Identification of Potential Secondary Metabolites in Extracted Cyanobacteria

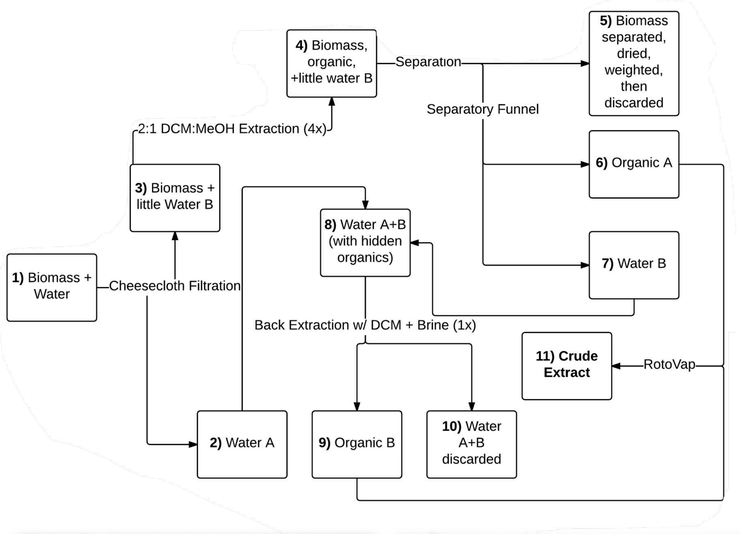
Raymond Banke, Dr. Evgenia Glukov, and Dr. William G. Gerwick

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography University of California San Diego, La Jolla, California 92093, United States

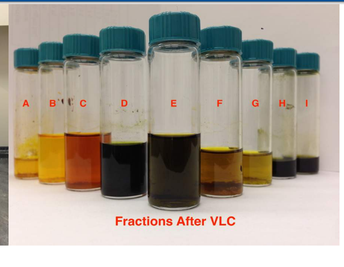
Abstract

The purpose of this study was to examine the secondary metabolites extracted from an assemblage of the cyanobacteria *Moorea sp.* and *Schizothrix sp* from American Samoa. Nine fractions of varying polarities were produced and tested using brine shrimp and cancer assays to determine toxicity. Two of these fractions, G and H, which had the greatest potential to contain useful peptides, were then tested via LCMS (Liquid Chromatography Mass Spectrometry), and suspect compounds were identified. This research is a stepping stone for someone to officially isolate compounds from these fractions and discover anti-cancer or anti-inflammatory properties in particular metabolic products produced by cyanobacteria.

Methods–Crude Extraction



Methods–VLC

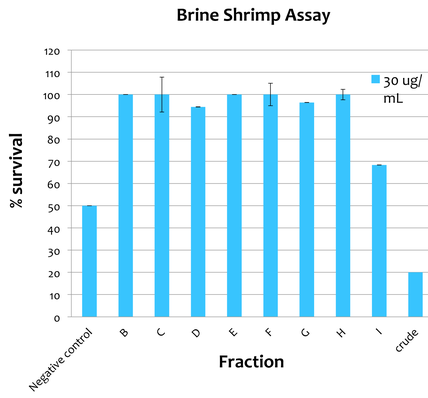


Nine fractions were created with varying parts of Hexane, Ethyl Acetate, and Methanol to create varying polarities (different compounds will move with different speed, which is related to their polarity). After first adding the crude extract to the packed VLC funnel, each fraction from least to most polar was run through.

Brine Shrimp Assay

Brine Shrimp Death				
	M1	M2	Avgerage	Error
Concentration for Shrimp	30 ug/mL	30 ug/mL	30 ug/mL	30 ug/mL
MeOH	0.67	0.33	0.50	0.24
B	0	0	0.00	0.00
C	0	0	0.00	0.00
D	0.11	0.00	0.06	0.08
E	0.00	0.00	0.00	0.00
F	0.00	0.00	0.00	0.00
G	0.00	0.07	0.04	0.05
H	0.00	0.00	0.00	0.00
I	0.30	0.33	0.32	0.02
crude	0.80	0.80	0.80	0.00

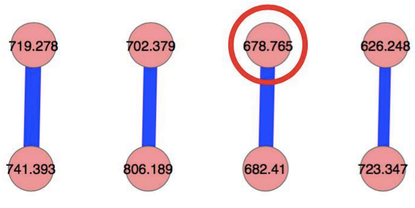
Brine Shrimp Survival		
Well	Average	
	% Surival for 30 ug/mL (%)	SD/% Error for 30 ug/mL (%)
Negative control	50	N/A
B	100	0
C	100	0
D	94	8
E	100	0
F	100	0
G	96	5
H	100	0
I	68	2
crude	20	0



Cytatoxicity Assay

In this assay, the fractions were diluted multiple times so as to be tested on human cancer cells. Concentrations of 1 ug/mL and 10 uq/mL for each fraction were used. However, only the 10 ug/mL concentration of fractions I and crude killed the cancer cells.

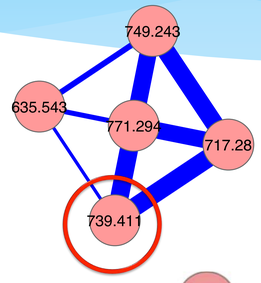
Molecular Networking & Metabolite Identification



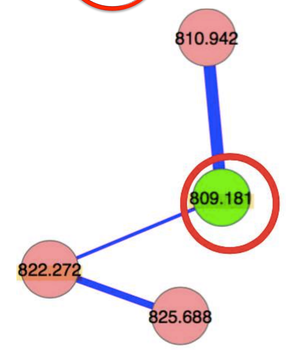
830. g/mol (not depicted) could potentially be the molecular weight of amphibactin S, which is produced by certain marine bacteria and contains useful fatty acids.

678.765 g/mol could potentially be the molecular weight of mayotamide B, iejimalide A, or veraguamide J. Mayotamides have been isolated from tunicates and have potential antitumor properties. Iejimalides are found in the Okinawan sea slug *Eudistoma cf. rigida* and have been found to be very effective at stopping cancer cell growth. Veraguamide J is found in the cyanobacterium *Oscillatoria margaritifera;* some of the veraguamide family have shown potential cytotoxicity to lung cancer cells.

Molecular Networking & Metabolite Identification cntd.



739.411 g/mol could be the molecular weight of antanapeptin B, nobilamide C, hantupeptin B, or batzelladine B. Antanapeptin A, which is related to B, has been found in the cyanobacterium *Lyngbya majuscula* and was found to have anti-cancer properties. Hantupeptin B has been isolated from the marine cyanobacterium *Lyngbya majuscula* and was found to be cytotoxic against leukemic and breast cancer cells. Batzelladine B is an anti-HIV alkaloid (secondary metabolite w/ nitrogen atoms).



809.181 g/mol could be the molecular weight of pitipeptolide A or viequeamide B. Pitipeptolide A was isolated from the marine cyanobacterium *Lyngbya majuscula* and possesses weak cytotoxicity against LoVo epithelial cancer cells. Viequeamide B was isolated from a marine button cyanobacterium. Interestingly, viequeamide A exhibited cytotoxicity against human lung cancer cells, but B did not.

Discussion and Conclusions

The brine shrimp assay revealed that majority of the fractions were nontoxic (fractions B, C, E, F, & H) or mostly nontoxic (fractions D, G, I, and crude). In the cancer cell assay, only fractions I and crude at 10 ug/mL concentration killed the cells. Nevertheless, we were unable to identify for sure what secondary metabolites were present in the G and H fractions, which went through the LCMS. We were though able to identify multiple potential compounds present via information from the LCMS graph peaks and by checking molecular weight and using molecular networking. Out of the possible compounds, majority had anti-cancer properties, such as iejimalide A and antanapeptin A.

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