Professional Development Grant

Project Title: Scorpion sodium toxin expression studies for proteomics.
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B. Abstract and Purpose/Objectives

Proteins are cellular macromolecules critical for cell and organismal function. They are the entities responsible for all characteristics of life: movement, energy production, and response to stimuli. The development of systems to artificially create a diverse protein array from a variety of genes is an important goal in the modern world of large scale genetic studies (genomics) and cellular protein studies (proteomics). Due to the cataloging and characterization of a multitude of genes through genomic research, methods to effectively produce the proteins associated with these genes are important to further study the complex interactions proteins are engaged during cellular activity. This project will provide materials to create a protein expression system that can effectively create cultured proteins from a wide variety of genes. In this research activity, I plan to target the genes and proteins associated with scorpion toxins.

Scorpion toxin gene evolution has been a major research focus for my laboratory. How toxin genes change and adapt to affect both prey and scorpion predators is an underdeveloped research area. My goal is to characterize the genetic changes and the protein (toxin) changes that occurred to create the scorpion's diverse venom arsenal. I also have interest to characterize the scorpion's toxin effects on human cells and how protein toxins can help medical researchers to better understand how human cell membranes function. This current research project will expand undergraduate and summer research work conducted to effectively create scorpion toxin proteins for further study and characterization through creating a robust system to isolate important genes and artificially produce their proteins.

My current research work with scorpion toxins stems from several summer research awards sponsored through the NIH-InBre program administered through UAMS. This work culminated in investigating variation in a sodium channel toxin gene among individuals from several populations across the striped scorpion's (*Centruroides vittatus*) geographic range. In the 2011 to 2013 spring semesters, undergraduate students expanded upon this work with additional population samples to produce a more robust data set for analysis. This scorpion's venom is intriguing because it is not considered as toxic to humans as the nearly identical western bark scorpion, *Centruroides sculpturatus* (Valdez-Cruz et al. 2004).

The most current project will develop a robust system to remove genes, as well as isolate and produce proteins from scorpion toxin genes. I plan to conduct activities with my summer collaborators to effectively isolate these genes and produce the subsequent proteins in culture. These activities are also pertinent as I plan to continue these activities during the fall and spring semesters with several undergraduate students.

C. Brief review of the research procedure

From the advent of modern genomic and proteomics research, new methodologies have been developed to quickly produce hundreds of proteins from many genes identified through genomic analyses. One of the new methods, Ligation Independent Cloning (LIC), is considered a robust alternative to standard methods to generate a wide variety of proteins from isolated genes. With the LIC technique, many different protein subregions (domains and domain combinations) can be created for protein expression. We have conducted gene cloning studies in summer 2010 with standard cloning methods. Although several suitable expression clones were created, some proteins from these clones proved difficult to purify. The generation of several types protein expression product with different affinity tags for purification would greatly assist due to more diverse protein expression products to screen for protein purification.

We have employed LIC cloning for several scorpion toxin genes to artificially create their proteins. I plan to further the protein production studies to finally produce the large amounts of protein needed for protein structural studies with different biochemical techniques. The project design is straightforward. We plan to purchase the necessary reagents to purify and isolate the proteins from scorpion toxin genes we are currently investigating in my lab. I plan to conduct the research activities in the following manner:

1. The creation of DNA with the gene of interest and DNA insertion into bacterial host cells.

Here I plan to isolate and remove the gene for the proteins with molecular techniques and place them into a circular piece of plasmid DNA for storage and then place the DNA into a bacterial cell for subsequent protein production. More specifically, the LIC primers will contain the appropriate sequences for T4 DNA polymerase digestion. After PCR amplification of the targeted insert regions, the gene insert and prepared vector will be annealed and then transformed into bacterial host cells. To verify transformed bacteria with the appropriate gene insert, we will isolate plasmid from transformed cells, amplify with PCR primers targeted to the gene insert and visualize products with agarose gel electrophoresis. In addition, purified plasmids will be sequenced for additional verification and insert sequence fidelity. This step has been finished, but I may incorporate other toxin genes and their proteins from this scorpion species. I have purchased components to expand our base of toxin genes, and I plan to conduct these experiments with undergraduates in Fall 2013 and Spring 2014.

2. Initial small scale protein production

After conformation of a robust plasmid with the gene of interest, the bacterial cells will be grown under conditions for protein expression. An initial check of protein production will occur with SDS gel electrophoresis of cellular extracts after expression induction. These gels can discriminate among many different proteins and allow the identification of expected protein production. If protein expression is seen with an appropriate size band to the targeted protein on the SDS gel, larger cultures will be created for large scale protein production and purification.

In the 2013 summer, I was able to successfully show toxin protein expression in the appropriate media fractions. I was then able to visit my collaborator's lab at UA-F and conduct a large scale expression study where we were able to successfully isolate the toxin fusion protein through affinity column purification. We plan to continue these experiments for structural analysis in several subsequent studies. This success has furthered our confidence in the LIC cloning and expression system. Several undergraduates currently working in the lab will also conduct further protein expression studies as proposed earlier in this project.

D. Summary of Findings:

The planned methods and experiments have shown that scorpion toxin expression can be conducted at a small and larger scale. These experiments suggest that the LIC methods are able to successfully create large amounts of protein toxins for further research and analysis.

E. Conclusions:

The PDG has allowed a large step in my current research through providing the components needed to successfully isolate scorpion toxins and artificially produce them in sufficient quantities for robust analysis. These studies will be expanded through collaboration with undergraduate research students and will provide meaningful projects that will improve their laboratory skills and hone their research skills.

I. Bibliography

Valdez-Cruz, N.A., Davila, S., Licea, A., Corona, M., Zamudio, F.Z., Garcia-Valdes, J., Boyer, L. & Possani, L.D. (2004) Biochemical, genetic, and physiological characterization of venom components from two species of scorpions: *Centruroides exilicauda* Wood and *Centruroides sculpturatus* Ewing. Biochime, **86**, 387-396.