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Paralytic Shellfish Toxin Profiles of the Dinoflagellate *Alexandrium* Species Isolated from Benthic Cysts in Jinhae Bay, Korea

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On the outbreak of paralytic shellfish poisoning in April 1993 in most of shellfish harvesting areas in Jinhae Bay, Korea, to clarify the toxin production of causative organism *Alexandrium* species, 19 axenic clonal isolates established from the benthic resting cysts in three different stations of those culture grounds were subjected to PSP toxin analysis by HPLC. Individual toxin content per cell was highly variable among the strains isolated from a sampling area and originated from an individual cyst. Average toxin contents in those areas revealed higher values of 54~70 fmol/cell. Toxin profiles included C1/C2 (epiGTX8/GTX8), GTX1/GTX4 and neoSTX as the major components, and GTX2/GTX3, GTX5, C4, dcSTX and STX as the minor or sporadic ones. neoSTX on the dominant toxins showed not only most diverse compositional changes comprising 5~54 mol% ranges but also no detection on the half of the strains examined, which were implicated in arising of heterogeneity with a genetic trait within a geographical region. When average toxin composition was compared, carbamate toxins comprised large proportions of 57%, 54% and 67% as total toxin in St. 1, St. 2 and St. 4, respectively. These results suggested that an extensive paralytic shellfish toxification in Jinhae Bay could be largely due to the production of highly potent carbamate toxins in the causative dinoflagellate *Alexandrium* species.

Key words : paralytic shellfish poisoning (PSP), Jinhae Bay, toxin production, *Alexandrium* species, resting cysts, HPLC, toxin profiles, carbamate toxins

Introduction

Paralytic shellfish poison (PSP) is one of the most serious problems in seafood poisoning. Since shellfish toxicity was found to be related to the blooms of *Alexandrium catenella* by Sommer and Meyer (1937), an array of chemically similar neurotoxins that differ in toxicity has been confirmed within several species of *Alexandrium* (Shimizu, 1987). These toxic algal blooms as well as the related regional toxic impacts have been increasing in frequency and intensity worldwide over the past 20 years (Sundstrom et al., 1990).

In Korea, the first PSP outbreak was reported in 1986 at Pusan. People who ate contaminated mussels (*Mytilus edulis*) showed fatal symptoms to result in two deaths (Chang et al., 1987). Since then, several works have been done to determine the causative organisms and shellfish toxification along the southeastern coast of Korea (Chang et al., 1987, 1988; Jeon et al., 1987, 1988; Lee et al., 1992; Han et al., 1993). Through these studies PSP toxins have been detected mainly in the wild and cultured mussels collected in the early spring, but they did not show so much high toxicity levels. In April 1993, an extensive PSP intoxification occurred in most of shellfish harvesting areas not only in Jinhae Bay but also in the western coast of Pusan, where toxicity of mussels exceeded up to ten times higher than regulatory concentration (80 μg/100 g). As a result the harvesting of the mussels were temporarily banned by the government. Based on the monitoring data on the outbreak of PSP, Kim (1995) suggested that *Alexandrium* spp. caused the regional shellfish toxification in the early spring sea-

son in Korea.

The main purpose of the present study is to clarify the toxin production from the germinants of the resting cysts in three areas of Jinhae Bay, and to compare their toxin content and profiles.

Materials and Methods

Organisms and Culture

Sediment samples were collected from three stations (St. 1, Sujeongri, Gusan; St. 2, Ukgokri, Jindong; St. 4, Taegokri, Hacheong) near the mussels culture grounds in Jinhae Bay from September 1993 to January 1994 (Fig. 1). *Alexandrium* cysts were individually isolated from the upper sediment (0~6cm) (Kim, 1995) and germinated in SWII medium (Iwasaki, 1961) at 18℃. The germinant cells through the first to third cell division were individually separated and their axenic clones were prepared by the micropipette washing method. All strains were isolated from the germinants of 13 cysts, of which 10 germinants were confirmed from the four cysts of C7, E9, A2 and G6 (Table 1). Cultures were examined for the presence of bacteria, using a microscope, liquid cultures [ST10^0 medium (Ishida et al., 1986)], and agar plates (1.5% of Bacto-agar in ST10^0 medium). All isolates were maintained and grown under 16L : 8D photocycle, provided by cool white bulbs (ca. 100 μE m^{-2} S^{-1}) at 18℃ in SWIIm medium (Kim et al., 1995). When the cultures reached the mid-exponential growth phase, approximately 10^6 cells were inoculated in 2L of SWIIm contained in a 3L flask. Cells (ca. 2×10^5) for toxin analyses were harvested at the late-point of the light cycle at the mid-exponential growth phase .

Toxin Analysis

The sampled cells were concentrated by centrifugation (2,000 g×5 min) at 18℃. They were rinsed once with cold distilled water and recentrifuged. The pelleted cells were resuspended in 0.05N acetic acid and were stored at -20℃ until extracted. The frozen samples were thawed and disrupted by mild sonication (10 KC, 2 min). After the sonication the cell debris was removed by centrifugation (10,000 g×10 min). The supernatant was filtered through a membrane filter (10,000 MW cut-off) (Ultrafree C3GC, Millipore) by centrifugation (8,000 g×5 min), and stored at -20 ℃ until HPLC analysis was conducted. Toxin analysis was carried out as described by Oshima et al. (1989) by means of a HPLC-fluorometric PSP toxin analyser (Waters 600E system, 470 fluoromonitor) on a Wakosil 5C8 column (4.6×250 mm; Wako Pure Chemical).

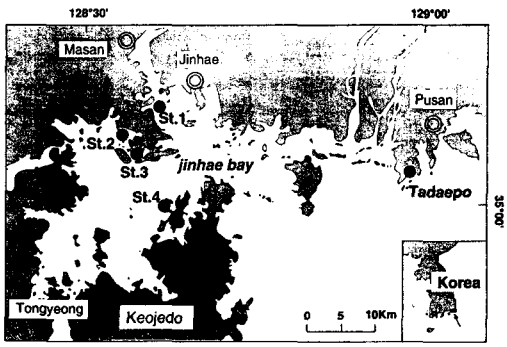

Fig. 1. Sampling stations of the benthic resting cysts of *Alexandrium* spp. in Jinhae Bay, Korea.

Results and Discussion

Table 1 shows a list and origin of *Alexandrium* isolates examined. Considering of the more important aspects of toxin variability both between the isolates of the same species (Anderson, 1990) and in the filial generation cells (Kim et al., 1993a, b), the clone cells were certainly picked up through the first to third cell divisions after the germination of each cyst. Individual toxin content per cell was expressed in Table 2, in which toxicity was highly variable among the strains isolated from same sampling site as well as the

Table 1. Origin of *Alexandrium* isolates germinated from the benthic cysts from Jinhae Bay

Strain code	Sediment sampling		Sediment fraction (cm)	Cyst code	Origin of clone cell
	Site	Date			
SC7-1	St. 1/Sujeongri	17 Jan. 1994	2~4	C7	F(c)
SC7-2	〃	〃	〃	〃	〃
SE9-2	〃	12 Oct. 1993	〃	E9	S(c)
SE9-3	〃	〃	〃	〃	〃
SE9-4	〃	〃	〃	〃	〃
JA7	St. 2/Ukgokri	14 Dec. 1993	0~2	A7	〃
JD1-1	〃	〃	〃	D1	〃
JD6-1	〃	〃	〃	D6	F(c)
TA2-2	St. 4/Taegokri	21 Sep. 1993	0~2	A2	S(c)
TA2-3	〃	〃	〃	〃	〃
TB1-1	〃	〃	〃	B1	F(c)
TC4-2	〃	〃	4~6	C4	T(c)
TD2-1	〃	〃	〃	D2	S(c)
TD3-3	〃	〃	〃	D3	〃
TG2-2	〃	〃	〃	G2	T(c)
TG6-2	〃	〃	2~4	G6	S(c)
TG6-3	〃	〃	〃	〃	St(c)
TG6-5	〃	〃	〃	〃	〃
TG8-4	〃	〃	〃	G8	S(c)

F(c): one of two clone cells through the first cell division from a planomeiocyte; S(c): one of four clone cells through the second cell division from a planomeiocyte; St(c): one of five clone cells through the second to third cell division from a planomeiocyte; T(c): one of eight clone cells through the third cell division from a planomeiocyte.

strains originated from an individual cyst. In the latter case, about twice the differences in total toxin content were observed between two isolates from C7 cyst and three times or more among three isolates from E9 and G6 cysts. The same tendency was found between two isolates, OF151 and OF152 originated from a cyst (Kim et al., 1993b), whereas it was suggested that toxin content variation could be largely due to the allocation of toxin to daughter cells during cell division. The variability of individual total toxin content observed in this study would be due to toxin allocation during cell division, as cell growth has shown a significant difference both among the strains mentioned above during the culture. Average total toxin contents in three stations revealed higher values of 54~70 fmol than those of 44 fmol reported by Kim et al. (1993a, b) in Ofunato Bay where is known as a representative area for PSP problems in Japan. Prevalent occurrence of *Alexandrium* spp. containing highly potent carbamate toxins have been well documented (Ogata et al., 1982; Oshima et al., 1982; Sekiguchi et al., 1989) in Japan.

The toxin profile of *Alexandrium* species revealed a similiar pattern within a geographical region, but it was not the case necessarily in different regions (Oshima et al., 1982; Maranda et al., 1985; Cembella et al., 1987; Kim et al., 1993b). From these view points, the toxin profiles and percent composition of total toxin among the strains established at three sites were compared (Table 2; Figs. 2~4). Toxin profiles

Table 2. Individual toxin content per cell of *Alexandrium* spp. analysed by HPLC

Isolates	Toxins, fmol/cell											Total
	GTX1	GTX2	GTX3	GTX4	GTX5	epiGTX8	GTX8	C4	neoSTX	dcSTX	STX	
SC7-1	18.18	-	0.30	4.26	5.33	4.91	34.43	Tr	22.01	1.06	1.57	92.05
SC7-2	5.16	-	0.26	15.26	0.72	1.80	8.10	-	Tr	Tr	-	41.30
SE9-2	13.20	-	0.35	30.25	3.02	4.68	35.55	-	4.27	-	-	91.32
SE9-3	28.44	-	0.50	37.98	-	3.61	23.43	-	-	-	-	93.96
SE9-4	-	-	0.07	12.31	-	0.80	12.25	-	6.78	-	-	32.21
JA7	2.19	-	1.45	8.61	1.31	0.89	15.75	Tr	18.16	0.93	2.66	51.95
JD1-1	1.91	-	0.31	8.65	0.96	0.92	17.85	Tr	-	-	-	30.60
JD6-1	-	-	2.61	38.49	1.08	1.67	32.33	Tr	4.65	Tr	-	80.83
TA2-2	16.08	-	0.83	38.26	6.24	3.70	24.01	-	-	-	-	89.12
TA2-3	22.64	0.13	0.49	73.08	8.28	1.22	17.06	-	-	-	-	122.90
TB1-1	7.91	-	0.50	25.91	2.99	2.53	34.09	Tr	-	-	-	74.93
TC4-2	2.07	-	-	8.49	-	1.37	7.55	-	22.95	-	-	42.43
TD2-1	6.69	0.07	0.10	12.85	-	14.56	9.65	-	-	-	-	43.92
TD3-3	8.16	-	0.15	14.54	-	2.69	14.61	-	16.61	-	-	56.76
TG2-2	-	-	0.31	5.07	-	0.65	12.50	-	13.28	Tr	-	31.81
TG6-2	-	-	-	4.30	-	0.33	1.87	-	Tr	-	-	6.50
TG6-3	2.38	-	-	8.81	-	0.67	3.84	-	13.04	-	-	28.74
TG6-5	10.66	-	0.24	22.82	3.99	1.28	6.79	-	8.36	-	-	54.14
TG8-4	6.41	-	0.17	15.17	-	2.46	18.29	-	15.81	0.62	-	58.93

Tr: <0.05; -: not detected.

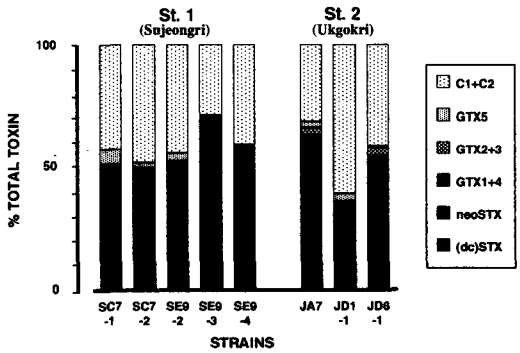

Fig. 2. Mole percentage of toxin composition of *Alexandrium* spp. germinated from the benthic resting cysts of Station 1 (Sujeongri area) and Station 2 (Ukgokri area) in Jinhae Bay.

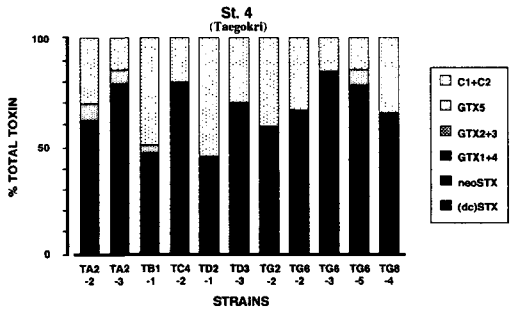

Fig. 3. Mole percentage of toxin composition of *Alexandrium* spp. germinated from the benthic resting cysts of Station 4 (Taegokri area) in Jinhae Bay.

of five strains in St. 1 and three in St. 2 included C1 %C2 (epiGTX8+GTX8), GTX1+GTX4 and neoSTX (only three strains of all) as the major components, and GTX3, GTX5, C4, and (dc) STX (with sporadic) as the minor ones (Table 2; Fig. 2). The same components as the major and minor ones also appeared in eleven strains of St. 4, in which only six strains contained neoSTX with comprising higher proportions of 15~54 mol% than those of 5~35 mol% in St. 1, 2 (Fig. 3). Toxin profiles arised from the strains in

Jinhae Bay would be largely grouped into two patterns of dominant toxins according to the presence of neoSTX. This difference in the toxin profiles was also found in the isolates originated from those of C7, E9 and G6 cysts. The same tendency was reported between two isolates germinated from a cyst of Ofunato Bay in which distinct deficiency in C1~C4 toxins in one isolate was observed throughout the growth (Kim et al., 1993a). Lee et al. (1992) observed the occurrence of highly different proportions of neoSTX contents between the mussels in 1989 and 1990 in Jinhae Bay, and they suggested that the causative organism should belong to the different strains. Diversity of toxin profiles of *Alexandrium* was well-documented on the west coast of North America, where some regional populations were found to be more genetically diverse than others. In that study, Cembella et al. (1987) presumed that the observed differences within a geographical region may account for some of the heterogeneity in toxin composition genetically fixed among the certain isolates. Therefore, the occurrence of other toxin profiles in half of the strains examined in this study might be explained by heterogeneity with a genetic trait than compositional changes observed in the culture studies (Boczar et al., 1987; Anderson et al., 1990).

Besides toxin composition difference among the strains, higher proportions of carbamate toxins occurred in each station. When average toxin composition was compared, as shown in Fig. 4, carbamate toxins (e.g., STX, neoSTX and GTX1-4) comprised in large proportions of 57%, 54% and 67% as total toxin in St. 1, St. 2 and St. 4, respectively. The same trend in toxin composition differences related to a regional toxicity elevation was also documented on the isolates of *A. tamarense* and *A. catenella* from the northern parts of Funka Bay and Ofunato Bay in Japan, comparable to the southern ones comprising higher proportions of N-sulforcarbamoyl toxins (Oshima et al., 1990; Kim et al., 1993b). Anderson (1990) reported that high toxicity of the northern isolates of *A. fundyense* was due to their production of the highly potent carbamate toxins, and the observed regional trend in toxicity could result from latitudinal differences in environmental parameters and their influence on the establishment of the genotypically different blooms. In Korea, since the first PSP was recorded in 1986, the occurrence of *A. tamarense* as a PSP causative organism was mainly defined in the cold waters from January to April (Lee et al., 1992; Han et al., 1993). Recently, Kim (1995)

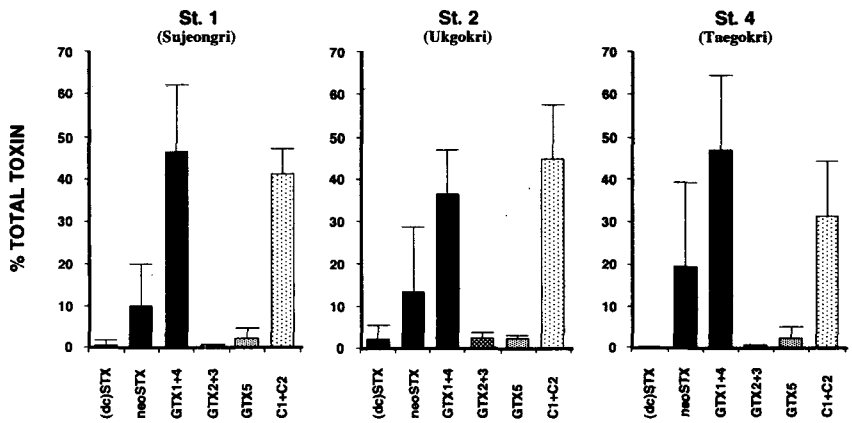

Fig. 4. Comparison of the average toxin composition (as % total toxin) of *Alexandrium* spp. germinated from the benthic resting cysts of the three regions in Jinhae Bay.

found a seasonal germinability of *Alexandrium* resting cysts collected from Jinhae Bay, and the motile cells occurred in the cold season could result from the germination of their benthic cysts which have been controlled by a seasonal rhythms instead of the general environmental factors such as temperature. Instead, cysts from shallow waters of Tadaepo station did not follow a seasonal rhythmic pattern and showed also high germination frequency as well as abundant swimming cells in spring and autumn. The similar trend in a germinability with an endogenous annual clock of *A. tamarense* resting cysts was documented from the deep coastal waters in the Gulf of Maine (Anderson and Keafer, 1987). Therefore, those defined occurrence of *Alexandrium* species with the same seasonality as a cyst germination might contribute to sporadic regional outbreaks of shellfish toxification with the advantage of environmental separation. Moreover, toxin production in *Alexandrium* was inherited in a normal Mendelian pattern with biparent type as well as with recombination type, and it was proposed that possible occurrence of the variability in toxicity in natural environments might be caused by these reasons (Ishida et al., 1990).

With the previous results of high density distribution and germination potential of the resting cysts in the cold season, it is suggested that the occurrence of higher proportions of carbamate toxins should be attributed to great possibility of PSP intoxification in Jinhae Bay.

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진해만산 와편모조류 *Alexandrium*속 휴면포자 발아체의 마비성패독 조성

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1993년 진해만 일원의 마비성패독 발생의 원인규명을 위한 모니터링의 일환으로 원인생물의 독생산과 성분조성을 조사하기 위하여 양식장 인근해역의 저서 휴면포자를 발아시켜 분리한 무균주의 독성분을 분석하였다.

분리된 전체주중에서 수정리산 (St. 1) 5주, 욱곡리산 (St. 2) 3주 및 대곡리산 (St. 4) 11주의 독조성 및 독함량을 비교하였을 때, 각 지점별 평균 독량은 약 54~70 fmol/cell의 높은 량을 나타내었고, 동일 휴면포자의 clone 분리주 뿐만 아니라 전체 분리주에서 개체별 독함량의 차이가 크게 나타났다. 독조성은 C1/C2 (epiGTX8 /GTX8), GTX1/GTX4 및 neoSTX가 주요 구성성분을 이루었고, GTX2/GTX3, GTX5, C4, dcSTX 및 STX성분은 미량 또는 산발적으로 출현하였다. 주요 성분중에서 neoSTX는 5~54 mol%로 변동이 컷으나, 전체 분리주의 절반은 출현을 보이지 않아 이 지역에서 조성이 다른 개체군의 출현이 시사되었다. 한편, 상대적으로 독성이 강한 GTX1-4 및 neoSTX와 같은 Carbamate군의 성분이 세 정점에서 각각 57%, 54% 및 67%의 높은 평균치를 나타내어 이 지역에서의 높은 독화율과 독화 가능성의 잠재력이 큰 것으로 시사되었다.