

## ISOLATION OF ASP TOXIN-PRODUCING *NITZSCHIA* FROM THAILAND

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### ABSTRACT

*Nitzschia navis-varingica* is recognized as the major ASP toxin producer other than *Pseudo-nitzschia* species. Surveys on the distribution and the toxin production of this diatom has been conducted in a few countries in eastern Asia and expanded to Thailand. Eighteen isolates from Thailand showed the positive result for the ASP toxin production is DA and IB, although the ratio of IB was much lower than those of the isolates from southern countries including Okinawa, Japan, the Philippines and Vietnam.

**Keywords:** *Nitzschia navis-varingica*, ASP, Domoic acid, Isodomoic acid, Pennate diatom

### INTRODUCTION

*Pseudo-nitzschia multiseriata* is well known as the major producer of domoic acid (DA), the causative compound of ASP accident in Canada (Bates *et al.*, 1989; Wright *et al.*, 1989). After this accident, other kinds of *Pseudo-nitzschia* were screened and found as DA producers, although the level of toxin production differs among species (Kotaki, 2002). However, little is known about the diatoms producing DA in eastern Asia, especially in subtropical and tropical areas. During the survey for DA-producing diatoms in eastern Asia, *Nitzschia navis-varingica* was first isolated as a new species from a shrimp culture pond in Do Son, Vietnam (Kotaki *et al.*, 2000). DA producing *Nitzschia* was screened and later isolated from wide but limited several areas of Japan (Tohoku district and Okinawa) and the Philippines (Kotaki *et al.*, 2004). During expanding this screening, some Philippine strains of *N. navis-varingica* were newly isolated and confirmed to produce isodomoic acids A (IA) and B (IB) instead

of DA (Kotaki *et al.*, 2005) (Fig.1). Re-observation of the HPLC chromatograms of toxin analyses of the Philippine isolates showed that many isolates produce DA and IB and the isolates from limited areas produce IA and IB. It seemed interesting whether *N. navis-varingica* outside the Philippine produces plural ASP toxins or not. Screening and ASP toxin analysis of *N. navis-varingica* was re-performed primarily in Japan, the Philippines and Vietnam. As a result, it was confirmed that major type of the ASP toxin production is DA-IB (all of the isolates from Japan and Vietnam, almost all the isolates from the Philippines), although the ratio of IB showed locality. The ratio of IB was higher in the southernmost isolates. And the minor type of the ASP toxin type was IA-IB that was limited to the isolates obtained from three areas near Manila Bay in the Philippines (Kotaki *et al.*, in press). Then, the survey was expanded to Thailand where no investigation on this topic has been performed. In the present paper, we preliminary report the isolation of pennate diatoms that produce DA and small amount of IB from Thailand.

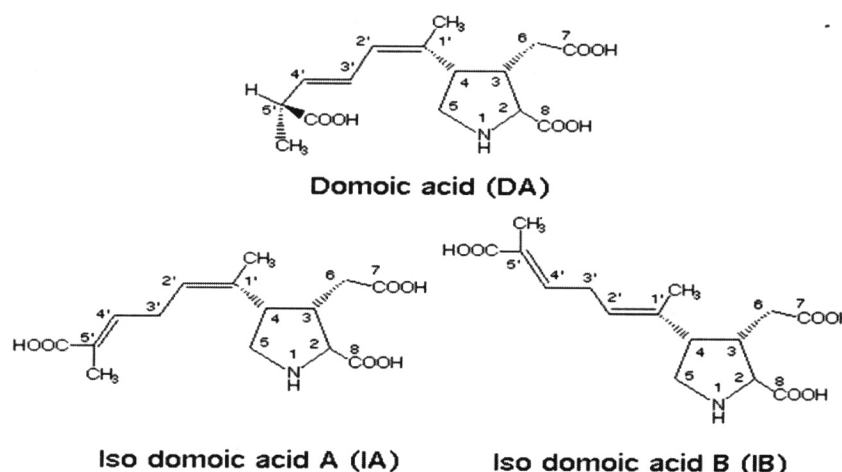


Figure 1. Structure of domoic acid (DA) and isodomoic acids A (IA) and B (IB)

## MATERIAL AND METHODS

### Collection and isolation of diatoms

Collection of *Nitzschia*-like diatoms were done using scoop net (20  $\mu$  m) at several sites in Chantaburi, Chonburi and Bangkok areas in April 2007. Collected diatoms were inoculated into tissue culture flask filled with *f*/2 medium (Guillard, 1983) and incubated for about 10 days at 25°C under irradiance level of 70-80  $\mu$  mol photons/m<sup>2</sup>/sec and L:D cycle of 14:10. Unialgal cultures of *Nitzschia*-like diatoms were established from the crude culture by capillary washing method under light microscope.

### Culture of the diatoms

Established pennate diatom isolates were tested for the ASP production by culture experiment using 50 ml tissue culture tube filled with 30 ml *f*/2 medium. Cell growth was monitored fluorometrically according to the *in vivo* chlorophyll *a* fluorescence (Koike *et al.*, 1994).

### Toxin analysis

Production culture was harvested 10 days after it reached the stationary growth phase. Harvested samples were extracted by boiling for three minutes in the test tube (Bajarias *et al.*, 2006). Toxin analysis was done for DA, IA and IB by HPLC with fluorescence detector (Kotaki *et al.*, 1999). Standard of these three toxins were

prepared from mass culture of *N. navis-varingica* and from the extract of red alga *Chondria armata* and calibrated by purchased Canadian standard toxin (DACS 1D) for DA and by weight (IA and IB). Confirmation of IA and IB were done by NMR analyses (Kotaki *et al.*, 2005). ASP toxin content was expressed by pg per cell basis.

### Morphological observation of the diatoms

Observation for the species identification was done on the representative strains positive for the ASP toxin production according to Lundholm and Moestrup (2000) and Lundholm *et al.*, (2002).

## RESULT AND DISCUSSION

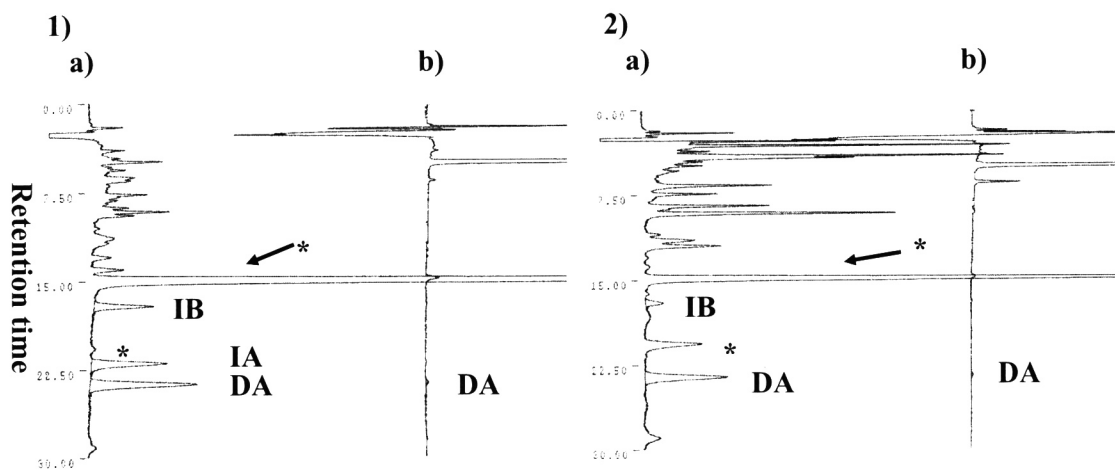
Twenty *Nitzschia*-like diatoms were obtained only from six sites near Bangkok, however any *Nitzschia*-like diatoms were not isolated from Chantaburi and Chonburi at all.

### Toxin level and profile of the isolated strains

As shown in Fig. 2, DA and IB were detected in the HPLC-fluorescence analysis, while IA was not detected. Total ASP toxin contents (DA+IB) of the 18 isolates from 6 sites ranged from 0.3 to 4.3 (average 2.1, DA; 2.0, IB; 0.1) pg/cell. Any other DA isomer peak was not observed in the chromatograms analyzed. The ratio of IB against total toxin content ranged from 0 to 25 (average 5 %) (Fig. 3). In the present paper, *Nitzschia*-like diatoms isolated from Thailand were newly confirmed to produce DA and IB. Total toxin levels

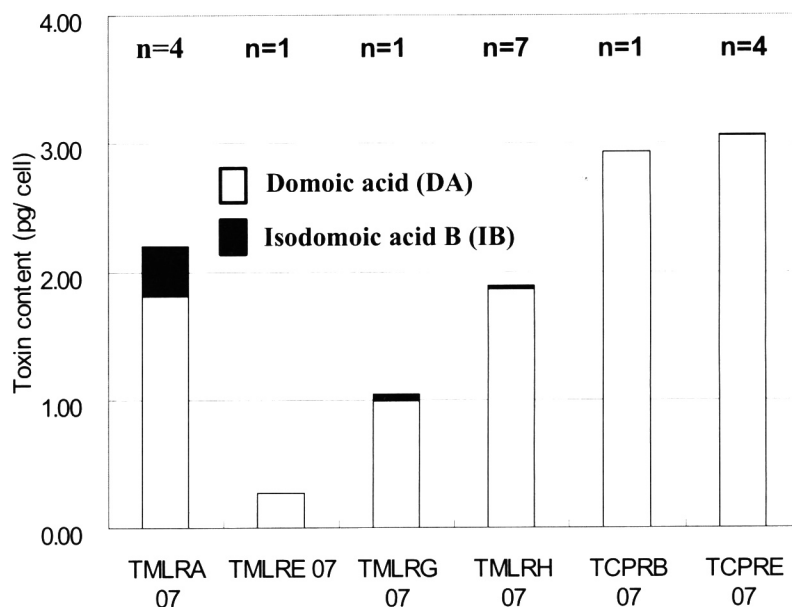
of the isolates from Vietnam (3.1 pg/cell) and Thailand (2.1 pg/cell) were comparable to those of the Tohoku district (1.5 pg/cell) and Okinawa, Japan (2.1 pg/cell), but lower than that of the Philippines (7.3 pg/cell). There was a locality in toxin profile with the tendency that the ratio of IB is higher in southern countries (Kotaki *et al.* in press). However in the present paper, the ratio of IB of the ASP toxins of Thai isolates is much lower than those of the isolates from southern countries such as Vietnam (35%), the Philippines (36%) and Okinawa (28%) and Japan (Kotaki *et al.*, in press). The average ratio is similar to that of the isolates

from northern Japan (Tohoku district) (6%). These tendencies suggest that toxin profile might not be affected by physical factors such as water temperature. One of the axenic strain isolated from the Philippines showed change of the toxin profile from parental non-axenic strain (DA-IB to IA-IB) but other two axenic strains did not. These suggest the possibility that ASP toxin profile might be affected by some bacteria or combination of bacteria (Kotaki *et al.*, in press). The effort tracing the bacteria that affect the toxin profile is now under way.



**Figure 2.** HPLC analysis of the ASP toxins in *Nitzschia*-like diatom isolated from Thailand.

- 1) Standard toxins (DAKS 04-1) including domoic acid (DA) and isodomoic acids A (IA) and B (IB)
  - 2) ASP toxins of TMLRA 07-2 strain. a) Analysis with pre-column derivatization of toxins using FMO-CI reagent. Fluorescence detection (Ex. 264 nm, Em. 313 nm). b) UV detection (242 nm).
- \* FMO-CI derivatives.



**Figure 3.** ASP toxin profile of the isolates from Thailand.

### Morphological observation of the diatoms

Morphological characteristics of the toxin-producing diatoms under light microscope was within those of *N. navis-varingica* described by Lundholm and Moestrup (2000). The cells were yellow-brown and possessed two chloroplasts at each end of the cells. Cells were lanceolate in valve view, 38-110 mm long and 9-11 mm wide, in girdle view rectangular, slightly indented in the middle. The perivalver axis is wider than the transapical axis. Most cells make ribbon-shaped colonies while growing. Observation under transmission microscope (TEM) is now under way.

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