Species Identification of Nontoxic *Alexandrium tamarense* (Dinophyceae) from Chinhae Bay, Korea, Using Molecular Probes

Eun Seob Cho*

South Sea Fisheries Research Institute, Coastal Environment Management, NFRDI, Yeosu 556-823, Korea

Received November 17, 2003 /Accepted February 15, 2004

The morphology of the apical pore complex, the first apical plate and the posterior sulcal plates in a new isolate of *Alexandrium tamarense* (Lebour) Balech from the Bay of Chinhae was compared with other that of toxic strains of *A. tamarense* previously isolated from Korean waters. Although this isolate was morphologically identical to these toxic strains, high performance liquid chromatography and mouse bioassay showed no evidence of toxin production. The nontoxic *A. tamarense* strain showed a strong positive binding activity with PNA lectin, indicating a high density of lactose and galactose residues on the cell surface, and in SDS-PAGE and Western blot analysis a unique protein of about 21-kDa molecular sizes was observed. These findings demonstrate that the use of PNA and immunobioassay could be used to discriminate between toxic and nontoxic strains of *A. tamarense*.

Key words – *Alexandrium tamarense*, detection, HPLC, immunobioassay, lectin, morphology, primer, toxic algae

Dinoflagellates make up one of the most important groups of marine phytoplankton. This is due to their biomass and their contribution to the trophic system and also to the fact that some species produce compounds which are toxic for humans, such as PSP toxins (paralytic shellfish poisoning). The PSP of shellfish by member of the genus *Alexandrium* has been well reported. At present, seven species of *Alexandrium* have been identified in Korean waters[7,32,46]. However, *Alexandrium tamarense* (Lebour) Balech has recognized as the only toxic dinoflagellate that caused PSP[24,28,29], a nerve disorder of impacting a economic and public health concern because of the development of aquaculture[3,9,15,37,48]. The occurrence of this species in Korea is usually from the middle of March to early July and mostly in Chinhae Bay[31].

All species within the genus *Alexandrium* are similar in morphological features and it is difficult to discriminate between them under the light microscope. The validity of morphological criteria in defining species within the genus *Alexandrium* has been debated on the last decade. Fukuyo *et al.*[18,19], Hallegraeff *et al.*[23] and Taylor *et al.*[50] suggested that the feature of the apical pore complex (APC), the shape of the first apical plate and the posterior sulcal plate played a useful role in discriminating *Alexandrium* species, though Fukuyo[19] reported slightly morphological difference under environmental conditions and growth stages. However, the introduction of molecular analysis to the taxonomic questions in *A. tamarense* has been much advanced and studied[2,16,44]. Molecular biological techniques, such as lectin-binding assay, specific immunological and DNA hybridization methods, have the potential to address more sensitive diagnostic methods for targeted phytoplankton[2,16,47]. Although it is highly sensitive and can be performed within a short time, the result is sometimes doubtful because labeling intensity may be different between stationary and exponential growth phase[4]. However, the specificity of molecular detection methods currently available is satisfactory. These methods can also provide simultaneous information on the localization of targeted phytoplankton in the cells.

Previously, we suggested that most of strains of *A. tamarense* occurring from Chinhae Bay were able to produce compounds, which were toxic for humans, PSP toxins (Paralytic Shellfish Poisoning), although variation of their toxicity was significantly different under the same environmental conditions[11]. However, all of toxic *A. tamarense* was reported to have similar morphological structure on the basis of thecal plates[11]. Recently, we isolated a new strain from the same region. The detection of PSP toxins in this new isolate and the subsequent morphological characteristics during the clonal culture were prompted the present investigation. We observed and confirmed the cell under light, epifluorescent and electron

***Corresponding author**
Tel : +82-61-686-8942, Fax : +82-61-686-1588
E-mail : eun-5657@hanmail.net

microscopes on the basis of the feature of APC, first and posterior sulcal plates. In addtion, we applied molecular probes to discriminate this species from toxic *A. tamarense* in cultures.

Materials and Methods

Cultures

Dr. B. S. Shin, Korea Oceanography Research and Development Institute provided seawater samples, which were obtained from Chinhae Bay, 2000. It was isolated using capillary pipette under microscope. Isolate was grown in f/2-Si medium[22] and incubated at 20℃ under 50 μmol^{-2} S^{-1} light intensity from fluorescent tubes with 14:10 h LD (light:dark) photoperiod. This strain was maintained in exponential growth phase by serial transfers of an inoculum to fresh medium once every month.

Staining

The thecal plate morphology was observed using phase contrast and UV epifluorescent microscopy after calcofluor staining[18] under a Nikon Optiphot (Tokyo, Japan) microscope attached with UV filter (excitation, 330-385 nM; emission, >420 nM).

Electron microscope

The cells were fixed with 2.5% glutaraldehyde and 1% osmium tetroxide. The material collected on the filters was subsequently washed with 0.1 M phosphate buffered saline (PBS, pH 7.2). Samples were dehydrated in a stepwise graded ethanol series upto 100% ethanol. Specimen were sputter-coated with deionized gold and examined under an Hitachi S-4200 (Tokyo, Japan) SEM.

Toxin analysis

Toxicity was examined by means of the mouse bioassay and HPLC (high performance liquid chromatography) according to previous methods[11].

Lectins

This study selected ten lectins from Sigma Chemical Company (Seoul, Korea), each of there having a different carbohydrate-binding specificity; lectins jack bean agglutinin (ConA, specific for Mannose/Glucose group), coral tree agglutinin (ECA, specific for N-Acetylgalactosamine/Galactose group), snail agglutinin (HPA, specific for D-glucosamine group), pea agglutinin (PEA, specific for Mannose/Glucose group), lima bean agglutinin (LBL, specific for N-Acetylgalactosamine group), peanut agglutinin (PNA, specific for Lactose/Galactose group), pokeweed agglutinin (PWM, specific for N-Acetylglucosamine group), soy bean agglutinin (SBA, specific for N-Acetylgalactosamine/Galactose group), gorse agglutinin (UEA, specific for Fucose group) and wheat germ agglutinin (WGA, specific for N-Acetylglucosamine group). Suspensions of lectin in filtered seawater (0.2 μm cartridge filter) were prepared at a final concentration of 50 $\mu g\ ml^{-1}$. A 100 μl percoll was added to the 1 ml (approximately 1×10^7 cells) culture for cell aggregation, and the cells were harvested by centrifugation. After centrifugation, the supernatant was removed and aliquots were mixed with lectins, and incubated for 1 h at room temperature. The supernatant was removed, and 1 ml PBS was added to resuspend the pellet. The cells were examined for binding activity under a Nikon Optiphot (Tokyo, Japan) microscope attached with blue light filter (Excitation, 450-490 nM; Emission, 529 nM). Binding was determined by direct observation and recorded as: ++ (strong green signal fluorescence on the cell surface), + (moderate green siganal fluorescence on the cell surface), *p*+ (weak green signal fluorescence on the cell surface), - (no fluorescence or autofluorescence). All assays were done three replicates.

Primary antibody

10 ml aliquots of *A. tamarense* collected by centrifugation ($250 \times g$, 10 min) were preserved in 4% formaline and rinsed in PBS (pH 7.4, ×4). Two Australian female rabbits were inoculated with purified *A. tamarense*. One subcutaneous injection containing Freund's complete adjuvant at 1 ml per rabbit was carried out. After 20 days, a subcutaneous booster containing Freund's incomplete adjuvant at 1 ml per rabbit was administrated. A schedule of four boosts was performed at 3 weeks interval. The rabbits were bled 5 days after the final booster. Serum was separated by centrifugation after clotting. The rabbits antibodies were purified using affinity chromatography (Pharmacia). The column (HiTrap) was washed and equilibrated with $Na_2HPO_42H_20$ 20 mM, pH 7.0. The fraction was specifically eluted in citrate buffer (0.1 M citric acid, pH 3.0). Fractions were collected in the presence of 1 M HCl Tris buffer, pH 9.0. Purified antibody was then aliquoted and stored at -20℃.

Western blot analysis

Electrophoresis of protein was performed routinely on

SDS (sodium dodecyl sulphate)-polyacrylamide gels by standard methods with a BioRad Mini Protean II apparatus according to the manufactuer's instructions. The sample buffer contained 10% sucrose, 10% SDS and 10% mercaptoethanol, and the samples were boiled for 5 min. For Western blotting, *c.a.* 10 μg of purified protein per lane were run on an SDS-PAGE (polyacrylamide gel electrophoresis) 10% gel and transferred to sheets of nitrocellulose filters according to the manufacturer's instructions. The blocking solution was PBS (pH 7.4) supplemented with bovine serum albumin (BSA) 5% and Tween-20 0.01%. Blots were incubated in sera diluted 1 in 200 in PBS for 2 h at room temperature. After being washed, the membranes were incubated at room temperature for 2 h in anti-rabbit IgG (whole molecule) alkaline phosphatase-conjugated secondary antibody (Sigma) diluted 1 in 20,000 in PBS. The colour reaction was developed with 5-bromo-chloro-3-indolyl phosphate 0.05 mg ml^{-1} (BCIP) and nitroblue tetrazolium 0.01% (NBT). Protein concentrations were determined by the BCA Protein Estimation Assay (Pierce, Rockford, USA) according to the manufacturer's instructions, with bovine serum albumin as standard.

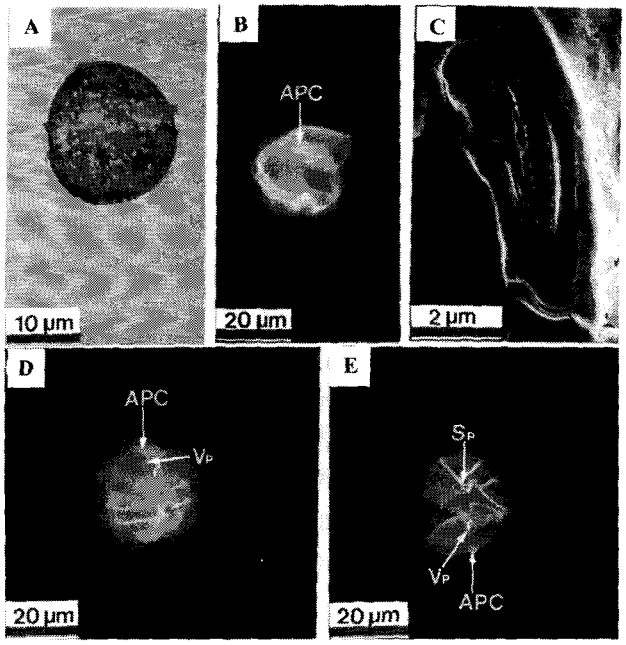

Fig. 1. Micrographs of *Alexandrium tamarense* cells in cultures. A, light microscope of ventral view of cell; B, epifluorescence micrograph of apical pore complex (APC) stained by calcofluor; C, APC by scanning electron microscope; D, epifluorescence micrograph of APC and ventral pore (Vp) in first apical plate; E, epifluorescence micrograph of APC, Vp and sulcal plate (Sp) in posterior plate.

Results

Cell ovoid, slightly longer than wide, circular in cross-section, 20-43 μm in long and 15-32 μm in wide ($n = 72$) during the culture were observed (Fig. 1a). In the ventral view, the shape was pentagonal and convex. Epitheca and hypotheca were nearly equal in altitude. The shape of epitheca was broadly conical and the hypotheca was irregularly trapezoidal. Cingulum was excavated and descended at the end of cell. Sulcus was relatively wide, running almost straightly. The APC was wide and angular, with an asymmetric bullet-shape (Fig. 1b, c). The upper segment of APC was nearly parallel to the main axis of the plate, whereas the lower was sloped inward. APC was connected with the first apical plate. Ventral pore (Vp) was presented in the first apical plate and attachment pore in posterior sulcal plate (Sp) was also shown in the cells (Fig. 1d, e). There was no presence of PSP toxin-complex by Mouse bioassay test and HPLC analysis. The nontoxic *A. tamarense* had a binding profile most like toxic *A. tamarense*, which showed a positive binding of ConA, ECA, HPA, PEA, PNA, SBA and WGA lectins (Table 1), except for the lack of reaction with LBL, PWM and UEA lectins (no fluorescence on the cell surface, Table 1, Fig. 2a, b, d). However, the nontoxic *A. tamarense* could be differentiated by a strong reactivity with PNA lectin, with remarkably fine green signal on the cell surface compared to toxic *A. tamarense* stained from even PNA lectin (Fig. 2c). The

Table 1. Binding response of toxic and non-toxic *Alexandrium tamarense* isolates isolated from Chinhae Bay to different lectins used as fluorescent probes. The symbols of ++, +, *p*+ and - represent to emit strong, moderate and weak green signal on the cell surface and no binding with lectins or autofluorescence, respectively

Strain	ConA	ECA	HPA	PEA	LBL	PNA	PWM	SBA	UEA	WGA
A. tamarense AT-2 (toxic)	+	+	+	+	-	+	-	+	-	+
A. tamarense AT-6 (toxic)	+	+	+	+	-	+	-	+	-	+
A. tamarense AT-B (toxic)	+	+	+	+	-	+	-	+	-	+
A. tamarense (non-toxic)	+	+	+	+	-	++	-	+	-	*p*+

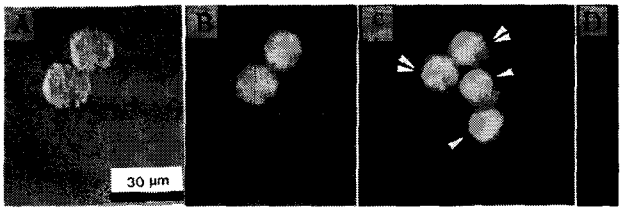

Fig. 2. Epifluorescence micrographs of *Alexandrium tamarense* in cultures. A, light microscope of *A. tamarense*; B, no binding of *A. tamarense* stained by LBL lectin; C, treatment of PNA lectin to toxic *A. tamarense* (two arrowheads), while to nontoxic *A. tamarense* (one arrowhead); D, no addition of lectins.

antibody targeted to nontoxic *A. tamarense* was tested against toxic *A. tamarense* isolates AT-2, AT-6, AT-B to discriminate nontoxic from toxic *A. tamarense* (Fig. 3). The nontoxic *A. tamarense* antiserum reacted strongly on the Western blots with non-toxic *A. tamarense*, whereas no binding of any toxic *A. tamarense* isolate observed. Reaction was found in the 21-kDa antigens with a high intensity of recognition.

Discussion

Morphological analysis

Alexandrium tamarense, *A. fundyense* (Lebour) Balech and *A. catenella* (Whedon & Kofoid) Balech are very similar in morphological features and are considerably confusing under the light microscope. However, the presence or absence of anterior attachment pore in 1′ plate plays an important role in differentiating *Alexandrium tamarense* from *A. catenella*/*A. fundyense*[7,19,23,50]. This study confirmed that this isolate was not regarded as *A. catenella* and *A. fundyense* because of the presence of ventral pore under the epifluorescence microscope. In cultures, the position of the anterior and posterior attachment pores was detected throughout the whole growth phases, which was present in solitary cells or chain-forming cells during cell division. Recently, Yoo *et al.*[52] reported a little variation in the anterior part of the first apical plate and its posterior end on the basis of original description. However, it is likely that this variation is considered within the range of the morphological variation of this taxon. Although the variability in the morphology of APC for *A. ostenfeldii* (Paulsen) Balech et Tangen was shown in New Zealand[35] and Canada[8], recently, the analysis of APC feature of nontoxic isolate showed also a similar thecal morphology as that described in toxic *A. tamarense*[11].

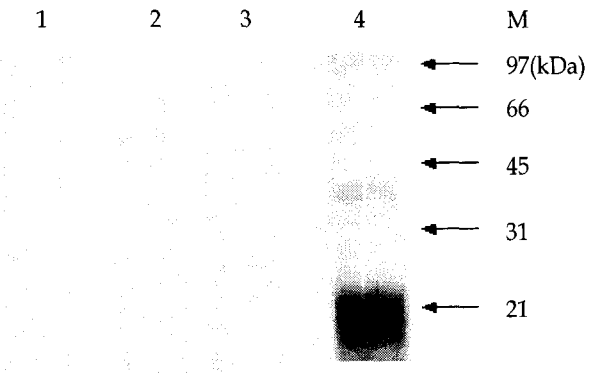

Fig. 3. Immunoblot of the approximately 21-KDa proteins from nontoxic *Alexandrium tamarense*. The protein was analyzed by SDS 10% PAGE, followed by blotting and Western screen with BioRad apparatus. Lane M (marker proteins): rabbit muscle phosphorylase (97-kDa), bovine serum albumin (66-kDa), ovalbumin (45-kDa), carbonic anhydrase (31-kDa) and trypsin inhibitor (21-kDa). Lane 1: Western blot analysis of *A. tamarense* AT-2, Lane 2: Western blot analysis of *A. tamarense* AT-6, Lane 3: Western blot analysis of *A. tamarense* AT-B, Lane 4: Western blot analysis of nontoxic *A. tamarense*.

Nontoxic A. tamarense

Adachi *et al.*[1] showed that genetic heterogeneity of crude protein band patterns existed between Japanese (toxic) and Thailand (nontoixc) strain of *A. tamarense*. Recently, Taroncher-Oldenburg and Anderson (2000) observed the differential display (DD) patterns of toxic and nontoxic *A. tamarense* and reported high variability between them. Consequently, characterization of toxic and nontoxic *A. tamarense* is quite different conception on the basis of genetic understanding. In Korea, the present study was first reported to exist nontoxic *A. tamarense* from Chinhae Bay. It is known that Chinhae Bay is a large waterbody with only one narrow avenue for exchange with oceanic waters and it is known that the strength and persistence of winds has an important effect on the residence time of seawater in the bay. The occurrence of nontoxic *A. tamarense* in Chinhae Bay as a heterogenesis species is unusual. The variation in toxin production and composition can be attributed to environmental conditions[36,51], nutrient limitation[6] and cell cycle events[5], with the genetic factors having less impact. Other researchers proposed that genetic factors might have an impact on the variation of toxin production and composition[27]. Anderson[3] and Ishida *et al.*[25] proposed that toxin production was di-

rectly contributed by intrinsically genetic basis. However, Kodama[30] suggested to be involved mobile genes from the endosymbiotic intracytoplasimic or intranuclear bacteria. Recently, the development of molecular analysis makes a possibility that toxin production better accorded with the endosymbiotic-origin hypothesis than with the intrinsic-origin hypothesis because PSP-producer species were randomly distributed[53]. This study have not tested the determination and occurrence of bacteria in cultures of this species, but is necessary to debate and study the interaction magnitude in future. Spatial distribution pattern of *A. tamarense* in Chinhae Bay is limited to widespread, almost homogenous population, because this studied region is difficult to be provided and mixed with outer waters on the basis of overall hydrogeographical perspective.

Previously, we suggested that *A. tamarense* in Chinhae Bay was composed by a unique population with its own physiological characteristics[11]. That indicates that Chinhae Bay exhibits a stable genetic population structure and forms a single phylogenetic group. Stable exchange of genetic solidarity within closely related groups can be explained by homologous recombination frequency. However, there are also other possible mechanisms for genetic exchange within genetically clustered groups. This is introduced by conjugation, simultaneous competence and recognition sequence[34]. The exchange of genetic information could not be a general mechanism. The observed heterogeneous distribution of the toxic and nontoxic strains may be a result of gene change at a number of times throughout evolution. Further studies addressing the mechanism of heterogeneous strain of *A. tamarense*, are necessary. It is known that *A. tamarense* can generate asexually or sexually against environmental and nutritional conditions following *in vitro* and *in vivo* studies[40]. The most reliable hypothesis is to be thought that some of the vegetative cells produce zygotic cysts by recombination with genetic material during the sexual reproduction. In an earlier study, we have compared gene sequences of cultured cells and germinated cells from resting cysts in *Gyrodinium impudicum* Fraga et Bravo and resulted to be identical nucleotides[14]. This species cannot produce seed source through sexual life cycle such as *A. tamarense*. In Chinhae Bay, where PSP is monitored, the large numbers of cysts found on shellfish culture are common[26]. Lee *et al.*[33] suggested that the relationship between the abundance of cells of *A. tamarense* and shellfish contamination was high correlated and reported no toxicity incidents in a decade in Chinhae Bay. This indicates that it is difficult for heterogeneous species to recruit and mix planktonic pool of toxic *A. tamarense* population, but this simply may be an issue of spatial scale.

Detection

Lectins (haemagglutinins, agglutinins) are sugar-binding proteins of non-immune origin that agglutinate or precipitate glycoconjugates and cells[21]. Due to the strong affinity and binding specificity that each lectin has toward a particular structure, they have been proposed as primary recognition molecules[45]. This data on lectin binding demonstrated the presence of glycoproteins that could act as identification tools (Fig. 2). This implies that non-toxic *A. tamarense* has more lactose and galactose residues on the cell surface than toxic *A. tamarense*, whereas acetylglucosame group on the cell surface of non-toxic *A. tamarense* was shown in a shortage. Both *A. tamarense* and *A. catenella* are considered toxic phytoplankton, however, previous studies showed the same binding activity[13,15]. Cho *et al.* [15] suggested that PNA lectin was useful to discriminate toxic *A. catenella* from nontoxic *A. frateculus*. However, three toxic *A. tamarense* isolates which were of different toxicity showed all positive PNA lectin binding[12]. Consequently, toxic and nontoxic *A. tamarense* and *A. catenella* from Chinhae Bay have common biochemical characteristics for the presence of PNA lectin on the cell surface, whereas in non-toxic *Alexandrium* species it possibly seems to be absent or shortage on the cell surface. It is also suggested that biochemical characteristics on the cell surface of toxic and nontoxic *A. tamarense* seem to be different and thus further expect the determination of protein-coding ribosomal RNA because of high degree of functional constancy.

During the last ten years, characterization of harmful marine algae by molecular probes techniques has become increasingly important and has now more or less replaced biochemical methods. Compared to biochemical analyses, DNA-based techniques have the additional benefit that they often provide significant information needed to verify or interpret the understanding of the taxonomy and systematics. At the generic level, polymerase chain reaction (PCR) amplification of the ribosomal DNA spacer has proved to be a very useful genetic mark for differentiating the species of *Alexandrium*[2,10,38,39,43,44]. More recently, however, Anderson *et al.*[4] suggested that oligonucleotide probe targeting rRNA had a variation of labeling intensity

depending on growth phase because of different concentrations of rRNA. In contrast to rRNA probe, immunofluorescent antibody targeting a cell surface protein, was less variable in signal intensity, although growth condition and step affected the results[4]. Similarly, Costas *et al.*[17] suggested that cell division cycle, culture media and environmental conditions had no impact of lectin binding activity. Considering in present study, it was certain that nontoxic *A. tamarense* showed an unknown molecule on the cell surface, indicating that it might play an important role in taxonomical tool for differentiating toxic from nontoxic *A. tamarense* and it also revealed to be different the biochemical molecular between them on the cell surface (Fig. 3). Consequently, this make us assure that the use of lectin and antibody probes targeting molecules on the cell surface will provide a reliable information for discriminating, enumerating and monitoring *Alexandrium*.

Future perspectives

Studies on phylogenies and evolutionary relationships are a long-standing topic debate[50]. A satisfactory resolution on this issue and clearer understanding of genetic status, will depend on the identification of defined populations and their interrogation with reliable genetic markers. The application of molecular tools has the potential to resolve much of the controversy over *Alxandrium* taxonomy, evolution and the species conception. Recent approaches studied to identify species-specific genetic markers for addressing taxonomic and biogeograhic questions in *Alexandrium*[2,41,42]. They demonstrated that small-subunit (SSU), large sub-unit (LSU) and internal transcribed spacer (ITS) rDNA regions were able to separate *Alexandrium* species or populations that exhibited two distinct genes, called "A gene" and "B gene"[2,41,42,44]. Interestingly, both strains of nontoxic *A. tamarense* isolated from Tasmania, Australia and Kushimoto, Japan, formed an independent molecular group and a unique evolutionary linkage[2,41,42]. The possible mechanism of distinguishing populations is evolutionally explained by the introduction of natural immigrations or human activity[44]. Since *Alexandrium* have a cosmopolitan distribution and dominates the populations of many environments, and it seems to be morphologically identical and to occur in geographically isolated environments world wide, they have a number of advantages for population analysis. Unfortunately, however, no study has actually tested the genetic character and possible population subdivision of the species within micro-geographic area in the Korean waters. Consequently, we will conduct to detect the possible existence of the genetic subdivision between micro-geographic locales and investigate the extent of DNA divergence within micro-geographic area.

Acknowledgements

This work was partially supported by a grant-in-aid from the Maritime Affairs and Fisheries Ministry of Korea. I thank Dr. C. S. Kim and Mr. H. J. Lee, National Fisheries Research and Development Institute, for analyzing toxicity. I also thank Mr. J. P. Kim, Korea Basic Science Institute Pusan Branch, for SEM.

References

1. Adachi, M., Y. Sako and Y. Ishida. 1993. The identification of conspecific dinoflagellates *Alexandrium tamarense* from Japan and Thailand by monoclonal antibodies. *Nip. Sui. Gak.* **59**, 327-332.
2. Adachi, M., Y. Sako and Y. Ishida. 1996. Analysis of *Alexandrium* (Dinophyceae) species using sequences of the 5.8S ribosomal DNA and internal transcribed spacer regions. *J. Phycol.* **32**, 424-432.
3. Anderson, D. M. 1990c. Toxin variability in *Alexandrium* species. In: Graneli, E., Sundstrom, B., Edler, L. Anderson, D. M. (eds) *Toxic Marine Phytoplankton*. Elsevier, New York, pp. 41-51.
4. Anderson, D. M., D. M. Kulis, B. A. Keafer and E. Berdalet. 1999. Detection of the toxic dinoflagellate *Alexandrium fundyense* (Dinophyceae) with oligonucleotide and antibody probes: variability in labeling intensity with physiological condition. *J. Phycol.* **35**, 870-883.
5. Anderson, D. M., D. M. Kulis, J. J. Sullivan and C. Lee. 1990b. Dynamics and physiology of saitoxin production by the dinoflagellates *Alexandrium* spp. *Mar. Biol.* **104**, 511-524.
6. Anderson, D. M., D. M. Kulis, J. J. Sullivan and S. Hall. 1990a. Toxin composition variations in one isolate of the dinoflagellate *Aleandrium fundyense*. *Toxicon* **28**, 885-893.
7. Balech, E. 1985. The genus *Alexandrium* or *Gonyaulax* of the *tamarensis* group. In: Anderson, D. M., White, A. W. and Baden, D. G. (eds). Elsevier Science Publishing, New York, pp. 33-38.
8. Cembella, A. D., N. I. Lewis and M. A. Quilliam. 2000. The marine dinoflagellate *Alexandrium ostenfeldii* (Dinophyceae) as the causative organism of spirolide shellfish toxins. *Phycolcgia* **39**, 67-74.
9. Chang, D. S., I. S. Shin, J. H. Pyeun and Y. H. Park. 1987. A study on paralytic shellfish poison of sea mussel, *Mytilus edulis*. *Bull. Kor. Fish. Soc.* **20**, 293-299.
10. Chang, F. H., I. Garthwaite, D. A. Anderson, N. Towers, R. Stewart and L. MacKenzie. 1999. Immunofluorescent

detection of a PSP-producing dinoflagellate, *Alxandrium minutum*, from Bay of Plenty, New Zealand. *NZ. J. Mar. Fresh. Res.* **33**, 533-543.
11. Cho, E. S. and H. J. Lee. 2001a. Thecal plates, toxin content and growth of five clones of *Alexandrium tamarense* (Dinophyceae) isolated from Jinhae Bay, Korea. *Phycologia* **40**, 435-439.
12. Cho, E. S., B. D. Choi, Y. C. Cho, T. J. Kim and H. G. Kim. 2001b. Discrimination of three highly toxic *Alexandrium tamarense* (Dinophyceae) isolates using FITC-conjugated lectin probes. *J. Plank. Res.* **23**, 89-95.
13. Cho, E. S., G. M. Seo, S. G. Lee, H. G. Kim, S. J. Lee, L. L. Rhodes and Y. K. Hong. 1998. Application of FITC-conjugated lectin probes for the recognition and differentiation of some Korean coastal red tide microalgae. *J. Fish. Sci. Tech.* **1**, 250-254.
14. Cho, E. S., G. Y. Kim, H. S. Park, B. H. Nam and J. D. Lee. 2001c. Phylogenetic relationship among several Korean coastal red tide dinoflagellates based on their rDNA internal transcribed spacer sequences. *Kor. J. Lif. Sci.* **11**, 74-80.
15. Cho, E. S., J. G. Park, H. G. Kim, C. H. Kim, L. L. Rhodes and C. S. Chung. 1999. The rapid differentiation of toxic *Alexandrium* and *Pseudo-nitzschia* species using fluorescent lectin probes. *J. Kor. Soc. Oceano.* **34**, 167-171.
16. Costas, E., R. Zardoya, J. Bautista, A. Garrido, C. Rojo and V. Lopez-Rodas. 1995. Morphospecies vs. genospecies in toxic marine dinoflagellates: An analysis of *Gymnodinium catenatum/Gyrodinium impudicum* and *Alexandrium minutum/A. lusitanicum* using antibodies, lectins, and gene sequences. *J. Phycol.* **31**, 801-807.
17. Costas, E., E. Hevia, Y. Carretero and V. Lopez-Rodas. 1996. Distinctive lectin labeling of the flagellar apparatus of *Tetraselmis* (Prasinophyceae) and *Dunaliella* (Chlorophyceae). *J. Phycol.* **32**, 847-849.
18. Fritz, L. and R. E. Triemer. 1985. A rapid simple technique utilizing calcofluor white M2R for the visualization of dinoflagellate thecal plates. *J. Phycol.* **21**, 662-664.
19. Fukuyo, Y. 1985. Morphology of *Protogonyaulax tamarensis* (Lebour) Taylor and *Protogonyaulax catenella* (Whedon and Kofoid) Taylor from Japanese Coastal waters. *Bull. Mar. Sci.* **37**, 529-537.
20. Fukuyo, Y., P. Pholpunthin and K. Yoshida. 1988. *Protogonyaula* (Dinoflagellate) in the Gulf of Thailand. *Bull. Plank. Soc. Jap.* **35**, 9-20.
21. Goldstein, I. J., R. Hughes, M. Monsigny, T. Osawa and N. Sharon. 1980. What should be called a lectin? *Nature* **285**, 66.
22. Guillard, R. R. L. and J. H. Ryther. 1962. Studies of marine planktonic diatoms 1. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Micro.* **8**, 229-239.
23. Hallegraeff, G. M., C. J. Bolch, S. I. Blackburn and Y. Oshima. 1991. Species of the toxigenic dinoflagellate Genus *Alexandrium* in Southeastern Australian waters. *Bot. Mar.* **34**, 575-587.
24. Han, M. S., J. K. Jeon and Y. O. Kim. 1992. Occurrence of dinoflagellate *Alexandrium tamarense*, a causative organism of paralytic shellfish poisoning in Chinhae Bay, Korea. *J. Plank. Res.* **14**, 1581-1592.
25. Ishida, Y., C. H. Kim, Y. Sako, N. Hirano and A. Uchida. 1993. PSP toxin production is chromosome dependent in *Alexandrium* spp. In: Smayad, T. J. and Shimizu, Y. (eds) *Toxic Marine Phytoplankton*. Elsevier, New York, pp. 881-887.
26. Kim, C. H. 1998. Development of PSP toxigenic dinoflagellates and toxin production in Korean coastal waters. In: Kim, H. G., Lee, S. G. and Lee, C. K. (eds) *Harmful algal blooms in Korea and China*. Kuduk Publshing, Pusan, pp. 69-86.
27. Kim, C. H., Y. Sako and Y. Ishida. 1993. Variation of toxin production and composition in axenic cultures of *Alexandrium catenella* and *A. tamarense*. *Nip. Sui. Gak.* **59**, 633-639.
28. Kim, C. H. and J. B. Shin. 1997. Harmful and toxic red tide algal development and toxins production in Korean coastal waters. *Algae* **12**, 269-297.
29. Kim, H. G., S. G. Lee, W. A. Lim, J. S. Lee and J. H. Kim. 1996. Environmental physiology of *Alexandrium tamarense* isolated from Chinhae Bay, the south sea of Korea. In: Yasumoto, T., Oshima, Y. and Fukuyo, Y. (eds) *Harmful and Toxic Algal Bloom*. Intergovernmental Oceanographic Commission of UNESCO, Paris, pp. 57-60.
30. Kodama, M. 1990. Possible links between bacteria and toxin production in algal blooms. *In*: (E. Graneli, B. Sundstrom, L. Edler and D. M. Anderson, eds) *Toxic Marine Phytoplankton*. Elsevier, Amsterdam, pp. 52-61.
31. Lee, S. G. 1991. The taxonomy and distribution of marine toxic flagellates in Chinhae Bay. Ph. D. thesis. University of Kyungsung, Pusan, 112 pp. (in Korean)
32. Lee, S. G., J. S. Park and H. G. Kim. 1993. Taxonomy of marine toxic flagellates occurring in the southern coastal waters of Korea. *Bull. Nat. Fish. Res. Dev. Agency* **48**, 1-23 (in Korean)
33. Lee, T. K., H. J. Lee, H. S. Byun, K. T. Son, E. K. Oh and J. H. Park. 1998. A study of sanitation in shellfish. *Nat. Fish. Res. Inst. Rep.* **28**, 517-533. (in Korean)
34. Lorenz, M. G. and W. Wackernagel. 1994. Bacterial gene transfer by natural genetic transformation in the environment. *Micro. Rev.* **58**, 563-602.
35. MacKenzie, L., D. White, Y. Oshima and J. Kapa. 1996. The resting cyst and toxicity of *Alexandrium ostenfeldii* (Dinophyceae) in New Zealand. *Phycologia* **35**, 148-155.
36. Ogata, T., T. Ishimaru and M. Kodama. 1987. Effect of water temperature and light intensity on growth rate and toxicity change in *Protogonyaulax tamarensis*. *Mar. Biol.* **95**, 217-220.
37. Oshima, Y., T. Yasumoto, M. Kodama, T. Ogata, Y. Fukuyo and M. Matsuura. 1982. Features of paralytic shellfish poison occurring in Tohoku district. *Nip. Sui. Gak.* **48**, 525-530.
38. Penna, A. and M. Magnani. 1999. Identification of *Alexandrium* (Dinophyceae) species using PCR and rDNA-targeted probes. *J. Phycol.* **35**, 615-621.
39. Penna, A. and M. Magnani. 2000. A PCR immunoassay method for the detection of *Alexandrium* (Dinophyceae) species. *J. Phycol.* **36**, 1183-1186.

40. Pfiester, L. A. and D. M. Anderson. 1987. Dinoflagellate reproduction. In: Taylor, F. J. R. (ed) *The Biology of Dinoflagellates*. Blackwell, Oxford, pp. 611-648.
41. Scholin, C. A. and D. M. Anderson. 1994a. Identification of group and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). I. RFLP analysis of SSU rRNA genes. *J. Phycol.* **30**, 744-754.
42. Scholin, C. A., M. Herzog, M. Sogin and D. M. Anderson. 1994b. Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. *J. Phycol.* **30**, 999-1011.
43. Scholin, C. A., D. M. Anderson and M. Sogin. 1993. The existence of two distinct small-subunit rRNA genes in the toxic dinoflagellate *Alexandrium fundyense* (Dinophyceae). *J. Phycol.* **29**, 209-216.
44. Scholin, C. A., G. M. Hallegraeff and D. M. Anderson. 1995. Molecular evolution of the *Alexandrium tamarense* 'species complex' (Dinophyceae): dispersal in the North American and West pacific regions. *Phycologia* 34, 472-485.
45. Sharon, N. and H. Lis. 1989. Lectins as cell recognition recognition. *Science* 177, 949-959.
46. Shim, J. H., E. Y. Shin and J. K. Choi. 1981. A taxonomy study on the dinoflagellates of the coastal waters in the vicinity of Yeosu, Korea. *J. Oceano. Soc. Kor.* **16**, 57-98.
47. Spalter, R. A., D. Walsh, R. A. Reeves, D. J. Saul, R. D. Gray, P. L. Bergquist, L. MacKenzie and P. R. Bergquist. 1997. Sequence heterogeneity of the ribosomal RNA intergenic region of *Alexandrium* species. *Bio. Syste. Ecol.* **25**, 231-239.
48. Stedidinger, K. A. and D. G. Baden. 1984. Toxic marine dinoflagellates. In: Spector, D. L. (ed) *Dinoflagellates*. Academic Press, San Diego, pp. 201-261.
49. Taroncher-Oldenburg, G. and D. M. Anderson. 2000. Identification and characterization of three differentially expressed genes, encoding S-adenosylhomocystein hydrolase, methionine aminopeptidase, and a histone-like protein, in the toxic dinoflagellate *Alexandrium fundyense*. *Appl. Envir. Micro.* **66**, 2105-2112.
50. Taylor, F. J. R., Y. Fukuyo and J. Lasen. 1995. Taxonomy on harmful dinoflagellates. In: Hallegraeff, G. M., Anderson, D. M., Cembella, A. D. (eds) *Manual on Harmful Marine Microalgae*. UNESCO, Paris, pp. 283-317.
51. White, A. W. 1978. Salinity effects on growth and toxin content of *Gonyaulax excavata*, a marine dinoflagellate causing paralytic shellfish poisoning. *J. Phycol.* **14**, 475-479.
52. Yoo, J. S., Y. Fukuyo, B. S. Cheun, S. G. Lee and H. G. Kim. 2000. Toxic algal bloom caused by dinoflagellate *Alexandrium tamarense* in Chindong Bay, Korea. *J. Fish. Sci. Tech.* **3**, 26-32.
53. Zardoya, R., E. Costas, V. Lopez-Rodas, A. Garrido-Pertierra and J. M. Bautista. 1995. Revised dinoflagellate phylogeny inferred from molecular analysis of large-subunit ribosomal RNA gene sequences. *J. Mol. Evol.* **41**, 637-645.

초록 : 진해만에서 분리한 무독성 *Alexandrium tamarense* (Dinophyceae) 동정을 위한 molecular probe 이용

조 은 섭*
(국립수산과학원 남해수산연구소)

진해만에서 분리한 무독성 *Alexandrium tamarense*와 이미 보고된 독성을 가진 *A. tamarense* 종 간의 접속공, 제1상판, 후속공의 형태을 상호 비교하였다. 형태적으로 독성종과 거의 일치되지만, HPLC나 생체실험에서 진해만에서 새롭게 분리한 종은 독성이 전혀 나타나지 않았다. lectin 반응결과 무독종 *A. tamarense*은 PNA와 강한 반응을 보여, 세포표면에 lactose나 galactose와 같은 물질이 많이 분포하고 있음을 보였다. 또한, 단백질 전기영동시 무독종은 유독종과 달리 21 kDa 정도 부위에서 종 특이적 밴드를 보였다. 따라서 PNA lectin이나 면역학적 방법을 이용하면 무독종 *A. tamarense*을 신속하게 동정하는데 이용될 수 있다.