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Domoic acid in small-sized plankton in Nha Phu Bay, Khanh Hoa Province, Vietnam

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Abstract: Recently, we observed that a significant level of domoic acid was detected in the plankton net samples (mesh size 20 μm) when a bivalve *Spondylus versicolor* accumulated high amount of domoic acid in Nha Phu Bay, Khanh Hoa Province, Vietnam. In order to know the domoic acid-producing plankton species in the bay, plankton cells in the plankton net sample were fractionated by successive filtration through sieves with different mesh sizes (100, 20, 10, 0.6 μm). More than 90% of domoic acid in the net sample was detected in the small-sized cell fraction which passed through 10 μm sieve. About 7% of total cells of phytoplankton was trapped in the fraction, around half of which was consisted of species belonging to *Nitzschia* and *Pseudo-nitzschia*. These results suggest that small-sized plankton species which is hardly collected by normal plankton net are involved in accumulation of domoic acid of tropical bivalves.

Keywords: *Domoic acid, Pseudo-nitzschia, Nitzschia, Vietnam*

1. Introduction

Domoic acid is an excitatory amino acid responsible for amnesic shellfish poisoning (ASP) which was first found in Prince Edward Island, Canada in 1987 (BATES *et al.*, 1989). Since the incident in Canada, accumulation of domoic acid in bivalve was reported from several areas in the world (AMZIL *et al.*, 2001 ; HONER and POSTEL, 1993). However, these areas are limited in temperate waters. There is few knowledge on domoic acid accumulation in bivalves in tropical waters. Recently, we observed that a significant level of domoic acid was detected in the plankton net samples (mesh size 20 μm) when a bivalve *Spondylus versicolor* accumulated high amount of domoic acid in Nha Phu Bay, Khanh Hoa Province, Vietnam (DAO *et al.*, 2009). These findings indicate that domoic acid-producing plankton species occur in the bay during the period when domoic acid level of *S. versicolor* is increasing. We report here that small-sized plankton species which are hardly trapped by plankton net with 20 μm mesh size are possibly involved in accumulation of domoic acid of *S. versicolor* in Vietnamese water.

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2. Materials and Methods

2.1 Sample collection

Specimens of *S. versicolor* were collected by diving in Nha Phu Bay, Khanh Hoa Province, Vietnam biweekly from March to April, 2007. When domoic acid level of *S. versicolor* started to increase, sampling frequency was increased to twice a week. Plankton samples were collected by repeated vertical net hauling (0–2 m depth, 20 times) using a plankton net (mesh

size : 20 μm, diameter : 30 cm) from the bay when bivalves were sampled. On April 17, two sets of plankton samples were collected.

2.2 Analysis of domoic acid in *S. versicolor* and plankton samples

The soft tissue of 5 specimens was homogenized individually with 4 volumes of 50% methanol and centrifuged (1,000 g, 20 min) to obtain the extract according to QUILLIAM *et al.*, (1989). One mL of the crude extract thus obtained is equivalent to 0.2 g of the soft tissue. After ultrafiltration through a membrane (NMWL 5,000, Millipore), the extract was analyzed for domoic acid by HPLC according to KODAMA and KOTAKI (2005).

The plankton samples collected by the net hauling were further condensed by centrifugation (1,000 g, 15 min). Cell pellets were extracted with equal volume of water under boiling water for 5 min. After centrifugation, domoic acid in the supernatant was analyzed by HPLC as described above. Domoic acid level of the plankton samples was expressed as ng L^{-1} of seawater.

Plankton cells in one plankton sample out of two samples collected on April 17 were fractionated to four subsamples with different cell sizes. The plankton net sample on April 17 was successively filtered through sieves with opening size of 200, 100, 10, and 0.6 μm. Cells on each sieve were washed with filtered seawater though GF/F filter (Whatman). For this process, plankton net cloth with opening size of 200, 100, and 10 μm and GF/F filter with opening size of 0.6 μm were used as sieves. A portion (1/10 to 1/20) of each fraction was fixed with formalin for the quantitative observation of phytoplankton species under a light microscope. The rest of plankton cells in larger than 200 μm, 100–200 μm and 10–100 μm fractions were centrifuged (1,000 g, 15 min) to obtain the cell pellets. Domoic acid in these pellets was extracted with equal volume of water under heating for 5 min. After centrifugation (1,000 g, 15 min), the supernatant was analyzed for domoic acid by HPLC as described above. Cells in the 0.6–10 μm fraction were harvested by filtration through GF/F filter (Whatman). The plankton cells in the 0.6–10 μm fraction retained on the filter were extracted together with the filter by 5 mL of water under heating for 5 min. After heating, the tube was centrifuged (1,000 g, 15 min) to obtain the extract. The supernatant was then ultrafiltered through a membrane (NMWL 5,000, Millipore), and then analyzed for domoic acid by HPLC as described above.

The minimum detectable concentration of domoic acid in the test solution required for S/N=3 at 20 μL injection was 9 ng mL^{-1} in HPLC applied in the present study. However, it is affected by chemical background which can vary between samples. In the present study, 90 ng mL^{-1} of the test solution was applied as practical quantitation limit at 20 μL injection.

3. Results and Discussion

Domoic acid level in *S. versicolor* increased from 8 ± 2 μg g^{-1} (March 29) to 11 ± 3 μg g^{-1} (April 13), and then to 17 ± 9 μg g^{-1} (April 17), showing that domoic acid in *S. versicolor* was increasing during this period. These findings indicate that domoic acid-producing plankton occur in the environmental water during the period.

In the analysis of the plankton sample of March 29, no domoic acid was detected. The sample on April 13 showed a low level of domoic acid in HPLC analysis (0.2 ng L^{-1} of seawater), showing that the domoic acid-producing plankton species were increasing. However, no domoic acid was detected in the sample on April 17, the cells of which were collected by centrifugation. These results indicated that domoic acid-producing plankton appeared and showed maximum growth on April 13, and decreased or disappeared on April 17.

In contrast, a significant amount of domoic acid was detected in the 0.6–10 μm fraction of the plankton net sample on April 17 as shown in Fig. 1. A small amount of domoic acid was also detected in the 10–100 μm fraction. In Table 1, the results of domoic acid analysis of all the particle fractions are summarized. The 0.06 and 0.6 ng L^{-1} concentrations of domoic acid were found to be contained in the 10–100 μm and the 0.6–10 μm fractions, respectively. These results indicate that most of the

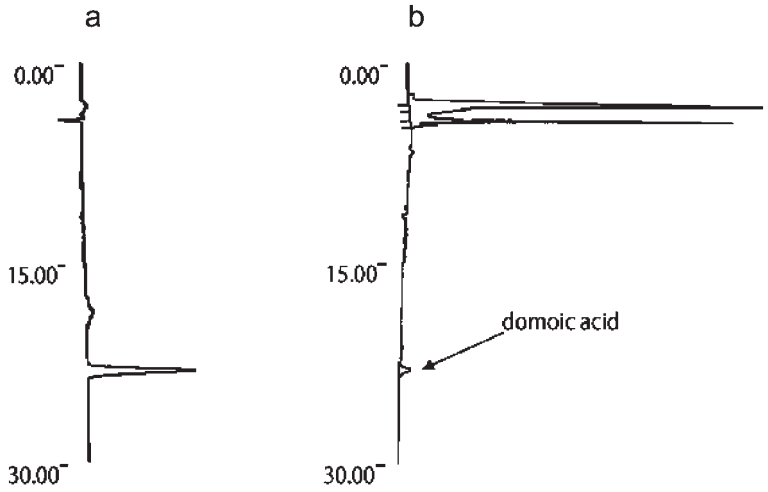

Fig. 1. HPLC chromatogram of domoic acid in the 0.6–10 μm fraction sample.
(a) Domoic acid standard (1 μ mL^{-1}) ; (b) 0.6–10 μm fraction sample.

Table 1. Domoic acid level in the different-sized fractions of the plankton net sample.

Particle size fraction	Volume of the extract (mL)	Level of domoic acid (ng L^{-1})
>200 μm	1.2*1	<0.04
100–200 μm	0.5*1	<0.02
10–100 μm	0.6*1	0.06
0.6–10 μm	5.0*2	0.6

*1 Plankton cells harvested by centrifugation were extracted with equal volume of water.
*2 Plankton cells harvested by filtration through GF/F filter were extracted with 5 mL of water.

plankton species containing domoic acid were trapped in the 0.6–10 μm fraction. In other words, the size of the toxic plankton species was small enough to pass through the sieve with 10 μm pore.

These results show that most of domoic acid-producing plankton cells can not be collected by hauling nets with mesh size of 20 μm. Only a small number of the cells could be collected probably due to the clogging of plankton net caused by repeated hauling. In the present survey, domoic acid was not detected in most of the plankton net samples while domoic acid level of *S. versicolor* was increasing. Possibly, the degree of clogging depends on the abundance and cell size of other plankton species in the seawater. The plankton cells of the sample on April 17 harvested by centrifugation showed no toxin, while those collected from the same sample by GF/F filter showed a significant level of toxin. These finding suggest that centrifugation process applied in the present study was not effective in separating small-sized domoic acid-producing plankton from other cells in the sample.

Since *P. multiseries* was identified as a causative species of amnesic shellfish poisoning (ASP) in Prince Edward Island, Canada (Bates *et al.*, 1989), several species of *Pseudo-nitzschia* have been reported to produce domoic acid (Bates, 2000 ; Bates and Trainer, 2006). *Pseudo-nitzschia* species are cosmopolitan species and often observed in plankton samples in tropical waters. However, little is known on the production of domoic acid of these species in tropical waters. On the other hand, a benthic diatom species *Nitzschia navis-varingica* isolated from Vietnamese water has been found to produce domoic acid (Kotaki *et al.*, 2000 ; Lundholm and Moestrup, 2000).

Table 2 shows the composition of *Pseudo-nitzschia* spp. and *Nitzschia* spp. in each size fraction. Cells belonging to both genera were observed in all the fractions. However, they are

Table 2. Cell number of total plankton, *Pseudo-nitzschia* and *Nitzschia* species in the different-sized fractions of the plankton net sample.

Particle size fraction	Cell density of total plankton (cells L^{-1})	Cell density of *Pseudo-nitzschia* spp. (cells L^{-1})	Cell density of *Nitzschia* spp. (cells L^{-1})
>200 μm	1798	71	71
100–200 μm	1335	149	7
10–100 μm	1451	170	71
0.6–10 μm	354	71	131

dominant in the 0.6–10 μm fraction in which most of domoic acid was concentrated, though at least 15 species of phytoplankton mostly consisting of small-sized diatom species were observed in the fraction (data not shown). These strongly suggest that small sized species belonging to *Pseudo-nitzschia* and/or *Nitzschia* observed in the 0.6–10 μm fraction are causative for domoic acid production in the bay. Studies on domoic acid production of small-sized plankton species, especially focused on the species of *Pseudo-nitzschia* and *Nitzschia*, is required to identify the species causative for domoic acid accumulation of *S. versicolor* in the bay.

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