Note

Paralytic Shellfish Poison Infestation to Oyster *Crassostrea gigas* Due to Dinoflagellate *Gymnodinium catenatum* in the Amakusa Islands, Kumamoto Prefecture

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At the end of January 1998, wild oysters, *Crassostrea gigas*, in Miyanokawachi Bay, Amakusa Islands, Kumamoto Prefecture were toxified with PSP probably due to *Gymnodinium catenatum*, whose cell density was 308 cells/mL. The toxicity score of the oysters ranged from 3.0 to 263 MU/g of the edible portion. Toxin compositions of the oysters and the dinoflagellate by high performance liquid chromatographic analysis for PSP resembled each other, consisting of C1 (PX1) and C2 (PX2) as the major toxins and, GTX5, GTX6, dcGTX2, dcGTX3 and dcSTX as the minor toxins.

From these results, it was concluded that toxic oysters in Miyanokawachi Bay, Amakusa, Kumamoto had been toxified by *G. catenatum* through the food chain.

Key words: PSP; paralytic shellfish poison; *Gymnodinium catenatum*; oyster; Kumamoto

Introduction

In Kyushu district, following the outbreak of paralytic shellfish poison (PSP) infestation of bivalves in Kamae, Oita Prefecture during 1996 to 1997, wild oysters in Amakusa, Kumamoto, were also toxified, probably due to *Gymnodinium catenatum*, at the end of January, 1998. As described in the previous paper, PSP infestation of bivalves in Kyushu district has recently occurred frequently due to *G. catenatum*, which was first reported in Japan as causative plankton of toxic oysters in Senzaki, Yamaguchi in 1989. In Japan, PSP infestation of bivalves due to *G. catenatum* was exceptional before 1995, while in other countries, such as Australia and Spain, only a few cases have been reported so far. However, in Kyushu district, two areas including Oita and Kumamoto were contaminated with *G. catenatum*, during 1996–98. In the contaminated areas, bivalves had been toxified due to *Alexandrium catenella* in Kamae, Oita in 1988 and due to *Alexandrium* sp. in Amakusa, Kumamoto in 1994. The causative plankton seems to be changing from *Alexandrium* sp. to *G. catenatum*.

In this study, the mechanism of PSP infestation of oysters in Amakusa, Kumamoto at the end of January 1998 was investigated from the viewpoint of the causative plankton as well as the causative agent.

Materials and Methods

Oyster sample

Five specimens of wild oyster *Crassostrea gigas* were collected at Miyanokawachi Bay, Amakusa, Kumamoto Prefecture at the end of January 1998 and immediately transported under cooling to our laboratory at Nagasaki University and kept frozen at −40°C until use.

In order to determine the toxicity score and to analyze the components of the toxin, the oyster samples were treated as follows. The whole body (3–5 g) of the shucked oyster was cut into
small pieces with scissors, mixed with an equal volume of 0.1 mol/L hydrochloric acid and heated in a boiling water bath for 5 min. After centrifugation at 5,000 rpm for 20 min, the supernatant was ultrafiltered through a Diaflo YM-1 membrane (Amicon; cut-off at 1,000 daltons). The filtrate was submitted to both mouse assay and high performance liquid chromatography (HPLC).

**Dinoflagellate sample**

One liter of seawater was collected from the surface of Miyanokawachi Bay on January 28, 1998 and immediately transported under cooling to our laboratory at Nagasaki University. Six hundred mL of seawater was filtered through a No. 1 filter paper (Advantec Toyo) and the residue obtained was extracted by sonication for 0.5 min, three times with 5 mL of 80% ethanol adjusted to pH 3.5 with acetic acid. The extracts were combined and concentrated under reduced pressure to 3 mL, followed by filtration through a 0.45μm cellulose acetate filter, and the filtrate was subjected to HPLC analysis. Cell density was determined by calculating the numbers of the toxic dinoflagellate per mL of seawater using a cell counting chamber. The toxic dinoflagellate was identified from its morphological properties under a microscope.

**Assay for toxicity**

The toxicity was determined by the Japanese official method for PSP, using male mice of ddY strain (18–20 g). One mouse unit (MU) is defined as the amount of toxin, which kills a 20 g mouse in 15 min after intraperitoneal injection.

**HPLC analysis**

Reverse-phase HPLC was performed on a Waters HPLC system. A LiChroCART RP-18 (e) column (φ4×250 mm, Merck) was used in combination with the following two mobile phases at the flow rate of 0.8 mL/min: (1) 2 mmol/L heptanesulfonic acid in 10 mmol/L ammonium phosphate (pH 7.3) for GTXs and (2) 2 mmol/L heptanesulfonic acid in 4% acetonitrile–30 mmol/L ammonium phosphate (pH 7.3) for STXs. Subsequently, the eluate was mixed with 50 mmol/L periodic acid and 0.2 mol/L KOH–1 mol/L ammonium formate–50% formamide at the flow rate of 0.8 mL/min. The intensity of the produced fluorescence was determined by means of a fluorescence detector (Tosoh, FS-8020) with excitation at 336 nm and emission at 392 nm. The concentration of each toxin component in the sample was determined from the relation between the peak area of each component and that of the standard. Identification of N-sulfocarbamoyl toxins (C1, C2, GTX5 and GTX6) was confirmed by their conversion to carbamoyl components (GTX2, 3, STX and neoSTX) by hydrolysis with 0.1 mol/L hydrochloric acid in boiling water for 10 min, followed by HPLC analysis.

**Results and Discussion**

**Toxicity of wild oysters**

The toxicity scores of the wild oysters (edible portion) collected from Miyanokawachi Bay, Amakusa, Kumamoto on January 28, 1998 were 3.0, 59, 106, 238, 240 and 263 MU/g from the result of bioassay for PSP. In 1995, bivalves such as oysters and a scallop *Chlamys nobilis* in this bay were reported to be contaminated with PSP by typical PSP-producing dinoflagellates, *Alexandrium catenella* and *A. tamarense*, differing from the present causative plankton, *G. catenatum*. As the bivalve toxin was not analyzed by HPLC, the toxin profile is uncertain. The dinoflagellate collected from Miyanokawachi Bay was identified as *Gymnodinium catenatum* from its morphological properties under a microscope.

**Toxin compositions of wild oysters and *G. catenatum***

HPLC analyses of the wild oysters and the causative plankton *G. catenatum* are shown in Figs. 1 and 2.
The oyster toxin consists mainly of carbamoyl-N-sulfo components, C1(PX1) (53.7 mol%), C2(PX2) (25.9 mol%), GTX6 (7.1 mol%) and GTX5 (3.8 mol%), and decarbamoyl (dc) components, dcGTX3 (5.6 mol%) and dcGTX2 (2.4 mol%).

The *G. catenatum* toxin consists mainly of carbamoyl-N-sulfo components: C1 (PX1) (25.2 mol%), C2 (PX2) (63.3 mol%), GTX6 (3.3 mol%), and GTX5 (2.5 mol%), and decarbamoyl components: dcGTX3 (3.5 mol%) and dcGTX2 (2.1 mol%).

The oyster toxin pattern is similar to that of *G. catenatum* (Table 1), in respect to the very high content (more than 90 mol%) of carbamoyl-N-sulfo components, although there is a small difference of toxin composition between them. In *G. catenatum*, C2 (PX2) was the main component, but not in the oyster. This result suggested that C2 may have been epimerized to C1 in the oysterootnote{11}.

The *G. catenatum* toxin pattern is similar to that of the same species of plankton collected from Kamae, Oitaootnote{1}, especially as to the abundance (more than 90 mol%) of carbamoyl-N-sulfo components. The oyster toxin profile is also similar to those of bivalves contaminated with *G. catenatum* in Kamae, Oita. The bioconversion of some PSP components is generally recognized in bivalves, being species specific. However, such conversion seems not to occur to any marked extent in fresh bivalves soon after collection.

From these results, it was concluded that the toxic oyster in Miyanokawachi Bay, Amakusa, Kumamoto had been toxified by *G. catenatum* through the food chain.

**Table 1.** Toxin Composition of Wild Oysters and *Gymnodinium catenatum*

<table>
<thead>
<tr>
<th>Component</th>
<th>Oysters</th>
<th><em>G. catenatum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mol%)</td>
<td>(mol%)</td>
</tr>
<tr>
<td>C1 (PX1)</td>
<td>53.7</td>
<td>25.2</td>
</tr>
<tr>
<td>C2 (PX2)</td>
<td>25.9</td>
<td>63.3</td>
</tr>
<tr>
<td>GTX5</td>
<td>3.8</td>
<td>2.5</td>
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<td>3.3</td>
</tr>
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<td>dcGTX2</td>
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<td>2.1</td>
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<td>dcGTX3</td>
<td>2.4</td>
<td>3.5</td>
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<tr>
<td>dcSTX</td>
<td>1.3</td>
<td>tr</td>
</tr>
<tr>
<td>neoSTX</td>
<td>0.3</td>
<td>0</td>
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tr: Trace (<0.1 mol%)
References