Collection and Analysis of Toxic Proteins in *Corydoras* Catfish

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Abstract

In the tropical fish pet trade, transportation and movement can subject fishes to prolonged stress. In response to these stressors, some species of catfishes are known to release defensive secretions which cause self-poisoning and death. The chemistry of these toxic secretions are unknown. We are interested in identifying and studying the venoms of Corydoras catfishes, a genus that is also a common household pet. Venom secretion was induced by stressful handling of the fish. Within minutes, the water turned cloudy, indicative of venom secretion. Signs of self-poisoning were evident when fish showed reduced vitality. Analysis of Corydoras venom through SDS-PAGE, Bradford Standard Assay (BSA), and Mass Spectrometry confirmed that the venom secretions consist of multiple protein compounds. Through Mass Spectrometry, a homology of certain polypeptides found in venom secretions of multiple Corydoras was found. The chemical diversity of the venom compounds is being explored in multiple Corydoras species. In conjunction with the chemical analysis, we are studying the anatomical structure of the venom glands through gross anatomy and histology analysis. Through gross anatomy, a gland was found proximal to an opening where secreted venom may empty through. Through histology analysis, we have been able to locate where ducts could potentially be at as well as nerve endings and vesicles possibly containing venom granules allowing us to elucidate the mechanism of venom secretion. While ongoing studies are still occurring, we have taken many steps that has allowed us to understand the unknown properties of Corydoras venom as well as the the general physiology and anatomy of Corydoras venom gland. With these studies taken together, our hope is to elucidate the functions of the venom and the degree of evolutionary homology within Corydoras genus.

Introduction

Corydoras is a genus of armored catfish from South America. They posses sharp fin spines and toxin-secreting skin glands used for self-defense. The toxin is secreted in stressful situations, such as when fish are chased or captured, and when transported commercially. When the toxin is secreted, the surrounding water becomes clouded.

The toxicological properties of *Corydoras* toxin are unknown, but the toxin can induce "self-poisoning," resulting in the rapid death of fish when captured and transported.

We are studying the phenomenon of self-poisoning, and the biochemistry of *Corydoras* toxin using two species: *C. sterbai* and *C. duplicareus*. Our approach is to induce toxin secretion for biochemical analysis, with the hypothesis that it is a protein. We are also studying the anatomical and histological relationships between the glands and the pectoral fin spines to assess the ability of the spines to act as an envenomation instrument.

C. sterbai

C. duplicareus

Methods

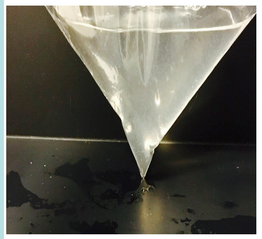
Sample Collection ⇨ Resolubilization & BSA quantitation

SDS-PAGE & In-Gel Digestion / In-Solution Tryptic Digestion

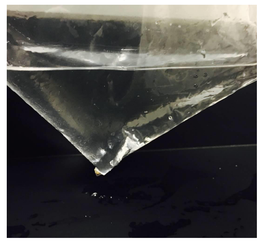
Desalting with C-18 Pipette Tips

Mass Spectrometry & ESI Analysis

PEAKS Bioinformatic Analysis



Toxin secretion



Tank water

Database Configuration and Bioinformatics

Fragmented and sequenced peptides were compared against a database using PEAKS Studio 8.5. This database contained all annotated peptides from the family Siluriformes, all known proteinaceous toxins and their targets, known proteomic contaminants, and all *E. coli* K12 proteins. Proteins were evaluated using an ALC value of 95 percent or higher, a FDR cutoff of 15 and a fragment mass error tolerance of 0.02 Da.

Toxin Collection

Five individuals of *C. sterbai* and *C. duplicareus* catfish were placed in separate bags containing 120ml of tank water for 10-30 minutes to simulate the stressful conditions. Fish remained in the bag until the water turned cloudy, indicating the presence of toxin. Signs of self-poisoning were evident; affected fish showed impairments such as decreased activity, slowed breathing or loss of equilibrium. The initial effects were reversed by quickly returning affected fish to clean water for recovery. Additional samples of tank water were collected at the same time as negative controls. After fish were removed, all water samples were immediately frozen in liquid nitrogen and lyophilized to dryness.

Sample Preparation

Lyophilized water samples were re-solubilized in 2 mL deionized water, divided into 50 uL aliquots, and stored at -80°C until subjected to SDS-PAGE or in-solution digestion.

Histology

Fish were euthanized in chilled benzocaine (1.5 mg/L water). Tissue samples including pectoral fins and postaxillary glands were dissected and preserved in 10% NBF. Tissues were decalcified for 8 days, then embedded in Technovit Resin. Three micron serial sections were cut and stained with Toluidine Blue. Slides were analyzed through light microscopy.

Results

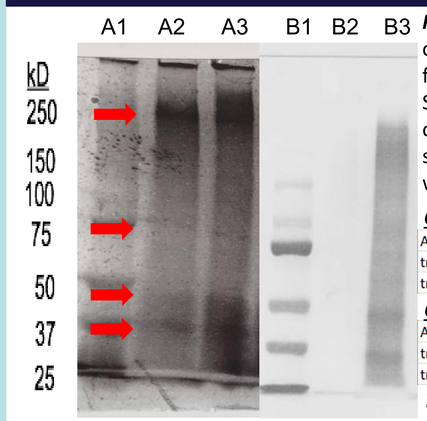


Figure 1: SDS-PAGE gels of both raw toxin extract and tank water control. Gel A contains resuspended toxin from *C. sterbai* while gel B contains resuspended toxin from *C. duplicareus*. Lane A1 and B1 are the ladder, Precision Plus Dual Color Standards. Lanes A2, A3, and B3 are all resuspended toxin in DI water at varying concentrations. B2 is the tank water control resuspended in DI water. Gels were stained with silver. Bands at for *C. sterbai* 250 kDa, 75 kDa, 45 kDa, and 37 kDa were all excised for in-gel tryptic digestion.

C. sterbai

Accession	-10lgP	Coverage	#Peptides	#Unique	Description
tr|W5UEZ8|W5UEZ8_ICTPU	143.02	11	12	7	Prostaglandin G/H Synthase 1 OS=Ictalurus punctatus
tr|W5UCU7|W5UCU7_ICTPU	131.64	7	7	2	Prostaglandin G/H Synthase 2 OS=Ictalurus punctatus

C. duplicareus

Accession	-10lgP	Coverage	#Peptides	#Unique	Description
tr|W5UEZ8|W5UEZ8_ICTPU	76.98	8	6	6	Prostaglandin G/H Synthase OS=Ictalurus punctatus
tr|E3TCK9|E3TCK9_ICTFU	21.64	3	1	1	Prostaglandin reductase 1 OS=Ictalurus furcatus

Figure 2: The screenshots above are proteins that have peptide matches in PEAKS. These proteins are involved in prostaglandin synthesis. An in-gel and in-solution tryptic digest was done for *C. sterbai,* while an in-solution digest was done for *C. duplicareus*. The peptides were filtered in PEAKS using a 95% ALC value, along with 90 percent or above confidence for each amino acid in the sequenced peptide.

C. sterbai

Accession	-10lgP	Coverage	#Peptides	#Unique	Description
E5L0E6|VSPCA_AGKPL	44.5	3	2	1	Protein C activator OS=Agkistrodon piscivorus leucostoma PE=2 SV=1
Q9XZC0|LCTA_LATTR	37.88	1	2	1	Alpha-latrocrustotoxin-Lt1a (Fragment) OS=Latrodectus tredecimguttatus PE=2 SV=2
t3db_target|P00742	25.64	1	1	1	Coagulation factor X
P0DJC8|SLA_BOTAS	24.18	10	1	1	Snaclec aspercetin subunit alpha (Fragment) OS=Bothrops asper PE=1 SV=1
P0DJC9|SLB_BOTAS	24.18	10	1	1	Snaclec aspercetin subunit beta (Fragment) OS=Bothrops asper PE=1 SV=1
P69929|TX9A_ANTMC	24.27	2	1	1	Delta-actitoxin-Amc1a OS=Antheopsis maculata PE=1 SV=1

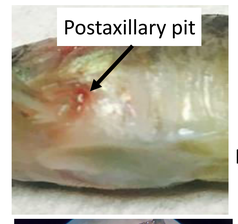
C. duplicareus

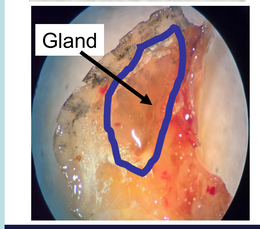
Accession	-10lgP	Coverage	#Peptides	#Unique	Description
Q9W7J6|3S37_PSETE	50.95	14	1	1	Short neurotoxin 7 OS=Pseudonaja textilis PE=1 SV=1

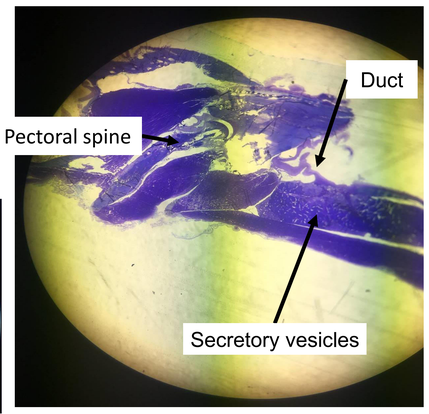
***Figure 3*:** These toxins are the proteins that have matches to peptide fragments. The peptide fragments were created in the same fashion as seen in Figure 2.

Anatomy/Histology

In *C. sterbai*, a postaxillary pit is visible on the skin surface overlying the gland, which is visible when the armor plating is dissected away from the body, exposing the gland and surrounding musculature. Histological analysis reveals a gland ~ 1-2 mm long underlying the armor, with a broad short duct leading from the gland to the postaxillary pit opening. The gland is filled with secretory vesicles and lacks an obvious secretory lumen.







Discussion

Looking first at Figure 1, it is evident that the toxin secretion is proteinaceous in nature; bands are clearly seen in both gels. From the in-gel digest data in Figure 3, the 250, 75, and 37 kDa bands all had peptide fragments that matched known toxins. The in-solution digest for *C. duplicareus* was a more complex sample, but still had one hit as seen in Figure 3. In Figure 2, both species had peptide fragments that matched to prostaglandin synthases, showing that both species had were stressed enough to secrete their toxin which may have caused inflamed tissues The high confidence of our peptide fragments in Figures 2 and 3 is a good sign that these hits are especially "real". One of our difficulties is our C18 desalting tips and C18 column can only bind hydrophobic peptides, therefore we are looking into other methods for desalting and cleanup of our samples.

Based on the relative position of the opening and the gland to the fin serrations, it appears that a method of envenomation from the fish is by using the pectoral fins to tear through the integument of its victim allowing for more exposure to the toxin.

Conclusion

We have found similarities in protein content of toxin from *C. duplicareus* and *C.* sterbai using multiple different analyses. Further separation of proteins and peptides while in-solution will allow us to hopefully discover the toxic substance that is secreted. We will use a high pH fractionation column next to further help separate our proteins in solution and to concentrate them. While an in-gel digest as well histological studies are still being conducted on *C. duplicareus*, the initial work on *C. sterbai* has given much insight on the possible general morphology of *Corydoras* toxin gland and duct. We are also expanding our toxin research on other *Corydoras* species to further understand the toxic properties of the genus's toxin.

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