

## 2009/2010 Professional Development Grant Report

Project Title: Protein expression studies from scorpion sodium toxin genes.  
Tsunemi Yamashita, Associate Professor of Biology

### B. Abstract and Purpose/Objectives

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 am also interested 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 summer research work conducted to effectively create scorpion toxin proteins for further study and characterization.

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. This scorpion's venom is intriguing as it is not considered as toxic to humans as the nearly identical western bark scorpion, *Centruroides sculpturatus* (Valdez-Cruz et al. 2004). Current research activities from the 2008 and 2009 summer resulted in creating a model system to artificially produce the scorpion's toxin protein. The research activities focused in this proposal will create potential toxin protein variants to further improve the success of the ultimate goal to create a functional toxin protein for continued research. The results from this project will serve as preliminary data and justification to enhance a proposed 2010 InBre research grant and equipment grant from UAMS and justify a 2010 NIH-Cobre grant from UA-Fayetteville. Additionally, as this project continues work from this summer, it will allow the purchase of small equipment items for the added benefit to enhance teaching and undergraduate research efforts in my upper level courses.

### C. Brief review of the research procedure

Scorpion venom toxicity occurs due to a range of venom components (peptides, lipids, nucleotides, and other compounds) (Possani et al. 2000). Most research has focused upon characterizing toxin proteins and their genes (Possani et al. 2000). These proteins have been shown to affect sodium, potassium, chloride, and calcium ion channels primarily in muscle or nervous tissue (Froy et al. 1999). These proteins are also known to disrupt cell communication through perturbation of ion channels, leading to membrane depolarization and then death of prey species (del Rio-Portilla et al. 2004).

The methods and procedures required to create additional toxin gene variants were initially tested in the 2008 summer and also tested in Tech's molecular genetics laboratory. An additional experiment that was not done in prior work was a protein expression study to test the toxin gene's activity. Continuing the work completed in the 2009 summer, I conducted these expression studies with the new toxin protein variants.

#### D. Summary of Findings:

An Honors undergraduate research student and I were able to complete all aspects of the project and presented the results at the 2010 ATU Undergraduate Research Conference. I plan to conduct additional studies to verify recombinant cells and further develop this system for recombinant protein expression with subsequent undergraduates. In the 2010 summer, I will conduct further protein expression research studies at UA-Fayetteville in the NIH Cobre program. This research activity was also included in a faculty research proposal to the AR NIH InBre program. Lastly, this project provided funds for equipment items necessary to extend the protein expression studies in the laboratory.

#### E. Conclusions:

The support provided through the Professional Development Grant significantly enhanced the ability for undergraduate students to conduct molecular genetics research activities. These activities improve learning opportunities for undergraduate students and provide skill development needed for a successful postgraduate career. Furthermore, this activity provides a valuable link to extend summer research work to teaching goals and student mentoring in the academic year.

#### F. Bibliography

- del Rio-Portilla, F. E Hernandez-Marin, G Pimienta, FV Coronas, FZ Zamudio, RC Rodriguez de la Vega, E Wanke, and LD Possani. 2004. NMR solution structure of Cn12, a novel peptide from the Mexican scorpion *Centruroides noxius* with a typical  $\beta$ -toxin sequence but with  $\alpha$ -like physiological activity. *Eur. J. Biochem.* 271: 2504-2516.
- Froy, O, T Sagiv, M Poreh, D Urbach, N Ziliberberg, and M Gurevitz. 1999. Dynamic diversification from a putative common ancestor of scorpion toxins affecting sodium, potassium, and chloride channels. *J Mol. Evol.* 48: 187-196.
- Possani, LD, E Merino, M Corona, F Bolivar, and B Becerril. 2000. Peptides and genes coding for scorpion toxins that affect ion-channels. *Biochimie* 82: 861-868
- 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. *Biochimie*, **86**, 387-396.