

WSG Project Number: **R/ES-42**
Project Title: Analysis of a Toxic Alga in Stasis

Project period: 2/1/2004 – 5/31/2008

Principal Investigator(s) and Affiliation:
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PROJECT COMPLETION SUMMARY REPORT

(Please provide your summary here. Character limit: 5,000 characters, including spaces):

WASHINGTON SEA GRANT PROJECT COMPLETION SUMMARY REPORT

INSTRUCTIONS: Please provide a lay summary for your completed project that includes the following project elements:

Objectives

Harmful and toxic algae often display two life history phases. The vegetative phase is most obvious. An abundance of actively growing algal cells in the water is often visible as a “brown” or “red” tide. On occasion, equally visible, is the unfortunate, catastrophic loss of finfish or shellfish that is caused by these algal blooms. In contrast, the second life history program often utilized by algae is virtually unseen. Some algal taxa have the ability to transform from the vegetative state into resting cells, cysts or statospores. This life history phase is one of metabolic stasis. It provides selective advantage to an organism for over-wintering, surviving nutrient stress, or relocating to a new geographic region either by natural currents or by ocean vessel transport. The temporary stasis afforded by an alternate life history phase often “seeds” coastal waters and thus acts as a source for later (vegetative) bloom events. The goal of this project is to study the cellular programs that allow an organism that must photosynthesize in order to live, to enter, survive and escape long-term metabolic stasis while residing in coastal sediments. The marine alga *Heterosigma akashiwo* that forms massive, destructive brown-tides in coastal regions world-wide will be used in these studies.

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Methodology

To analyze the impact of altering physiological cues on resting cell induction, *Heterosigma akashiwo* cultures that have been subject to a known stress (cold and darkness) were monitored using micro-cinematography. Gene expression and HAB

species identification was measured using quantitative PCR. Genetic bar coding of *Heterosigma akashiwo* populations has been initiated using primers designed via the comparative sequence analysis of single nucleotide polymorphisms in both mitochondrial and chloroplast genes. Toxicity of *H. akashiwo*, toxicity caused by the presence of reactive oxygen species, was quantified by measuring hydrogen peroxide levels fluorometrically using the Amplex® Red reagent, (AR: 10-acetyl-3,7-dihydroxyphenoxazine and superoxide concentrations using the luciferin analog 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (MCLA) in a luminescence assay. For bacterial counts, cells were fixed with 2% formaldehyde and visualized with acridine orange. Protein modeling allowed the identification of a putative G-protein-coupled receptor.

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Rationale

The ability of many toxic algae to enter stasis provides a survival mechanism that remains essentially unstudied at the molecular and biochemical level. Because these metabolically quiescent cells can form "seed beds" in coastal sediments, as well as move to new geographic locations via natural currents or ship ballast water, their occurrence presents a significant challenge in coastal ecosystem management. Understanding the biology of vegetative and resting cells as well as the transformation of *H. akashiwo* between these two life history states will allow the development of new strategies for harmful algal bloom control.

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Major findings

(1) *Identification of geographically distinct but morphologically identical H. akashiwo populations.* Both mitochondrial and chloroplast DNA from two *H. akashiwo* strains (CCMP 452 ;West Atlantic and NIES 293; West Pacific) have been sequenced in their entirety. Analysis shows that the two strains show extensive genetic divergence. Also of interest is the fact that both genomes contain unique genes that are new to the entire genome data bases and that both chloroplast and mitochondrial protein-encoding genes contain inserts not seen in any other terrestrial plant or chloroplast genome that has been sequenced to date. The *H. akashiwo* chloroplast sequence represents the first raphidophyte genome to be completed and only the third available (both published chloroplast genomes are from diatoms). Additionally, we have finger printed 24 *H. akashiwo* strains using the putative Rubisco activase gene *cfxQ*, and have shown that sequence divergence that is associated with the geographic location of the strains. Work has now begun using the mitochondrial sequences to genetically fingerprint the extensive *H. akashiwo* culture collection maintained in the laboratory (over 50 strains).

(2) *Analysis of metabolic state which allows an organism to survive either vegetatively or in stasis.* We are now in an excellent position to address questions of algal response to environmental challenges. Through our chloroplast and mitochondrial sequencing effort, we now have an extensive library of genes including those responsible for Calvin cycle function, photosynthesis, transport, signal transduction, ATP generation, cytochrome

function, etc. Using our newly established qPCR methodology preliminary experiments show that the two *H. akashiwo* strains respond differently in gene expression when challenged by similar environmental cues. We are now in apposition to analyze the metabolic programs that permit survival of long-term stasis (cell survival in the dark and cold).

(3) Specific environmental cues impact *H. akashiwo* survival and genetic identity. Our data show that specific environmental stresses (eg. iron deficiency, heavy metal stress) will cause a *H. akashiwo* population to pass through a genetic bottleneck wherein a new *H. akashiwo* population will emerge that is of new genetic identity. We show that this new population appears to have a restructured stress tolerance. For example, a population that survives one type of stress, has a much better capacity to survive a second, different stress. Significantly, our results show that hydrogen peroxide is produced in *H. akashiwo* cultures predominantly through indirect, light-driven reactions of extracellular substances excreted by the algae. Moreover, the bacterial population that co-exists with the algae influences ROS production. We initiated experiments to determine *H. akashiwo* lipid synthesis, for it has been reported that lipid production synergistically influences reactive oxygen species toxicity.

Significance of results

- 1) We have established a primary data-base by generating both chloroplast and mitochondrial DNA sequences from the first raphidophyte analyzed to date. These sequences can be used for a broad spectrum of studies, ranging from population analysis to research related to cellular function. It is important to note that with National Science Foundation support, we are continuing to expand this data-base to include additional HAB species such as *Chattonella*, *Aureococcus*, and *Auroumbra*.
- 2) We show that populations of *H. akashiwo* found world-wide have different genetic signatures. This data is in marked contrast to those reported previously, which failed because of limited sequence data sampling used in their analysis.
- 3) We showed that genetic change can be driven in *H. akashiwo* populations as a result of an environmental stress.
- 4) Data verify that *H. akashiwo* produces reactive oxygen species that cause the organism to be toxic. That the production of this material is not only intracellular, but is also extracellular and that bacterial eco-cohorts can impact the generation of this product.
- 5) That *H. akashiwo* can be used as a model system to study the progression of cells through the life history phases of vegetative growth and long-term stasis.
- 6) The new technology of plating fragile algae has allowed a new approach to analyzing genetic divergence and diversity of algal populations.

Students supported

Post-doctoral students: Melinda Duplessis

Graduate students: Michael Lakeman, Elizabeth Tobin; Michael Nichizaki, Jean Veleluppillai

Undergraduates:

Husen Husen, Victor Tran, William Hardin, Mary Menichol, Thien Vo, Loc Ngo, Karen Avery, Jamie Ballantine, Nick Birk, Trieu Dang, Amanda Hoyt, Kun Lee, Kelly Kalulau, Vicky Lockheart, Erica Moore, Sarah Tinkham, Quynh Tu, Lanaya Waldron, Kojo Marfo, Megan Berger, Dave Keith, Shalana O'Brien-LaBayen, Jeremy Vargas+

outreach activities

- Gave opportunity for minority students to participate in research.