

# *Pseudo-nitzschia fukuyoi* (Bacillariophyceae), a domoic acid-producing species from Nha Phu Bay, Khanh Hoa Province, Vietnam

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Received: 9 January 2015 / Accepted: 21 February 2015 / Published online: 18 March 2015  
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**Abstract** Two strains of *Pseudo-nitzschia fukuyoi* isolated from Vietnamese waters produce domoic acid, a toxin responsible for amnesic shellfish poisoning. Species identification was based on detailed morphological observation using a transmission electron microscope and also molecular data on large subunit (LSU) and the second internal transcribed spacer (ITS2) with NCBI nucleotide Blast (blastn). Toxin productivity of the two strains was confirmed and their range were 3.85–4.54 pg/cell by analyses using LC–MS/MS. This is the first report of occurrence of *P. fukuyoi* in Vietnamese waters, and the first confirmation of productivity of domoic acid of the species.

**Keywords** Domoic acid · Amnesic shellfish toxin · *Pseudo-nitzschia fukuyoi* · Vietnam

## Introduction

Species of the cosmopolitan genus *Pseudo-nitzschia*, some of which produce the neurotoxin domoic acid (DA), are often observed in tropical waters. Recently, several

potentially toxic *Pseudo-nitzschia* species were reported in the tropics, including Malaysia [1–5] and Vietnam [6, 7]. We recently documented that one of relatively small-sized species, *P. cf. caciantha*, from Nha Phu Bay, Khanh Hoa Province, Vietnam, was a DA producer [8]. Furthermore, we suspected that the species was most likely the source of the DA contamination in thorny oyster *Spondylus verisicolor* in the bay. However, DA levels in plankton showed peaks in different seasons, which were around April [8] and August [9]. In the previous studies, during DA peak in plankton in August, DA producing species could not be detected successfully. Therefore, this study aims to investigate potential producers of DA in Nha Phu Bay in around August, with emphasis on species of the genus *Pseudo-nitzschia* using unialgal cultures established from the bay.

## Materials and methods

### Sampling and algal cultures

On 17 July 2013, 5 l of seawater were collected at a site near Hon Thi Island, Nha Phu Bay (12°38'42"N, 109°22'06"E), Khanh Hoa Province, Vietnam, using a Van Dorn sampler from a 2-m depth. This sample was kept in the dark, without any preservative, and brought back to the laboratory for single-cell isolation.

Single cells of *Pseudo-nitzschia* species from the water sample were isolated using a fine-drawn Pasteur pipette under an inverted microscope (Nikon TMS-F MFA 20100). Cells were rinsed several times with 0.2 µm filtered seawater before transferring into a 24-multiwell plate containing 1 ml of f/2 medium [10] with pH of 7.8–8.0 at a salinity of 30. Cells that grew successfully were inoculated into 50 ml culture flasks containing 30 ml of f/2 medium, and finally

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