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Characterization of Jellyfish Toxins and Their Effect on Fish

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1. INTRODUCTION

- Jellyfish are equipped with stinging cells called nematocysts, which comprises a capsule that 'explodes' upon physical contact with a prey.
- During the explosion, a barbed tubule penetrates the prey with an acceleration of 5.4 × 106 g and a pressure of 7.7 GPa (Tibballs et al, 2011). Upon penetration the barbed tubule deposits powerful venom.
- The effects of the venom on the prey depend on the species of jellyfish, and in humans, effects can vary from mild skin irritation to the lethal Irukanji syndrome.
- The Jellyfish toxin is a cocktail of poorly characterized proteins or peptides. Very few toxins have been characterized and their toxicological effects have been largely neglected (Lee et. al. 2016).

2. SPECIFIC AIMS

This project proposes to characterize the composition of toxins from jellyfish common in the Gulf of Mexico, and assess their toxicological effects on fish.

Specific Aims:

1. Isolate and characterize toxins from two jellyfish species (*Aurelia* sp. and *Chrysaora quinquechirra*) common to the Gulf of Mexico).

2. Expose zebrafish to extracted jellyfish toxins and assess toxicological effects using whole-genome transcriptomics (RNA-seq) analysis.

3. Use bioinformatics and network biology approaches to identify diagnostic effects on signaling, metabolic and physiological systems in zebrafish exposed to toxins.

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3. METHODS

3.1 Jellyfish nematocyst extraction

- Remove tentacles from live *Chrysaora* or *Aurelia* spp. jellyfish and store at -80°C until extraction.
- Thaw tentacles on ice and gently homogenize using a pestle and mortar in cold SuFi solution (300 mM sucrose containing 50% percoll (Weber et al. 1987).
- Keep the homogenate at 4°C for 30 minutes, then pass it through a 2 mm stainless steel strainer.
- Centrifuge the filtrate at 3000 g for 10 minutes at 4°C, remove supernatant.
- Reconstitute the pellet in 200 µL of 50 mM of triethylammonium bicarbonate (TEAB) buffer.
- Sonicate the reconstituted material in a water bath for 15 min.
- Centrifuge the tubes at 10,000 g for 5 min at 4°C.
- Collect supernatant and discard pellet.

3.2 Quantify protein

- Perform Bradford Assay to quantify the protein concentration.

3.3 Nematocyst protein purification and in-gel trypsin digestion

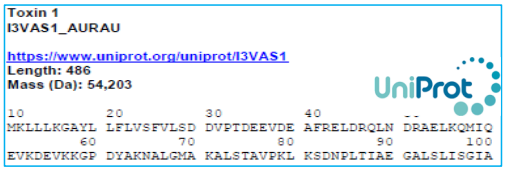
- Mix 20 µg of protein sample with Laemmli and 2-mercaptoethanol buffer to a final volume of 45 µL.
- Load samples onto a 4-15% 12x2 precast gel.
- Run the gel with 1X Tris/Glycine/SDS running buffer.
- Stain gel using Bio-safe Coomassie stain solution.
- Cut out band corresponding to 50.7kDa or 54kDa (molecular mass of *Chrysaora* toxin or *Aurelia* toxin respectively), and place in individual tubes.
- Add 200 µL of acetonitrile, shake vigorously for 45 min to destain, then dry in a speedvac for 60 minutes.
- Re-suspend in 1 mL of 25 mM ammonium bicarbonate (NH_4HCO_3).
- Add 20 µg of Promega sequence-grade trypsin in 100 µL of Promega sequence trypsin buffer to each tube; incubate for 18 hours at 37°C for digestion.
- Add 1 mL of 50% acetonitrile in water with 0.5% trifluoroacetic acid to each vial and vortex for 45 min.
- Transfer supernatants to new vials for analysis by LC-MS/MS.

4. RESULTS

- Medusae were collected from the Gulf of Mexico; a protocol for toxin extraction was developed.
- Toxins were extracted from tentacles

a) Jellyfish toxin peptide analysis

The Uniprot database was used to identify and obtain the peptide sequence belonging to the jellyfish toxin of interest



b) Predict trypsin-digested peptide fragments

Systems biology toolboxes were used predict trypsin-digested peptide fragments.

#	Pos	Mass	pI	Peptide
1	1	277.38	8.50	MK
2	2	485.67	8.75	LLLK
3	3	3047.36	3.62	GAYLLFLVSFVLSDDVPTDEEVDEAFR
4	4	531.57	4.37	ELDR
5	5	644.69	5.84	QLNDR
6	6	459.54	6.05	AELK

Trypsin-digested peptide fragments

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Mass/Charge Table

	Mass	
	Mono	Avg
(M)	1681.74316	1682.71793
$(M+H)^{+}$	1682.75044	1683.72520
$(M+2H)^{2+}$	841.87888	842.36626
$(M+3H)^{3+}$	561.58836	561.91328
$(M+4H)^{4+}$	421.44310	421.68679

Identify likely $(M+H)^{n+}$ mass fragments likely to be identified by liquid chromatography – mass spectrometry (LC-MS/MS)

5. NEXT STEPS

- Studies are underway to continue the extractions of jellyfish venom toxins.
- An IACUC approved Animal Use Protocol (AUP) will be used to test the toxicity effects of[venom toxins on zebrafish (*Danio rerio*).