California Sea Grant Sea Grant
Final Project Progress Report
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The Role of Symbiotic Bacterial Siderophores in the Development of Toxic Phytoplankton Blooms

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Project Hypotheses
Research Hypothesis. The working hypothesis of this proposal is that a) phytoplankton growth can be controlled by the availability of the essential micronutrient iron b) symbiotic bacteria produce iron-binding compounds (siderophores) that can be utilized by the plankton to provide the iron needed for prolific growth, c) bacterially produced boron containing molecules may also contribute to control of phytoplankton growth d) a more complete understanding of this process could provide a means to predict where, when and under what conditions heavy growth of these organisms would occur.

Project Goals and Objectives
Project Objectives. The overall project objectives are:
1) To determine if bacteria known to be symbionts of toxic phytoplankton species such as Gymnodinium and Scrippsiella produce iron binding compounds known as siderophores.
2) To determine the structure and iron binding characteristics of the new siderophores.
3) To determine if the phytoplankton can utilize (transport) the iron from the siderophores produced by their own symbiotic and/or other bacteria. There are several possible hypotheses:
- phytoplankton use siderophores only from symbiotic bacteria to directly to acquire iron
- phytoplankton can acquire iron from many different siderophores via (presumably) an indirect route such as reduction
- phytoplankton acquire iron only from photoreactive siderophores either through their transient formation of Fe(II) or by uptake of the resulting new decarboxylated Fe(III) siderophore complex

4) To determine if the availability of iron from their symbiotic bacterial partners can trigger rapid outgrowth of dormant phytoplankton.
5) To determine if the phytoplankton can utilize (transport) boron from “boronophores” produced by their own symbiotic bacteria.
6) To determine if boron-containing molecules produced by symbiotic bacterial partners are involved in the rapid outgrowth of dormant phytoplankton. There are several hypotheses:

- iron acquisition in concert with bacterially produced boron containing quorum sensing molecules regulates phytoplankton growth
- in addition to siderophores, symbiotic bacteria produce "boronophores" to provide the diatoms with the needed micronutrient boron which itself controls growth
- there is a relationship between Fe and B metabolism in these organisms

Briefly describe project methodology

Isolation and Characterization

Our plan of work encompasses two basic parts. Part 1 is the isolation and structural and “metal” binding characterization of new siderophores/boronophores from bacteria symbiotic with various species of phytoplankton such as G. catenatum. Part 2 involves testing the ability of these new siderophores/boronophores to supply iron/boron to actively growing phytoplankton and/or to stimulate outgrowth of dormant cells.

Part 2: Phytoplankton Experiments

Consistent with the concept of a symbiotic relationship between certain bacteria and marine algae, we have been unable to establish genuinely bacteria-free cultures of dinoflagellates. Hence we will use `engineered' dinoflagellate cultures which have a single, well-characterized bacterium living in association with the dinoflagellate. In terms of primary productivity and carbon cycling in the marine ecosystem, diatoms and coccolithophores may be ecologically more important than dinoflagellates. The availability of axenic diatom (Phaedactylum tricornutum) and coccolithophore (Emiliana huxleyi) cultures at SAMS, makes it possible to examine directly the hypothesis of whether phytoplankton can utilize iron chelated to bacterial siderophores.

Describe progress and accomplishments toward meeting goals and objectives

a) We have found that vibrioferrin, VF, a previously discovered dicitrate siderophore, is widely present among bacteria specifically associated with toxic phytoplankton species.

b) The physiochemical characteristics of VF as well as its Fe-complex have been explored. We find that VF binds Fe(III) weakly as compared to other siderophores.

c) Fe loaded VF is very photosensitive with a half life under low illumination of 24 hrs at pH 8. The products of this photolysis are 1) an organic molecule which no longer binds Fe(III) or functions as a siderophore for the producing bacteria and 2) free Fe(II) which is oxidized to transiently soluble Fe(III)'.

d) We have found that BOTH the phytoplankton and their bacterial partners show a significant enhancement in iron uptake after photolysis suggesting that the associated bacteria may be "sharing" their newly bioavailable iron.

The implications of our findings suggest a redefining of algal-bacterial interactions as a key component in phytoplankton nutrient acquisition that ultimately promotes algal growth whether it is primary productivity or harmful algal blooms.

Project modifications

An unexpected finding is that VF and all other dicitrate as well as dicatecholate siderophores are capable of chelating boron in addition to Fe(III).

Due to large conformational changes a siderophore molecule will exhibit when bound to B vs. Fe(III), we are examining the biological effect boron and/or iron can play in inter- as well as intra-species communication.

Project outcomes
n/a

Impacts of project
n/a

Benefits, commercialization and application of project results
n/a

Economic benefits generated by discovery
n/a

Issue-based forecast capabilities
The relationship we have uncovered between particular bacterial species, iron and bloom forming dinoflagellates opens the door for better forecasting of bloom events.

Tools, technologies and information services developed
n/a

Publications
Conference papers, proceedings, symposia
(text) Title: "The Role of Symbiotic Bacterial Siderophores in the Development of Toxic Phytoplankton Blooms"
Authors: Shady A. Amin, Frithjof C. Küpper, David Green, and Carl J. Carrano
Date: February 4-9, 2007
Conference Title: ASLO 2007
Location: Santa Fe, New Mexico

Title: “The Role of Symbiotic Bacterial Siderophores in Promoting Marine Algal Blooms”
Authors: Shady A. Amin, David H. Green, Mark C. Hart, Frithjof C. Kuepper, Carl J. Carrano.
Date: March 2-7, 2008
Conference Title: ASLO Ocean Sciences Meeting,
Location: Orlando, Florida

Title: "Share the Wealth" The role of iron in bacterial agal interactions.
Authors: C.J. Carrano, S.A. Amin, F.C. Kuepper, D. Green
Date:01/25/2008-01/29/2008
Conference Title:Metals in Biology- Gordon Research Conference
Location: Ventura, CA

Title: Coordination Chemistry of Fe(III)-Vibrioferrin and its Implication to Bacterial-Algal Interactions
Authors: Shady A. Amin, David H. Green, Mark C. Hart, Frithjof C. Kuepper, Carl J. Carrano
Date: June 15-20, 2008
Conference Title: Gordon Research Conference, Environmental Bioinorganic Chemistry
Location: Waterville Valley Resort, New Hampshire

Peer-reviewed journal articles or book chapters
Title: "Boron Binding by a Siderophore Isolated from Marine Bacteria "Associated" with the Toxic Dinoflagellate G. catenatum".
Authors: Shady A. Amin, Frithjof C. Küpper, David H. Green, Wesley R. Harris and, Carl J. Carrano
Date: 2007.
Journal Name: J. Am. Chem. Soc.
Issue/Page Numbers: 129, 478-479

Title: "Borate-binding to Siderophores: Structure and Stability",
Authors: Wesley R. Harris, Shady A. Amin, Frithjof C. Küpper, David H. Green, and Carl J. Carrano
Date: 2007
Journal Name: J. Am. Chem. Soc.
Issue/Page Numbers: 129(40) 12263-12271.

Title: Bacterial-Algal Interactions: Siderophore Based Photochemical Iron "Sharing"
Authors: Shady A. Amin, David H. Green, Mark C Hart, Frithjof C. Küpper, William Sunda and Carl J. Carrano
Date: Feb. 2008
Journal Name: Nature
Issue/Page Numbers: submitted

Title: Vibrioferrin, an Unusual Marine Siderophore: Iron Binding, Photochemistry, and Biological Implications
Authors:Shady A. Amin, David H. Green, Frithjof C. Küpper, and Carl J. Carrano
Date: Feb. 2008
Journal Name: Journal of the American Chemical Society
Issue/Page Numbers: in revision
Media coverage coverage

Media
KPBS, public radio broadcast of "These Days"
City: San Diego
State: CA
Date of publication/broadcast: 05/01/2007
Headline or topic: HAB, "Killer Algae"

KPBS, public television broadcast of "Full Focus"
City: San Diego
State: CA
Date of publication/broadcast: 05/01/2007
Headline or topic: Harmful Algal Blooms

"The Daily Aztec" and "SDSU Universe"
City: San Diego
State: CA
Date of publication/broadcast:
Headline or topic: Harmful Algal Blooms, SeaGrant Research

Please list any workshops/presentations given
n/a

Dissemination of results
n/a

Students
Shady A. Amin
San Diego State University
Department of Chemistry and Biochemistry
Degree program enrolled in: Ph.D.
Theses/dissertation title: n/a
Supported by Sea Grant funds? [x] yes [] no
Start date: 03/01/2006
End date: 05/31/2010

Madura Rane
San Diego State University
Department of Chemistry and Biochemistry
Degree program enrolled in: M.S.
Theses/dissertation title: An Electron microscopic Study of Bacterial Algal Interactions
Supported by Sea Grant funds? [x] yes [] no
Start date: 01/01/2008
End date: 12/31/2008

Sukh Singh
San Diego State University
Department of Chemistry and Biochemistry
Degree program enrolled in: M.S.
Theses/dissertation title: Proteomics of Algal Associated Marinobacters
Supported by Sea Grant funds? [] yes [x] no
Start date: 01/01/2008
End date: 12/31/2008

How many student volunteers were involved in the project? 1

Cooperating organizations
None listed

International implications
This proposal represents a truly international, multidisciplinary collaboration. In broad outlines the PI Carrano, as a chemist and siderophore expert, is responsible for all the natural product (siderophore) isolation, characterization and structural determinations using facilities available to him in his San Diego laboratory. In addition, those biological experiments that involve only the isolated bacterial systems, i.e. real time PCR, transposon mutagenesis, PCR screening and quorum sensing are also done in his laboratory. Experiments involving phytoplankton require the specialized facilities and expertise made available by Dr. Green and Dr. Küpper at SAMS. We will also be collaborating with Dr. Christopher Bolch at the University of Tasmania. The SAMS and Tasmania groups will be responsible for uptake and growth studies with the phytoplankton/bacterial systems and assistance with the comparative genomics and annotation of bacterial symbiont genomes.

Awards
Graduate Student Shady Amin, Ph.D. in Chemistry (Carl Carrano, advisor) was awarded first place in the Biological Sciences Divison of the 2nd Annual Student Research Competition held at SDSU March 2 and 3rd and subsequently took a second place for his research presentation entitled "The Role of Symbiotic Bacterial Siderophores in the Development of Primary Productivity in the Ocean" at the Twenty-Second Annual California State University Student Research Competition held May 2 and 3, 2008, at California State University, East Bay. This system-wide competition showcased excellent research conducted by CSU undergraduate and graduate students in the full range of academic programs offered by the CSU. Student participants made oral presentations before juries of professional experts from major corporations, foundations, public agencies, colleges and universities in California. Shady has also received an invitation to present one of the featured talks at the inaugural Graduate Research Conference/Gordon Research Conference on "Oceans and Human Health" to be held this June in New Hampshire. This is an unprecedented honor.

Keywords
iron, bacteria, phytoplankton, siderophores