

## Toxicity of diatom *Pseudo-nitzschia* (Bacillariophyceae) analyzed using high performance liquid chromatography (HPLC)

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**Abstract** Amnesic shellfish poisoning (ASP) is a type of shellfish poisoning due to the consumption of shellfish mollusks contaminated with domoic acid (DA). The toxin was first reported in the chain-forming pennate diatom, *Pseudo-nitzschia* and subsequently in other diatom species. In this study, clonal cultures of *Pseudo-nitzschia* were established from plankton samples collected from Sarawak and Sabah coastal waters. Clonal cultures were maintained in SWII medium with the addition of silicate at 25 °C, 30 PSU and under 12:12 light-dark photoperiod. Fifteen milliliters of late exponential phase cultures were collected for toxin analysis and subsamples were taken for cell count. Cellular toxin was extracted by boiling in medium at 100 °C for 5 minutes. The extracts were filtered to remove cell debris before being analyzed with HPLC using standard domoic acid, isodomoic A and B as reference toxins. All the 32 strains of *Pseudo-nitzschia* sp. analyzed in this study showed the absence of peaks corresponding to the three ASP toxins. This implies that non-toxic strains of *Pseudo-nitzschia* sp. are common in Malaysian waters. Further study will be carried out to include more strains along the coastal waters of Borneo as well as selected sites with shellfish farming activities in Peninsula Malaysia.

**Keywords** domoic acid – amnesic shellfish poisoning (ASP) – *Pseudo-nitzschia* sp. – HPLC

### INTRODUCTION

Amnesic shellfish poisoning (ASP) was first reported in Prince Edward Island, Canada in 1987 with density of the pennate diatom *Pseudo-nitzschia multiseriata* (previously described as *Nitzschia pungens*) reaching up to  $15 \times 10^6$  cells/L [1]. The shellfish poisoning caused three deaths and 105 cases of acute human intoxication after the consumption of contaminated blue mussels (*Mytilus edulis*) containing 900 µg/kg domoic acid [2]. A toxic bloom of *Pseudo-nitzschia pseudodelicatissima* was later reported at mussel cultivation areas in 1989 [3, 4].

In subsequent years, bloom event of *Pseudo-nitzschia australis* ( $7 \times 10^5$  cells/L) in Monterey Bay, California in 1991 was reported with mortality of 100 brown pelicans and cormorants [5]. In the same year, dozens of cases of human illness were reported along

the Pacific coasts of Washington and Oregon, where razor clams, Dungeness crabs, blue mussels and oyster were found to contain up to 154 µg/g of domoic acid [6]. The victims exhibited gastrointestinal disorders after digestion of any contaminated shellfish. The symptoms include nausea, vomiting abdominal cramps, headache, diarrhea, and memory loss [6]. The loss of memory in patients intoxicated with ASP appeared to be similar to patients with Alzheimer's disease [7].

Domoic acid (DA) is the compound responsible for ASP (Fig. 1). It is a water soluble tricarboxylic amino acid with a molecular weight of 311.14, (C<sub>15</sub>H<sub>21</sub>NO<sub>6</sub>). DA acts as an analogue of the neurotransmitter glutamate and is a potent glutamate receptor agonist [8] that has high binding affinity towards both kainate and AMPA (L-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid) [9].