Investigating Algicidal and Growth-inhibiting Bacteria associated with Seagrass and Macroalgae beds in Puget Sound

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Investigating Algicidal and Growth-inhibiting Bacteria associated with Seagrass and Macroalgae beds in Puget Sound

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¹Plankton Laboratory, Graduate School of Fisheries Sciences, Hokkaido University
²Laboratory of Marine Environmental Microbiology, Graduate School of Agriculture, Kyoto University.
³Friday Harbor Laboratories, University of Washington
⁴Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA
Introduction

*Heterosigma* red tides and PSP (Paralytic Shellfish Poisoning)
Problems in Puget Sound, WA

- Continuous *Heterosigma* red tides in recent years
- Mortality of wild and farmed salmon $2 million (farmed Atlantic salmon) in 2006 (Rensel 2007)

- Probable mass mortality of juvenile sockeye salmon by *H. akashiwo* in Puget Sound before migrating to Bering Sea (Rensel *et al.*, 2010)

- Frequent occurrences of high levels of PSP has increased since 1957 (WDOH) (Shellfish harvesting closures)

Urgent need to establish mitigation strategy of HABs
Introduction

Using algicidal bacteria as Bio-control of HABs

What is Algicidal Bacteria?

**Definition:** bacteria which not only compete for nutrients, but actively attack and kill microalgae in order to use their organic matter for growth (Imai 2011)

Algicidal bacteria increase drastically at the end of a red tide (Hallegraeff *et al.*, 2002; Imai 1998b; Kim *et al.*, 1998)

Suggesting

- Algicidal bacteria play important role in decay process of red tide
- Potential use as ecologically friendly counter-measure for HABs
Killed

Algicidal bacteria from coastal water around the world
- Algicidal range (specific to certain HABs)
- Density to cause algicidal activity
- Killing mechanism
  (Direct attack or indirect attack)
- Phylogenetic characterization

However,
Very few ecological studies of algicidal bacteria in natural seawater

Where are algicidal bacteria most abundant??
## Introduction

Seagrass bed as enormous source of algicidal bacteria in Japan

<table>
<thead>
<tr>
<th>HAB species</th>
<th>(CFU / g wet weight)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Heterosigma akashiwo</em></td>
<td>$4.30 \times 10^5$</td>
<td>(Kuroda 2011)</td>
</tr>
<tr>
<td><em>Chattonella antiqua</em></td>
<td>$5.12 \times 10^7$</td>
<td>(Inaba 2013)</td>
</tr>
<tr>
<td><em>Cochlodinium polykrikoides</em></td>
<td>$2.76 \times 10^7$</td>
<td>(Imai <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td><em>Karenia mikimotoi</em></td>
<td>$6.43 \times 10^7$</td>
<td>(ibid.)</td>
</tr>
<tr>
<td><em>Alexandrium tamarense</em></td>
<td>$4.60 \times 10^5$</td>
<td>(Onishi 2010)</td>
</tr>
</tbody>
</table>

*The loss of habitat for algicidal bacteria may have contributed to increased outbreaks of HABs in the coastal zones in recent decades.*

## Objective

To investigate algicidal bacteria associated with seagrass and macroalgae beds in Puget Sound: seeking prevention strategies for HAB problems
Material and Methods

Puget Sound, WA, USA
2012 (June - July)
2013 (July)

Sampling

Seagrass

- Z. marina
- Z. japonica
- P. scouleri

Green algae

- U. lactuca
- Enteromorpha sp.

Brown algae

- S. sessile
- F. distichus
- N. luetkeana

Locations:
- ① Jackles Lagoon
- ② North Padilla Bay
- ③ Samish Bay
- ④ South Padilla Bay
- ⑤ Shallow Bay, Sucia
- ⑥ Beach Heaven
- ⑦ North Bay
- ⑧ Barlow Bay
- ⑨ South Carkeek
- ⑩ Dumas Bay
- ⑪ Lynch Cove
- ⑫ Potlatch State Park
- ⑬ Cornet Bay
- ⑭ Holmes Harbor
- ⑮ ES10
- ⑯ JPN001
- ⑰ Offshore
- ⑱ Blaine Bay Kelp Bed
- Heaven's Beach

Map locations:
- Puget Sound
- Seattle
- Seattle

Samples:
- Z. marina
- Z. japonica
- P. scouleri
- U. lactuca
- Enteromorpha spp.
- S. sessile
- F. distichus
- N. luetkeana

Eelgrass Beds:
- ① Jackles Lagoon
- ② North Padilla Bay
- ③ Samish Bay
- ④ South Padilla Bay
- ⑤ Shallow Bay, Sucia
Materials and Methods

Isolation of bacteria

Seawater sample

1/10 …… 1/1000 fold

Dilution

Particle-associated bacteria (PAB)
Free-living bacteria (FLB)

*Glutaraldehyde fixation for direct counts (DAPI count)

Z.marina

1/10 …… 1/10000 fold

Stationary culture (20°C)

Shake 500times (Autoclaved seawater)

Dilution

Isolation
Materials and Methods

Co-Culture Experiment

- **Algal culture**
- **Modified SWM-3**
- **Dilution of algal culture** (about $10^3$ cells/mL)
- **Incubate 1 or 2 days**
- **Detecting**
  - Algicidal bacteria
  - Growth-Inhibiting Bacteria (GIB)
- **Inoculation of bacteria**
- **Inoculation of bacteria**
- **16S ribosomal RNA Analysis**

*Condition of Incubation*
- $20^\circ$C
- Light: 14h
- Dark: 10h
- about 100 μmol photons/m$^2$/sec
Results  Density of algicidal bacteria (AB) and Growth-inhibiting bacteria (GIB) detected in 2012

(Target species: *H. akashiwo*)

![Graph showing density of AB and GIB in Padilla Bay and Dumas Bay.](image)

<table>
<thead>
<tr>
<th>Location</th>
<th>AB</th>
<th>GIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Padilla Bay</td>
<td>1.8 x 10⁶ CFU/g wt</td>
<td>3.3 x 10⁷ CFU/g wt</td>
</tr>
<tr>
<td>Dumas Bay</td>
<td>6.3 x 10² CFU/mL</td>
<td>7.5 x 10² CFU/mL</td>
</tr>
</tbody>
</table>

(Target species: *A. tamarense*)

![Graph showing density of AB and GIB in Padilla Bay and Dumas Bay.](image)

<table>
<thead>
<tr>
<th>Location</th>
<th>AB</th>
<th>GIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Padilla Bay</td>
<td>2.8 x 10⁶ CFU/g wt</td>
<td>8.3 x 10⁶ – 1.5 x 10⁷ CFU/g wt</td>
</tr>
<tr>
<td>Dumas Bay</td>
<td>6.3 x 10² CFU/mL</td>
<td>7.5 x 10² – 4.1 x 10³ CFU/mL</td>
</tr>
</tbody>
</table>

*H. akashiwo*
Algicidal bacteria (AB)
*Z. marina* 2.8 x 10⁶ CFU/ g wt
Seawater 1.8 x 10¹ – 1.6 x 10³ CFU/ mL

Growth-inhibiting bacteria (GIB)
*U. lactuca* 3.3 x 10⁷ CFU/ g wt
Seawater 4.2 x 10² – 2.8 x 10³ CFU/ mL

*A. tamarense*
Algicidal bacteria (AB)
Seawater 6.3 x 10² CFU/ mL

Growth-inhibiting bacteria (GIB)
*Z. marina* 8.3 x 10⁶ – 1.5 x 10⁷ CFU/ g wt
Seawater 7.5 x 10² – 4.1 x 10³ CFU/ mL
Results AB and GIB from different seagrass and macroalgae in 2013
(Target species: \textit{H. akashiwo} 893)

\begin{itemize}
  \item \textbf{AB} \textit{Z. marina} and \textit{U. lactuca} >10^8 \text{CFU / g wt}
  \item \textbf{GIB} \textit{Z. japonica} and \textit{U. lactuca} ca. 10^6 - 10^8 \text{CFU / g wt}
\end{itemize}

(Target species: \textit{A. tamarense})

\begin{itemize}
  \item \textbf{GIB} \textit{Z. marina}, \textit{Z. japonica} and \textit{U. lactuca} ca. 10^5 - 10^8 \text{CFU / g wt}
\end{itemize}
Results  Phytoplankton composition at Seagrass Beds in 2013

- **Centric diatoms**
- **Pennate diatoms**
- **Dinoflagellates**
- **Raphidophytes**

**Composition (%)**

- Padilla Bay
- Cattle point
- Sucia
- FHL
- Potlatch

**Cell density (x10^5 cells L^-1)**

- **Pennate diatoms**
  - Licmophora spp.
  - Cylindrotheca closterium

**Dinoflagellate**

- Dinophysis spp.

**Centric diatoms**

- Detonula pumila
- Odontella spp.

**Amphiprora spp.**

Cell dimensions:

- 20μm
- 25μm
- 50μm
Results

Investigating *H. akashiwo* and *Alexandrium* Cyst at eelgrass beds in 2013

* Westcott Bay (Seagrass bed recently disappeared)

Estimated by MPN method

<table>
<thead>
<tr>
<th>sampling station</th>
<th>cells/g wet sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Westcott Bay</td>
<td>3400</td>
</tr>
<tr>
<td>Sucia</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Padilla Bay</td>
<td>&lt;200</td>
</tr>
<tr>
<td>North Berlinham Bay</td>
<td>&lt;200</td>
</tr>
<tr>
<td>FHL</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Potlatch</td>
<td>&lt;200</td>
</tr>
</tbody>
</table>

Cyst of *H. akashiwo* detected only from Westcott Bay where eelgrass bed recently disappeared
(3400 cells/g wet sediment)
Summary

- First report on algicidal and growth-inhibiting bacteria associated with seagrass and macroalgae in Puget Sound
- Algicidal and growth-inhibiting bacteria were detected high density from *Z. marina, Z. japonica* and *U. lactuca*
- Bacteria isolated from eelgrass at Padilla Bay showed algicidal or growth-inhibiting activity against all tested harmful algae
- Very few harmful algal species observed in the seawater at eelgrass beds
- *Heterosigma* cyst were only detected from Westcott Bay where eelgrasses recently disappeared
Preservation and restoration of seagrass and macroalage beds to create potential environments for the prevention of harmful algal blooms.
Thank you very much for listening!!

Acknowledgement
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Friday Harbor Labs, UW
Marco Hatch (Northwest Indian College)

Nobuharu Inaba, Hokkaido University
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## Results

### List of phytoplankton

<table>
<thead>
<tr>
<th>Phytoplankton</th>
<th>Padilla Bay</th>
<th>Cattle point</th>
<th>Sucia</th>
<th>FHL</th>
<th>Potlatch</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cerataulina pelagica</em></td>
<td></td>
<td></td>
<td><em>Lauderia</em> spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Leptocylindrus danicus</em></td>
<td></td>
<td></td>
<td></td>
<td><em>Pseudo-nitzschia</em> spp.</td>
<td><em>Bacillaria</em> spp.</td>
</tr>
<tr>
<td><em>Melosira</em> spp.</td>
<td></td>
<td></td>
<td></td>
<td><em>Diploneis</em> spp.</td>
<td><em>Cylindrotheca / Nitzschia</em></td>
</tr>
<tr>
<td><em>Paralia sulcata</em></td>
<td></td>
<td></td>
<td></td>
<td><em>Cymbella</em> spp.</td>
<td><em>Rhizosolenia</em> spp.</td>
</tr>
<tr>
<td><em>Skeltonema</em> spp.</td>
<td></td>
<td></td>
<td></td>
<td><em>Pleurosigma</em> spp.</td>
<td></td>
</tr>
</tbody>
</table>
Puget Sound 2014

・アマモ場海水にH.a人工赤潮添加によるマイクロコズム実験
・Padilla Bay にて経時的な殺藻・増殖阻害細菌、植物プランクトン組成の調査
（＊二者培養試験に用いる細菌数を多くする、アマモ数個体を用い検証）
・NOAAのH.akashiwo有菌株から優占細菌株を分離し、増殖速度を蛍光値で測定
Heterosigma-associated bacteriaの藻類株に対する影響を評価。殺藻細菌や増殖阻害細菌といった細菌との3者培養を通して現場海水中で起きている現象をより現実的に再現
・アマモ場の規模と殺藻細菌の供給量の推定
・殺藻及び増殖阻害細菌の遺伝子解析
・アマモ場の泥（アマモ消失エリアの調査）
・強力な殺藻細菌の分離（詳細な性状解析）

今後
・人工デトライタス添加による殺藻能の検証
・殺藻細菌のクオラムセンシング機構解明（AI-1及びAI-2両方を検証）
Materials and Methods

Cyst detection of *H. akashiwo* and *Alexandrium* by MPN

1. Mixing

2. Suspend into sterile filtered seawater

3. Serial dilutions
   (Modified SWM-3 + GeO₂ 1 mg/L)

4. Incubation
   (14 days, 20°C, 14 h Light:10 h Dark, 50 μmol photons m⁻² s⁻¹)

5. Identification
   (Inverted light microscope)
   Vegetative cells = Positive
   → Count the number of positive wells (each dilution)

6. Calculation MPN cells cm⁻³ wet sediment
   By statistical tables
   (Throndsen, 1978; Itoh and Imai, 1987).
Results (2012)

Algicidal bacteria (AB) and Growth-inhibiting bacteria (GIB) detected from seawater at eelgrass beds and offshore

(Target species: *H. akashiwo* 893)

**Eelgrass Beds**

1. 6/9 Jackles Lagoon
2. 6/18 North Padilla Bay
3. 6/20 Shallow Bay, Sucia
4. 6/22 Barlow Bay
5. 7/2 South Carkeek
6. 7/3 Dumas Bay
7. 7/4 Potlatch Sate Park
8. 7/5 Cornet Bay
9. 7/4 Potlatch Sate Park

**Offshore**

10. 6/11 ES10
11. JPN001
12. 6/22 Offshore

Algicidal bacteria (AB) and Growth-inhibiting bacteria (GIB)

- **AB**
  - 6.3 x 10^2 CFU/mL
  - 7.5 x 10^2 – 4.1 x 10^3 CFU/mL

- **GIB**
  - 4.2 x 10^2 – 2.8 x 10^3 CFU/mL
  - 7.5 x 10^2 – 4.1 x 10^3 CFU/mL
Results

GIB detected from seagrass and macroalgae

(Target species: *K. mikimotoi*)

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/3</td>
<td>Padilla Bay</td>
<td><em>Zostera marina</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Z. japonica</em></td>
</tr>
<tr>
<td>7/8</td>
<td>Cattle Point</td>
<td><em>Z. marina</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>U. lactuca</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Phyllospadix scouleri</em></td>
</tr>
<tr>
<td>7/10</td>
<td>Shallow Bay, Sucia</td>
<td><em>Z. marina</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>U. lactuca</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Enteromorpha spp.</em></td>
</tr>
<tr>
<td>7/12</td>
<td>Pier at FHL</td>
<td><em>Z. marina</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Fucus distichus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Saccharina sessile</em></td>
</tr>
<tr>
<td>7/25</td>
<td>Potlatch state park</td>
<td><em>Z. marina</em></td>
</tr>
</tbody>
</table>

GIB

*Z. marina* 3.8 x 10^7 CFU/ g wt

*Z. japonica* 4.7 x 10^7 CFU/ g wt

Seawater (PAB) 4.3 x 10^3 CFU mL^-1

*U. lactuca* 7.0 x 10^5 CFU/ g wt
Results

GIB detected from seagrass and macroalgae

(Target species: *A. tamarense*)

<table>
<thead>
<tr>
<th>Species</th>
<th>CFU/g or mL</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Z. marina</em></td>
<td>10^8</td>
<td>Killed and lost motility</td>
</tr>
<tr>
<td><em>Z. japonica</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Z. marina</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>U. lactuca</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. scouleri</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Z. marina</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>U. lactuca</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteromorpha spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAB</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Z. marina</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. sessile</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>F. distichus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Z. marina</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GIB detected from seagrass and macroalgae
Material and Methods

Sampled species

<table>
<thead>
<tr>
<th>Seagrass</th>
<th>Green algae</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Z. marina</em></td>
<td><em>U. lactuca</em></td>
</tr>
<tr>
<td><em>Z. japonica</em></td>
<td><em>Enteromorpha sp.</em></td>
</tr>
<tr>
<td><em>P. scouleri</em></td>
<td></td>
</tr>
</tbody>
</table>

Brown algae

| *S. sessile* | *F. distichus* | *N. luetkeana* |