OCCURRENCE OF THREE ALEXANDRIUM SPECIES, A. affine, A. tamutum and A. tamiyavanichii IN KUCHING WATERS

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Abstract

A field survey was carried out in Kuching waters to monitor harmful microalgae in Kuching waters. Samples were collected fortnightly from Semariang Batu and Santubong estuaries during high tide. Live samples were used for culture establishment, while preserved samples were processed for morphological observation under epifluorescence microscopy. The occurrence of Alexandrium affine, A. tamutum and A. tamiyavanichii is reported for the first time in the coastal waters of Sarawak, with A. tamutum as a new record in Malaysian waters, which increased the number of Alexandrium species found to eight species. They are A. affine, A. leei, A. minutum, A. peruvianum, A. tamarense, A. tamiyavanichii, A. tamutum and A. taylori. This study has provided further information to the microalgae species inventory of the country.

Keywords: Alexandrium affine, A. tamutum, A. tamiyavanichii, Kuching.

1. INTRODUCTION

Since the first outbreak of harmful algal blooms (HABs) related shellfish poisoning event in 1976, paralytic shellfish poisoning (PSP) has caught the attention of researchers in the county. Besides the well-known PSP-toxin producer, Pyrodinium bahamense var. compressum, other PSP-toxin producing organisms, particularly species in the genus Alexandrium has become the main focus in local research (Usup et al., 2002a, b; Lim et al., 2003, 2004, 2005a, 2006, 2007; Lim and Ogata, 2005; Leaw et al., 2005). Five species of Alexandrium had been reported from the coasts of Malaya (Usup et al. 2002b, Lim et al. 2005). They were A. affine, Alexandrium leei, A. minutum, A. peruvianum, A. tamarense, A. tamiyavanichii and A. taylori. Distribution of each species is scattered, and not all species reported were present throughout the waters. Alexandrium affine was found in the northern and southern of the Straits of Malacca. On the other hand, A. tamiyavanichii was found only in the central to southern of the Straits of Malacca (Anton et al., 2000; Usup et al., 2002b). Two Alexandrium species were reported previously from Kuching waters, A. peruvianum and A. taylori (Lim et al., 2005). The occurrence of these toxic species in Sarawak will certainly pose a threat to the aquaculture industries and public health if blooms of these species occur. In this field survey, we aim to document the occurrence of this genus and to provide reference micrographs for country HAB monitoring purposes.

2. MATERIALS AND METHODS

2.1 Samples

Field samplings were undertaken fortnightly at two sampling sites in the estuary of Kuching, Sarawak. Qualitative plankton samples were collected using a 20 µm mesh size plankton net. Concentrated samples were preserved in Lugol’s iodine solution. Live samples were collected for isolation and
establishment of clonal cultures. Single cell isolation technique was used to obtain clonal cultures. Cultures were maintained in ES-DK medium (Kokinos & Anderson, 1995) at 25°C under 12h: 12h light: dark photoperiod.

2.2 Morphological observation

Field and cultured samples were examined under an Olympus IX51 inverted microscope (Olympus, Japan) using normal light under 200 to 600×. For epi-fluorescence microscopy, cells were fixed in 2% formaldehyde and the theca plates were stained with Fluorstain (Fluka, Japan) for 1 min. Samples were transferred onto a microscope slide and covered with slips. Samples were then viewed under Olympus IX51 epifluorescence microscope equipped with UV filter set. Digital images were captured using XC30 CCD camera (SIS, Germany). Identification was based on Balech (1995).

2.3 Toxin Analysis

Cultures at exponential phase were harvested by centrifugation (2,000×g, 5 min). Cell pellets were then extracted using 5% acetic acid and tested on SKit ELISA for PSP (Shin Nihon Kentei Kyokai, Japan) according to the manufacturer's instruction.

3. RESULTS AND DISCUSSION

3.1 Alexandrium affine (Inoue and Fukuyo) Balech (1985)

3.1.1 Morphology

Alexandrium affine is a chain forming species which usually forms chains of 2-8 cells long (Figure 1A). The cells are normally longer (29-66 μm) than wide (23-65 μm) and convex-pentagonal. The epitheca is longer than the hypotheca, and is conic-convex. The first apical plate (1°) is directly connected with apical pore plate and a small ventral pore (vp) is present toward the anterior of 1° along the right margin (Figure 1C). The sulcal plate of the A. affine is longer than wide, with both anterior ends well projected (Figure 1D).

The posterior sulcal plate (s.p) is wider than long (Figure 1E). The main diagnostic feature of this species is the location of the anterior attachment pore in the APC. The anterior attachment pore of the A. affine is located at the dorsal half of APC (Figure 1F).

3.1.2 Distribution

Alexandrium affine was previously reported in Japan, Korea, Spain, Portugal, Gulf of Thailand and Philippines (Balech, 1995). It was also found in Vietnam (Nguyen-Ngoc, 2004). In Malaysia, the species was previously reported from Sebatu and Pulau Aman in the Straits of Malacca (Usup et al. 2002). A. affine was found in Semariang Batu, Kuching in this study.

3.1.3 Toxicity

Toxicity test showed undetectable value from the extract.
**3.2 Alexandrium tamutum** Montresor, Beran and John (2004)

### 3.2.1 Morphology

*Alexandrium tamutum* is a solitary single cell, oval shape, relatively small, 24-35 µm long and 26-32 µm wide (Figure 2A). The *A. tamutum* cells have a short and wide posterior sulcal plate (SP) (Figure 2F). The first apical plate (1’) is directly connected with apical pore plate and a small ventral pore is present which located toward the anterior of 1’ along the right margin (Figure 2D-E). It also lacks of the anterior attachment pore at the APC (Figure 2E). The kidney-shape nucleus of the cell is located at middle of the cell (Figure 2C). The non-toxic *A. tamutum* cells are morphologically similar to the PSP toxin producer *A. minutum*. The only distinctive feature between *A. tamutum* and *A. minutum* is the 6’’ precingular plate. *A. tamutum* has a wider (width: length, ≈1) precingular plate (6’’) while *A. minutum* has a narrower (width: length, ≈0.5) precingular plate (6’’) (Figure 2D).

### 3.2.2 Distribution

*Alexandrium tamutum* was first reported from Adriatic, Tyrrhenian, Mediterranean Sea (Montresor et al. 2004), Northwest Pacific (Yoshida, 2002) and Sea of Okhotsk, Russia (Selina & Morozova, 2005). In Malaysia, the species was first discovered from Semariang Batu, Kuching and Kudat, Malaysia. This represents a new record of *Alexandrium* species occurrence in Malaysia.

### 3.2.3 Toxicity

No detectable value was shown in the extract of *A. tamutum*. 
3.3 Alexandrium tamutum (AuKA01) from Kuala Abai, Sabah. LM. (A) Solitary cell with relatively oval shape. (B) Auto-fluorescence of the cell showing the arrangement of chloroplast. Scale bar = 100 μm. (C) Fluorescence of kidney-shape nucleus. Scale bar = 100 μm. (C) Ventral view of cell, vp: ventral pore. 1', 4': first, forth apical plates. 1'', 5'', 6'': first, fifth, sixth precingular plates. S.a: anterior sulcal plate. (E) Apical view of the cell, APC: apical pore complex. (F) Antapical view of the cell. 2''', 3''', 4'''': second, third, fourth postcingular plates. 1'''', 2'''': first, second antapical plates. Sp: posterior sulcal plate. Scale bars= 10 μm.

3.3.1 Morphology

Under light microscope, cells are round and heavy pigmented (Figure 3A, B). Cells in chains of 2, 4 and 8-cells are frequently observed (Figure 4A). Calcofluor-stained thecal plates are easily diagnosed under a fluorescence microscope. First apical plate (1') is rhomboidal and link directly to the apical pore complex (APC) (Figure 4B-E). The ventral pore (vp) is present on the anterior right margin of the 1' (Figure 4B). The apical pore (po) is oval with a large anterior attachment pore located at the anterior right margin of the attachment pore in which this characteristic were specifically observed in certain Alexandrium species including A. tamutum (Figure 4C). However, there is absent of anterior attachment pore in some specimens (Figure 4D).

The long anterior sulcal plate (s.a) had a triangular to trapezoidal precingular part (p.pr.) which attached to slightly concave posterior margin of 1'. The posterior sulcal plate (s.p.) is longer than wide (Figure 4F), with a posterior pore connected by an irregular furrow to the right margin of the plate. The second antapical plate (2'''') is wide (Figure 4G).

3.3.2 Distribution

Alexandrium tamutum was reported from Japan (Ogata et al. 1990; Nagai et al. 2005), Brazil (Menezes et al. 2010) , South Africa (Ruiz Sebastian et al. 2005) , Thailand (Kodama et al. 1988) and
peninsular Malaysia (Lim et al. 2006). *Alexandrium tamiyavanichii* was reported from Semariang Batu in this study (New record).

### 3.3.3 Toxicity

Extract showed positive result in the ELISA assay.

**Figure 3** *Alexandrium tamiyavanichii, AcSm01* from Semariang, Sarawak. LM. (A) A chain of two vegetative cells. (B) Red autofluorescence showing the chloroplast content. Scale bar = 10 µm.

**Figure 4** *Alexandrium tamiyavanichii* (AcSm01) from Semariang, Sarawak. (A) Chain of four vegetative cells. (B) Apical-ventral view of cell. pr.p: precingular part. s.a: anterior sulcal plate. There’s an oblique posterior end of 1’ and a triangular shape of the p.pr. Apical plates 1’- 4’ and precingular plates 1’’, 2’’, 4’’ – 6’’. (C) Dorsal-apical view showing apical pore (Po) and precingular plates 2’’ – 5’’. (D) Close-up of Po. (E) Apical view showing the ventral pore (v.p). (F) Antapical-ventral view showing postcingular plates 1’’’, 4’’’, 5’’’ and antapical plate 1’’’’. (G) Dorsal-antapical view showing postcingular plates 2’’’, 3’’’, 4’’’ and antapical plate 2’’’’. Scale bars = 10 µm.
4. CONCLUSION

The new discovery of *A. affine*, *A. tamiyavanichii* and *A. tamutum* in this study has increased the known number of *Alexandrium* species in Malaysian waters to eight species. Total of five species were reported from Sarawak waters thus far, namely *A. taylori*, *A. peruvianum* (Lim et al. 2006), *A. affine*, *A. tamiyavanichii* and *A. tamutum* (this study). The increasing number of toxic species in Malaysian waters should be taken seriously by the monitoring agencies. Clonal cultures deposited in the University Malaysia Sarawak (UNIMAS) culture collection will be useful for further study in the future.

REFERENCES


