

ABSTRACT

Pseudo-nitzschia and *Alexandrium* are harmful algal blooms (HAB) genera that commonly found in the Malaysian waters. Both genera were reported as toxic species and associate with the shellfish contamination in the Southeast Asia region. *Pseudo-nitzschia* is a worldwide pennate diatom that is known to contain domoic acid (DA) and cause amnesic shellfish poisoning (ASP). On the other hand, *Alexandrium pseudogonyaulax* is one of the toxins goniodomin producer from the dinoflagellate group. These potent antifungal toxins associated to aquatic invertebrate mortalities. The objectives of this study were to investigate the potential toxic of *Pseudo-nitzschia* in environmental condition and taxonomic study of *A. pseudogonyaulax* in Malaysian waters. In March 2018, a *Pseudo-nitzschia* algal bloom was discovered for the first time in Miri, Sarawak, Malaysia Borneo. The plankton samples were collected for cell enumeration and species identification by electron microscopic and molecular characterisation. The neurotoxic domoic acid (DA) was detected and quantified in both plankton and shellfish samples using liquid chromatography-mass spectrometry (LC-MS/MS). The abundance of *Pseudo-nitzschia* cells was found in the range of 5.6×10^5 to 3.5×10^6 cell L⁻¹ during the bloom event. Morphological observation of the cells by transmission electron microscopy showed that the plankton samples comprised a single *Pseudo-nitzschia* morphotype resembled *P. cuspidata*. The ITS2 sequence-structure phylogenetic inference further supported the species identify as *P. cuspidata*. DA was detected in the plankton samples, with cellular DA, particulate DA, and dissolved DA of 257–504 fg DA cell⁻¹, 676 ng/L, and 15 ng/L, respectively. The amount of DA, 8 µg g⁻¹ tissue, was found present in the oyster sample (*Crassostrea*). Dinoflagellate *Alexandrium pseudogonyaulax* from the subgenus *Gessnerium* is one of the goniodomin. The subgenus *Gessnerium* possesses common thecal characteristics like indirect connection of first apical (1') plate to the apical

pore complex (Po) and oblique posterior sulcal plate (Sa). The group species identification is relied on the minute differences in the shape of the 1' and 6" plates. Seven strains of *Alexandrium*-like cell were established in this study. All the clonal cultures were undergone microscopic observation and molecular characterisation. *A. pseudogonyaulax* was identified and two new morphotypes, herein designated as *Alexandrium limii* nom. nud. and *Alexandrium ogatae* nom. nud. *Alexandrium limii* differs from *A. taylorii* in length of the suture and 1', 6", Sa and position of ventral pore. *Alexandrium ogatae* differs from *A. foedum* by the shape of plate 1', 6", 2''', and Sp. The species delineations were further supported by the phylogenetic inferences using two ribosomal gene markers, i.e., the large subunit (LSU) rDNA and internal transcribed spacer (ITS), including the complementary base changes (CBCs) analysis. The results showed *A. limii* and *A. ogatae* were clustered together with *A. pseudogonyaulax*, *A. taylorii*, and *A. hiranoi*. The species lineages were also supported by high genetic divergences. The pairwise comparisons of the ITS2 transcripts revealed the presence of CBCs between the *A. limii* and *A. ogatae* with the closely related species. This is first time for *Pseudo-nitzschia cuspidata* bloom in Southeast Asia, and it has been connected to shellfish domoic acid pollution. This study also documented for the first time of subgenus *Gessnerium* in Malaysia water based on the detail morphology and molecular with description of the two new species.

Keywords: *Alexandrium*, CBCs, domoic acid, ITS2 secondary structure, *Pseudo-nitzschia*

Morfologi, Molekular dan Toksikologi Pseudo-nitzschia cuspidata dan Alexandrium pseudogonyaulax dengan Penerangan Dua Spesis Alexandrium Baharu dalam Perairan Terpilih di Malaysia

ABSTRAK

Diatom marin Pseudo-nitzschia dan Dinoflagelat Alexandrium merupakan genera ledakan algal berbahaya (HABs) yang kerap dijumpai dipersisiran perairan Malaysia. Kedua-dua genera ini telah dilaporkan sebagai spesis yang bertoksik and penyebab kepada pencemaran toksin kerang-kerangan di rantau Asia Tenggara. Pseudo-nitzschia merupakan diatom pena yang diketahui kerana mengandungi asid domoik dan penyebab ke atas keracunan kerang-kerangan amnesik (ASP). Selain daripada itu, Alexandrium pseudogonyaulax merupakan salah satu penghasil toksin alga jenis goniodomin daripada kumpulan dinoflagelat. Toksin antikulat ini telah dikaitkan dengan kematian invertebrata akuatik. Objektif kajian dijalankan adalah untuk menyiasat keupayaan Pseudo-nitzschia bertoksik dalam keadaan persekitaran dan kajian taksonomik A. pseudogonyaulax di perairan Malaysia. Pada Mac 2018, satu kejadian ledakan alga Pseudo-nitzschia telah ditemui pertama kali di Miri, Sarawak, Malaysia Borneo. Sampel plankton telah diperolehi untuk tujuan penentuan kepadatan sel, pengecaman spesis menggunakan mikroskopik elektron dan karakter molekular. Neurotoksik asid domoik telah dikesan dan dikuantitikan daripada sampel plankton dan sampel kerang-kerangan menggunakan Kromatografi cecair berspektrometri jisim (LCMS/MS). Kepadatan sel Pseudo-nitzschia ketika ledakan adalah dalam julat 5.6×10^5 to 3.5×10^6 sel L^{-1} . Pengamatan morfologi sel menggunakan transmisi elektron mikroskop menunjukkan sampel plankton terdiri daripada morfotip menyerupai P. cuspidata. Jujukan struktur filogeni inferens (ITS2) turut menyokong pengecaman spesis sebagai P. cuspidata. Asid domoik telah dikesan dalam sampel plankton melalui selular asid domoik, zarah asid domoik dan asid domoik terlarut iaitu 257–504 fg DA sel⁻¹, 676 ng/L,

dan 15 ng/L, masing-masing. Sejumlah 8 $\mu\text{g g}^{-1}$ tisu asid domoik telah dijumpai dalam sampel tiram (*Crassostrea*). Dinoflagelat *Alexandrium pseudogonyaulax* daripada subgenus *Gessnerium* merupakan salah satu penghasil goniodomin. Subgenus *Gessnerium* mempunyai ciri-ciri teka umum, seperti hubungan tidak langsung apikal pertama (1') dengan liang kompleks apikal (Po) dan plat sulkus posterior yang serong. Pengecaman spesis ini bergantung pada berbezaan kecil pada bentuk plat 1' dan 6". Tujuh strain *Alexandrium* telah didirikan dalam kajian ini. Kesemua klon kultur telah menjalani pengamatan mikroskopik dan pencirian molekular. *A. pseudogonyaulax* telah dikenal pasti dan dua morfotip baharu telah ditentukan sebagai *Alexandrium limii* nom. nud. dan *Alexandrium ogatae* nom. nud. *A. limii* berbeza daripada *A. taylorii* pada panjang sutur dan plat 1', 6", Sa dan posisi liang ventral. *A. ogatae* berbeza daripada *A. foedum* pada bentuk plat 1', 6", 2"" dan Sp Persempadanan spesis turut disokong dengan inferens filogenetik berdasarkan dua penanda gen iaitu subunit besar (LSU) rDNA dan spacer transkrip dalaman (ITS), termasuk analisis perubahan bes gentian (CBCs). Keputusan menunjukkan *A. limii* dan *A. ogatae* berkelompok bersama dengan *A. pseudogonyaulax*, *A. taylorii* dan *A. hiranoi*. Keturunan spesis juga disokong dengan lencongan genetik yang tinggi. Perbandingan secara berpasangan bagi transkripsi ITS2 menunjukkan kewujudan dalam CBCs antara *A. limii* dan *A. ogatae* dengan spesis yang berhubung rapat dengannya. Ini merupakan ledakan pertama *Pseudo-nitzschia cuspidata* di Asia Tenggara yang telah dikaitkan dengan pencapahan kerang-kerangan oleh asid domoik. Kajian ini juga adalah pertama kali untuk mendokumentasikan subgenus *Gessnerium* di perairan Malaysia berdasarkan morfologi dan molekular secara terperinci dengan penerangan dua spesis baharu.

Kata kunci: Asid domoik, *Alexandrium*, CBCs, ITS2 struktur sekunder, *Pseudo-nitzschia*