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Harmful Algae 4 (2005) 391–400
HARMFUL ALGAE
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First report of *Alexandrium taylori* and *Alexandrium peruvianum* (Dinophyceae) in Malaysia waters

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Received 31 March 2004; received in revised form 28 June 2004; accepted 7 July 2004

Abstract

The occurrence of *Alexandrium taylori* and *Alexandrium peruvianum* is reported for the first time in Malaysia waters. The Malaysian *A. taylori* isolates were pyriform in shape with a transdiameter range of 36–40 μm and a cell length range of 33–37 μm. The first apical plate (1′) was pentagonal with two distinctive anterior margins. No direct connection between 1′ and the apical pore complex was observed. The posterior sulcal plate (S.p.) was large, elongated and oblique to the right with anterior projections. The ventral pore (vp) was relatively large and situated at a confluence point of 1′, the second apical (2′) and the fourth apical (4′) plates. Cells of *A. peruvianum* were slightly anteriorly and posteriorly compressed. S.p. had an irregular pentagonal shape, with the anterior margin divided into 2 portions. 1′ was boomerang-shaped with a large and truncated ventral pore in the middle right margin. The anterior right margin of 1′ was straight. The sixth precingular plate (6″) was wider than long. The anterior sulcal plate (S.a.) was triangular and lacked a left portion extension. In laboratory cultures, both *A. taylori* and *A. peruvianum* produced paralytic shellfish toxins, with GTX4 and GTX6 as the predominant toxin, respectively. This is the first report of PSP toxins production for both species as well as the occurrences in Malaysia waters.

Keywords: *Alexandrium taylori*; *Alexandrium peruvianum*; PSP; Sarawak; Malaysia

1. Introduction

Malaysia is one of several countries affected by harmful algal bloom (HAB) events and associated shellfish toxicity. At present, the most significant HAB-related problem in the country is paralytic shellfish poisoning (PSP), dating back to 1976 on the west coast of Sabah (Roy, 1977). Since 1991, PSP has also been

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doi:10.1016/j.hal.2004.07.001

reported from the west and east coasts of Peninsula Malaysia. At least three PSP-toxin producing marine dinoflagellate species have been confirmed in Malaysian waters, namely *Pyrodinium bahamense* var. *compressum*, *Alexandrium minutum* and *Alexandrium tamiyavanichii* (Usup et al., 2002a). Plankton samples have been routinely collected from various locations in Malaysian coastal waters as part of monitoring and research activities. Analysis of those samples revealed the presence of several proven and potentially harmful microalgal species, including those that could cause diarrhetic shellfish poisoning, amnesic shellfish poisoning and ciguatera fish poisoning.

Phytoplankton monitoring activities in Malaysia have concentrated primarily on the west coast of Sabah, and recently the monitoring programme has been expanded to the coastal areas of Peninsula Malaysia due to the augmented HAB events in the peninsula. In September 2001, a case of PSP in north east of the peninsula caused a casualty (Usup et al., 2002b) and there were massive blooms in the southern part of the peninsula (Usup et al., in press). Very few studies have been carried out in Sarawak, which is somewhat surprising considering that it is the state with the second longest coastline in Malaysia. As such, the occurrence of HAB species in the waters of Sarawak is unknown. To date, no cases of HAB-related human intoxication or fish mortality have been reported from the state, although this is no proof of absence. We report here the presence of two additional *Alexandrium* species, *Alexandrium taylori* Balech (1994) and *Alexandrium peruvianum* (Balech and Mendiola, 1977; Balech and Tangen, 1985) found in Malaysia waters. The toxicity of both species have been documented and discussed.

2. Materials and methods

Plankton samples were collected from Kuching Bay and the Sarawak River in Sarawak in September 2003 (Fig. 1). Samples were collected during high tide with a 20 μm mesh size plankton net. Some samples were fixed in Lugol's iodine solution and some were brought back live to the laboratory for cell isolation and culturing. For observation and identification of dinoflagellates, samples were stained with 1% calcofluor white and viewed under epifluorescence on an Olympus

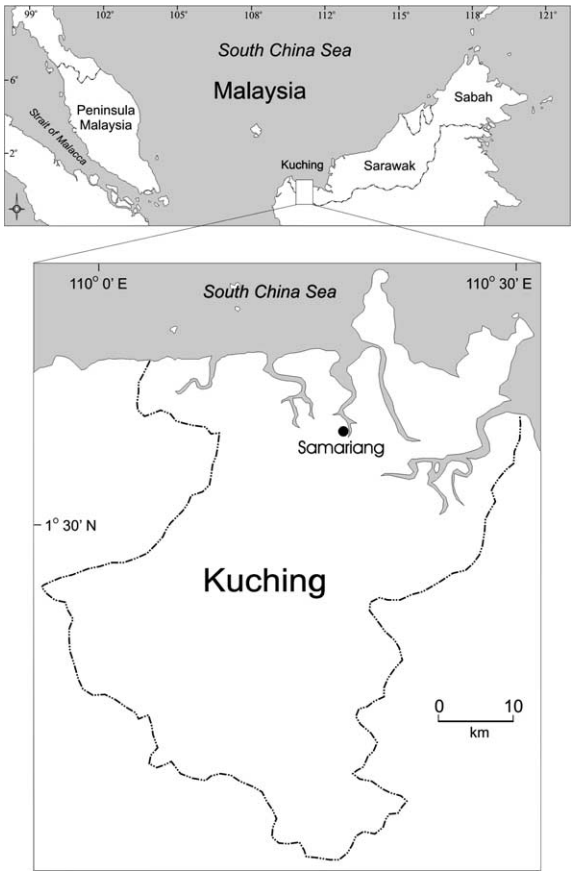

Fig. 1. Map showing location of Samariang, Kuching from where *A. taylori* and *A. peruvianum* were collected.

BX51 microscope. Images were captured using a ColorView F12 cooled CCD camera (Soft Imaging System GmbH, Germany). For culturing, individual cells were isolated by micropipeting and placed in individual wells of a 96-well tissue culture plate containing ES-DK medium (Kokinos and Anderson, 1995). The cells were allowed to divide in the wells and then transferred into culture tubes. Cultures were maintained at 26 °C under a light intensity of 70 μE m^{-2} s^{-1} on a 14:10 h light:dark cycle.

For toxin analysis, mid exponential phase cells were harvested by centrifugation at 2000 × *g* for 5 min. Cell pellet was resuspended in 0.05 M acetic acid. Cells were lysed by sonication. The slurry was further purified using Ultrafree MC centrifugal filter unit (Milipore; 5000 NMWL). HPLC analysis was carried out using the isocratic, post-column derivati-

zation method of Oshima (Oshima, 1995) on a JASCO HPLC system fitted with a fluorescence detector. The samples were run through a Wakosil C8 column (4.6 mm i.d. × 15 cm, 120 Å, 4 μm). The chromatographic conditions were as follows: for the STXs, the mobile phase was 2 mM heptanesulfonate in 30 mM ammonium phosphate buffer and 6% acetonitrile (v/v), pH 7.1; for the GTXs, the mobile phase was 2 mM heptanesulfonate in 30 mM ammonium phosphate buffer, pH 7.1; and for the C toxins, the mobile phase was 2 mM tetrabutyl ammonium in acetate buffer, pH 5.8. The post-column oxidizing reagent was 7 mM periodic acid in 80 mM sodium phosphate buffer, pH 9.0, while the acidifier was 0.5 M acetic acid. Sample injection volume was 10–20 μl. Flow rates for the mobile phases were 0.8 ml min^{-1} and 0.4 ml min^{-1} for each post-column reagent. The reaction coil temperature was kept at 70 °C water bath for all runs. Detection wavelengths were set at 330 nm excitation and 390 nm emission. Toxins identification and quantification were determined by comparison with authentic toxin standards. Further toxins identification was carried out in non-oxidizing condition by replacing the oxidizing reagent with distilled water and kept the reaction coil in ice bath. All other running conditions were identical. This simple HPLC procedure was carried out to remove 'imposter' toxin peaks. Hydrolysis of samples was carried out in 0.1N HCl in boiling water for 10 min (Hall and Reichardt, 1984).

3. Results

Several dinoflagellate species were present in the samples, but of most significance were *A. taylori* and *A. peruvianum*. Living cells of the two species were found about 3 km upstream of the mouth of the Sarawak river. Water salinity, where the cells were found, was 28 psu. Clonal laboratory cultures of the species have been established. The morphological descriptions given here were based on both wild and cultured cells.

3.1. Morphology of Alexandrium taylori

Cells of *A. taylori* were of medium size and pyriform in shape (Fig. 2A). Typical cell dimensions were 33–37 μm in length and 36–40 μm in transdiameter. The epitheca was equal in height to the hypotheca. The cingulum has no lists. The first apical plate (1′) was pentagonal in shape with two distinctive anterior margins. There was no direct connection between 1′ and the apical pore complex (APC). The ventral pore (vp) was relatively large and situated at a confluence point of the first (1′), second (2′) and fourth (4′) apical plates in most specimens (Fig. 2B). In some specimens, the vp was located between the margins of 2′ and 4′ (Fig. 2C). The 4′ was generally wide but tapered toward the sixth precingular plate (6″). The 6″ was longer than wide with a curved posterior left margin that was attached to the relatively narrow anterior sulcal plate (S.a.). There were two types of apical pore (Po) observed. The Po was typically oval to triangular in shape, although a long and narrow Po was also observed in some specimens (Fig. 2C). The posterior sulcal plate (S.p.) was large, elongated and oblique to the right. It had anterior projections on both sides that extended unequally into the sulcus (Fig. 2D). The antapical plates (1‴ and 2‴) were relatively larger (Fig. 2E). Based on these morphological characters, the species was designated as *A. taylori*.

Different stages of life cycle of this species were also documented. The resting cysts were oval to spherical in shape with a typical orange body (Fig. 2F). Gametes, which were pale in color, were observed in culture. Fusion of these gametes produced planozygotes with two transverse flagella (Fig. 2G). Vegetative cells underwent binary division as dinoflagellates generally do (Fig. 2H). Temporary cysts were formed from cells that underwent ecdysis (Fig. 2I). In some of these ecdysed cells the protoplasm was somewhat contracted (Fig. 2J). These ecdysed cells were able to divide into daughter cells (Fig. 2K). Occasionally, some of these ecdysed cells returned to vegetative cell with theca plate without undergoing cell division (Fig. 2L).

3.2. Morphology of Alexandrium peruvianum

Cells were slightly compressed in the anterio-posterior axis with dimensions in the range of 20–29 μm (transdiameter) and 20–29 μm (length). Generally, S.p. was wider than long (Fig. 3A) but some cells had longer S.p. (Fig. 3B). The S.p. was asymmetrical with a longer anterior right margin compared to the posterior right margin. The posterior left margin was

slightly curved and bent to the left. Second antapical plate, 2′′′′ was equal in dorsoventral and transverse axes (Fig. 3B). First apical plate, 1′ was boomerang-shaped with a large and truncated ventral pore in the middle of the right margin. The anterior right margin of 1′ was straight to slightly curved in some cells. 6′′ was wider than long (Fig. 3C). The Po was oval-shaped and the foramen was clearly visible with an obvious comma-head shape. Po touched the 1′ directly with a wide border between Po and 1′ (Fig. 3D). Both S.s.p. and S.d.p. were longer than wide and narrow toward the posterior margin (Fig. 3E). The S.a. was triangular in

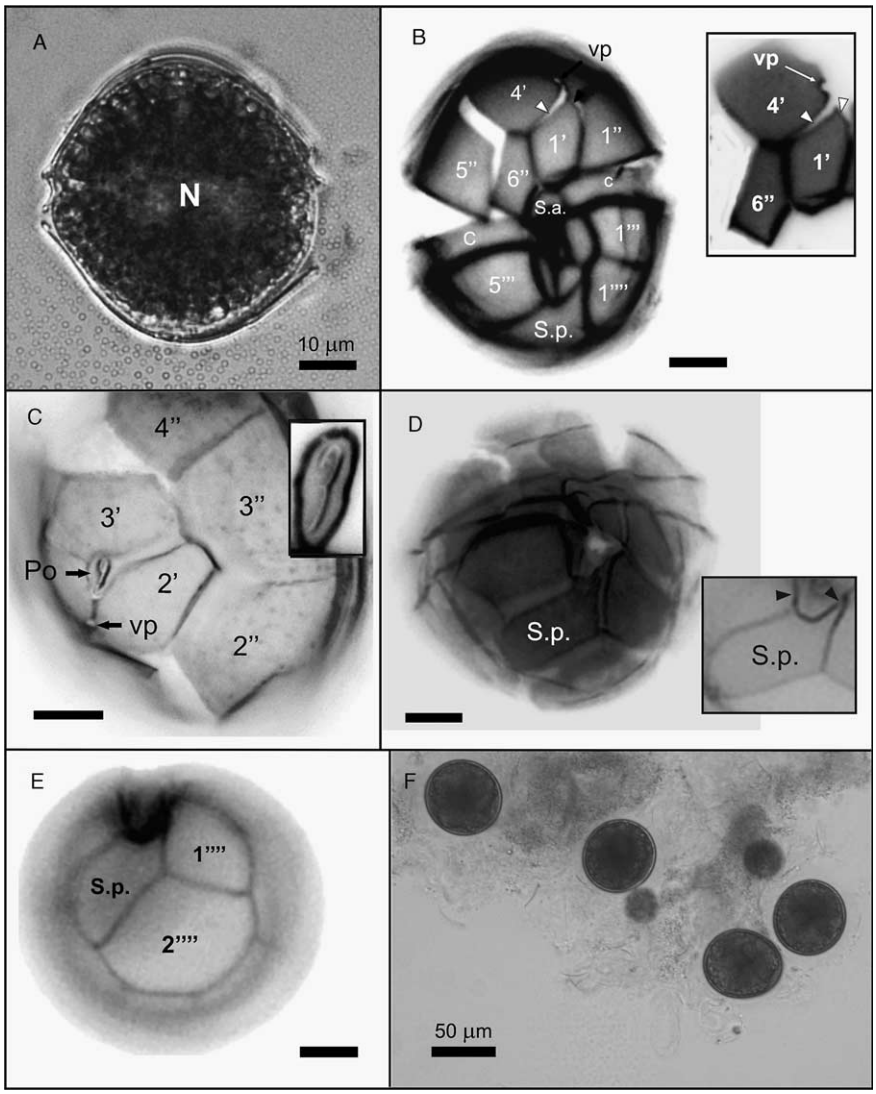

Fig. 2. Micrographs of *Alexandrium taylori*. (A) Vegetative cell with pyriform shape. (B) Ventral view of cell showed the position of ventral pore (vp) and the anterior margins (arrowheads) of first apical plate (1′) (Insert). (C) Partial apical view. Insert: a long and narrow apical pore complex. (D) Partial antapical view showed elongated posterior sulcal plate (S.p.). The S.p. is twisted to the right. Insert: The anterior projections that projected unequally into the sulcus (black arrow heads). (E) The antapical plates (1′′′′ and 2′′′′). (F) Resting cysts of *A. taylori* in culture. (G) Planozygote with two transverse flagella (arrows). (H) Binary division of vegetative cell. Arrowhead showed the cingulum position. (I) Temporary cysts formed after the vegetative cell shaded the theca plates. (J) Cell division of temporary cyst (N, nucleus). (K) Division of temporary cyst into two cells. (L) The ecdysed cell returned to vegetative cell with theca plates without undergoing cell division.

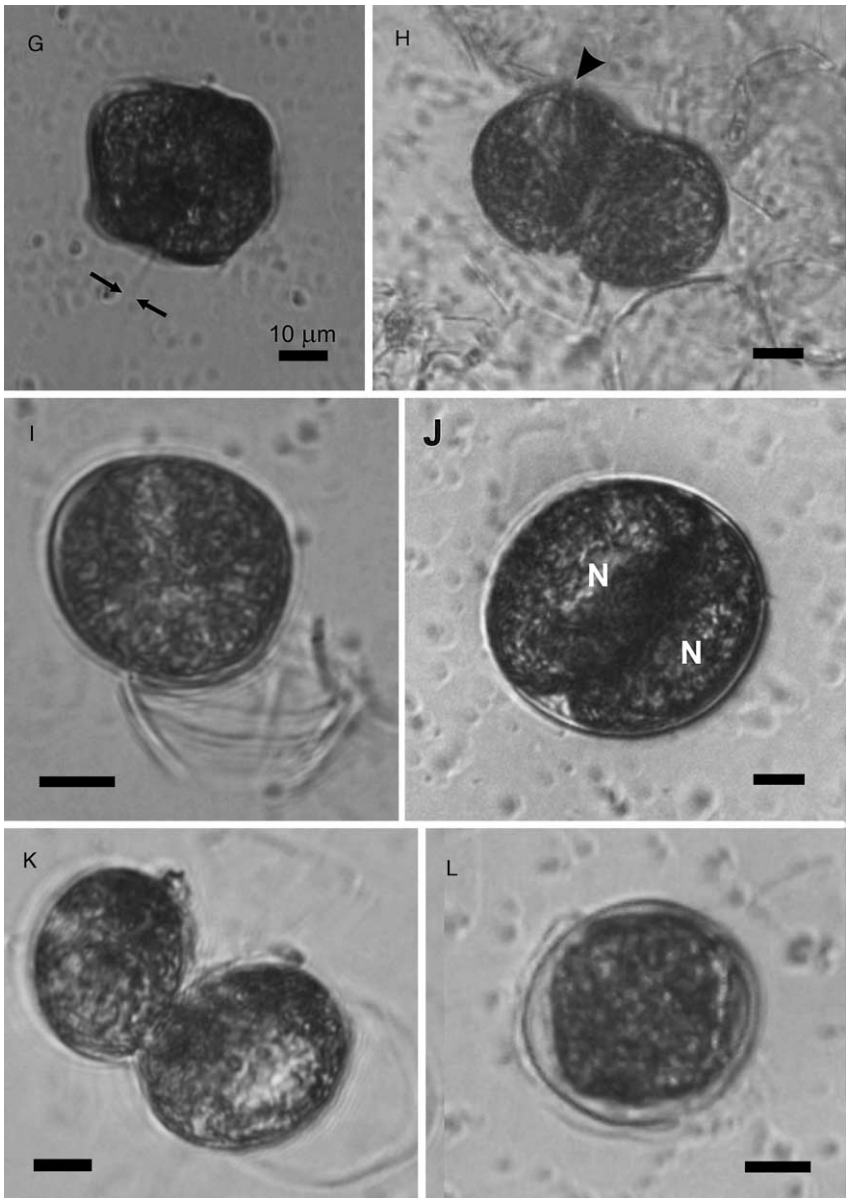

Fig. 2. (*Continued*).

overall shape and as wide as it was long. The anterior margin was very short. The plate was connected to 1′ on the right anterior margin (Fig. 3E). The hooked posterior projections formed a wide and low posterior sinus (Fig. 3E). Comparison of cell dimension and other morphological character of these two species from different localities are shown in Table 1.

3.3. Toxicity

Preliminary toxin analysis carried out using HPLC showed that both *A. taylori* and *A. peruvianum* produced PSP toxins. *A. taylori* contained dcSTX, neoSTX, STX, GTX6, GTX5, GTX4, GTX3, and GTX2, with GTX4 as the predominant toxin congener

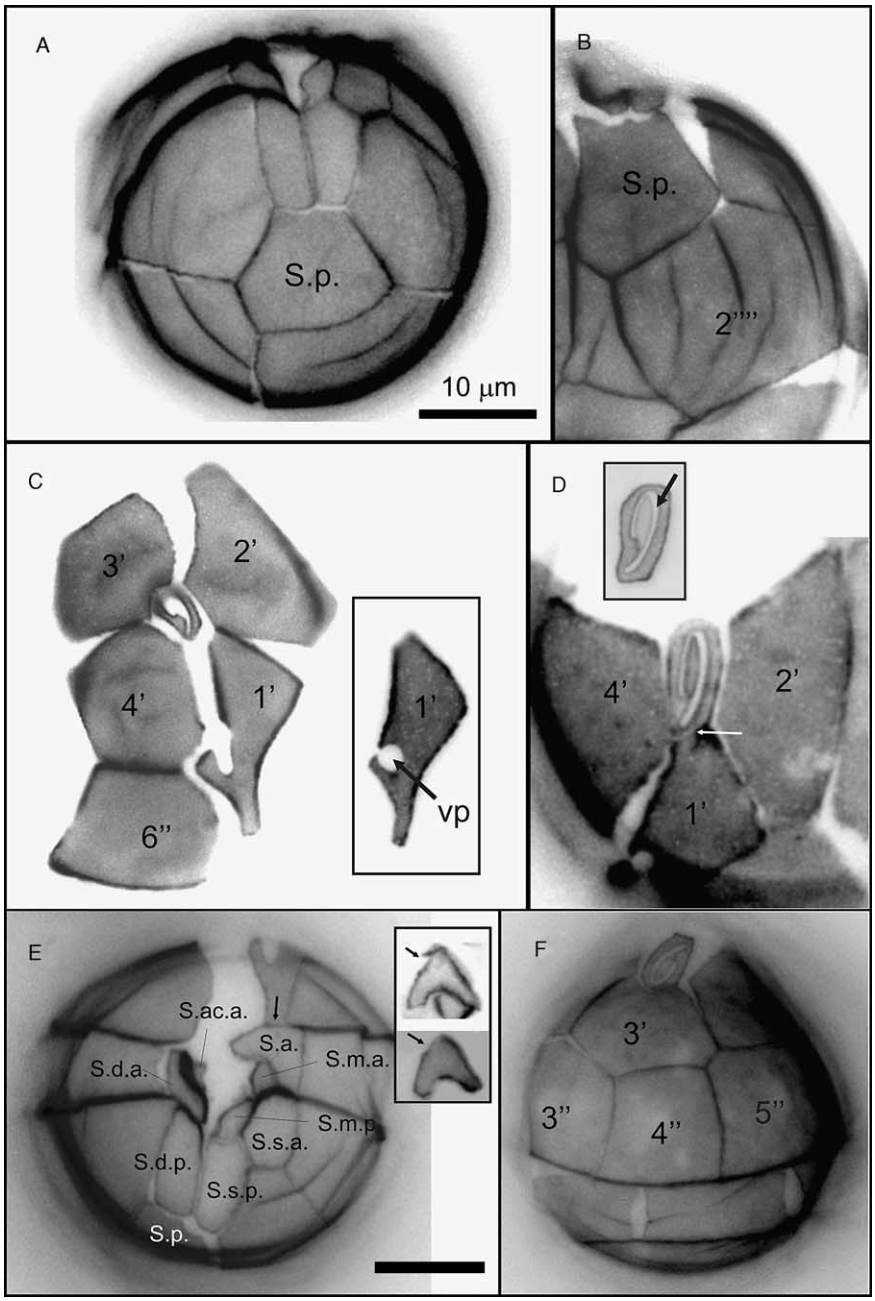

Fig. 3. Micrographs of *Alexandrium peruvianum*. (A) Posterior sulcal plate (S.p.) showing irregular shape with left posterior margin bended toward and to the left. (B) Second antapical plate ($2''''$) is longer in the dorsaventral axis than the width. (C) The first apical plate ($1'$) with truncated big ventral pore (vp) on the right margin and the slightly curved right anterior margin (Insert). (D) Apical pore complex (APC) without anterior attachment pore. Connection between first apical plate ($1'$) and APC (white arrow). Insert: arrow indicates a narrow foramen with an obvious comma shape. (E) Details of sulcal plates. S.s.p. and S.d.p. are longer than wide and narrow toward posterior margin. Note the border of right anterior margin of S.a. and $1'$ (arrow). Insert: triangular shape of S.a. without left extension. (F) Dorsal epitheca view showing asymmetry third apical plate ($3'$).

Table 1
Comparison of *A. peruvianum* and *A. ostenfeldii* found in different biogeographical locations

Species	Locality	Dimension[a]	Main morphological characters	Toxicity	References
A. peruvianum	Malaysia	*T*: 20–29 μm, *L*: 23–29 μm	Curved and straight 1′ right margin, triangular S.a.	GTX 6, GTX4, GTX1, GTX5 dcSTX, STX	This study
A. peruvianum	Peru	*T*: 29–41 μm, *L*: 25–40 μm	Curved 1′ right margin, triangular S.a.	N.A.	Balech (1995)
A. peruvianum	North America	*T*: 33–44 μm, *L*: 33–44 μm	Curved 1′ right margin, triangular S.a.	N.A.	Balech (1995)
A. ostenfeldii	Denmark	N.A.	N.A.	GTX6, C1, C2, Trace: C2 and 3	Hansen et al. (1992)
A. ostenfeldii	Denmark	N.A.	N.A.	GTX6, GTX4, C3, C4	Ravn et al. (1995)
A. ostenfeldii	Denmark	*L*: 30–36 μm	Straight 1′ right margin	N.A.	Jensen and Moestrup (1997)
A. ostenfeldii	Canada	*T*: 50.5 ± 5.8 μm, *L*: 48.7 ± 5.3 μm (natural samples), *T*: 29.4 ± 3.5 μm, *L*: 29.8 ± 3.6 μm (culture)	Straight 1′ right margin	Spirolide (no PSP)	Cembella et al. (2000)
A. ostenfeldii	New Zealand	*T* (*L*): 26–40 μm	Straight 1′ right margin; triangular S.a. with left extension	GTX3, GTX5, STX	Mackenzie et al. (1996)

[a] *T*, transdiameter; *L*, length; N.A., not available.

(up to 40% mole) (Fig. 4B and E). Toxin profiles remained consistent over the study period. However, toxin composition varied in these analysis. *A. peruvianum*, on the other hand, produced STX, dcSTX (≤20% mole), neoSTX (≤20% mole), GTX6 (≤45% mole), GTX4, GTX1, GTX5, and GTX2, with GTX6 as the major toxin component (Fig. 4C and F). Similar to *A. taylori*, toxin profile remained a stable character but the toxin composition varied. The respective toxin peaks disappeared in the absence of oxidizing reagent. Presence of GTX5 and GTX6 was further confirmed by disappearance of the peaks and increase in STX and neoSTX peak areas after hydrolysis by 0.1N HCl.

4. Discussions

Morphological differences observed between the Malaysian isolates and previously described *A. taylori* (Balech, 1995) were minor. The ventral pore in some of our specimens was located between the 2′ and 4′ plates and not just between the 1′, 2′ and 4′ plates as described by Balech (1995). In addition, the existence of more than one ventral pore in other specimens (Balech, 1994; Delgado et al., 1997) was not observed in the Malaysian isolates. The importance of the ventral pore in taxonomic diagnostics of *Alexandrium* species has recently been questioned (Hansen et al., 2003). Most probably minor morphological differences exist between different geographical isolates of the species.

Both temporary and resting cysts were produced in clonal cultures of *A. taylori*. Since resting cysts were products of sexual reproduction, it can be concluded that the species was homothallic. The dormancy period of the resting cysts has not been determined. There were two types of cell division observed in the cultures, in cells that were still thecate and in ecdysed cells. Interestingly, binary division involving thecate cells was not as common as divisions involving ecdysed cells. Observations of living cells showed that temporary cysts were the first products of ecdysis and that these temporary cysts were able to undergo cell division or eleutheroschisis. A similar observation has been reported for other isolates of the species in earlier studies (Garcés et al., 1998; Garcés et al., 2002). Eleutheroschisis is not a new phenomenon as it has been described in several other dinoflagellate species (Silva and Faust, 1995; Montresor, 1995).

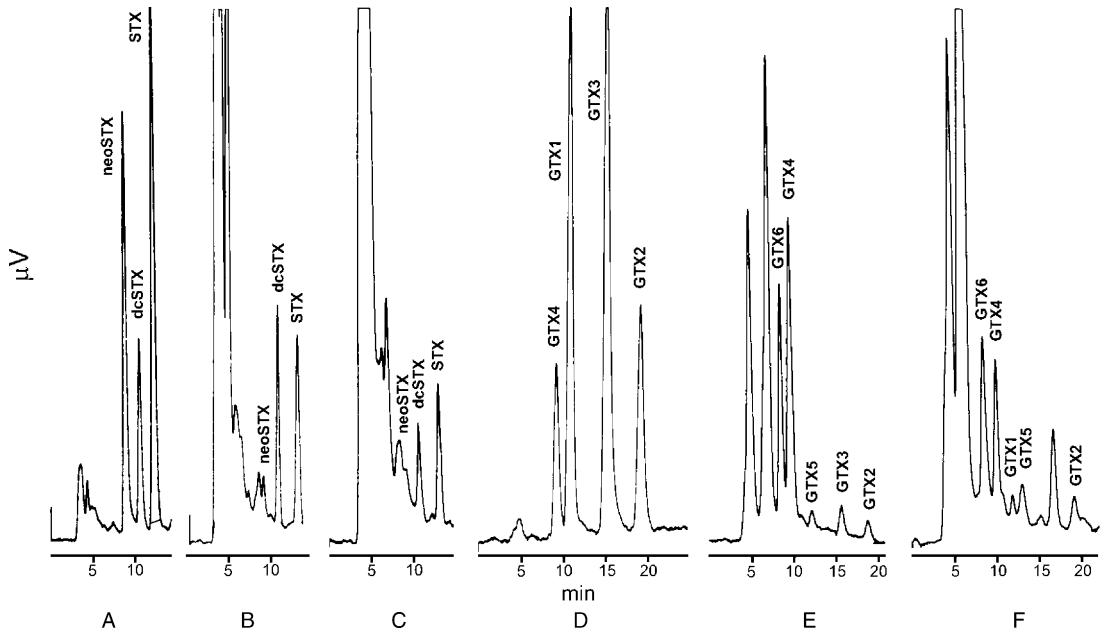

Fig. 4. Toxin profiles of *Alexandrium taylori* and *A. peruvianum*. (A–C), STXs run: (A) STXs standard, (B) *A. taylori*, (C) *A. peruvianum*. (D–F), GTXs run: (D) GTXs standard, (E) *A. taylori*, (F) *A. peruvianum*.

Identification of wild and cultured specimens of *A. peruvianum* in this study was not a straightforward task. Preliminary morphological observations of the first apical plate, large ventral pore, and anterior as well as posterior sulcal plates indicated that the specimens could have been either *A. ostenfeldii* or *A. peruvianum*. One of the main features that has been used to distinguish *A. ostenfeldii* from *A. peruvianum* is the shape of the first apical plate. Mackenzie et al. (1996) differentiated New Zealand strains of *A. ostenfeldii* from *A. peruvianum* by the straight anterior right margin of the 1′ plate in the former. In our wild and cultured specimens, both straight and slightly curved anterior margins of 1′ were observed. Balech (1995) also showed that both straight and curved anterior right margins of 1′ were present in various specimens of *A. peruvianum* he examined. However, detailed observation of cultured specimens in the present study showed that a straight anterior margin of 1′ was more common than a curved margin. This observation suggested that the characteristic of the anterior right margin of 1′ might not be a useful character to differentiate between *A. ostenfeldii* and *A. peruvianum*. Some workers also suggested that these two species might be conspecific (Faust and Gulledge, 2002).

Balech (1995) in his description of *A. peruvianum* emphasized that the S.a. should be the primary morphological characteristic to distinguish *A. ostenfeldii* from *A. peruvianum*. In the latter, the S.a. is triangular in shape with a sharp anterior margin and low posterior sinus while in the former, the S.a. always exhibits a left portion extension. Observation of the S.a. in our specimens consistently showed that this plate was triangular and lacked a left portion extension. For this reason, the isolates were identified as *A. peruvianum*.

Another feature that could differentiate the two species is the S.p. Both *A. peruvianum* and *A. ostenfeldii* have a wide, asymmetrical S.p. The difference is in the shape of the left posterior margin of the plate and the length of the plate. Balech (1995) characterized *A. peruvianum* as having a longer S.p. with the left posterior margin bent forward and to the left, while *A. ostenfeldii* has a wider S.p. with a fairly straight left posterior margin. Observation of the S.p. in our specimens also indicated similarity to typical *A. peruvianum*. Finally, cell sizes of our specimens were also typical of *A. peruvianum*, which tend to be smaller than *A. ostenfeldii* (Balech, 1995).

Very few studies on the toxicity of *A. taylori* have been reported. Blooms of *A. taylori* have been reported

in the Mediterranean Sea, causing serious impact on the marine ecosystem and loss of recreational value of coastal regions (Penna et al., 2000). These events, however, were not related to PSP toxin production. In fact, as far as we were aware there has been no previous report of PSP toxin production in this species. A study carried out on a Japanese strain of *A. taylori* showed that this species was capable of producing a proteinaceous exotoxin with hemolytic activity (Emura et al., 2004). Thus, the present study provides the first evidence of PSP toxin production in *A. taylori* and also in the subgenus *Gessnerium*. However, the toxin content in this species was extremely low at less than 1 fmole per cell.

The presence of PSP toxins in A. *peruvianum* was less surprising considering the very close phylogenetic affiliation of the species to *A. ostenfeldii*, if the two are in fact not the same species. PSP toxin profiles of *A. ostenfeldii* have been documented by several workers (Hansen et al., 1992; Ravn et al., 1995; MacKenzie et al., 1996). Likewise, non-PSP toxin producing strains produced spirolide, a lipophilic fast acting toxin (Cembella et al., 2000). Further indication of the genetic relatedness of the two species was provided by their highly similar toxin profiles (Table 1).

Conclusive evidence for the presence of PSP toxins in the two species studied could only be provided by structural analysis using NMR or LC/MS. Previous studies have shown that 'imposter' PSP toxin peaks could be generated in shellfish and phytoplankton extracts analyzed by HPLC (Genenah and Shimizu, 1981). Unfortunately, there was insufficient culture material for LC/MS analysis, although this issue should be addressable in the near future. Nonetheless, results of non-oxidizing procedure as well as hydrolysis with 0.1N HCl provided strong evidence for the presence of PSP toxins in the two species.

This discovery of *A. taylori* and *A. peruvianum* brings the currently known number of *Alexandrium* species in Malaysian waters to at least seven. The other species are *A. affine*, *A. leei*, *A. minutum*, *A. tamarense*, *A. tamiyavanichii*. There is a good possibility that more *Alexandrium* species could be present considering that in the Vietnam region of the South China Sea at least 15 species have been reported (Nguyen-Ngoc, 2002). Meanwhile, the number of currently known PSP toxin-producing dinoflagellate species in Malaysia is five. Results of this study also indicated that the current shellfish toxicity-monitoring program in Malaysia should be expanded to at least include the localities where *A. taylori* and *A. peruvianum* were found. Clonal culture of *A. peruvianum* and *A. taylori* established in this study also provide valuable material for further comparative study of these species.

Acknowledgements

This work was funded in part by Universiti Malaysia Sarawak (UNIMAS) through the short-term research grant. Lim was supported by scholarship from UNIMAS and Malaysian Government. Thanks to Joanne, BoonKoon Ng and MeiYee Cheah for assistance in culture establishment.

References

Balech, E., 1994. Three new species of the genus *Alexandrium* (Dinoflagellata). Trans. Am. Micros. Soc. 113, 216–220.

Balech, E., 1995. The Genus *Alexandrium* Halim (Dinoflagellata), Sherkin Island Marine Station, Cork, Ireland.

Balech, E., Mendiola, B.R., 1977. Un Nuevo *Gonyaulax* productor de hemotalasia en Peru. Neotropica 23, 49–54.

Balech, E., Tangen, K., 1985. Morphology and taxonomy of toxic species in the *tamarensis* group (Dinophyceae) *Alexandrium excavatum* (Braarud) com. nov. amd *Alexandrium ostenfeldii* (Paulsen) comb. nov. Sarsia 70, 333–343.

Cembella, A.D., Lewis, N.I., Quilliam, M.A., 2000. The marine dinoflagellate *Alexandrium ostenfeldii* (Dinophyceae) as the causative organism of spirolide shellfish toxins. Phycologia 39 (1), 67–74.

Delgado, M., Garcés, E., Vila, M., Camp, J., 1997. Morphological variability in three populations of the dinoflagellates *Alexandrium taylori*. J. Plankton Res. 19 (6), 749–757.

Emura, A., Matsuyama, Y., Oda, T., 2004. Evidence for the production of a novel proteinaceaous hemolytic exotoxin by dinoflagellate *Alexandrium taylori*. Harmful Algae 3 (1), 29–37.

Faust, M.A., Gulledge, R.A., 2002. Identifying Harmful Marine Dinoflagellate, Smithsonian Institution.

Garcés, E., Delgado, M., Masó, M., Camp, J., 1998. Life history and *in-situ* growth rate of *Alexandrium taylori* (Dinophyceae, Pyrrophyta). J. Phycol. 34, 880–887.

Garcés, E., Masó, M., Camp, J., 2002. Role of temporary cysts in the population dynamics of *Alexandrium taylori* (Dinophyceae). J. Plankton Res. 24 (7), 681–686.

Genenah, A.A., Shimizu, Y., 1981. Specific toxicity of paralytic shellfish poisons. J. Agric. Food Chem. 29, 1289–1291.

Hall, S., Reichardt, P.B., 1984. Cryptic paralytic shellfish toxins. In: Ragelis, E.P. (Ed.), ACS Symposium Series 262, American Chemical Society, Washington DC, pp. 113–123.

Hansen, P.J., Cembella, A.D., Moestrup, Ø., 1992. The marine dinoflagellate *Alexandrium ostenfeldii*. Paralytic shellfish toxin concentration, composition, and toxicity to a tintinid ciliate. J. Phycol. 28, 597–603.

Hansen, G., Daugbjerg, N., Franco, J.M., 2003. Morphology, toxin composition and LSU rDNA phylogeny of *Alexandrium minutum* (Dinophyceae) from Denmark, with some morphological observations on other European strains. Harmful Algae 2, 317–335.

Jensen, M., Moestrup, Ø., 1997. Autoecology of the toxic dinoflagellate *Alexandrium ostenfeldii*: life history and growth at different temperature and salinities. Eur. J. Phycol. 32, 9–18.

Kokinos, J.P., Anderson, D.M., 1995. Morphological development of resting cysts in cultures of the marine dinoflagellate *Lingulodinium polyedrum* (= *L. machaerophorum*). Palynology 19, 143–166.

Mackenzie, L., White, D., Oshima, Y., Kapa, J., 1996. The resting cyst and toxicity of *Alexandrium ostenfeldii* (Dinophyceae) in New Zealand. Phycologia 35, 148–155.

Montresor, M., 1995. The life history of *Alexandrium pseudogonyaulax* (Gonyaulacales Dinophyceae). Phycologia 34, 444–448.

Nguyen-Ngoc, L., 2002. Biology and taxonomy of dinoflagellates in Vietnamese waters. PhD. Thesis. Botanical Institute, University of Copenhagen, Copenhagen.

Oshima, Y., 1995. Post-column derivatization HPLC methods for paralytic shellfish poisons. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (Eds.), Manual on Harmful Marine Microalgae, IOC/UNESCO (IOC Manual and Guides, 33)pp. 81–94.

Penna, A., Giacobbe, M.G., Andreoni, F., Garces, E., Bertuli, S., Magnani, M., 2000. Molecular characterization of Mediterranean isolates of the HAB dinoflagellate *Alexandrium taylori*: preliminary intra- and interspecies analysis. In: Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J. (Eds.), Harmful Algal Blooms, IOC/UNESCO, Paris, pp. 218–221.

Ravn, H., Schmidt, C.U., Sten, H., Anthoni, U., Christophersen, C., Nielsen, P.H., 1995. Elicitation of *Alexandrium ostenfeldii* (Dinophyceae) affects the toxin profile. Comp. Biochem. Physio. 111C (3), 405–412.

Roy, R.N., 1977. Red tide and outbreak of paralytic shellfish poisoning in Sabah. Med. J. Malays. 31 (3), 247–251.

Silva, E.S., Faust, M.A., 1995. Small cells in the life history of dinoflagellate (Dinophyceae): a review. Phycologia 34, 396–408.

Usup, G., Leaw, C.P., Ahmad, A., Lim, P.T., 2002a. *Alexandrium* (Dinophyceae) species in Malaysian waters. Harmful Algae 1, 265–275.

Usup, G., Leaw, C.P., Lim, P.T., Ahmad, A., 2002b. Probable toxin producer responsible for the first occurrence of paralytic shellfish poisoning on the east coast of Peninsula Malaysia. Malays. Appl. Biol. 31 (2), 29–35.

Usup, G., Cheah, M.Y., Rozirwani, Ng, B.K., Leaw, C.P., Othman, M., Faazaz, A.L., in press. Identification of the species responsible for the harmful algal blooms event in Tebrau Strait in 2002. Malays. Appl. Biol.