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Diatom *Nitzschia navis-varingica* (Bacillariophyceae) and its domoic acid production from the mangrove environments of Malaysia

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ABSTRACT

The distribution of the toxic pennate diatom *Nitzschia* was investigated at four mangrove areas along the coastal brackish waters of Peninsular Malaysia. Eighty-two strains of *N. navis-varingica* were isolated and established, and their identity confirmed morphologically and molecularly. Frustule morphological characteristics of the strains examined are identical to previously identified *N. navis-varingica*, but with a sightly higher density of the number of areolae per 1 μm (4–7 areolae). Both LSU and ITS rDNAs phylogenetic trees clustered all strains in the *N. navis-varingica* clade, with high sequence homogeneity in the LSU rDNA (0–0.3%), while the intraspecific divergences in the ITS2 data set reached up to 7.4%. Domoic acid (DA) and its geometrical isomers, isodomoic A (IA) and isodomoic B (IB), were detected in cultures of *N. navis-varingica* by FMOC-LC-FLD, and subsequently confirmed by LC–MS/MS, with selected ion monitoring (SIM) and multiple reaction monitoring (MRM) runs. DA contents ranged between 0.37 and 11.06 pg cell^{-1}. This study demonstrated that the toxigenic euryhaline diatom *N. navis-varingica* is widely distributed in Malaysian mangrove swamps, suggesting the risk of amnesic shellfish poisoning and the possibility of DA contamination in the mangrove-related fisheries products.

1. Introduction

Domoic acid (DA), a neurotoxin that binds irreversibly to the glutamate receptor in the vertebrate central nervous system (Pulido, 2008), was first discovered from the red alga *Chondria armata* (Kützing) Okamura (Takemoto and Daigo, 1958). The toxin was responsible for amnesic shellfish poisoning (ASP) (reviewed in Lelong et al., 2012; Fernandes et al., 2014) that can be fatal to humans after consuming DA-contaminated fishery products. The source of DA contamination was diatoms of the genus *Pseudo-nitzschia* (e.g., Bates and Bird, 1989; Lelong et al., 2012; Dao et al., 2014). The massive bloom of *Pseudo-nitzschia* along the west coast of North America in 2015 caused a closure of crab and molluscan shellfisheries for an unprecedented time. This affected not only the local economy but also the fishing communities (McCabe et al., 2016). DA has also disrupted the marine food web, resulting in mass mortalities of marine mammals (sea lions and whales) at higher trophic levels (Lefebvre et al., 2016).

Diatoms of the genus *Nitzschia*, on the other hand, have so far never been associated with any human poisonings, even though two species have been confirmed to produce DA: *N. navis-varingica* Lundholm and Moestrup (Lundholm and Moestrup, 2000) and *N. bizertensis* Bouchouicha Smida, Lundholm, Sakka and Hadj Mabrouk (Bouchouicha Smida et al., 2014). The toxigenic *N. navis-varingica* was discovered for the first time from shrimp-culture ponds in Vietnam (Kotaki et al., 2000), and subsequently was found in brackish waters and estuaries throughout the Southeast Asian region (Kotaki et al., 2004, 2005, 2006, 2008; Bajarias et al., 2006; Romero et al., 2008, 2011, 2012; Takata et al., 2009; Thoha et al., 2012; Suriyanti and Usup, 2015). Conversely, the distribution of *N. bizertensis* was limited to Bizerte Lagoon, Tunisia, the type locality (Bouchouicha Smida et al., 2014).

While most toxigenic *Pseudo-nitzschia* species are planktonic, *N. navis-varingica* was frequently found in the benthic brackish

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