Paralytic Shellfish Toxins in the Mussel *Mytilus edulis* and Dinoflagellate *Alexandrium tamarense* from Jinhae Bay, Korea

Jong-Soo LEE · Joong-Kyun JEON* · Myung-Soo HAN**
Yasukatsu OSHIMA*** and Takeshi YASUMOTO***

Department of Fisheries Processing, Tong-Yeong Fisheries Technical College, 650-160 Chungmu, Kyeongnam, Korea

**Korea Ocean Research and Development Institute, P. O. Box 29, 425-600 Ansan, Kyeonggi-do, Korea*

***Department of Biology, College of Natural Sciences, Hanyang University, 133-791 Seoul, Korea*

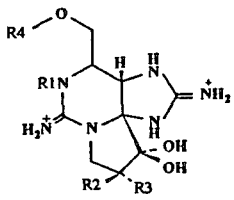
****Faculty of Agriculture, Tohoku University, 1-1, Tsutsumidori, Amamiya, Aoba-ku 981, Sendai, Japan*

Paralytic shellfish toxins in mussels *Mytilus edulis* and dinoflagellate *Alexandrium tamarense* from Jinhae Bay, south coast of Korea were investigated. The mussels collected in March-April, 1989 showed toxicities of 7.5 MU/*g* of whole meat(31~88 MU/*g* of the digestive gland), and those collected in 1990 showed toxicity level of 1.9~9.9 MU/*g* of whole meat by the standard mouse bioassay. Analysis of toxins by high performance liquid chromatography revealed the presence of gonyautoxin 1~4(48~76%), gonyautoxin 8 and *epi*-gonyautoxin 8(C1~C2, 14~39%), saxitoxin(1~10%), neosaxitoxin(1~7%) and trace amount of decarbamoylgonyautoxin 2 and 3(dcGTX2, dcGTX3) in the mussels of 1989. While, Mussels collected in 1990 contained a significantly larger proportion of neosaxitoxin (44~50%) than did those of 1989. *A. tamarense* isolated in April 1989 produced the same toxins in culture with slightly higher proportion of C1, C2, dcGTX2 and dcGTX3 than in the mussels. The difference was within a range of toxin change during accumulation by shellfish and during sample preparation for analysis. It was thus concluded that the dinoflagellate was the cause of toxins in the mussels.

Introduction

Paralytic shellfish poisoning(PSP) is a notorious marine food poisoning occuring worldwide due to the ingestion of toxic shellfish which accumulated neurotoxins of dinoflagellates origins. Three genera of dinoflagellate, *Alexandrium*(*Gonyaulax, Protogonyaulax*) spp., *Gymnodinium catenatum* and *Pyrodinium bahamense var. compressa* are known to produce the paralytic shellfish toxins(Taylor, 1984). As the causative toxins, more than a dozen analogues of saxitoxin(STX, Fig. 1) have been so far identified from several sources such as dinoflagellates and contaminated shellfish(Oshima et al., 1989).

In Korea, the first food poisoning accident involving two death took place in 1986 at Pusan due to ingestion of mussels(Jeon, 1986; Chang et al., 1987). Since then PSP has become not only a potential threat to public health but also a vital problem to shellfish farming industries. There have been a few report on paralytic shellfish toxins in mussels in Korea(Chang et al., 1988a, 1988b; Jeon et al., 1987, 1988, 1989; Arakawa et al., 1989), but none on the causative organism. Recently, Lee(1991) and one of



	R1	R2	R3	R4	Specific toxicity* (MU/μ mole)
saxitoxin(STX)	H	H	H	$CONH_2$	2,045
neosaxitoxin(neoSTX)	OH	H	H	$CONH_2$	1,038
decarbamoylsaxitoxin(dcSTX)	H	H	H	H	1,220
gonyautoxin-1(GTX1)	OH	H	OSO_3^-	$CONH_2$	1,638
gonyautoxin-2(GTX2)	H	H	OSO_3^-	$CONH_2$	793
decarbamoylgonyautoxin-2(dcGTX2)	H	H	OSO_3^-	H	530
gonyautoxin-3(GTX3)	H	OSO_3^-	H	$CONH_2$	2,234
decarbamoylgonyautoxin-3(dcGTX3)	H	OSO_3^-	H	H	990
gonyautoxin-4(GTX4)	OH	OSO_3^-	H	$CONH_2$	673
gonyautoxin-5(GTX5)	H	H	H	$CONHSO_3^-$	350
gonyautoxin-6(GTX6)	OH	H	H	$CONHSO_3^-$	180
C1(gonyautoxin-8, PX1)	H	OSO_3^-	H	$CONHSO_3^-$	430
C2(*epi*-gonyautoxin-8, PX2)	H	H	OSO_3^-	$CONHSO_3^-$	16
C3(PX3)	OH	H	OSO_3^-	$CONHSO_3^-$	18
C4(PX4)	OH	OSO_3^-	H	$CONHSO_3^-$	57

Fig. 1. Structures and specific toxicities of paralytic shellfish toxins(*Oshima et al., 1989).

the author identified *Alexandrium tamarense* in the Jinhae Bay, south coast of Korea as a possible culprit for PSP(Han et al., 1992).

In this study, we wish report the occurrence of toxic mussels in Jinhae bay, and toxin compositions of the mussel as well as *A. tamarense* isolated from the same area determined by means of fluorometric high performance liquid chromatography(HPLC).

Materials and Methods

Materials

Cultured mussels, *Mytilus edulis*, were collected at stations shown in Fig. 2 between March and April in 1989 and 1990, and stored under -20 ℃ until used. Two clonal cultures of *A. tamarense* were established from the motile cells collected at the stations S-1 and B-4 in April 1989. They were cultured for 2 weeks in 20 *l* carboys using slightly modified f-2 medium consist of 30‰ salinity seawater at 20 ℃ under 200 μEm^{-2} sec^{-1} light of 12 hour L:D cycle.

Bioassay

Whole edible parts or the digestive glands of the mussels were adopted for toxicity test according to the standard mouse bioassay(AOAC, 1984). Toxicity was expressed in mouse unit(MU) per gram of the sample, where 1 MU means a toxicity to kill a mouse of 20 *g* weight in 15 min.

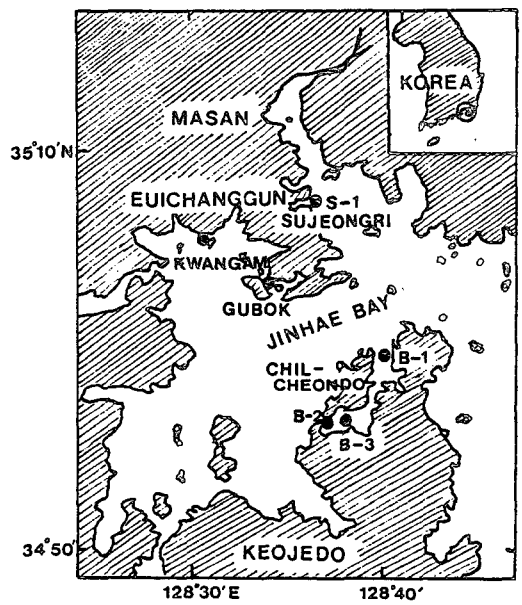


Fig. 2. Sampling stations of mussels and *Alexandrium tamarense*.

HPLC analyses of toxins

Mussel extracts prepared for the mouse bioassay

were passed through a Sep-Pak ODS cartrige column(Waters) which had been washed forehand with 10 *ml* each of methanol and water, and the elute between 1.5~2.0 *ml* was applied to a ultrafiltration kit(Ultrafree GC3C, millipore) 5,000 *g* centrifugation. Cultured *A. tamarense* was collected on a filter paper and lyophilized. The cells were suspended in 0.5 N acetic acid and sonicated for 1 min with Nihonseiki UH-50 sonicator(50 W, 3 *mm* probe). The supernatant obtained by centrifuging the cell suspension at 10,000 *g* was applied to ultrafiltration as mentioned above. Each 10 *μl* of the filtrate was subjected to analysis.

Toxin analysis was carried out by a fluorometric HPLC system with a C-8 bonded silicagel column as reported previously(Oshima et al., 1989), with slightly modification. Three mobile phases were used for different toxin groups. Details of analytical conditions are shown in Table 1. The following abreviations of toxins are used hereafter; STX, saxitoxin, neoSTX, neosaxitoxin; GTX1~6, gonyautoxin 1~6; dcGTX2 and dcGTX3, decarbamoylgonyautoxin 2 and 3.

Table 1. HPLC conditions for analysis of PSP toxins.

Column: Develosil C8-5(4.6×250 *mm*, Nomura Chemical Co.)
Mobile phases: flow rate at 0.8 *ml/min.*
(a) for GTX group;
2 mM sodium 1-heptanesulfonate in 10 mM phosphoric acid adjusted to pH 7.1 with ammonium water
(b) for C1-C4;
1 mM tetrabutylammonium phosphate adjusted to pH 5.8 with acetic acid
(c) for STX group;
(b): acetonitrile(96:4)
Oxidizing reagent: flow rate at 0.4 *ml/min.*
7 mM periodic acid in 50 mM sodium phosphate buffer(pH 9.0)
Reaction: at 65 ℃ in teflon tube(0.5 *mm*, i.d.×10 *m*)
Acidifying reagent: flow rate at 0.4 *ml/min.*
0.5 M acetic acid
Detection: excitation and emission wavelength 330 *nm*, 390 *nm*

Results and Discussions

Toxicities of mussels

Toxicities of the samples are summarised in Table 2. In early spring in 1989 and 1990, mussels all over Jinhae Bay became toxic. The toxicity levels were not so high but most of the samples exceeded 4 MU/*g*, a quarantine limit adopted in many countries. Compared with the whole meat, the digestive glands showed much higher toxicity, as observed in most of shellfish. Although the number of samples tested was small, periodical change of toxicity at Sujeongri(S-1) indicated that the causative dinoflagellate proliferated in mid April resulting in toxicity increased 2 to 9 MU/*g* during April 7 to 19, 1990.

Toxicity levels of cultured *A. tamarense* were varied between 19~132 MU/10^6 cells.

Table 2. Toxicities of mussels collected at Jinhae Bay.

Stations	Date	Toxicity (MU/*g*)
Sujeongri(S-1)*	3/31/1989	35.9
〃 〃	4/07/1990	1.9
〃 〃	4/19/1990	8.8
〃 〃	4/28/1990	6.0
Gubok	4/19/1990	3.3
Kwangam	4/19/1990	9.9
Chilcheondo(B-1)*	3/17/1989	31.0
〃 (B-2)*	3/25/1989	87.6
〃 (B-3)	4/07/1989	7.5
〃 (B-3)	3/30/1990	5.2
〃 (B-3)	4/28/1990	6.6

*: MU/*g* of digestive glands.

Toxin composition of mussels and dinoflagellate

Toxin contents(nanomole/*g*) and toxin composition(mole%) in the digestive glands of mussles are summarized in Table 3. Typical chromatograms are shown in Fig. 3. All the samples showed complicate toxin profiles with significant difference between the mussels collected in 1989 and those of 1990. In 1989 samples, major toxins were GTX1~GTX4, comprising 48~76%, of total toxin. A slightly large proportion of C1 and C2(14~39%), small amounts of STX, neoSTX and dcGTX, and absence of GTX5, GTX6, dcSTX, C3 and C4 were characteristic in all the samples. Comparison between two pairs of the interconvertible epimers of 11-hydroxysulfate(GTX 1 and GTX4 against GTX2 and GTX3) indicated a higher proportion of combined GTX1 and GTX4 and lower proportion of combined GTX2 and GTX3 when the shellfish toxicity was high. This toxin profile might reflect conversion from GTX1/4 to GTX2/3 or selective degradation of GTX1/4 during depletion of toxins in the mussels as observed previously(Oshima et al., 1989). In contrast to the 1989 samples, samples collected in 1990 showed completely different toxin profiles in which neoSTX comprised nearly half of total toxins.

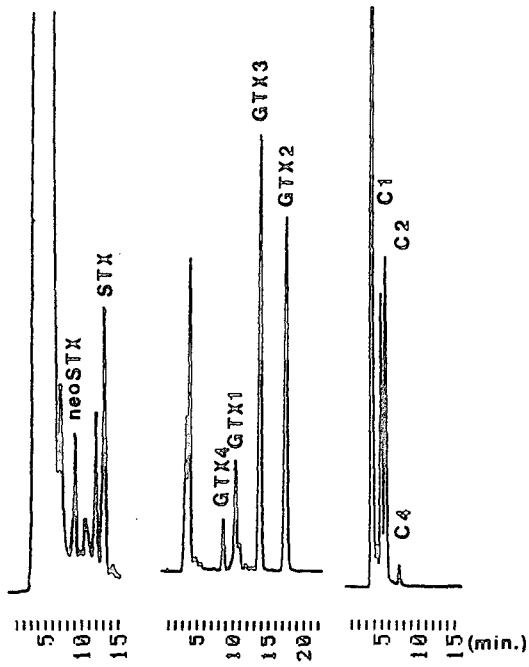


Fig. 3. Typical fluorometric HPLC chromatograms of mussel extracts collected at B-3 (4/7/1989).

Two isolated of *A. tamarense* from Jinhae Bay were similar to each other in the toxin profile(C1

Table 3. Toxin compositions of mussels collected at Jinhae Bay*.

Station Date	S-1 1989 Mar. 31		B-1 1989 Mar. 17		B-2 1989 Mar. 25		B-3 1989 Apr. 7		S-1 1990 Apr. 7		B-3 1990 Mar. 30	
STX	1.10	(3.3)	0.24	(1.3)	0.67	(4.4)	0.15	(9.7)	0.06	(1.5)	0.06	(2.4)
neoSTX	2.36	(7.0)	0.27	(1.4)	0.81	(5.3)	0.05	(3.2)	1.77	(43.6)	1.25	(50.2)
dcSTX	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-
GTX1	15.46	(45.7)	5.79	(30.4)	0.80	(5.2)	0.05	(3.2)	0.31	(7.6)	0.05	(2.0)
GTX2	3.62	(10.7)	2.88	(15.1)	3.56	(23.3)	0.56	(36.1)	0.64	(15.8)	0.22	(8.8)
GTX3	0.70	(2.1)	1.04	(5.5)	1.07	(7.0)	0.09	(5.8)	0.15	(3.7)	0.09	(3.6)
GTX4	5.92	(17.5)	3.20	(16.8)	4.01	(26.3)	0.04	(2.6)	0.57	(14.0)	0.27	(10.8)
GTX5	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-
GTX6	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-
dcGTX2	0.04	(0.1)	0.03	(0.2)	0.04	(0.3)	ND	-	0.02	(0.5)	ND	-
dcGTX3	0.03	(0.1)	0.03	(0.2)	0.02	(0.1)	ND	-	0.02	(0.5)	0.03	(1.2)
C1	3.82	(11.3)	3.79	(19.9)	2.92	(19.1)	0.52	(33.5)	0.43	(10.6)	0.43	(17.3)
C2	0.78	(2.3)	1.79	(9.4)	1.37	(9.0)	0.09	(5.8)	0.09	(2.2)	0.09	(3.6)
C3	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-
C4	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-
Total	33.83		19.06		15.27		1.55		4.06		2.49	

* Unit: nanomole/*g*. Numbers in parentheses indicate relative abundance in mole%.
ND: not detected.

/C2 44%; GTX1/4 31~33%; GTX2/3 9~10%, dcGTX2/3 12~14%) as shown in Table 4. A high proportion of dcGTX2 and dcGTX3 might have resulted from the degradation of C1 and C2 during at high pH(Oshima et al., in preparation). The dinoflagellate produces a high proportion of C1 and C2 in culture than in nature(Oshima et al., 1989). Hence the toxin profile of *A. tamarense* was reasonably said to be similar to those of contaminated mussels and thus support that the dinoflagellate was actually the causative organism in the bay. The high contents of neoSTX in the 1990 mussels suggested that the causative organism belonged to the different strain from the one isolated in 1989. The diversity of toxin profiles among species or strains in *A. tamarense* and *A. catenella* have been well established in North America(Cembella et al., 1987) and in Japan(Oshima et al., 1989, 1990).

Arakawa et al.(1989) reported almost the same toxin components(GTX1~4, C1, C2, STX and neoSTX) in the mussels related to human poisoning at Pusan in 1986, though their analysis was quantitative.

Table 4. Toxin compositions in *Alexandrium tamarense* from Jinhae Bay*.

Station	S-1		B-3	
Date	April	1989	April	1989
STX	0.02	(0.3)	0.02	(0.5)
neoSTX	0.09	(1.3)	0.08	(2.2)
dcSTX	ND	-	ND	-
GTX1	1.26	(17.7)	0.75	(20.3)
GTX2	0.53	(7.5)	0.26	(7.0)
GTX3	0.15	(2.1)	0.07	(1.9)
GTX4	0.96	(13.5)	0.46	(12.5)
GTX5	ND	-	ND	-
GTX6	ND	-	ND	-
dcGTX2	0.82	(11.5)	0.36	(9.8)
dcGTX3	0.16	(2.3)	0.07	(1.9)
C1	2.50	(35.2)	1.29	(35.0)
C2	0.61	(8.6)	0.33	(8.9)
C3	ND	-	ND	-
C4	ND	-	ND	-
Total	7.10		3.69	

* Unit: $\times 10^{-4}$ picomole/cell. Numbers in parentheses indicate mole%.
ND: not detected.

In this paper, occurrence of toxigenic *A. tamarense* in Jinhae Bay was confirmed and toxin profiles of them and those of mussels were first clarified by analysis of cultured cells and mussels. Mussels are a favorite shellfish next to osyter in Korea and are consumed as cooked or dried and sometimes as raw additives to pickles. Although mussels are widely cultured for both domestic use and for export, little attention has been paid to the possible contamination of shellfish by paralytic shellfish toxins. Dissemination of information about the potential risks seems to be urgent to prevent another outbreak of poisoning. In North America, Europe and Japan, the monitoring programs on shellfish toxicity have been conducted in shellfish producing areas to prevent harvesting and marketing of shellfish exceeding a quarantine limit(4 MU/*g* or 80 *μg* STX equivalent /100 *g*). From the view point of public health and the development of marine culture industries, it is urgent to establish a monitoring program and impose a regulation to secure the safety of shellfish.

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진해만산 진주담치, *Mytilus edulis* 및 와편모조, *Alexandrium tamarense*의 마비성패독

이종수 · 전중균* · 한명수** ·
Yasukatsu OSHIMA*** and Takeshi YASUMOTO***
통영수산전문대학 수산가공과 · *한국해양연구소 응용생물연구실
**한양대학교 생물학과 · ***日本 東北大学 농학부

1989년 및 1990년도 진해만산 진주담치(*Mytilus edulis*)와 1989년도산 유독와편모조(*Alexandrium tamarense*) 배양 조체의 마비성패독 성분의 조성과 독성을 mouse assay법과 post column을 이용한 형광고속액체 chromatography법으로 조사하였다. mouse assay법으로 조사한 진주담치의 독성은 1989년산(3~4월)이 중장선 1g당 31~88 MU, 1990년도산이 가식부 1g당 1.9~9.9 MU이었다. 독조성은 1989년도산의 경우, gonyautoxin1-4(48~76%), C1~C2(14~39%), saxitoxin(1~10%), neosaxitoxin(1~7%) 그리고 미량의 decarbamoyl gonyautoxin 2,3가 포함되어 있었으며, 1990년도의 경우는 neosaxitoxin의 함량이 44~50%의 비율을 나타내어 년도에 따라 큰 독조성의 차이를 나타내었다. 한편, *A. tamarense*의 배양조체의 독조성은 1989년도산 진주담치와 유사하였으나, C1, C2, decarbamoyl gonyautoxin 2,3의 비율이 다소 높았다.