


BIOCHEMISTRY OF CONE SNAIL TOXIN ACTIVATION

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INTRODUCTION

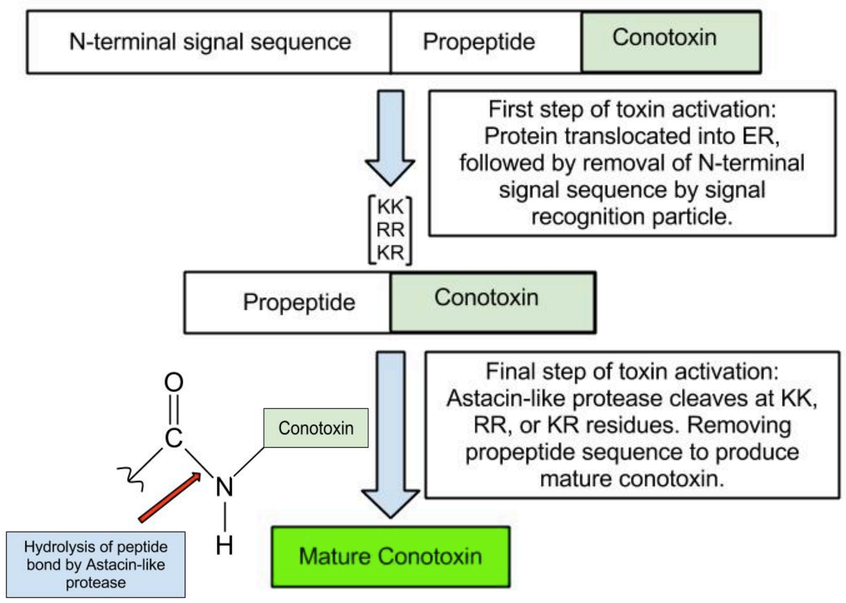
Conus geographus

Cone snails use venom to capture prey for food and for defense against predators. The venom is composed of over 100 active peptides that target specific receptors in the nervous system. Several of these peptides have the potential to become medicine for treatment of pain, depression, seizures, and neurological disorders such as Alzheimer's disease and MS.

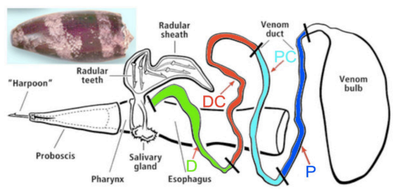
PROJECT GOAL: In our efforts to understand how cone snails make toxins, we want to purify the Astacin-like protease thought to execute the final steps in toxin activation. Initial trials indicated that purification from bacteria expressing the protease would be challenging. We applied a sparse matrix search to find buffer conditions (pH, salt, metal ions, additives) that maximized yield of soluble Astacin-like protease.

CONE SNAIL TOXIN ACTIVATION:

- The production of each active peptide/toxin involves several steps beginning with translating RNA to protein.
- The N-terminal signal sequence directs the protein to be secreted, which is removed upon translocation into the ER.
- A propeptide sequence helps with folding of the toxin.
- The mature toxin is released from the propeptide segment by action of an Astacin-like protease.



VENOM DUCT OF *C. geographus*



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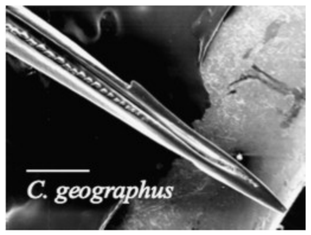

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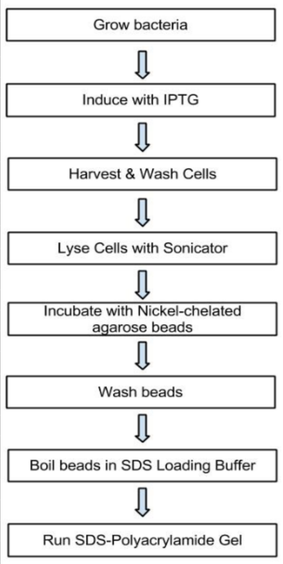
METHODS

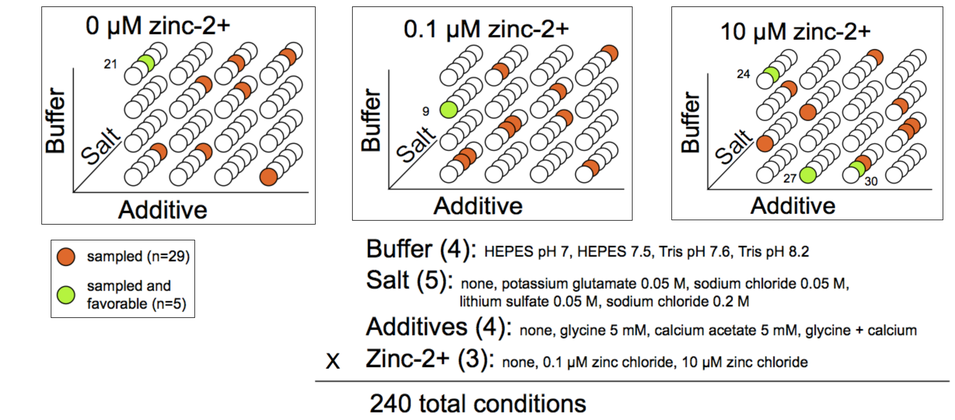
TABLE 1: ARCHANA EXTRACTION BUFFERS (AEB)

AEB #	Buffer	Salt	Metal	Additive
2	HEPES pH=7.5	NaCl 50 mM	Zn 0.1 uM	Gly 5 mM
3	HEPES pH=7.5	None	Zn 10 uM	None
4	TRIS pH=8.2	NaCl 200 mM	Zn 0.1 uM	Both
5	HEPES pH=7.5	NaCl 50 mM	Zn 10 uM	Both
6	TRIS pH=8.2	NaCl 50 mM	None	CaAc 5 mM
7	TRIS pH=8.2	$LiSO_4$ 50 mM	None	Both
8	TRIS pH=7.6	Glu 50 mM	Zn 10 uM	Both
9	TRIS pH=7.6	None	Zn 0.1 uM	None
10	TRIS pH=7.6	NaCl 200 mM	None	Gly 5 mM
11	HEPES pH=7.5	$LiSO_4$ 50 mM	Zn 10 uM	Both
12	TRIS pH=7.6	$LiSO_4$ 50 mM	None	CaAc 5 mM
13	TRIS pH=7.6	NaCl 200 mM	Zn 10 uM	None
14	TRIS pH=8.2	Glu 50 mM	Zn 0.1 uM	Gly 5 mM
15	HEPES pH=7.5	NaCl 200 mM	Zn 0.1 uM	CaAc 5 mM
16	HEPES pH=7	NaCl 50 mM	Zn 10 uM	CaAc 5 mM
17	HEPES pH=7	Glu 50 mM	Zn 0.1 uM	Both
18	TRIS pH=7.6	$LiSO_4$ 50 mM	Zn 0.1 uM	CaAc 5 mM
19	HEPES pH=7	NaCl 200 mM	None	Gly 5 mM
20	TRIS pH=7.6	None	Zn 10 uM	Gly 5 mM
21	TRIS pH=8.2	NaCl 50 mM	None	None
22	HEPES pH=7	$LiSO_4$ 50 mM	Zn 0.1 uM	None
23	HEPES pH=7	NaCl 50 mM	Zn 0.1 uM	None
24	TRIS pH=8.2	Glu 50 mM	Zn 10 uM	None
25	HEPES pH=7	None	None	Both
26	TRIS pH=8.2	NaCl 200 mM	Zn 10 uM	CaAc 5 mM
27	HEPES pH=7	None	Zn 10 uM	Gly 5 mM
28	HEPES pH=7.5	$LiSO_4$ 50 mM	Zn 0.1 uM	Gly 5 mM
29	HEPES pH=7	NaCl 200 mM	None	None
30	HEPES pH=7	Glu 50 mM	Zn 10 uM	CaAc 5 mM

PROTOCOL

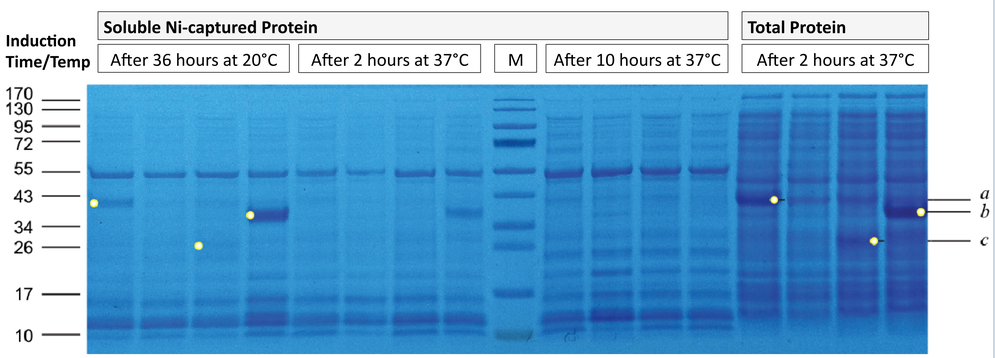


SPARSE MATRIX TEST GENERATED BY SAMBA COMPUTER PROGRAM



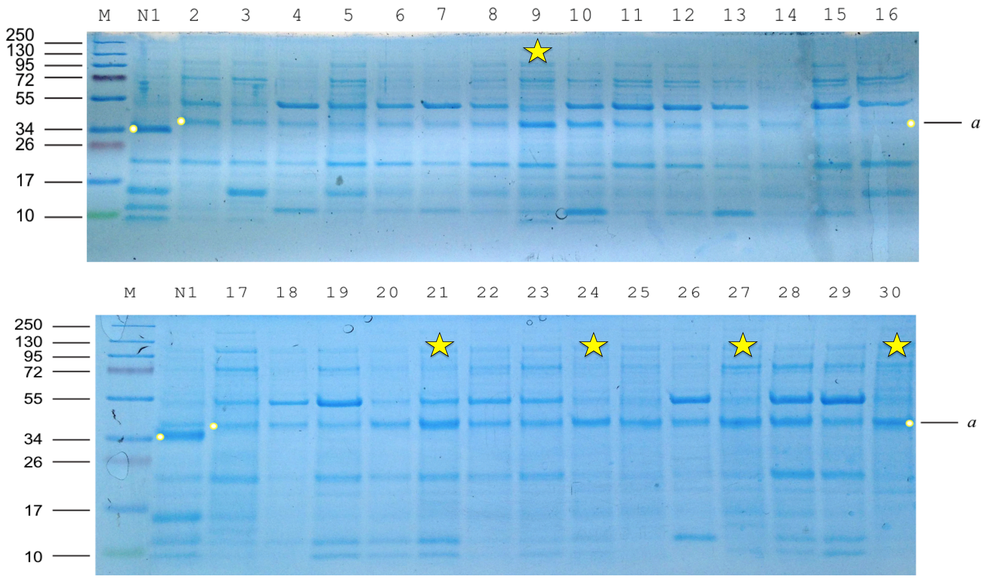
Sparse Matrix Approach. Four variables (pH, salt, zinc-2+, and additives) were assigned one of several possible values by the Samba server: http://iqs-server.cnrs-mrs.fr/samba.
Twenty-nine of the 240 possible combinations were tested (orange or green dots). Five of the twenty-nine combinations generated better than average yield of soluble protein (green dots).

RESULTS



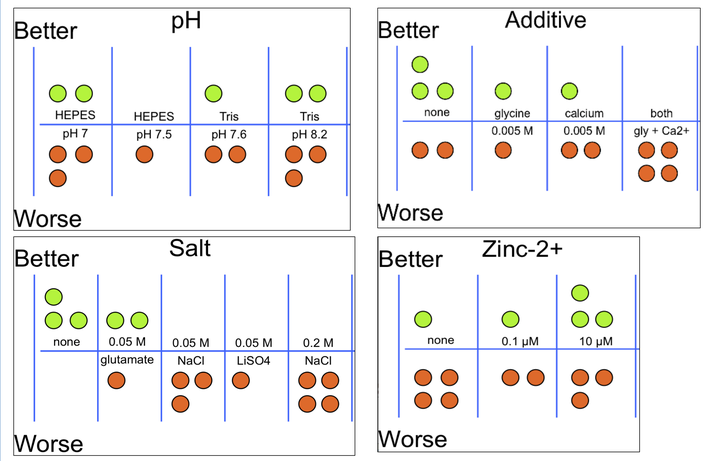
Gel 1: SDS-PAGE Gel showing a) Inactive zymogen form of Astacin-like protease, b) NR1 positive control, c) active Astacin-like protease.

RESULTS (CONT.)



Gels 2 & 3: SDS-PAGE Gel showing test of AEB #2-30 to determine highest yield of Astacin-like protease. M=Protein Ladder, N1=NR1 positive control. Extraction Buffers #9, 21, 24, 27, and 30 (highlighted with yellow stars) produced the highest yield of the protease. Extraction Buffers #4, 5, 6, 7, 13, 16, 19, and 29 produced the lowest yield of the protease.

ANALYSIS



CONCLUSION

The two variables that mattered most were additive and salt. Future experiments will attempt to purify the Astacin-like protease with use of a more ideal buffer containing HEPES pH=7 buffer, no salt, 10 µM Zinc metal, and no additive.

ACKNOWLEDGEMENTS

This work was funded in part by the University of Utah through the Undergraduate Research Opportunities Program. This project started as a collaboration with Helena Safavi, who made the initial discovery of the Astacin-like protease in cone snails and who provided the plasmid DNAs for expression of the protease in bacteria.