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Screening of diatoms producing domoic acid and its derivatives in the Philippines

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Abstract—Domoic acid is the known causative agent responsible for amnesic shellfish poisoning (ASP). Although there is only one documented ASP case in the world, there is a potential of its occurrence in Southeast Asian countries. However, limited information on domoic acid producing diatoms is available except for *Nitzschia navis-varingica*, which is known to produce significant levels of domoic acid. In order to obtain fundamental data on domoic acid producing diatoms, screening of *Pseudo-nitzschia* and *Nitzschia* species were primarily performed in the Philippines. Two source areas, i.e. Manila Bay and Iba estuary of Luzon Island, were selected for observation of these diatoms. Fifty eight isolates of *Pseudo-nitzscia* and 18 isolates of *Nitzschia*-like diatoms were prepared from Manila Bay and Iba estuary, respectively. These isolates were cultured and tested for the production of domoic acid and its derivatives. *Pseudo-nitzscia* strains did not show any signs of domoic acid production. Five out of 18 *Nitzschia* isolates were confirmed to produce isodomoic acids A and B. Comparison of sonication and boiling in water bath as extraction methods was investigated and results showed that both methods yielded comparable amounts of domoic acid. Stability of domoic acid extracted by boiling was also investigated and was found out to be stable at room temperature for ten days. Results implied an advantageous and convenient way of sample preparation and preservation for international transport.

Key words: domoic acid, isodomoic acid, amnesic shellfish poisoning, *Pseudo-nitzschia*, *Nitzschia navis-varingica*, diatom, Philippines

Introduction

The very first and so far the only documented outbreak of amnesic shellfish poisoning (ASP) occurred in Canada. During this incident the causative agent was identified as domoic acid (DA) (Wright et al. 1989). DA was first isolated from the red macroalga *Chondria armata* (Takemoto and Daigo 1958). It belongs to a class of compounds known as kainoids and known to act in the central nervous system as an agonist to glutamate receptors. Structures of DA and some of its derivatives are illustrated in Fig. 1.

Consequently, the causative organism that produces DA was traced to the diatom *Pseudo-nitzschia multiseries* (Bates et al. 1989) which is formerly known as *P. pungens* f. *multiseries*. This discovery prompted a wide research for other *Pseudo-nitzschia* species that produce DA. As a result, several other *Pseudo-nitzschia* species that produce significant amount of DA, such as *P. australis* and *P. seriata*, had been reported (Cho et al. 2001, Garrison et al. 1992, Kotaki et al. 1999, Lundholm et al. 1994, Martin et al. 1990, Rhodes et al. 1998, Sarno and Dahlman 2000). *P. australis* is famous especially for its bloom that caused mass mortality of sea birds and sea lions (Scholin et al. 2000).

Recently, *Nitzschia navis-varingica* was reported to produce significant amount of DA (Kotaki et al. 2000, Lundholm and Moestrup 2000). Also reported as well is its wide distribution in Southeast Asian waters (Kotaki et al. 2004). Thus there is potential ASP incidents in Southeast Asian waters. Information however on DA producing diatoms in this particular region except for *N. navis-varingica* is very limited (Hasle 2002). In order to establish a fundamental strategy against potential ASP in South East Asian countries, fundamental data on DA producing diatoms, including both *Pseudo-nitzschia* and *Nitzschia* species, is needed.

DA is the main chemical compound found in toxic shellfish and causative organisms *Pseudo-nitzschia* spp. Some isomers (isodomoic acids D, E, F and 5′-epi-DA) have been also detected in *Pseudo-nitzschia* and shellfish samples together with DA. All the isomers were understood as artifacts and their amounts in the extracts of shellfish and planktons were very low (Wright et al. 1990). Other isomers of DA (isodomoic acids A, B, C, G, and H) were isolated from macroalga *C. armata* with DA (Maeda et al. 1987, Zaman et al. 1997). Some strains of *P. australis* found in New Zealand have been confirmed to produce isodomoic acid C with DA

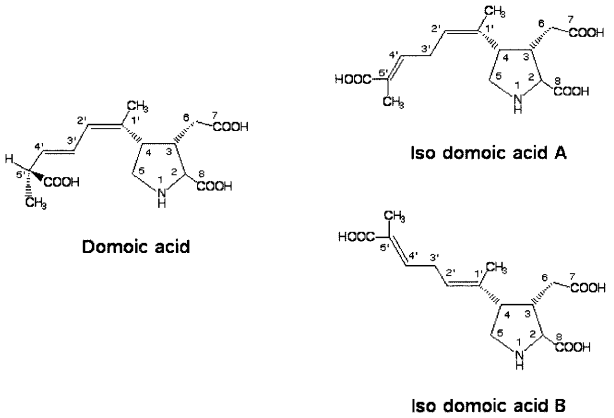

Fig. 1. Structures of domoic acid and some of its derivatives.

(Holland et al. 2003, Holland et al. 2005). They were also found contaminating in shellfish (Rhodes et al. 2003). Most recently, some strains of *N. navis-varingica* isolated from Bulacan Estuary in northern Manila Bay produced significant amount of isodomoic acids A and B instead of DA, and same species from several estuaries in southern parts of Manila Bay produced isodomoic acid B together with DA (Kotaki et al. this bull.).

In order to address the negative economic and health impacts of possible ASP incidents in South East Asia, international cooperative activity is also essential. In a joint international endeavor, several restrictive factors are present. Some of which are equipment availability and international transport of samples. To address problems posed by limited equipment availability, an alternative means of DA extraction that can be used as a satisfactory substitute for the conventional method of disruptive sonication should be investigated. In addition, sending of whole culture samples for DA analysis present the problem of international transport. Diatom cultures must arrive at the laboratory in a viable state, use of cooling gel or dry ice is often required. This is often an expensive way of transport and most of the time rejected by international couriers. Therefore in conjunction with finding alternative extraction method, DA stability relative to preservation and storage should be also investigated.

This study aimed to isolate and determine toxic *Pseudo-nitzschia* and *Nitzschia*-like diatoms and to quantify the DA components in selected Philippine waters. This study also aimed to examine DA extraction procedures favorable for international transport of samples necessary for result confirmation.

Materials and Methods

Comparison of boiling and sonication methods for domoic acid extraction

Two extraction methods were investigated. The first one is the conventional method of sonication (Kotaki, et al. 1999). In this method DA from diatom culture sample were investigated. *P. multiseries* (strain OFPm 032-1) isolated from Ofunato Bay in October 2003 was used. The strain was cultured in 1 L f/2 medium (Guillard 1983) in 2 L Erlenmeyer flask for 1 week at 15°C under irradiance level of 70 μmol photons/m^2/sec, with light : dark cycle of 16 : 8. Ten mL of the whole culture was extracted by sonication and was used as control. The second method was extraction by boiling. DA was extracted from the same strain of *P. multiseries* described above. In this method four 10 mL of whole culture was put in a screw cap glass vial and placed in boiling water bath and heated at different times. The different boiling time applied were 3, 6, 9 and 12 min. Experiments were conducted in triplicate.

Domoic acid Analysis

Extracts both from sonication and boiling were subjected to ultrafiltration using Ultrafree-MC (Millipore Corporation, Bedford, MA. USA mw 10,000 cut-off). The filtrate was then analyzed for DA concentration by HPLC-FD with pre-column derivatization using 9-fluorenylmethylchloroformate (FMOC-Cl) according to Pocklington et al. (Pocklington et al. 1990) in which a Develosil ODS-5 column (4.6× 250 mm, Nomura, Seto, Aichi, Japan) and a mobile phase of 40% acetonitrile in 20 mM phosphate buffer (pH 2.5) were used (Kotaki et al. 2004).

Domoic acid stability investigation towards temperature

The same strain of *P. multiseries* was cultured for 3 weeks for the investigation of stability of DA in boiled extract. Three 10-mL aliquots of the whole culture was put into screw-cap tubes and heated in a boiling water bath. Boiling period was set at 3 levels of 1, 3 and 5 min. The boiled extract was then maintained under the two conditions namely room temperature (ca. 23°C) and in a refrigerator (ca. 4°C). Extracts kept at room temperature were analyzed for DA content at a two-day interval. Extracts kept at 4°C were analyzed at a weekly interval. This experiment was performed in triplicate.

Collection of *Pseudo-nitzschia* and *Nitzschia*

Screening of *Pseudo-nitzschia* was primarily done in Manila Bay. Collection sites were illustrated in Fig. 1 and these sampling stations were located along the coastal waters of four provinces namely Bataan, Bulacan, Cavite and Metro Manila (Navotas). *Pseudo-nitzschia* samples were collected by vertical hauling using a 20 μm mesh sized plankton net at an average depth of 5 m, 10 m, 3 m and 5 m in Cavite, Bataan, Bulacan and Navotas, respectively. Sample collection was performed during March 2004 to January 2005. *Pseudo-nitzschia* was isolated from the net sample by capillary washing under a light microscope. Preliminary *Pseudo-nitzschia* uni-algal culture was made in a 100 mL Erlenmeyer flask

containing 40 mL f/2 medium for 5 days at 27°C under irradiance level of ca. 60 μmol photons/m^2/sec with light : dark cycle of 12 : 12. A part of a well grown culture was inoculated into a 50 mL tissue culture tube filled with 30 mL f/2 medium and maintained for 16 days or more under the above cited conditions. Cell growth of the *Pseudo-nitzschia* culture was observed under a light microscope. Ten mL of each *Pseudo-nitzschia* culture was taken out at around 21st day and DA was extracted by 3-min boiling and filtrated by membrane filter (3 μm pore size, Advantec). The filtrate was analyzed by HPLC as described above.

Screening of isodomoic acid-producing *N. navis-varingica* was conducted at Iba estuary, in Zambales. *Nitzschia*-like samples were collected by a scoop net of 20 μm mesh size. Collection were done on the surfaces of mangrove root or bamboo net constructed at the waters of Iba estuary, Iba, Zambales on March 2005. Collection area was illustrated in Fig. 2. Net samples were inoculated in the f/2 medium and maintained at 25°C under irradiance level of ca. 60 μmol photons/m^2/sec with light : dark cycle of 16 : 8 until individual cells were isolated by capillary washing method. Preliminary uni-algal culture was prepared by inoculating *Nitzschia* isolates in 100 mL Erlenmeyer flask containing 40 mL f/2 medium maintained for 5 days under similar conditions for Manila Bay samples. A part of the well-grown culture was inoculated into 50 mL tissue culture tube filled with 30 mL f/2 medium for starting the production culture. Cell growth was monitored by *in vivo* chl. *a* fluorescence using a handmade fluorometer (Koike et al. 1990). Ten mL of each *Nitzschia* culture was taken out 10 days after the growth reached the stationary phase. DA was extracted by ultra-sonication as described above (out put 7, 2 min). The extract was ultra-filtrated and the filtrate was analyzed for DA concentration by HPLC described above.

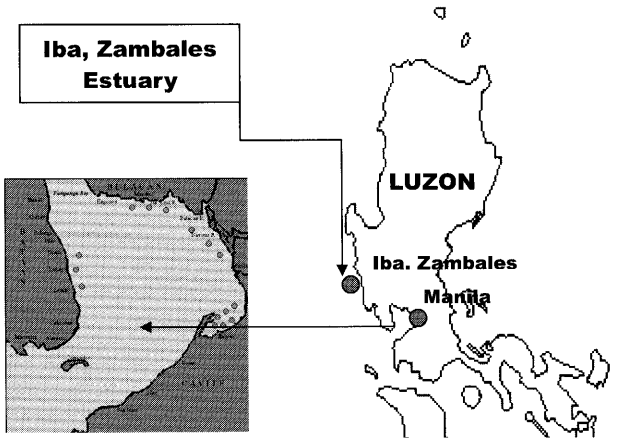

Fig. 2. Location of Sampling area used for collection of *Nitzschia navis-varingica*.

Monitoring of amnesic shellfish poison was conducted to complement the screening of *Pseudo-nitzschia* that produce DA in Manila Bay. Monitoring parameters used were shellfish toxicity and *Pseudo-nitzschia* cell density. Plankton samples were collected by vertical towing using a 20-μm plankton net at Cavite, Bataan, Bulacan and Navotas at an average depth of 5 m, 10 m, 3 m and 5 m, respectively. Shellfish samples were collected at areas corresponding to the site from which plankton were collected. Shellfish samples were analyzed for DA using the method of Kotaki et al. (1999). In this method DA was analyzed by HPLC using a reversed phase ODS RP-18 column (Agilent), mobile phase of 20 mM phosphate buffer at pH 2.5 containing 10% acetonitrile and UV detection at 242 nm. *Pseudo-nitzschia* was observed under light microscope.

Species identification

Species identification of diatoms was conducted by observing morphological characteristics of *Pseudo-nitzschia* under scanning electron microscope (SEM) according to Fryxell and Hasle (Fryxell and Hasle 1993). Those of *Nitzschia* were preliminary observed under light microscope according to Lundholm and Moestrup (2000).

Results and Discussion

Comparison of boiling and sonication methods for domoic acid extraction

Results of the comparison of the extraction efficiency between sonication and boiling were summarized in Table 1. Extraction by boiling showed a slightly lower DA recovery compared to that of usual extraction by sonication. DA recoveries for the different boiling times of 3, 6, 9 and 12 min

Table 1. Evaluation of boiling as an extraction method for domoic acid in *Pseudo-nitzschia* culture.

	Control*	3 min boiling**	6 min boiling**	9 min boiling**	12 min boiling**
DA content*** (ng/ml)	79.7	75.6[b]	73.6[b]	77.9[a]	77.6[a]

* The conventional sonication method was used as control.
** Values are average of three trials.
*** Means are significantly different using ANOVA at α=0.05 level of significance.
[a] values are not significantly different from control using t-test at α=0.05.
[b] values are significantly different from control using t-test at α=0.05.

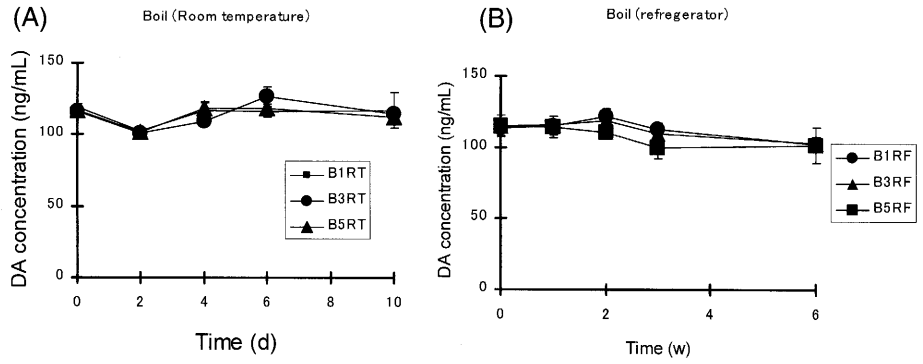

Fig. 3. Stability of DA. (A) Obtained by boiling stored at room temperature. (B) Obtained by boiling stored at refrigerator ca. 4°C. B1RT=extraction by boiling for 1 minute, B3RT=extraction by boiling for 3 minutes, B5RT=extraction by boiling for 5 minutes.

were 95%, 92%, 98% and 97%, respectively. DA recovery test by boiling was conducted in triplicate for each boiling time. DA extracted by sonication was used as control. Statistical analysis of the obtained data showed significant difference by analysis of variance at $\alpha=0.05$ however, values for 9 and 12 min boiling did not show significant difference compared to the control using t-test at $\alpha=0.05$. However, the 95% ratio DA recovered by 3-min boiling to that of usual extraction by ultra-sonication is enough recovery of DA from *Pseudo-nitzschia* culture. Prior to this experiment, it was confirmed that 93% DA was exist in the cells of *P. multiseries* culture.

Storage stability investigation

Storage stability was also evaluated using boiling method for DA extraction. Figure 3a shows DA concentration of extract obtained by boiling, stored at room temperature (ca. 23°C) and for a period of 10 days with DA measured at a two-day interval. Prior to the examination, it was confirmed that 72% DA exist in the cells of *P. multiseries*. Different boiling times of 1, 3 and 5 min employed showed the same trend of DA concentration in the 10-day storage period and within this time almost 100% of DA remained. Statistical test using t-test at $\alpha=0.05$ was performed using the 0 day storage as reference. Based on statistical results, boiling by three minutes showed no significant differences consistently compared to the control for the 10-day period observation. In general, DA extracted through boiling method showed stability at room temperature.

Figure 3b shows the DA concentration of the extract obtained by boiling, stored in refrigerator for six weeks and measured at a one-week interval. About 90% DA was remained after 6 weeks storage. And furthermore if the sample is kept in refrigerator, DA will still be detected even if storage time is more 6 weeks.

In determining the appropriate boiling time to be used in successive analysis, consistency relative to the control was a chosen as the basis. It is recommended that a *Pseudo-nitzschia* sample is put into test tube with screw cap, extracted by boiling for 3 min and sent under ambient temperature.

This chosen method renders sample suitable for international transport which may sometimes require a week to complete delivery. Shipment of whole culture samples were often met with difficulty for the samples must be kept at a low temperature during transport. This requires the use of considerable amount of cooling gel or dry ice and such is relatively costly and may be rejected by international couriers. With this alternative DA extraction method, the above cited impediment is effectively addressed since DA extract is to be sent in place of whole culture samples.

Identification and DA content analysis of *Pseudo-nitzschia* samples

The first ASP incident was attributed to DA produced by *P. multiseries* and since then, several other species of *Pseudo-nitzschia* has been discovered to contain DA, its derivatives or combination of DA and its derivatives. *Pseudo-nitzschia* has been detected in Manila Bay in the past and therefore a good area to survey the presence of toxic *Pseudo-nitzschia* species. During the one year collection period, 58 *Pseudo-nitzschia* were successfully isolated, cultured and investigated for DA production. Table 2 shows the result of the screening. All isolates were negative for DA production. Forty four isolates out of 58 showed the morphological characteristics that coincide well with those of *P. pungens*. Pseudonodule is absent; frustule has 9–15 keel puncta and 9–15 transapical costae in 10 μm; intercostal membrane is perforted by two rows of poroids which have 3–4 pores in 1 μm; the length is 70–120 μm; and the width is 3.0–4.5 μm. The remaining 14 isolates were not able to observe because of bad conditions of fixation. *P. multiseries* and *P. australis* that is famous for their strong DA production were not found in Manila Bay during the conducted screening, showing that there is less potential of ASP accident there. However, screening of this kind of *Pseudo-nitzschia* might be important to give a quick response when food poisoning by shellfish might happen.

In relation to screening of toxic *Pseudo-nitzschia*, a sup-

Table 2. Screening of domoic acid producing *Pseudo-nitzschia* in Manila Bay.

Collection Site	Collection Date	DA Production	Species
MBC2 (Cavite)	24 March 2004	ND*	*P. pungens*
	10 April 2004	ND	*P. pungens*
	10 April 2004	ND	*P. pungens*
	16 April 2004	ND	*P. pungens*
	16 April 2004	ND	*P. pungens*
	25 June 2004	ND	*P. pungens*
	25 June 2004	ND	*P. pungens*
	25 June 2004	ND	*P. pungens*
	25 June 2004	ND	*P. pungens*
MBB1 (Bataan)	20 August 2004	ND	*P. pungens*
	20 August 2004	ND	Unidentified**
	20 August 2004	ND	Unidentified
	20 August 2004	ND	Unidentified
	20 August 2004	ND	Unidentified
	20 August 2004	ND	Unidentified
	20 August 2004	ND	Unidentified
	20 August 2004	ND	Unidentified
	20 August 2004	ND	Unidentified
	20 August 2004	ND	Unidentified
	04 September 2004	ND	*P. pungens*
	04 September 2004	ND	Unidentified
	25 January 2005	ND	*P. pungens*
	25 January 2005	ND	Unidentified
	25 January 2005	ND	*P. pungens*
	25 January 2005	ND	*P. pungens*
	26 January 2005	ND	*P. pungens*
	26 January 2005	ND	*P. pungens*
	26 January 2005	ND	*P. pungens*
	26 January 2005	ND	*P. pungens*
	26 January 2005	ND	*P. pungens*
MBB2 (Bataan)	14 December 2004	ND	*P. pungens*
	14 December 2004	ND	*P. pungens*
	14 December 2004	ND	*P. pungens*
	14 December 2004	ND	*P. pungens*
	14 December 2004	ND	*P. pungens*
	14 December 2004	ND	*P. pungens*
MBBL1 (Bulacan)	25 January 2005	ND	*P. pungens*
	28 January 2005	ND	*P. pungens*
	28 January 2005	ND	*P. pungens*
	28 January 2005	ND	*P. pungens*
	28 January 2005	ND	*P. pungens*
	28 January 2005	ND	*P. pungens*
MBBL2 (Bulacan)	04 October 2004	ND	*P. pungens*
	05 October 2004	ND	*P. pungens*
	07 October 2004	ND	*P. pungens*
	07 October 2004	ND	*P. pungens*
MBN2 (Navotas)	05 October 2004	ND	*P. pungens*
	05 October 2004	ND	*P. pungens*
	05 October 2004	ND	*P. pungens*
	05 October 2004	ND	*P. pungens*
	05 October 2004	ND	*P. pungens*
	05 October 2004	ND	*P. pungens*
	05 October 2004	ND	*P. pungens*
	05 October 2004	ND	Unidentified
	05 October 2004	ND	Unidentfied
	05 October 2004	ND	*P. pungens*
	05 October 2004	ND	*P. pungens*

* ND=DA less than 0.3 ng/mL.

** Species identification was not done due to poor sample condition.

Table 3. Domoic acid monitoring data for shellfish from Manila Bay.

Date Collected	Sampling Area	Sampling Station	DA content (ng/g)	*Pseudonitzschia* Cell Density
January 10, 2005	Cavite	Las Piñas	ND*	6 cells/Liter
		Parañaque	ND	63
		Pelayo	ND	30
January 11, 2005	Bulacan	Santa Cruz	ND	0
January 14, 2005	Bataan	Luz	ND	4
		Orion	ND	8
		Planters	ND	13
February 1, 2005	Bulacan	Masokol	ND	22
February 2, 2005	Bataan	Luz	ND	3
		Orion	ND	2
		Planters	ND	6
February 11, 2005	Cavite	Las Piñas	ND	12
		Parañaque	ND	7
		Pelayo	ND	3
March 2, 2005	Cavite	Las Piñas	ND	0
		Parañaque	ND	0
		Pelayo	ND	0
March 3, 2005	Bataan	Luz	ND	0
		Orion	ND	0
		Planters	ND	0
March 4, 2005	Bulacan	Santa Cruz	ND	15
April 4, 2005	Bataan	Luz	ND	13
		Orion	ND	19
April 5, 2005	Bulacan	Santa Cruz	ND	0
April 7, 2005	Cavite	Las Piñas	ND	0
		Parañaque	ND	0
		Pelayo	ND	0
May 2, 2005	Cavite	Las Piñas	ND	0
		Parañaque	ND	0
		Pelayo	ND	0
May 4, 2005	Bataan	Luz	ND	5
		Orion	ND	3
		Planters	ND	8
June 6, 2005	Cavite	Las Piñas	ND	21, 054
		Parañaque	ND	41, 932
		Pelayo	ND	21, 122
June 6, 2005	Bataan	Luz	ND	0
		Orion	ND	0
		Planters	ND	0

* ND: Not Detected.

plemental monitoring of possible amnesic shellfish poison was also conducted in Manila Bay. The monitoring scheme involves the collection of both shellfish and plankton samples. Shellfish was analyzed for the presence of DA by HPLC-UV according to the method of Kotaki et al. (1999). Present *Pseudo-nitzschia* was determined by light microscopy and subsequently quantified expressed in cells per Liter. ASP monitoring period covers the first half of 2005 and results are summarized in Table 3. Low *Pseudo-nitzschia* cell count was recorded except in June where cell densities for the three Cavite sampling stations ranged from 21,000 to 42,000 cells/L. DA was not detected in all of the shellfish samples even at *Pseudo-nitzschia* cell count of 21,000 to 42,000 cells/L. This indicates that shellfish in Manila Bay is free from DA contamination as far. However, more monitoring should be performed continuously together with the monitoring for PSP to obtain the reliable data for the safe consumption of shellfish. Monitoring of ASP will be continued

Table 4. Toxin composition of *Nitzschia*-like diatom culture from Iba, Zambales estuary.

	pg/cell				ng/mL			
Strain name	DAs	IA	IB	DA	DAs	IA	IB	DA
IBE 05-02	9.5	4.0	5.5	ND	71.8	30.2	41.6	ND
IBE 05-11	3.1	2.2	0.9	ND	14.0	9.9	4.1	ND
IBE 05-12	3.0	1.1	1.9	ND	31.4	11.7	19.7	ND
IBE 05-13	5.4	2.8	2.6	ND	37.3	19.4	17.9	ND
IBE 05-14	0.5	0.3	0.1	ND	7.4	5.5	1.9	ND
Average	4.3	2.1	2.2	ND	32.4	15.3	17.0	ND

IA; isodomoic acid A, IB; isodomoic acid B, DA; domoic acid.
DAs; total amount of DA and isodomoic acids A and B, ND; non-detectable.

by the Philippine's Bureau of Fisheries and Aquatic Resources as it is to become an integral part of the Bureau's action in ensuring food safety and safeguarding public health.

Identification and DA content analysis of *Nitzschia* samples

Eighteen isolates of *Nitzschia*-like diatoms were established from Iba estuary. Among them, 5 isolates showed positive result for production of isodomoic acids A and B. Table 4 shows the toxin composition of 5 positive isolates. Production of isodomoic acid A of these five isolates ranged from 0.3 to 4.0 pg/cell (average of 2.1 pg/cell) and isodomoic acid B production ranged from 0.1 to 5.5 pg/cell (average of 2.2 pg/cell). DA however was not detected. This toxin composition was similar to that of *N. navis-varingica* isolated from Bulacan estuary, northern Manila Bay (Kotaki et al. this bull.). Figure 4 shows an example of HPLC-fluorescence analysis of isolates (strain IBE 05-12). Isodomoic acids B and A appeared before DA. Morphological characteristics of the 5 isolates positive for DA production coincided well with those of *N. navis-varingica* under light microscope. Cells were yellow-brown and possessed two chloroplasts at each end of the cells. Cells were lanceolate in valve view, 30–120 μm long and 9–13 μm wide, in girdle view rectangular, slightly indented in the middle. These indicate that these 5 isolates are *N. navis-varingica*, although detailed observation by SEM is under way. The remaining *Nitzschia*-like isolates negative for DA and isodomoic acids production have not been identified yet.

N. navis-varingica has been isolated in Manila Bay particularly in Bulacan, and Bacoor estuaries. It was also isolated in San Roque, Samar and San Pedro, Leyte estuaries located in the southern part of the Philippines (Kotaki et al. this bull.). DA production of *N. navis-varingica* isolated from Iba, Zambales were compared to DA production from the above mentioned areas. *N. navis-varingica* strains from Iba, Zambales and Bulacan estuaries showed similar DA production which is combination of isodomoic acid A and isodomoic acid B in contrast to strains obtained from Bacoor, Samar and Leyte estuaries which showed production of combination of DA and isodomoic acid B (Kotaki et al. this bull.). The difference in toxin composition of strains from areas north of Manila Bay from those of the south was noted. These indicate that there is a possibility of toxin composition difference among locality that includes the strain difference or some environmental difference.

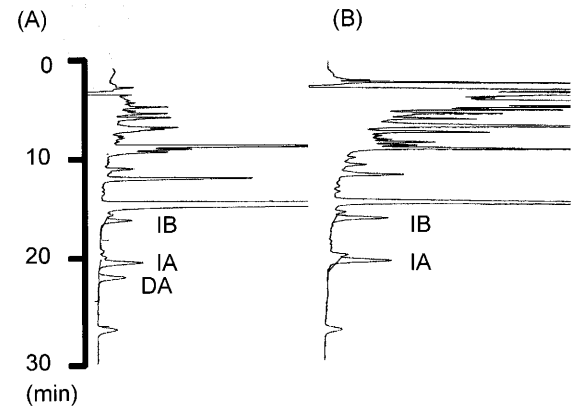

Fig. 4. HPLC- fluorescence analysis of *Nizschia navis-varingica* isolates from Iba Estuary showing chromatograms from DA standard (A) and IBE 05-12 (B). DA: domoic acid, IA: isodomoic acid A, IB: isodomoic acid B.

Nuero-toxicity of isodomoic acids A and B are uncertain as far. Isodomoic acids A and B were first isolated from macro-alga *C. armata* as an insecticidal agent that are 12-fold less toxic than DA and same as DDT against American cockroach (Maeda et al. 1984). Isodomoic acid C was isolated from New Zealand shellfish (Holland et al. 2003) and *P. australis* (Holland et al. 2005, Rhodes et al. 2003) simultaneously with DA but its insecticidal toxicity is 20-fold less than DA and the affinity to glutamate receptor is much lower than DA (Holland et al. 2005). However, Hampson et al. (1992) reported that isomers (like isodomoic acid C) that lack a 1′-2′ double bond with the Z configuration show very low affinity to glutamate receptor. Isodomoic acid A does have this

structure (1′-2′ double bond with the Z configulation), indicating that neuro-toxicity of isodomoic acid A might be much higher than isodomoic acid C. It is not known how high the *N. navis-varingica* might bloom at the estuary area and how high isodomoic acids A and B might be accumulated in brackish water animals. Observation of *N. navis-varingica* in brackish water area and toxin screening of brackish water animals is needed to estimate the potential of *N. navis-varingica* against ASP.

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