin immunoassays for deployment on field-portable and autonomous, in-water sensors.*. 5th International Conference on Harmful Algae, Changwon, Republic. Doucette, G.J., Asong, J., Marshall, N., Smith, J.. Status = OTHER; Acknowledgement of Federal Support = Yes

Bratcher, A R, P Millard and L B Connell (2011). *Portable surface plasmon resonance detection of the toxin-producing dinoflagellate Alexandrium*. Sixth Symposium on Harmful Algae. Austin, TX. Status = OTHER; Acknowledgement of Federal Support = Yes

Inventions
Nothing to report.

Journals
Duy, J, R L Smith, S D Collins and L B Connell (2012). A field-deployable colorimetric bioassay for the rapid and specific detection of ribosomal RNA. *Biosensors and Bioelectronics*. on line 1. Status = PUBLISHED; Acknowledgment of Federal Support = Yes ; Peer Reviewed = Yes ; DOI: 10.1016/j.bios.2012.05.039

Licenses
Nothing to report.

Other Products
Nothing to report.

Other Publications

Patents
Nothing to report.

Technologies or Techniques

WE have developed a number of probes and antibodies for detection of our target organisms. In addition we have developed several techniues and used these probes on a handheld instrument. Non of the technologies developed were submitted for patent protection.

Thesis/Dissertations

Websites
Nothing to report.

Participants/Organizations

What individuals have worked on the project?

Name	Most Senior Project Role	Nearest Person Month Worked
Connell, Laurie	PD/PI	2
Doucette, Gregory	Co PD/PI	2
Smith, Rosemary	Co PD/PI	1
Marshall, Nicholas	Postdoctoral (scholar, fellow or other postdoctoral position)	2
Mikulski, Christina	Technician	1
Bratcher, Amber	Graduate Student (research assistant)	4
Duy, Janice	Graduate Student (research assistant)	4

Full details of individuals who have worked on the project:

Laurie B Connell
Email: laurie.connell@umit.maine.edu
Most Senior Project Role: PD/PI
Nearest Person Month Worked: 2

Contribution to the Project: Directed biological side of sensor development

Funding Support: NSF

International Collaboration: Yes, Ireland
International Travel: Yes, Ireland - 0 years, 0 months, 3 days; United Kingdom - 0 years, 0 months, 3 days; Ireland - 0 years, 0 months, 3 days; United Kingdom - 0 years, 0 months, 3 days; United Kingdom - 0 years, 0 months, 3 days

Gregory J Doucette
Email: greg.doucette@noaa.gov
Most Senior Project Role: Co PD/PI
Nearest Person Month Worked: 2

Contribution to the Project: Directed antibody section of the project

Funding Support: NOAA/NOS

International Collaboration: Yes, Ireland
International Travel: Yes, Ireland - 0 years, 0 months, 3 days; United Kingdom - 0 years, 0 months, 3 days; Ireland - 0 years, 0 months, 3 days; United Kingdom - 0 years, 0 months, 3 days; United Kingdom - 0 years, 0 months, 3 days

Rosemary L Smith
Email: rosemary.smith@maine.edu

Most Senior Project Role: Co PD/PI
Nearest Person Month Worked: 1

Contribution to the Project: directed development of sensor platform

Funding Support: University of Maine

International Collaboration: Yes, Ireland
International Travel: Yes, Ireland - 0 years, 0 months, 3 days; Ireland - 0 years, 0 months, 3 days

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Nicholas Marshall
Email: nicholas.marshall@noaa.gov
Most Senior Project Role: Postdoctoral (scholar, fellow or other postdoctoral position)
Nearest Person Month Worked: 2

Contribution to the Project: design/develop SPR-based multi-PST detection assay, including surface chemistry component

Funding Support: NOAA Postdoctoral fellowship

International Collaboration: No
International Travel: No

Christina Mikulski
Email: christina.mikulski@noaa.gov
Most Senior Project Role: Technician
Nearest Person Month Worked: 1

Contribution to the Project: Technical staff. prepared serum and tested biochemically

Funding Support: NOAA

International Collaboration: No
International Travel: No

Amber Bratcher
Email: amber.bratcher@umit.maine.edu
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 4

Contribution to the Project: Amber works on application of PNA probes to SPR surfaces

Funding Support: NSF

International Collaboration: Yes, United Kingdom
International Travel: Yes, United Kingdom - 1 years, 2 months, 16 days

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Janice Duy
Email: janice.duy@umit.maine.edu
Most Senior Project Role: Graduate Student (research assistant)
Nearest Person Month Worked: 4

Contribution to the Project: worked on PNA dye-based assay and development of hand-held instrument platform

Funding Support: Functional Genomics

International Collaboration: Yes, United Kingdom
International Travel: No

What other organizations have been involved as partners?

Name	Type of Partner Organization	Location
Dublin City Univeristy	Academic Institution	Dublin, Ireland
Queen's University Belfast	Academic Institution	Belfast UK

Full details of organizations that have been involved as partners:

Dublin City Univeristy

Organization Type: Academic Institution
Organization Location: Dublin, Ireland

Partner's Contribution to the Project:
Collaborative Research

More Detail on Partner and Contribution: DCU is the Irish portion of our US Ireland Partnership program. Their major role in the overall project was to develop specific epitope antibodies to be used as reagents in all of the antibody based assays

Queen's University Belfast

Organization Type: Academic Institution
Organization Location: Belfast UK

Partner's Contribution to the Project:
Collaborative Research
Personnel Exchanges

More Detail on Partner and Contribution: QUB was our Northern Ireland Partner in the US-Ireland Partnership program. They worked on SPR development of antibodies and initiated the movement of the SPR assays to the mBio platform

What other collaborators or contacts have been involved?

YES

Impacts

What is the impact on the development of the principal discipline(s) of the project?

These findings have resulted in increased detection of water contaminating organisms and their toxins. The techniques have shown to be suitable for the level of detection needed. The use of the portable SPR instrument has been shown to be a viable option for field based detection of both toxin and organism. This is a significant finding since typically only one or the other system is developed. The ability to detect multiple types of analytes from the same sample is a boon to any detection device. Further development of the dye based chemistry using PNA had not previously been accomplished and is a new technique for rapid organism detection.

What is the impact on other disciplines?

Water quality managment and public health could be significantly be imroved with the use of the PNA-based chemisty developed here.

What is the impact on the development of human resources?

The development of both the analyites (PNA probes and antibodies) as well as further development of the SPR as well as the dye-based assay have significantly moved our field forward to detection of toxins and the organisms that make those toxins.

What is the impact on physical resources that form infrastructure?
Nothing to report.

What is the impact on institutional resources that form infrastructure?
Nothing to report.

What is the impact on information resources that form infrastructure?
Nothing to report.

What is the impact on technology transfer?
Nothing to report.

What is the impact on society beyond science and technology?

The development of both the analyites (PNA probes and antibodies) as well as further development of the SPR as well as the dye-based assay have significantly moved our field forward to detection of toxins and the organisms that make those toxins.

Changes/Problems

Changes in approach and reason for change

Initially we were planning to develop organism detection using the MBio based waveguide platform, however the company was difficult to collaborate with in that they would not help development in our laboratory but wanted all work done in CO. After our intial contac with them they decided they would work with us on a fee based system rather than a collaboration. This was not practical for several reasons for example the cost of shipping each step of the probe development would be large and they were planning on charging us the full price for a fully developed assay even though this is a development. Also, development would be so slow that we would take several years to do what we finally accomplished in a short amount of time. Our group then decided that we needed to use two other platforms for organism detection, the field based SPR and our field based colorimeter. The antibody work was also much slower because of this lack of good collaboration with MBio but since the QUB had already printed some slides with antibodies that work could continue, although much slower than anticipated. We now do not rely on MBIo for any future work.

In addition, problems in acquiring toads from Australia for saxiphilin testing as a probe instead of the antibody work

done by our collaborators in Ireland and the UK (Northern Ireland). Given the unfortunate delay in the saxiphilin component of this project, as an alternative approach we adopted an antibody-based strategy using immunoreagents produced by our QUB partners and made reasonable progress towards developing a PST detection assay formatted for use on a field-portable SPR instrument. More work remains to be done in order to accommodate limitations specific to this field-portable instrument (i.e., use of miniature SPR chips and development of surface chemistries) and to achieve acceptable sensitivity for detection of multiple PSTs. This effort will be expanded upon and taken up during a follow-on project.

A third section where we did not accomplish what we porposed was in cell disruption. The methods suggested were attempted but we in effective for large volume samples as might be encountered for water monitoring.

Actual or Anticipated problems or delays and actions or plans to resolve them

Development of the saxifillin analyte was delayed because of difficulty in obtaining serum from toads in Austrailia. At this point we have the serum in storage and will contiune in a followup project.

WE have designed an additional method for cell disrution and will work on it in a followup project.

We now have the capability to print our own cassettes so can continue with the work intially to be done in collabortation with MBio.

Changes that have a significant impact on expenditures
Nothing to report.

Significant changes in use or care of human subjects
Nothing to report.

Significant changes in use or care of vertebrate animals
Nothing to report.

Significant changes in use or care of biohazards
Nothing to report.