Background

Tetrodotoxin (TTX) is a powerful neurotoxin that binds to voltage-gated sodium channels, blocking the channels from sending or receiving signals. It was originally thought that the rough skinned newt, *Taricha granulosa,* produced its own TTX due to reports that *T. granulosa* increase levels of toxicity while in captivity (Hanifin, Brodie, and Brodie 2002), *T. granulosa* can regenerate TTX levels after secretion while in long term captivity (Cardinal *et al*. 2004), and that no bacteria were found in the glands that are thought to produce, harbor, and secrete TTX or in other TTX containing tissue (Lehman *et al.* 2004). However, recent research (Salisbury, Stokes and Szick 201x) suggest that cutaneous bacteria may be the source of TTX in the rough skinned newt, finding multiple species of TTX producing bacteria on the skin of *T. granulosa.*

Newts are not the only organisms that use the toxin, TTX is commonly known for its presence in multiple species of puffer fish. Puffer fish have been found to acquire their TTX from external sources and have also been found to lose their toxicity in captivity (Lago *et al.* 2015). Knowing that the source of toxicity is external, researchers have investigated the mechanism in which puffer fish accumulate this toxin. Two very similar binding proteins identified from the plasma of puffer fish, Matsu *et al.* (2000) have found a protein, tetrodotoxin binding protein (TBP), that bind to tetrodotoxin in the plasma of *Takifugu niphobles.* Yotsu-Yamashita *et al.* (2001) have also found a binding protein, puffer fish saxitoxin and tetrodotoxin binding protein (PSTBP), that binds to saxitoxin and tetrodotoxin in the plasma of the puffer fish *Fugu pardalis.* These binding proteins are thought to assist in the accumulation and secretion of the potent neurotoxin that puffer fish harbor. Although the origin of TTX in *T. granulosa* is till under investigation, it is known that the rough skinned newt can have large amounts of TTX. To survive such large amounts of TTX, the newts must have a mechanism for preventing the negative effects of the toxin. Therefore, I hypothesize that *T. granulosa* will possess a gene for a tetrodotoxin binding protein.

Description of proposed study

The focus of this study will be investigating the presence of a TTX binding protein gene in the DNA of *T. granulosa..* DNA has already been extracted from multiple newt livers. Using polymerase chain reaction (PCR) the extracted DNA will be matched against primers designed after newt genes in order to confirm the effectiveness of extraction. PCR is a method to exponentially amplify DNA using primers that serve as a matching point for amplification. Primers are short single stranded sections of DNA that bind to the existing DNA if there is a match in the target gene. PCR will also be used to target possible TBP/PSTBP genes in the newt liver. If the genes for a binding protein are present they will be amplified using PCR. The amplified binding protein DNA will then be cloned into plasmids and introduced into bacteria. Plasmids are circular pieces of DNA that can be introduced and easily taken up by bacteria. The purpose of this is to introduce only one variation of binding protein genes into bacteria so the gene can be multiplied as the bacteria grow. DNA from bacterial isolates will then be extracted and matched against the same primers during PCR. Once purified, the amplified genes will then be sent for sequencing. From sequencing I expect to find TBP genes with multiple allelic differences.

Justification, Importance, and Significance

Tetrodotoxin has been investigated for its status as a potent neurotoxin. Currently the only remedy to tetrodotoxin poisoning is supportive care as the lung and heart experience the effects of tetrodotoxin. Identifying the genes for a TTX binding protein in newts may lead the way to a mode of synthesizing the protein for potential medical use. An activated binding protein could potentially reduce the amount of tetrodotoxin and saxitoxin in the blood before all the toxin reached the voltage-gated sodium channels of the subject.

Expected outcomes

I expect to be able to identify and isolate, genes responsible for the production of a tetrodotoxin binding protein from the DNA of *T. granulosa.* These findings may also aid in the understanding of the mechanism newts and puffer fish may use to accumulate toxic compounds. Findings in this study may also allow for the in vitro synthesis of a functioning binding protein that can be used in future research.

Table 1. Proposed Research Timeline.

	Spring 2017	Summer 2017
DNA Extractions	X	
Confirmation of DNA	X	
TBP Primers	X	
Plasmid Cloning	X	X
PCR Clone DNA		X
Sequencing		X
Manuscript preparation		X

Literature cited

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