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Occurrence of Toxic *Alexandrium* and Intoxification of Two Mollusk Species by Paralytic Shellfish Poisoning Toxins on the Southeastern Coast of Korea

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We analyzed the paralytic shellfish poisoning (PSP) toxins of the toxic marine dinoflagellate *Alexandrium tamarense* collected from Dadaepo and Gaduck-do in Busan and from Sujeong-ri in Jinhae Bay, Korea, in April 2003. We also analyzed the PSP toxin of mussels (*Mytilus galloprovincialis*) and oysters (*Crassostrea gigas*) collected around Busan and Jinhae Bay. PSP toxin analyses were conducted by high performance liquid chromatography (HPLC). Fifteen cultured *A. tamarense* isolates contained 2.78 to 57.47 fmol/cell, with nearly identical toxin profiles: major components C2, GTX4; minor components C1, GTX1, NEO; and trace components GTX2, GTX3, STX. PSP toxin contents were 0 to 492 μg STXeq/100 g in mussels and 0 to 48 μg STXeq/100 g in oysters. Mussels at Gijang and Sujeong-ri contained the most PSP toxin contents (492 μg STXeq/100 g and 252 μg STXeq/100 g, respectively), exceeding the quarantine level (80 μg STXeq/100 g). Their dominant toxin components were C2, C1, GTX2, and GTX3; the minor components GTX1, GTX4, GTX5, and NEO were sporadically detected. Phytoplankton contained 0.774 fmol/L seawater and 1.228 fmol/L seawater at Gijang and Sujeong-ri in April. At that time, *Alexandrium* cells were present in the water column at Gijang at 2,577 cells/mL and at Sujeong-ri at 6,750 cells/mL. Overall, we found the high and similar PSP toxin contents in *Alexandrium* isolates and mussels, and a correlation between occurrence of toxic *Alexandrium* cells in the water column and mussel intoxification. High densities of toxic *Alexandrium* cells in the water column immediately preceded shellfish intoxification at Gijang and Sujeong-ri in April.

Key words: *Alexandrium*, intoxification, mollusk, paralytic shellfish poisoning (PSP) toxins

Introduction

Two paralytic shellfish poisoning (PSP) accidents resulting in human deaths were reported from Busan in 1986 and Geoje-do in 1996 on the southeastern coast of Korea (Chang et al., 1987; Jeon et al., 1988; Lee et al., 1997). Chang et al. (1988) first mentioned the correlation between the occurrence of *Alexandrium tamarense* (as *Protogonyaulax tamarensis*) and shellfish intoxification. PSP toxin production by *A. tamarense* and *Gymnodinium catenatum* have been demonstrated by several research groups (Han et al., 1992; Kim and Lee, 1996; Park et al., 2004; Kim et al., 2005). *Alexandrium tamarense* forms vernal blooms on the southeastern coast of Korea (Chang et al., 1988; Han et al., 1992; Kim, 1995; Yoo et al., 2000) and is thought to cause shellfish intoxification.

A number of studies have been performed over the last two decade to verify the PSP toxin contents and toxin profiles of commercial shellfishes (Chang et al., 1988; Jeon et al., 1988; Lee et al., 1997; Jeon and Han, 1998; NFRDI, 2002) and the PSP causative dinoflagellates (Han et al., 1992, 1993; Kim, 1995; Kim and Lee, 1996; Park et al., 2004; Kim et al., 2005). However, few studies have compared PSP toxin profiles between PSP causative microorganisms and intoxified mollusks (Lee et al., 1992). In this study, we assessed the presence of the causative microorganism and its toxin profile, and determined the correlation between these two factors and shellfish intoxification.

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Materials and Methods

Mollusks

Natural and cultured mussels (*Mytilus galloprovincialis*) and oysters (*Crassostrea gigas*) were sampled along the southeastern coast of Korea in April, May, September and October 2003 (Fig. 1). After transporting the samples to the laboratory, the edible part of the shellfishes were immediately dissected, treated with 0.1 N hydrochloride, and preserved at -20°C until analysis.

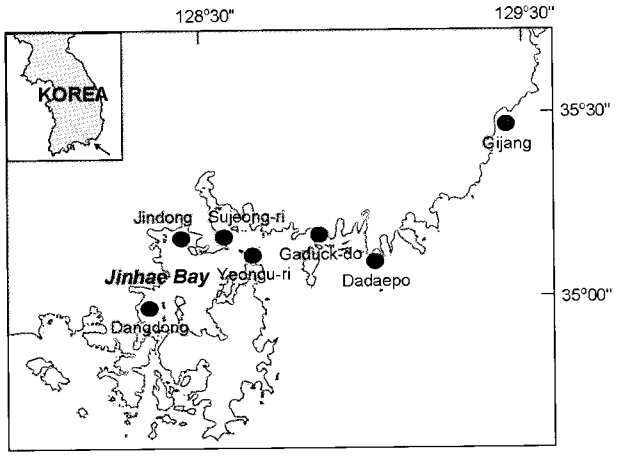

Fig 1. Sampling sites of *Alexandrium tamarense* cells, phytoplankton concentrates and mollusks.

Phytoplankton

Alexandrium tamarense cells were sampled and their abundance was measured along the southeastern coast of Korea in April, May, September and October 2003 (Fig. 1). Individual *A. tamarense* cells were isolated from net-haul (20 μm mesh) samples from Dadaepo, Gaduckdo and Sujeong-ri in April 2003 using a micropipette under a stereomicroscope (Olympus SZ40, Japan). These cells were inoculated in f/2 medium (Guillard and Lyther, 1962) and grown at 17°C under a 14:10 light:dark cycle with a light intensity of 50 $\mu E/m^2/s$ in a 300 mL conical flask.

Phytoplankton for concentration measurements were collected in 5 L of surface seawater at each sampling station. The water was filtered with a 20 μm sieve (Mueller gauze); the recovered cells were put into a 50 mL centrifuge tube and centrifuged (2,000 ×*g* for 5 min). These pelleted cells were stored at -20°C until analysis. Cell abundance in the water column was counted using a Sedgwick-Rafter chamber in triplicate.

Alexandrium isolates were identified by using a species-specific DNA probe (Kim, 2005) with a fluorescent microscope (Carl Zeiss, Axiovert 200, fluorescein isothiocyanate [FITC]: Ex, 490 nm. Em, 525 nm).

PSP toxin analysis

Alexandrium tamarense cultures ($1.5\text{-}2.5\times10^5$ cells) were harvested in the mid-exponential growth phase by brief centrifugation. The pelleted isolates and phytoplankton concentrates were washed with distilled water, suspended in 1 mL of 0.05 N acetic acid, and disrupted with mild ultrasonication (Daigger Ultrasonic Processor GE750, Japan) while on ice. The extracts were filtered through a membrane filter (cut-off, 10,000 molecular weight; Ultrafree C3GC, Millipore) by centrifugation (8,000×*g* for 5 min) to remove cell debris and were then stored at -20°C until analysis. The frozen mollusks were thawed at room temperature and disrupted by sonication while on ice. The supernatant was centrifuged (3,000×*g* for 10 min) and passed through a Sep-Pak ODS cartridge column (Waters, USA). The filtrate was filtered through a membrane filter as described above.

Filtrate from each sample was analyzed by HPLC and a fluorometric analyzer (Hewlett Packard 1100 System, USA) using the three-step isocratic elution method of Oshima (1995). A Wakosil 5C8 column (4.6×250 mm, Wako Pure Chemical Industry, Japan) or a Zorbax Eclipse XDB-C8 (4.6×250 mm, Agilent Technology, USA) was used to separate each toxins. PSP toxin contents were measured by comparing the peak area of each toxin to that of the standard. The standard toxins used in this study (STX, NEO, GTX1-5, C1-4, dcSTX, dcGTX2-3) were kindly provided by Professor Y. Oshima of Tohoku University, Japan.

Results and Discussion

Alexandrium occurrence and PSP toxin profile

Fifteen isolates from Dadaepo, Gaduck-do and Sujeong-ri were identified as *A. tamarense* by DNA hybridization cdata not shown). All were toxic, with PSP toxin contents from 2.78 to 57.47 fmol/cell (Table 1). In general, their major components were C2 and GTX4, their minor components were C1, GTX1 and NEO, and their trace components were GTX2, GTX3 and STX (Fig. 2). This PSP toxin profile was identi-cal to those reported previously for samples from the southeastern coast of Korea (Han et al., 1992, 1993; Kim, 1995; Kim and Lee, 1996; Kim et al., 2005). The PSP toxin compositions of *Alexandrium* spp. are known to be stable over a broad range of physico-chemical conditions under homologous growth con-ditions; they are therefore

Table 1. The paralytic shellfish poisoning (PSP) toxin profiles of *Alexandrium tamarense* isolates established in this study in April 2003

Strain code[1]	Toxin content (fmol/cell)										
	Carbamate						N-sulfocarbamoyl			dc	Total toxin
	STX[2]	NEO	GTX1	GTX2	GTX3	GTX4	GTX5	C1	C2	GTX3	
DDPW0304	-	0.83	0.87	-	tr	4.82	-	5.66	11.08	tr	23.27
GDDW0304-1	0.10	0.29	0.05	0.04	2.29	0.93	1.57	4.13	33.46	-	42.86
GDDW0304-2	-	0.41	0.77	0.11	2.37	10.43	-	2.14	31.82	tr	48.06
GDDW0304-4	-	0.05	0.54	-	0.05	3.50	-	0.62	11.06	-	15.82
GDDW0304-5	-	tr	0.25	-	0.09	0.91	-	0.67	6.63	-	8.58
GDDW0304-7	-	0.10	0.38	-	tr	1.72	-	0.34	3.04	-	5.60
GDDW0304-8	0.10	1.30	0.22	tr	1.50	13.24	-	1.18	27.33	-	44.77
GDDW0304-9	-	1.81	3.35	0.10	0.52	11.88	-	5.23	34.58	tr	57.47
SJW0304-1	-	0.96	1.63	0.42	3.01	9.54	-	3.38	26.13	tr	45.41
SJW0304-2	-	-	1.50	-	tr	3.20	-	0.88	2.69	-	8.31
SJW0304-3	-	0.06	0.88	-	tr	2.26	-	0.79	2.60	-	6.62
SJW0304-4	-	0.30	0.24	0.01	0.05	0.52	-	1.21	2.10	-	4.45
SJW0304-5	-	0.49	0.15	0.01	0.07	5.27	-	3.70	4.97	tr	14.67
SJW0304-11	-	0.06	1.58	-	-	2.93	-	3.56	18.10	-	26.97
SJW0304-12	-	tr	0.43	-	tr	0.36	-	0.89	1.08	-	2.78

[1]DDP, Dadaepo; GDD, Gaduckkdo; SJ, Sujeong-ri
[2]STX, saxitoxin; NEO, neosaxitoxin; GTX, gonyautoxin; dcGTX, decarbamoyl gonyautoxin
[3]tr, trace (<0.05 fmol/cell); -, not detected.

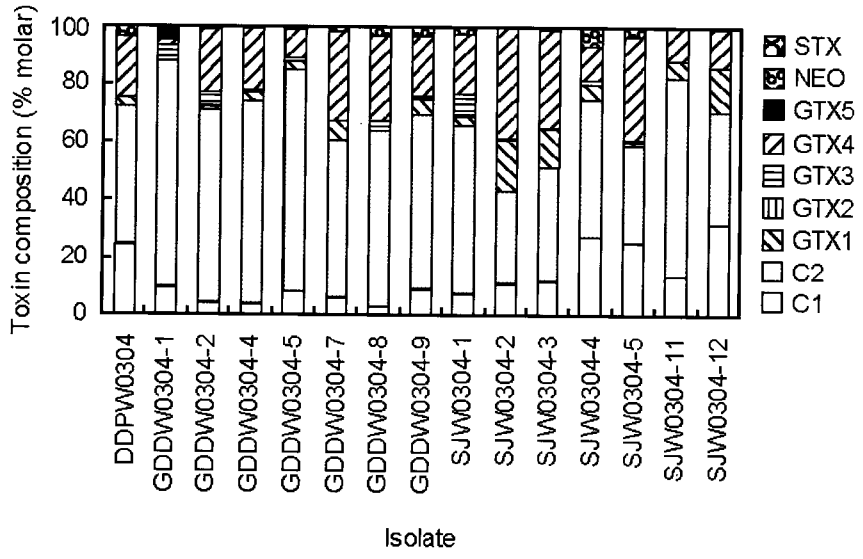

Fig. 2. The paralytic shellfish poisoning (PSP) toxin contents of *Alexandrium tamarense* isolates collected in April 2003.

used as biochemical indicators of regional populations (Oshima et al., 1982; Cembella et al., 1987; Kim et al., 1993; Anderson et al., 1994). Changes in toxin compositions over the life cycle or under varying physiological conditions been recently documented (Boczar et al., 1988; Anderson et al., 1990; MacIntyre et al., 1997). Thus, the identical PSP toxin profile among *A. tamarense* isolates in our study and among many isolates in previous studies indicates that the environ-mental regime affecting *Alexandrium* toxin produc-tion has not changed greatly over the last two decade.

The PSP toxin contents of phytoplankton concentrates collected in April 2003 were 0.024 to 1.228 fmol/L seawater (Table 2), but the toxin contents of those collected on the other sampling date were below 0.024 fmol/L (data not shown). The toxin profiles were C2 and GTX4 as major components, and C1, GTX1 and GTX3 as minor components. These PSP toxin compositions were similar to those

Table 2. Abundance and the paralytic shellfish poisoning (PSP) toxin profiles of *Alexandrium* spp. collected in April 2003

Sampling site	Abundance (cells/mL)	Toxin composition (fmol/L seawater)[1]					Toxin content
		GTX1	GTX3	GTX4	C1	C2	
Dadaepo	100	-		0.024	-	-	0.024
Gaduck-do	15	0.003	0.002	0.106	0.035	0.087	0.230
Gijang	6,750	-	0.021	0.374	0.004	0.828	1.228
Dangdong	-	-	-	0.024	-	-	0.024
Jindong	133	-	tr	0.045	0.009	0.004	0.058
Sujeong-ri	2,327	-	0.048	0.583	0.023	0.118	0.774
Yeongu-ri	311	-	-	-	-	0.066	0.066

[1] tr, trace; -, not detected

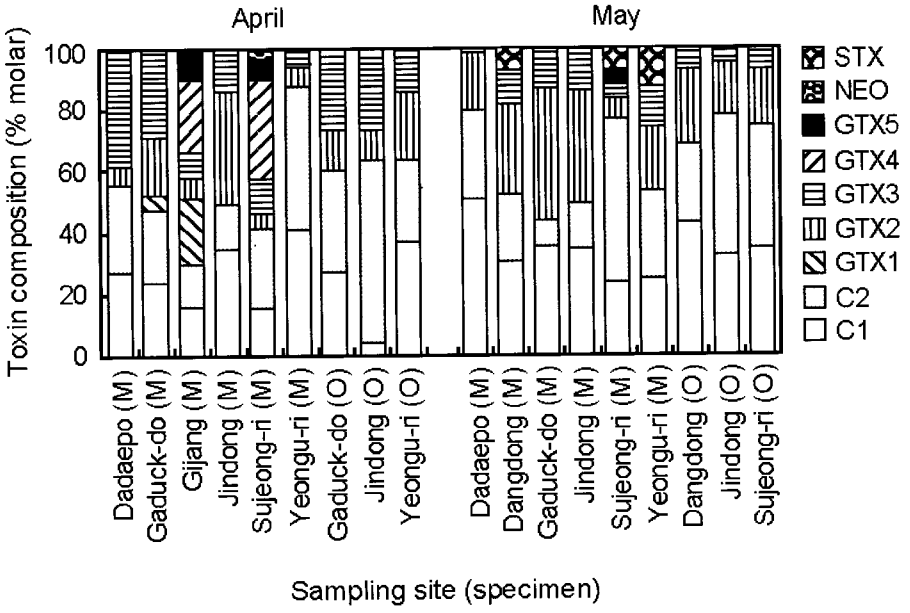

Fig. 3. The paralytic shellfish poisoning (PSP) toxin contents of two mollusk species collected in April and May 2003 (M, mussel; O, oyster).

of the *A. tamarense* isolates except for the absence of minor and trace components (e.g., GTX2 and NEO; Fig. 2). This simpler toxin composition probably resulted from the diversity of the phytoplankton assemblage.

Alexandrium spp. occurred in the water column at 6,750 cells/mL at Gijang and 2,327 cells/mL at Sujeong-ri in April (Table 2), but on the other sampling date and at the other sites, the abundance was below 100 cells/mL (data not shown). The higher cell densities of *Alexandrium* spp. coincided with the higher toxicity of the mussels (Fig. 4).

The PSP toxin profiles of mussels and oysters

Mussels (*Mytilus galloprovincialis*) collected at Gijang and Sujeong-ri in April 2003 contained the highest PSP toxin levels we recorded; 492 μg STXeq/100 g and 252 μg STXeq/100 g, respectively, exceeding the quarantine level (80 μg STXeq/100 g; Table 3). The PSP toxin contents of other mussels and oysters (*Crassostrea gigas*) in April, May, September and October were below the quarantine level. The highest levels of toxin content in mussels, measured in the spring, were consistent with previous reports from the southeastern coast of Korea (Chang et al., 1988; Lee et al., 1992, 1997; Kim and Lee, 1996; Kim et al., 2005). These levels have resulted in a prohibition on shellfish harvesting in spring (NFRDI, 2002). On the same sampling dates at the same sites, mussels had much higher toxin contents than oysters (Table 3). This difference probably related to the higher filtration rates, uptake rates and detoxification rates of mussels compared to oysters (Bricelj and Shumway, 1998). The different proportions of toxin compositions in mussels between

Table 3. The paralytic shellfish poisoning (PSP) toxin contents of mussels and oysters determined by HPLC

Sampling site	Organism	Toxicity (μg STXeq/100 g)[1]				Remark
		Apr	May	Sep	Oct	
Dadaepo	mussel	4	2	nd	nd	wild
	oyster	-	-	nd	nd	wild
Gaduck-do	mussel	70	76	nd	nd	cultured
	oyster	48	-	nd	nd	cultured
Gijang	mussel	492	-	-	-	wild
Dangdong	mussel	tr	28	nd	tr	cultured
	oyster	nd	4	nd	tr	wild
Jindong	mussel	26	62	nd	tr	cultured
	oyster	12	4	nd	nd	cultured
Sujeong-ri	mussel	252	26	nd	tr	cultured
	oyster	-	4	-	-	wild
Yeongu-ri	mussel	2	18	nd	tr	wild
	oyster	4	-	-	-	cultured

[1]tr, trace; nd, not detected; -, not analyzed

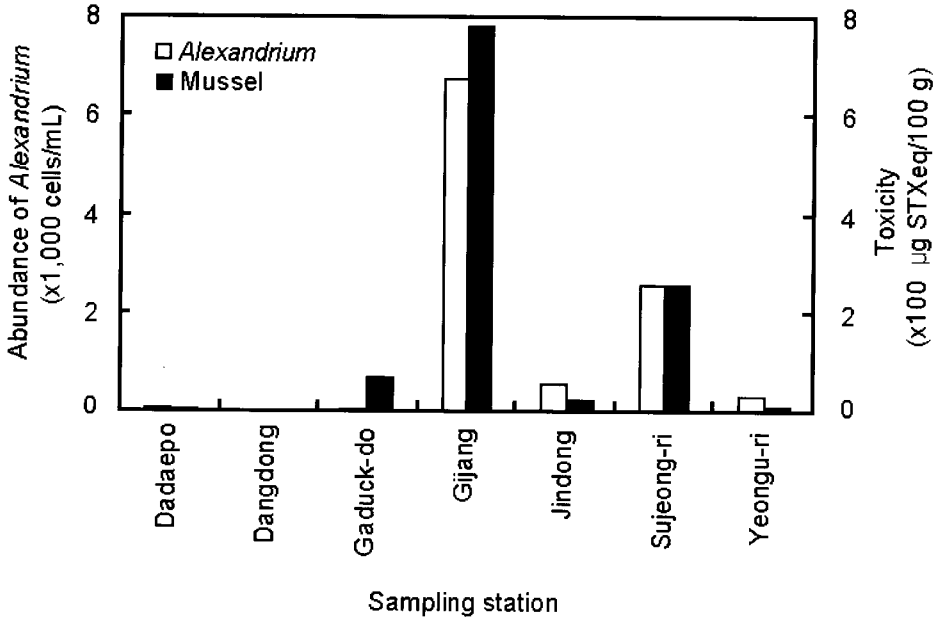

Fig. 4. Abundance of *Alexandrium* cells and the paralytic shellfish poisoning (PSP) toxin contents of mussels collected in April 2003.

the April and May samples suggest that toxin conversion depends on detoxification kinetics within their tissue (Sullivan et al., 1983; Bricelj and Shumway, 1998).

The toxin profiles of C1, C2, GTX1-5, STX and NEO in mussels and oysters (Fig. 3) were similar to those in previous reports (Lee et al., 1992, 1997). This similarity suggests that intoxification of these two mollusk species has been induced by the same regional population of toxic *A. tamarense* at least since 1992. Although toxic *A. catenella* (Kim et al., 2005) and *Gymnodinium catenatum* (Park et al., 2004) have also been reported on the southeastern coast of Korea, the former was a summer species (Kim et al., 2002) and the latter has a distinct toxin composition that of *A. tamarense* (Park et al., 2004). Therefore, these toxic species may have no relation to the spring PSP event in the study area.

The similar PSP toxin compositions of *Alexandrium* cells and the two mollusks (Fig. 2) is likely due to intact *Alexandrium* cells in the guts of the mollusks (Bricelj et al., 1990; Bricelj and Shumway, 1998). Despite their overall decreasing toxicity during the study period, some mussels sampled in May contained STX (Fig. 3). Mollusks usually transform toxins when they ingest toxic dinoflagellates con-

taining high proportions of *N*-sulfocarbamoyl toxins; these toxins are more labile than carbamoyl toxins (Sullivan et al., 1983; Cembella et al., 1987; Lee et al., 1992; Bricelj and Shumway, 1998). Therefore, *A. tamarense* may convert *N*-sulfocarbamoyl toxins such as C1 and C2 to carbamate toxins such as STX.

In conclusion, we found that (1) the occurrence of toxic *A. tamarense* in the water column readily induces PSP intoxification in commercial mollusks and (2) the annual PSP episode on the southeastern coast of Korea is caused by the same toxic regional population.

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