Toxicon 216 (2022) 65–72

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

First record of the diatom *Nitzschia navis-varingica* (Bacillariophyceae) producing amnesic shellfish poisoning-toxins from Papua New Guinea

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ARTICLE INFO

Handling Editor: Dr. Ray Norton

Keywords:
Nitzschia navis-varingica
Domoic acid
Isodomoic acid B
ITS2
LSU rDNA
Phylogeny

ABSTRACT

To determine the species distribution of an amnesic shellfish poisoning (ASP) toxins-producing diatom *Nitzschia navis-varingica* outside its current restricted geographical distribution range in Asian coastal waters, samples were collected from two sites of Bootless Bay, located on southwest coast of Papua New Guinea near Port Moresby. A total of twenty-one strains of *N. navis-varingica* were isolated and the clonal cultures established. The species identity was confirmed by molecular characterization based on the ribosomal DNA markers. The LSU rDNA phylogenetic inference revealed a monophyletic clade of all strains, clustered with *N. navis-varingica* with high bootstrap supports. ASP toxin production in the strains was investigated by HPLC with fluorescence detection and subsequently confirmed for the representative isolates by LC-MS/MS with multiple reaction monitoring (MRM) mode. All eleven strains from site A showed presence of domoic acid (DA) and isodomoic acid (IB); the toxin quota ranged from 0.70 to 4.63 pg cell^{-1} (average 2.75 ± 1.26 pg cell^{-1}, $n = 11$), with the composition of DA and IB of 21 DA: 79 IB. While for strains from site B, four out of ten strains showed presence of DA and IB, with the toxin quota ranged from 1.40 to 3.84 (average 2.57 ± 1.17 pg cell^{-1}, $n = 4$); the composition was 52 DA: 48 IB. The strains examined in this study were divided into toxic and probably non-toxic groups in ITS2 phylogeny. This represents the first record of domoic acid-producing *Nitzschia navis-varingica* from Papua New Guinea.

1. Introduction

Domoic acid (DA) was first recognized as the causative compound that was responsible for Amnesic Shellfish Poisoning (ASP) events that occurred in Canada nearly four decades ago (Wright et al., 1989). In addition to DA, some isomers, isodomoic acid D (ID), E (IE), and F (IF), have been identified as minor components of the DA toxin family (Wright et al., 1990). And the primary organism that produces DA was identified as the diatom *Pseudo-nitzschia multiseries* (Bates et al., 1989). After the finding, many other *Pseudo-nitzschia* spp. were found to produce DA (Trainer et al., 2012; Bates et al., 2018), although the toxin level varies depending on the species or culture conditions. Among them, three species (*P. multiseries*, *P. australis*, *P. seriata*) are known for their high toxicity levels (e.g., Bates et al., 1989; Martin et al., 1990; Garrison et al., 1992; Lundholm et al., 1994; Rhodes et al., 1996; Rhodes, 1998; Orsini et al., 2002). Presence of isodomoic acid C (IC) simultaneously with DA was later reported in *P. australis* and the shellfish that fed on *P. australis* (Holland et al., 2003, Holland et al., 2005; Rhodes et al., 2003). Before the recognition derived from the Canadian ASP incidents, DA and its derivatives (e.g., isodomoic acids A, B, C, G, and H) had been isolated and identified as an insecticidal agent from the red-alga *Chondria armata* (Takemoto and Daigo, 1958; Maeda et al., 1986; Zaman et al., 1997).

During the screening of harmful microalgae in tropical waters, one diatom was isolated from the resting shrimp pond in Do Son, Vietnam, and it was later confirmed to produce high level of DA by culture

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https://doi.org/10.1016/j.toxicon.2022.06.016
Received 30 March 2022; Received in revised form 26 June 2022; Accepted 27 June 2022
Available online 2 July 2022