Title
Harnessing the Pharmaceutical Potential of Marine Cyanobacteria

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Harnessing the Pharmaceutical Potential of Marine Cyanobacteria

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Project Hypothesis
We hypothesize that the secondary metabolite pathways in cyanobacteria can be manipulated by applying transcriptional promoters, either of a genetic or chemical nature (elicitors), to produce more secondary metabolites with potential pharmaceutical utility.

Project Goals and Objectives
1. To complete our use of a reporter gene assay (beta-galactosidase) to determine regulatory DNA sequences for biosynthetic genes, and to discover protein transcriptional activators of these regulatory domains (finished).
2. Using four different cultured cyanobacteria, to evaluate a variety of putative elicitor conditions for upregulated transcription of secondary metabolites.
3. Large scale elicitation or upregulation of secondary metabolites, and isolation, identification and biological characterization of compounds.
4. Outreach and Dissemination of Results.

Briefly describe project methodology
Objective 1. The beta galactosidase assay has been used successfully to determine promoter and ribosomal binding site regions in the jamacamide pathway. In addition, the upstream DNA sequence of Jam A was amplified and used as the bait region in protein pull-down assays, using a protein extract made from the *Lyngbya majuscula* JHB strain. Two proteins that bound to this bait region were isolated and their identities determined by mass spectrometry. The binding of these proteins to these DNA sequences has been reconfirmed by gel shift electrophoresis.
Objective 2. During the second face of this grant we have focused on the elicitation experiments. Ten different cyanobacteria, *Lyngbya majuscula* JHB, *Lyngbya majuscula* 3L, *Lyngbya bouillonii*, Phormidium, PAL 8/17/08-11 (Lyngbya), PAB 21/June 06-1, PNG 4/22/06-1, PAL 8/1/09-3, PAL 8/1/09-3(2), 3L Oscillatorium, were cultured and identical cultures were set up in 24 well plates. Ten different elicitors were added to the different wells.

Elicitors used in the elicitation experiment

Elicitor	Hypothesized mode of Action	Concentration	Molecular Weight (g/mol)	Cost ($)
DMSO	Chemical Stress Response / HDAC inhibitor	0.3%, 3.0%	78.01	4.25/g
Sodium butyrate	HDAC inhibitor	3/30 mM	110.09	0.30/g
5- Azacytidine	DNA Methyl Transferase	20/200µM	244.21	130.73/mg
2,4-D (auxin that controls growth)	Growth regulator	2/20µM	221.04	0.19/g
Heat Killed Bacteria/LPS: *E. coli*	Infection mimic	1/10 µg/mL	-	6.09/mg
Jasmonic acid	Growth regulator	1/10 µM	210.27	0.53/mg
2-oxo-Glutarate*	Krebs cycle intermediate, Nitrogen transporter	25/50mM	146.1	0.46/g
Ascorbate	Antioxidant	5/50 mM	198.11	0.23/g
Metadione sodium bisulfite	Vitamine K analog, Auxin metabolism	0.2/2 µm	276.2	0.37/g
Cumene hydroperoxide	Oxidative stress	2/20 µM	152.19	0.25/g

* α-Ketoglutaric acid

Objective 3. Several large scales cultures are now being worked up in an effort to isolated enough of the elicited compound for structure elucidation work.

Describe progress and accomplishments toward meeting goals and objectives.
We learned that several of the potential elicitors that we added were toxic but we also learned that in particular that several of the elicitors, the HDAC inhibitor *Sodium butyrate* and a combination of LPS (bacterial component) and jasmonic acid (growth regulator) worked well. The capacity for cyanobacteria to produce new compounds is a way to be able to find new entities that can potentially be used has human medicines. Several manuscripts have been published during this period but we expect several more to be published within a year based on the results obtained. First, the elicited molecules need to be isolated in enough quantity so that structures can be elucidated.

PROJECT MODIFICATIONS:
We increased the number of cyanobacterial strains and the number of elicitor to ten each, hence a much larger experiment. this was done in an effort to find several new ways to elicit new compounds.

PROJECT OUTCOMES:
None listed.

IMPACTS OF PROJECT:
None listed.

BENEFITS, COMMERCIALIZATION, AND APPLICATION OF PROJECT RESULTS:

None listed.

ECONOMIC BENEFITS generated by discovery

Issue-based forecast capabilities:
None listed.

Tools, technologies and information services developed:
None listed.

Publications
Peer-reviewed journal articles or book chapters
Engene N., Choi H., Esquenazi E., Rottacker E.C., Ellisman M.H., Dorrestein P.C., Gerwick W.H. 2011. Underestimated Biodiversity as a Major Explanation for the Perceived Prolific Secondary Metabolite Capacity of the Cyanobacterial Genus Lyngbya. *Environ. Microbiol.* 13(6): 1601-10. doi: 10.1111/j.1462-2920.2011.02472.x. Epub

Engene N., Rottacker E.C., Kastovsk, J.H.; Komrek, J.; Gerwick W.H. *Moorea producta* gen. nov., sp. nov. and *Moorea bouillonii* comb. nov., tropical marine cyanobacteria rich in bioactive secondary metabolites. *J. Syst. Evol. Microbiol.* In Review.

Jones, A.C., E.A. Monroe, S. Podell, W.R. Hess, S. Klages, E. Esquenazi, S. Niessen, H. Hoover, M. Rothmann, R.S. Lasken, J.R. Yates III, R. Reinhardt, M. Kube, M.D. burkart, E.E. Allen, P.C. Dorrestein, W.H. Gerwick and L. Gerwick. 2011. Genomic insights into the physiology and ecology of the marine filamentous cyanobacterium *Lyngbya majuscula*. PNAS 108 (21) 8815-8820. doi:10.1073/pnas.1101137108

Esquenazi E., Jones A.C., Byrum T., Dorrestein P.C., Gerwick W.H. 2011. Temporal dynamics of natural products biosynthesis in marine cyanobacteria. PNAS. doi: 10.1073/pnas.1012813108.

MEDIA COVERAGE:
The two PNAS manuscripts were written up as press releases and both of them were featured on the California Sea Grant website.

DISSEMINATION OF RESULTS:
Results have been communicated at several meetings and most of the work has been published.

COOPERATING ORGANIZATIONS:
None listed.

Students
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http://www.escholarship.org/uc/item/0dq568hh
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