Molecular characterization of the ITS2 transcript and saxitoxin biosynthetic genes in two toxic dinoflagellates, *Alexandrium* (Dinophyceae) from Malaysia

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ABSTRACT

The genus *Alexandrium* is a widely distributed dinoflagellate, and has the ability to produce potent neurotoxins, saxitoxin (STX). Taxonomy of this genus still remains uncertain and species identification is yet confusing. In this study, the second internal transcribed spacer (ITS2) transcript was used to infer the phylogenetic relationships of *Alexandrium* species distributed worldwide. A total of 33 ITS2 transcript of *Alexandrium* spp. were successfully modeled *in silico*, with one each from *Pyrodinium bahamense* var. *compressum*, *Coolia malayensis* and *C. monotis* as outgroups. The models showed conserved four universal helices of ITS2 transcript. The phylogenetic inference based on sequence-structural information revealed nearly similar phylogenetic framework as inferred in the Large Subunit (LSU) rDNA phylogeny. However, the results showed possible phylogeographic break in the *A. minutum* clade where the Asia Pacific and New Zealand *A. minutum* formed a distant group from the Australian and European group. Genetics of the STX biosynthesis pathway has recently become one of the major focuses in Paralytic Shellfish Poisoning (PSP) toxin-related studies after the discovery of STX biosynethetic genes in toxic cyanobacteria, and later in the toxic dinoflagellates. In the present study, two domains of a saxitoxin biosynthetic gene *sxtA*, S-adenosyl-L-methionine (SAM)-dependent methyltransferase coding gene (*sxtA1*) and the class II aminotransferase coding gene (*sxtA4*) were characterized from a toxic *A. tamiyavanichii* from Samariang, Sarawak. A saxitoxin biosynthetic gene encoding the *O*-carbamoyltransferase (*sxtI*) was also characterized in the toxic *A. minutum* from Tumpat, Kelantan. The partial coding sequences of saxitoxin starting gene, *sxtA1* and *sxtA4* of *A. tamiyavanichii* were 432 bp and 639 bp, respectively. While the deduced amino acid sequences of *sxtA1* and *sxtA4* were 144 and 213 amino acid residues, respectively. Sequences comparison revealed high similarity and identity to other PSP toxins-producing dinoflagellates (82-98% and 84-99%). In contrast, protein phylogenetic analyses revealed close relationship of both *A. tamiyavanichii sxtA1* and *sxtA4* to others PSP toxins-producing dinoflagellates, with *sxtA* of PSP toxins-producing cyanobacteria and putative toxin-related genes forming the sister clade. On the other hand, the coding sequence of *O*-carbamoyltransferase (*sxtI*) of *A. minutum* was 1,920 bp long, and the deduced amino acid sequence revealed a polypeptide of 639 amino acids. Structural sequence alignment revealed high similarity and identity (50-52% and 87-89%) to *sxtI* from the toxic cyanobacteria. Sequence comparison of *A. minutum sxtI* revealed highly conserved pattern, with five phosphorylation motifs, two catalytic regions, and a zinc finger detected. Even though homology between *A. minutum sxtI* and other cyanobacterial *sxtI* was observed, protein phylogenetic analysis inferred a distant relationship with the cyanobacterial SxtI, suggesting a paralog of SxtI in dinoflagellates.

Keywords: *Alexandrium*, paralytic shellfish poisoning (PSP), internal transcribed spacer region (ITS), phylogeny, secondary structure modeling, saxitoxin biosynthetic gene, *sxtA*, *sxtI*

Pencirian molekul transkrip ITS2 dan gene-gene biosintesis saxitoksin dalam dua dinoflagellates toksik, Alexandrium (Dinophyceae) dari Malaysia

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ABSTRAK

Genus Alexandrium bertaburan luas dan berkeupayaan untuk menghasilkan neurotoksin, saksitoksin (STX). Taksonomi dalam genus ini tidak mantap dan pengecaman spesis masih mengelirukan. Dalam kajian ini, struktur sekunder transkripsi spacer dalaman (ITS2) telah digunakan untuk mengkaji hubungan filogenetik spesis Alexandrium. Sebanyak 33 struktur transkrip ITS2 daripada Alexandrium spp. telah berjaya dimodel secara in siliko, dan Pyrodinium bahamense var. compressum, Coolia malayensis dan C. monotis digunakan sebagai taksa kumpulan luar. Model analysis menunjukkan struktur sekunder empat heliks yang terpelihara dalam transkrip ITS2. Kesimpulan filogenetik berdasarkan maklumat jujukan-struktur menunjukkan rangka kerja filogenetik yang hampir sama dalam filogeni Subunit Besar (LSU) rDNA. Walau bagaimanapun, keputusan menunjukkan pemisahan filogeografi dalam A. minutum di mana kumpulan Asia Pasifik dan New Zealand membentuk satu kumpulan yang terpisah dari kumpulan Australia dan Eropah. Genetik molekul tapak jalan biosintesis STX telah menjadi tumpuan utama kebelakangan ini dalam kajian toksin keracunan kerang-kerangan lumpuh (PSP) selepas penemuan gen biosinthesis STX di kalangan spesis toksik sianobakteria dan dinoflagelat. Dalam kajian ini, dua domain dalam gen biosintesis STX sxtA, gen yang mengekod S-adenosyl-L-methionine (SAM)-dependent methytransferase (sxtA1) dan aminotransferase kelas II (sxtA4) telah dicirikan dari A. tamiyavanichii dari Samariang, Sarawak. Gen biosintesis STX pengekodan O-carbamoyltransferase (sxtI) juga dicirikan dari A. minutum dari Tumpat, Kelantan. Jujukan gen separa sxtA1 dan sxtA4 dari A. tamiyavanichii adalah 432 bp dan 639 bp masing-masing. Manakala urutan asid amino polipetida bagi sxtA1 dan sxtA4 adalah 144 dan 213 aa. Keputusan kajian ini mendedahkan identiti dan persamaan yang tinggi berbanding dengan dinoflagelat penghasil-toksin PSP yang lain (82-98% and 84-99%). Analisis filogenetik protein juga mnunjukkan hubungan yang rapat kedua-dua sxtA1 dan sxtA4 dengan dinoflagelat penghasil toksin PSP yang lain, manakala sxtA dari sianobakteria penghasil-toksin PSP membentuk kumpulan adik-beradik. Sebaliknya, gen pengekod O-carbamoyltransferase (sxtI) dari A. minutum mempunyai jujukan sepanjang 1920 bp, dan urutan asid amino polipeptida sebanyak 639 aa. Penjajaran jujukan-struktur menunjukkan kesamaan dan identiti yang tinggi (50-52% and 87-89%) berbanding dengan sxtI dari sianobakteria toksik. Perbandingan jujukan sxtI A. minutum dengan kehadiran lima motif pemfosforilan, dua kawasan pemangkin dan satu zinc finger telah berjaya ditemui. Walaupun terdapat homologi antara sxtI untuk A. minutum dan sianobakteria, analisis filogenetik protein telah menunjukkan kewujudan hubungan yang jauh dengan SxtI sianobakteria, dan SxtI dalam dinoflagelat adalah dianggap sebagai paralog.

Kata Kunci: Alexandrium, Keracunan kerang-kerangan lumpuh (PSP), transkripsi spacer dalaman (ITS), filogenetik, permodelan struktur sekunder, gen biosintesis saksitoksin, sxtA, sxtI