Paralytic shellfish toxins
OUTLINES

• The toxins
• The route of toxins in nature
• Current technology in toxin detection
What we know about

The toxins
What we know...

• The structures and chemistry

(Oshima 1995)
Chemistry of Saxitoxin

- Trialkyl tetrahydropurine
- Can be substituted at various positions, leading to more than 57 naturally occurred STX derivatives (e.g. Oshima, 1995; Lim et al., 2007; Wiese et al., 2010).
- The variable positions:
  - Hydroxylated
  - Sulfated
  - decarbamoylated
- Saxitoxin and its analogs are highly potent neurotoxins.
- (STXs) - bind to the Na⁺ channel at neuron and inhibit channel open.
- Paralytic shellfish poisoning (PSP)
### Specific toxicities of saxitoxin (STX)

<table>
<thead>
<tr>
<th>Toxins</th>
<th>Toxin Specific toxicity (MU/μmol)</th>
<th>Toxicity equivalency factor (TEF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STX</td>
<td>2483</td>
<td>1.000/1</td>
</tr>
<tr>
<td>neoSTX</td>
<td>2295</td>
<td>0.924/1</td>
</tr>
<tr>
<td>GTX1</td>
<td>2468</td>
<td>0.994/1</td>
</tr>
<tr>
<td>GTX2</td>
<td>892</td>
<td>0.359/0.4</td>
</tr>
<tr>
<td>GTX3</td>
<td>1584</td>
<td>0.638/0.6</td>
</tr>
<tr>
<td>GTX4</td>
<td>1803</td>
<td>0.726/0.7</td>
</tr>
<tr>
<td>dcSTX</td>
<td>1274</td>
<td>0.513/1</td>
</tr>
<tr>
<td>GTX5</td>
<td>160</td>
<td>0.064/0.1</td>
</tr>
<tr>
<td>C1</td>
<td>15</td>
<td>0.006/-</td>
</tr>
<tr>
<td>C2</td>
<td>239</td>
<td>0.096/0.1</td>
</tr>
</tbody>
</table>

Oshima (1995)
New STX derivatives

• At least 57 derivatives have been discovered thus far (Wiese et al. 2010).
Saxitoxin Producers

- Organisms that have the ability to produce saxitoxins (STXs):
  - Marine Species, eukaryote (Dinoflagellates)
    - *Alexandrium*
      - *A. minutum* (GTX1/4, 2/3, dcSTX)
      - *A. tamiyavanichii* (GTX1/3, 4/5, C2)
    - *Pyrodinium*
      - *Pyrodinium bahamanse* (STX, neoSTX and GTX5/6)
    - *Gymnodium*
      - *Gymnodinium catenatum* (STX, GTX5/6)
  - Freshwater species, prokaryote (Cyanabacteria)
    - *Anabaena circinalis* (dcSTX, GTX3/ 2/dcGTX3/2, C1/2)
    - *Aphanizomenon* sp. (neoSTX)
    - *Cylidrospormopsis raciborskii* (neoSTX, dcSXT, GTX5)
## The producers

### Western Pacific
- *Pyrodinium bahamense*
- *Alexandrium tamiyavanichii*
- *Alexandrium minutum*
- *Alexandrium tamarense*
- *Alexandrium catenella (A. pacificum?)*
- A. ostenfeldii
- A. taylori
- Gymnodinium catenatum

### Other regions
- *Pyrodinium bahamense*
- *Alexandrium fundyense*
- A. minutum
- A. tamarense
- A. catenella? (Chilean)
- A. ostenfeldii
- A. taylori
- G. catenatum
Toxin profiles and composition of the toxic dinoflagellates

- as a taxonomic or biogeographical marker
- Source of toxins in the contaminated shellfish

Wiese et al. (2010) Marine Drug
Vulnerability of tropical shellfishes against PSP contamination during bloom of *Pyrodinium bahamense var. compressum*

Ulysses M. Montojo, Marc Lawrence J. Romero, Valeriano M. Borja, Miriam F. Cayme, Shigeru Sato, Masaaki Kodama and Yasuwo Fukuyo

Fig. 2. Seasonal toxicity of shellfish (A, B) and the occurrence of *P. bahamense var. compressum* (C, D) in Sorsogon Bay.
• Distinct toxin profiles
• *P. bahamense* –
  — most toxic; GTX1/4 and C toxins absent
• *A. minutum* – GTX5/6 absent
• Toxicity level differs:
  *A. tamiyavanichii>*A. minutum>*A. cf. tamarense*
Comparison of toxin profiles of dinoflagellates and contaminated shellfish

A. minutum

Polymesoda sp.

Distinctive toxin profiles of contaminated shellfish and toxic dinoflagellates ~ predictive value
Toxicity and the mechanism

What we know...

• Molecular target site and pharmacology
• The cause of human illness (symptoms)
Medicinal Use of STXs

Pathmell et al. (2015) Anesthesiology
STX biosynthesis

• Shimizu et al. (1985) first proposed the STX biosynthesis pathway based on feeding experiments with $^{13}$C- and/or $^{15}$N-labeled amino acids and acetic acid.
• Kellman et al. (2008) discovered a putative STX biosynthetic gene cluster in *Cylindrospermopsis*.
• Stüken et al. (2011) first reported the *sxt* genes in two PSTs-producing dinoflagellate strains (*Alexandrium fundyense* and *A. minutum*).
Saxitoxin (sxt) biosynthetic gene cluster

- The sxt gene cluster from cyanobacteria (*Anabaena circinalis*, *Aphanizomenon flos-aquaea* and *Cylindrospermopsis raciborskii*) have been discovered (Kellmann and Neilan, 2007; Kellmann et al. 2008).
sxtA in *Alexandrium* spp.

- sxtA of *Alexandrium* spp. (*A. minutum, A. fundyense, A. catenella, A. tamarense and Gymnodinium catenatum*) has been discovered (Stüken et al., 2011).
sxtG in *Alexandrium* spp.

Recently, sxtG of *Alexandrium* sp. also been reported in both non-toxin and toxin *Alexandrium* spp (Orr et al., 2013).
O-carbamoyltransferase

SxtI (O-Carbamoyltransferase) will transfers a carbamonyl group to the hydroxymenthyl side chain of saxitoxin precursors.

- *AmsxtI* was highly induced only in the P-depleted.
- It was suspected to be involved in the metabolic P-recycling system by increasing or reusing intracellular P.
Recent breakthrough...

- Tsuchiya et al. (2014, 2015, 2016)
- Saxitoxin intermediates were found in both cyanobacteria and dinoflagellates.
- Further strengthen the biosynthetic route.
What we know about

THE ROUTE OF TOXINS IN NATURE
The route of toxins in nature

Toxic plankton → Primary vectors → Non-traditional vectors → Human
PSTs in shellfish

- Quote from Deed et al. (2008)
  [The fate and distribution of STXs in bivalves varies by the environmental conditions; prior history of exposure; species, intrapopulation, and individual variability; uptake dynamics and detoxification mechanisms; anatomical localization and retention; physiological breakdown or biotransformation mechanisms; and differences in initial toxicity of dinoflagellates]
Toxin composition in contaminated shellfish

(Lim et al. 2007)

Changes of toxin compositions in benthic clam, *Polymesoda* sp. after feeding with toxic *A. minutum*.

Total toxin contents in the clams (µg STXeq. /100g tissue) throughout the experiment.
Cell density <100 cells/L for at least 2 weeks

Closure threshold
80 μg STX in 100g meat

Law et al. (in prep)
DETECTION METHODS
Detection tools

- Analytical – HPLC-FLD (pre-column/post-column oxidation), LC-MS/MS
- Bioassay – RBA, ELISA
- Toxin gene detection
Oshima (1995) HPLC-FLD

Post-column derivatization of STXs

(Courtesy of Ogata, T.)
Analysis of paralytic shellfish poisoning toxin congeners by a sodium channel receptor binding assay

Gires Usup*, Chui-Pin Leaw, Mei-Yee Cheah, Asmat Ahmad, Boon-Koon Ng

SPECIAL GUEST EDITOR SECTION

Quantitative ELISA Kit for Paralytic Shellfish Toxins Coupled with Sample Pretreatment

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Toxin gene (sxtA) qPCR assay

Saxitoxins-producing population in the field can be confirmed by the assay.
Gap sto address

• The STX biosynthesis route has been partly discovered, but the complete route remained to be resolved.

• Genetic basis of toxin production in relation to environmental parameters is worthwhile for further investigation.

• Availability of reference materials is always the hindrance in the toxin analysis (issues to prioritize: how to source for toxin materials? How to develop technical knowhow on toxin purification? How to avoid CWC requirement for transfer of materials?)
Gaps to address

- Effectiveness and efficiency of the existing high throughput screening assay need further improvement.
- Cost-effective and public health considerations (e.g.: USD500 for a kit to test shellfish worth <USD50)
- Technology transfer is crucial to reduce the cost
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