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Toxic Algal Bloom Caused by Dinoflagellate *Alexandrium tamarense* in Chindong Bay, Korea

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Monospecific red tide by a toxic dinoflagellate belonging to the genus *Alexandrium* occurred at Chindong Bay in the southern coast of Korea and continued from April 6th to 15th in 1997. The ratio of its cell number to total phytoplankton cell number was much higher than 95%. This organism was identified as *Alexandrium tamarense*, although slight morphological differences were found comparing to the original and successive descriptions of the species. We found neither anterior nor posterior attachment pores in these cells of the bloom population. The occurrence of red tide caused by *A. tamarense* was first reported in Korea. Its plate formula is Po, Pc, 4', 6"c, 8s, 5"' and 2"". Thecal plates are thin with pore-like ornamentation. In those plates, the anterior part of the first apical plate (1') is narrower and its posterior end has sometimes a block-like accessory, but this variation was considered within the range of the morphological variability of this taxon. The cell density during the red tide exhibited a wide range of variation by the depth of water column, ranging from 2×10^6 cells$\cdot\ell^{-1}$ to 5×10^6 cells$\cdot\ell^{-1}$. Water temperature varied from 11.8 to 12.3 ℃. Toxicity of *A. tamarense* during red tide was measured as 8.8×10^5 MU$\cdot$cell^{-1} by mouse bioassay.

Key wards: toxic dinoflagellate, *Alexandrium tamarense*, red tide

Introduction

After the 1970's, in proportion to the concentration of population in cities and the rapid growth of the Korean economy, there has been an increase in house and industrial waste water containing a high concentration of nutrients and organic materials (Lee and Kwak, 1986; Shim, 1991; Lee et al., 1994). The quality of sea water and bottom in mariculture areas on the southern coast of Korea decreased rapidly during the last two decades. At the same time, eutrophication also accelerated remarkably (Park et al., 1988; Park, 1991).

These conditions are sufficient to provide an adequate habitat for harmful algae. Recently, the red tide occurred frequently in shallow embayments all over the country. Red tide occurrences have been mainly concentrated in areas where intensive shellfish and fish farming is practiced. The red tide problem is currently having an economic impact on the mariculture industry (Park, 1991).

Paralytic shellfish poisoning (PSP) is caused by potent neurotoxins produced by some marine dinoflagellates; e.g. *Alexandrium catenella, Alexandrium tamarense, Gymnodinium catenatum, Pyrodinium bahamense*. Now the occurrences of red tide are widespread throughout the world including tropical waters (Hallegraeff, 1993; Maclean, 1993; Shumway, 1995). In Korea, the first record of paralytic shellfish poisoning took place at Kamchon Bay (near Pusan) of southern coastal waters in April, 1986 due to the ingestion of the mussel *Mytilus edulis* (Chang et al., 1987; Arakawa et al., 1989). At that time, 15 people became ill and two of them died. In 1993, contamination of mussel with paralytic shellfish poisoning occurred in most of the

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aquaculture area of Chinhae Bay and the adjacent regions. The measured toxicity of mussels and oysters was much higher than the regulatory level (80 μg STX eq/100g). Thus harvesting and marketing of mussels were banned by the Ministry of Health and Welfare. The organism responsible for the toxin contamination in this area was determined to be *A. tamarense* according to field and culture studies (Kim et al., 1996).

Since the 1980s, more frequent and long-lasting red tides by harmful dinoflagellates have occurred regularly every year in Chinhae Bay and the adjacent areas, e.g., *Gymnodinium sanguineum, Cochlodinium polykrikoides, Noctiluca scintillans, Ceratium furca, Prorocentrum miminum, P. dentatum* (Park, 1991; Kim et al., 1997). However, monospecific red tide of *A. tamarense* has not yet been reported in Korea. Also, monospecific red tide caused by this toxic species has rarely occurred elsewhere in the world.

Until now, seven species of *Alexandrium* have been identified in the coastal waters of Korea (Shim et al., 1981; Balech, 1985; Lee et al., 1993). Among these species, only *A. tamarense* was found to be toxic (Han et al., 1992; Kim et al., 1996; Kim and Shin, 1997). The detailed characteristics of *A. tamarense* in Korean waters, including morphological variation, had not yet been described, although it was identified at Chinhae Bay by Lee et al. (1993).

Some *Alexandrium* species including the *tamarense* group are not easy to identify because of their similarity to each other in cell shape (Taylor, 1975; Steidinger, 1983; Balech, 1995; Taylor et al., 1995). Therefore the identification of these species needs detailed observation of thecal plates of the following: cell shape and size, chain formation, shape of the apical pore plate (Po), position and presence or absence of ventral pore, position and presence or absence of anterior and posterior attachment pores (p.a.p.), shape of sulcal anterior plate (s.a) and sulcal posterior plate (s.p) (Fukuyo, 1985; Fukuyo et al., 1988; Balech, 1985, 1995; Steidinger and Tangen, 1996). In these characteristics, Taylor et al. (1995) emphasized the importance of these three key diagnostic plates for taxonomy of harmful *Alexandrium* species: the apical pore complex(Po), the first apical plate(1′), and the posterial sulcal plates.

The purpose of this study is to report on the monospecific red tide caused by toxic *A. tamarense* in Korean coastal waters and to describe morphological variation of this species during the bloom.

Materials and Methods

The study was carried out on April 8th, 1997, in Chindong Bay, the southern coast of Korea (Fig. 1). The red tide species were identified and counted under a light microscope with differential interference contrast optics. The thecal plates were observed after dissecting a theca using sodium hypochlorite solution and staining the same theca utilizing the chloralhydrate KI-I_2 (Fukuyo et al., 1985).

Sea water samples were collected at surface, middle and bottom waters of Chingdong Bay; water temperature, salinity and the amount of chlorophyll *a* were measured. Water temperature and salinity were measured with a portable S-T-C meter (YSI model III). Chlorophyll *a* was collected on Whatman GF/F filters and then the amount of the extraction from 90% aceton solution was measured

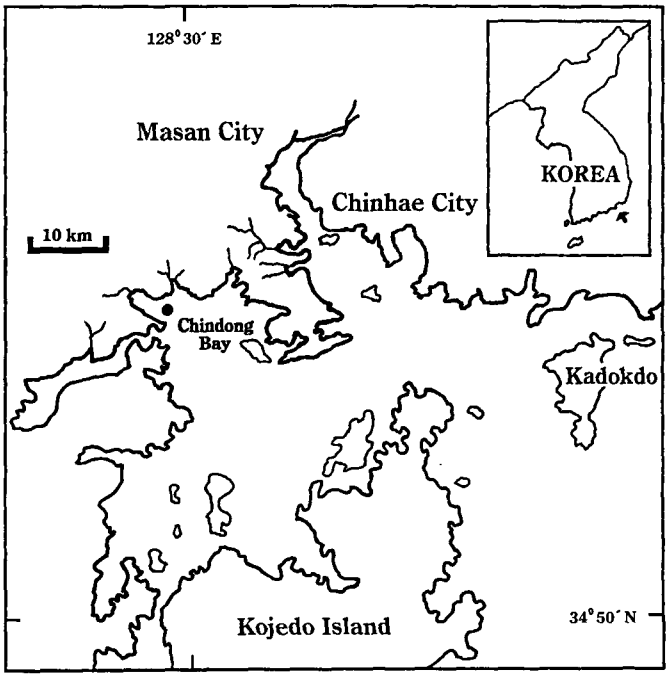
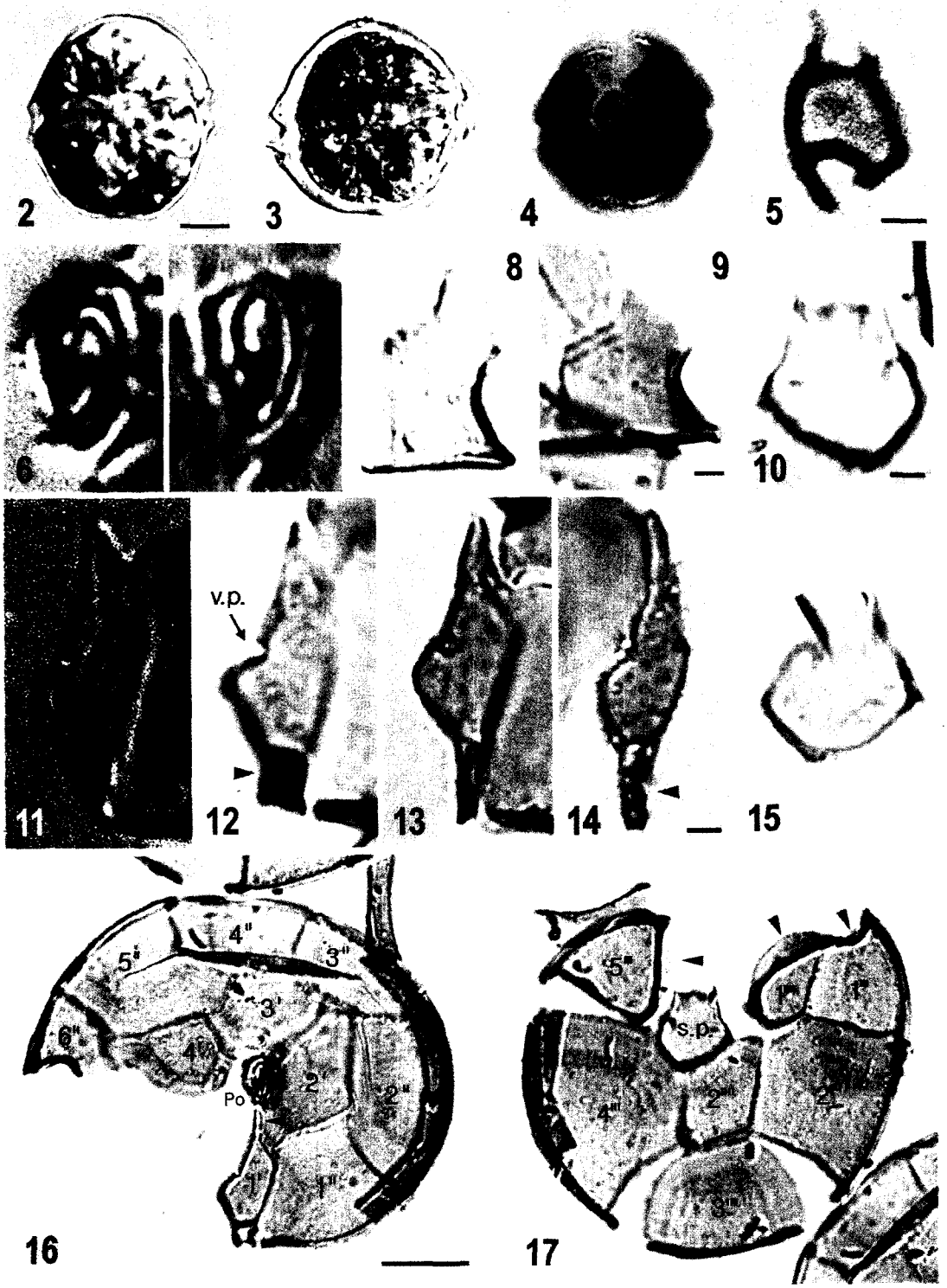


Fig. 1. Map showing sampling station in Chindong Bay, Korea.



Figs. 2~17. *Alexandrium tamarense*. Figs. 2~4. Ventral view of cell. Figs. 5. Sulcal anterior plate (s.a.). Figs. 6~7. Apical pore plate (Po). Figs. 8 and 9. Sixth precingular plates has a pore-like ornamentation. Figs. 10 and 15. Sulcal posterior plate (s.p.). Figs. 11~14. First apical plate (1′) with ventral pore (v. p.) and block-like accessary (arrowheads). Fig. 16. Epitheca. The thread-like projection on the anterior tip of the first apical plate (arrowhead). Fig. 17. Hypotheca. The first postcingular plate (1‴), fifth postcingular plate (5‴) and first antapical plate (1⁗) has a list (arrowheads). Scale bar: Figs. 2, 5, and 16=10 μm, Figs. 9, 10, and 14=2 μm.

with a UV-VIS spectrophotometer (Parson et al., 1984).

Toxin extraction was carried out as described by Oshima (1995) by means of the post-column derivatization HPLC(high performance liquid chromatography) method. Toxicity was expressed in mouse units (MU) per cell.

Results

Identification of species

Alexandrium tamerense (Lebour) Balech 1985

Figures 2~17.

Syn: *Gonyaulax tamraensis* Lebour; Lebour, 1925, p. 95, pl. 14 fig. 1a~1d; Sousa E Silva, 1962, p. 5, pl. 2, figs. 4~13, pls. 3~6, pl. 7, fig. 1; Balech, 1977, p. 119, pl. 1, figs. 15~25, pl. 2 figs. 26~35; Turpin, Dobell and Taylor, 1978, p. 235, fig. 1; Anderson and Wall, 1978, p. 226, figs. 16, 27~40, 43~50; Anderson, 1980, p. 167, figs. 1~16; Dodge, 1983, p. 216, fig. 25A~C, pl. 6e: *Gonyaulax tamarensis* var. *excavata Brraarud;* Brraarud, 1945, p. 10, pl. 2, fig. 5A: *Gonyaulax excavata* (Braarud) Balech; non Balech, 1971, p. 28, figs. 119~124; Fukuyo, 1979, p. 62, figs. 1~5; Toriumi and Takano, 1979, p. 58, fig. 3: *Gonyaulax catenella* Whedon and Kofoid; non Whedon and Kofoid, 1936, p. 25, figs. 1~7; Postek and Cox, 1976, p. 88, figs. 1~11: *Gonyaulax* sp.; Murano, 1975, p. 36, figs. 5~6: *Gessnerium tamarensis* (Lebour) Loeblich III and Loeblich; Loeblich III and Loeblich, 1979, p. 44: *Protogonyaulax tamarensis* (Lebour) Taylor; Taylor, 1979, 51; Fukuyo, 1985, p. 531. fig. 2. A~G; : *Alexandrium excavatum* (Braarud) Balech and Tangen; Balech and Tangen, 1985, p. 334, fig. 1 (A~L) and fig. 2.

The cell is small in size and is slightly longer than its width, sometimes isodiametric. Epitheca and hypotheca are nearly equal in altitude. The shoulders of the cells are either lacking or scarcely noticeable. In the ventral view, the shape is irregularly pentagonal and convex. The shape of epitheca is broadly conical but slightly convex. The hypotheca is irregularly trapezoidal and posteriorly concave; it has somewhat irregular sides. Its posterior margin is very often asymmetric. A concavity is usually located on the left side of the hypotheca. It is noticeable, but not deep. The cingulum is deeply excavated and has a descending end; the right end of the cingulum is situated lower than the left end, with both ends touching each other.

The sulcus is variably deep and posterior width is one to one-half times wider than anterior width. It has a moderately developed sulcal list. Plate formula is Po, Pc, 4′, 6″, 6c, 8s, 5‴, and 2⁗. Thecal plates are thin with pore-like ornamentations on the surface (Figs. 2~4, 16, 17).

The apical pore complex (Po) is wide and angular, sometimes with an asymmetric bullet-shape. Its anterior margin is relatively extensive and nearly straight or slightly convex. The posterior end is pointed or sometimes obliquely truncated, and it forms a direct connection with the first apical plate (1′). The right margin of Po is composed of two segments, sometimes regularly convex. The upper segment is generally straight and roughly parallel to the main axis of the plate. The lower one is sloped inward and straight; however it can sometimes be slightly concave or convex. The left margin of Po is regularly or irregularly convex. The drop-like apical pore with canopy is located on the left side of the apical pore complex and has a fully developed callus. Small marginal pores were observed in some cells. The apical attachment pore in this population can not be observed because of sealing (Fig. 6, 7, and 16).

The 1′ is irregularly rhomboidal, but its morphological variation is the largest among the plates. Usually, the anterior and posterior ends are pointed; more frequently, the posterior end is rather abruptly truncated. Sometimes a block-like accessory (Figs. 12 and 14) exists between the posterior end of the first apical plate and the anterior margin of the cingulum. The anterior right margin is slightly concave; frequently, this margin is suddenly angled at the area under the ventral pore (v.p.). The small ventral pore is always located in the center of the right margin or postmedially. The pore occurs generally on the suture between 1′ and 4′. But the degree of its indentation on the plate varies from the bare penetration to more than half way through the plate (Figs. 11~14). The sixth precingular plate (6″) is longer than its wide; in rare cases length and width are the same. Its sulcal margin is very concave, but its right margin is

generally straight. Its contact margins with 1′ and 4′ are either slightly convex or nearly straight (Figs. 8 and 9).

The first postcingular plate (1‴) has a low list (Fig. 17), which continues to the right margin of the first antapical plate (1⁗). The fifth postcingular plate (5‴) has a right sulcal list along its left margin. The second and fourth postcingular plates (2‴ and 4‴) are the largest in the hypothecal plate. The second antapical plate (2⁗) is generally longer than it is wide; sometimes they are equal. The cingulum's six plates are nearly the same in size. In the sulcal area, between the sulcal anterior plate (s. a.) and the sulcal posterior plate (s.p.), there are six plates: sulcal right anterior plate (s.r.a.), two medial plates (sulcal anterior midean plate, sulcal posterior midean plate), sulcal left anterior plate (s.l.a.), sulcal right posterior plate (s.r.p.), sulcal left posterior plate (s.l.p.). The sulcal anterior plate (s. a.) is generally a little longer than its width, sometimes of equal length and width, and has a sinus and two branches. The anterior margin is more or less straight, sometimes with two small projections. Also this margin is situated at or a little above the level of the anterior left margin of the cingulum (Fig. 4). The s.p. is usually longer than its width but its shape varies widely; some cells are slightly wider than their length. Its anterior area has more than one fold, generally two. The right and left anterior ends are pointed (Figs. 10 and 15). The anterior attachment pore (a.a.p.) and posterior attachment pore (p.a.p.) are sealed.

The cells are always solitary. Only once a two-celled chain was observed. The thecal plate is thin, frequently with pore-like ornamentation or sometimes lacking any ornamentation. Most of the thecal plates have an intercalary marginal zone. The length and width varied from 23 to 51 μm and 22 to 50 μm respectively. A cell 27 to 44 μm in length and 27 to 40 μm in width is most common. The nucleus is located just inside the cingulum.

Abundance of *A. tamarense* and physiochemical conditions during red tide

The water temperature and salinity during *A. tamarense* red tide had range, i.e. 12.3~12.7℃ and 32.4~32.8 psu. The cell number per litter of *A. tamarense* in Chindong Bay exhibited a wide range of variation by depth, ranging from 2×10^6 cells·ℓ^{-1} to 5×10^6 cells·ℓ^{-1}. And the average of chlorophyll *a* concentration was 11.24 μg·ℓ^{-1}. The number of species in the plankton community during the bloom was very low, including some diatoms and tintinneda. The ratio of *A. tamarense* to total phytoplankton cell number during the bloom was much higher than 95%. Toxicity of *A. tamarense* in the water column in Chindong Bay was measured to be 8.8×10^{-5} MU·cell^{-1}.

Discussion

Except for a few species with some peculiar characteristics, the shape of species belonging to the genus *Alexandrium* is notably homogeneous; it is very difficult to identify specific species in the genus only by their cell shape. Therefore, for the identification of these species, it is necessary to extend the observations to taxonomic details (Fukuyo, 1985; Fukuyo et al., 1988; Balech, 1985, 1995; Taylor et al., 1995; Steidinger and Tangen, 1996).

Although we found neither anterior (a.a.p.) nor posterior attachment pore (p.a.p.) in the cells of the bloom population (more than 1000 cells were observed). The cell could be identified as *A. tamarense* according to the several characteristics. The *A. tamarense* cell is similar to the other species in the *tamarense* group except *A. tropicale*, e.g. *A. fundyense* and *A. acatenella* (Balech, 1995). *A. tropicale* is distinguishable from *A. tamarense* by its lacks of anterior and posterior attachment pores; especially in the first apical plate, the size of the ventral pore is larger than in the other species and the apical portion is pointed. *A. fundyense* is different from *A. tamarense*; in that it lacks a ventral pore. *A. acatenella* is only dubiously separated from *A. tamarense* by the proportions of the epitheca to hypotheca; the length of the epitheca is greater than that of the hypotheca (Whedon and Kofoid, 1936). Furthermore, the anterior margin of the sulcal anterior plate is irregular and slightly curved (Balech, 1995).

Fukuyo (1985) reported that the most reliable key characteristics used to differentiate *A. tamarense* and *A. catenella* is the ventral pore, because other morphological differences are so slight and

changeable depending on environmental conditions and cell growth stages. This bloom species is certainly different from *A. catenella* because of the presence of a ventral pore (Balech, 1985; Fukuyo, 1985; Taylor et al., 1995). *A. leei*, which was originally found in Korea by Balech (1985), is distinguishable from *A. tamarense* by its narrow and elongated Po complex and the presence of a ventral pore inside the first apical plate.

Compared with the cells described in previous reports (Fukuyo, 1985; Balech, 1985; Han et al., 19 93; Lee et al., 1993), the cells in this study are different in that the anterior part of the 1′ is narrower and the posterior end of it has sometimes a block-like accessory. There was also a observation of a thread-like projection on the anterior tip of the 1′ (Fig. 16). These accessories are believed to be formed during cell expansion, but the existence of these accessories varies according to the sampling areas (Fukuyo, 1985; Hollegraeff et al., 1991; Lee et al., 1993; Balech, 1995). In addition, there are many cases in which these accessories are not found in the expanded cells. Therefore, the morphological variation should not be ignored. By observing these characteristics, *A. tamarense* in Korea is slightly different from other geographical strain of *A. tamarense* (Fukuyo, 1985; Hallegraeff et al., 1991; Balech, 1995). But these differences were considered to be within the acceptable range of natural morphological variation for this taxon.

Even though numerous cells were observed in this study, the apical and posterior attachment pores were not found. But the two-celled chain was rarely observed in the field, which leads one to believe in the existence of attachment pores. This belief is also supported by the observation of marks indicating the location of the closed attachment pores. In conclusion, all characteristics support that the bloom of dinoflagellate in Chindong Bay, Korea is truly caused by *A. tamarense* (Lebour) Balech.

In Korea, the PSP incident by the mussels *Mytilus edulis* occurred for the first time in 1986. One year after, the lethal potency of PSP and the cell density of the causative organism *A. tamarense* were observed as high as 680 MU·g^{-1} and 5×10^5 cells·ℓ^{-1} (Han et al., 1993). Later, this species was observed in Masan Bay, Chinhae Bay, Chilchundo Island and Yosuhae Bay (Han et al., 1992; Kim et al., 1996; Kim et al., 1997). Han et al. (1993) suggested that the distribution of toxic dinoflgaellates *A. tamarense* through the previous reports disperse more and more in Korean coastal waters; but there are no experimental evidence on this matter yet.

It is a rare phenomenon for toxic *A. tamarense* cause a monospecific red tide with 5×10^6 cells·ℓ^{-1} as it did at the southern coast of Korea (ratio of cell number of *A. tamarense* > 95%). Since the PSP incident in 1986, although research for the monitoring and control for toxic dinoflagellates has been continued by NFRDI (National Fisheries Research & Development Institute) and other organization, the increasing occurrence of harmful dinoflagellate in the southern coast of Korea become an undeniable fact (Han et al., 1992; Han et al., 1993; Lee et al., 1993; Kim, 1995; Kim et al., 1996; Kim et al., 1997). Since the 1986's PSP incident, it is believed that the frequency of the occurrence of *A. tamerense* was increased by spreading out to neighboring areas or by certain environmental changes of each habitat (Han et al., 1992; Lee et al., 1993; Kim et al., 1996). However the further study is needed on the taxonomic investigation of geological strain and cyst to clearly understand the spreading of *A. tamerense.*

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