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Pseudo-Nitzschia pseudodelicatissima Isolated From Hurun Bay: Salinity Tolerance and Domoic Acid Content

Pseudo-Nitzschia pseudodelicatissima Hasil Isolasi Dari Teluk Hurun: Toleransi Terhadap Salinitas dan Kandungan Asam Domoat

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Abstract

***Pseudo-nitzschia pseudodelicatissima* dari sampel perairan Teluk Hurun, Lampung berhasil diisolasi dan dikultur dalam medium F/2 dengan penyinaran 2500 luks 12 jam per hari pada suhu 24-25^{0}C. Perlakuan salinitas menunjukkan bahwa *P. pseudodelicatissima* tidak toleran terhadap salinitas kurang dari 15 PSU dan sangat toleran terhadap salinitas yang tinggi hingga mencapai 45 PSU (Pressure Salinity Unit). Salinitas optimum untuk pertumbuhan *P.pseudodelicatissima* yaitu pada 30 dan 35 PSU. Analisis ekstrak *P. pseudodelicatissima*, 9 hari sesudah inokulasi, menggunakan HPLC-UV tidak menunjukkan adanya kandungan asam domoat.**

Kata kunci: *Pseudo-nitzschia pseudodelicatissima*, toleransi salinitas, asam domoat, teluk Hurun

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Introduction

The diatoms species *Pseudo-nitzschia* is a cosmopolitan genus in coastal waters around the world. Several *Pseudo-nitzschia* species are important because they produce domoic acid (DA), the cause of the outbreaks of amnesic shellfish poisoning (ASP). ASP toxin (domoic acid) has given a great fright to the fisheries since the first ASP outbreak in Prince Edwards Islands, Canada (1987) was traced to be produceed by the bloom of *Pseudo-nitzschia multiseries* (Hasle) which was formerly identified as *P. pungens* f. *multiseries* (Bates *et al.*, 1989) Since then nine more *Pseudo-nitzschia* species mostly from temperate waters were reported to produce DA (Bates, 2000, Lundholm, *et al*., 2003), i.e. *P. australis*; *P. caliantha, P. delicatissima*; *P. fraudulenta*; *P. multiseriata*;. *P. pseudodelicatissima*, Lundholm, *P. pungens*; *P. seriata* and *P. turgidula*. There was little information on the toxicity of *Pseudo nitzschia* from tropical areas such as Philippines (Bajarias *et al*., 2005), Vietnam (Kotaki *et al*., 2000) and Indonesia (this paper).

Pseudo-nitzschia live in abundance in coastal waters of a wide range of salinities. Therefore salinity is considered to be one of the important abiotic factor affecting growth. Here the effect of salinity on the growth of *Pseudo-nitzschia pseudodelicatissima* isolated from Hurun Bay, Lampung was examined.

Bates *et al*., (1989) atau 89?? reported that *Pseudo-nitzschia* species from different locations do not always content domoic acid, such as *P. pungens* of the Pacific coast was found toxic in one location in New Zealand, but mostly nontoxic in Atlantic coast of Canada, Atlantic coast of USA, the Gulf of Mexico, Monterey Bay, Europe and New Zealand. Further results of laboratory studies indicated that level of DA production varies depending on *Pseudo-nitzschia* spp. and location. Therefore the isolate was analyzed for the confirmation wether *Pseudo-nitzschia*

pseudodelicatissima found in Hurun Bay, Lampung Indonesia produces DA or not.

Materials and Methods

Isolation and Culture

Phytoplankton samples were collected from mangrove estuary at Hurun Bay, Lampung by vertically towing planktonnet at a depth of only 2 m. Two kinds of *Pseudo-nitzschia* (one belongs to *seriata* and one was *delicatissima* group) were isolated by capillary method and inoculated singly into 1 mL F/10 (Guilard, 1983). Isolates were then transferred to 40 mL F/2 medium in erlenmeyer flask and maintained at 24-25^{o}C under 3000 lux at 12 hrs/day illumination. Of the two isolates, one smaller (*delicatissima* group) grew well, but the larger group (*seriata*) failed to grow. The remaining culture was acclimatized to different salinities, i.e. 5, 10, 15, 20, 25, 30, 35, 40 and 45 PSU. They were grown to reach early stationary phase and used as inoculum. Cultures acclimatized to 5 and10 PSU failed to grow. Consequently, treatments for salinities in three replicates started only from 15 to 45 PSU.

Analysis for DA and Species Identification

Aliquots or 10 mL samples at day 9 were sampled from the batch at 30 PSU, added 5 mL methanol and sent to National Centre for Fishery Quality Control and Processing Technology Development, Ministry of Marine and Fisheries Affair for domoic acid UV-HPLC analysis according to Quilliams (2003). Remaining cells of inoculum were fixed in lugol solution then rinsed and treated with HCl 10% and H2SO4 30%, boiled about one minute and rinse again, then mounted using MGK solution. Species identification of the "clean' diatom was conducted by observing morphological characteristics using light microscope. References for species identification were Fryxel and Hasle (2003) and Hasle and Sylvertsen (1997).

Results and Discussions

Species Description

Cells elongate, stepped chain united by very short overlap of valve ends. In active growth condition, chains were loosed to single cells. Valve slightly lanceolate with slightly rounded ends. Central interspace present. Apical axis 38-60 μm. Transapical axis 2.2-2.4 μm. Striae of the 'clean cells' of the diatom were difficult to observe with light microscope (Figure 1). Based on the shape and size only, the species was similar to *Pseudo-nitzschia delicatissima* according to Fryxel and Hasle (2003). The strain was then brought to Japan by Dr. Kotaki for further SEM observation, and identified as *Pseudo-nitzschia pseudodelicatissima*. Further description will be consulted later with diatom taxonomist.

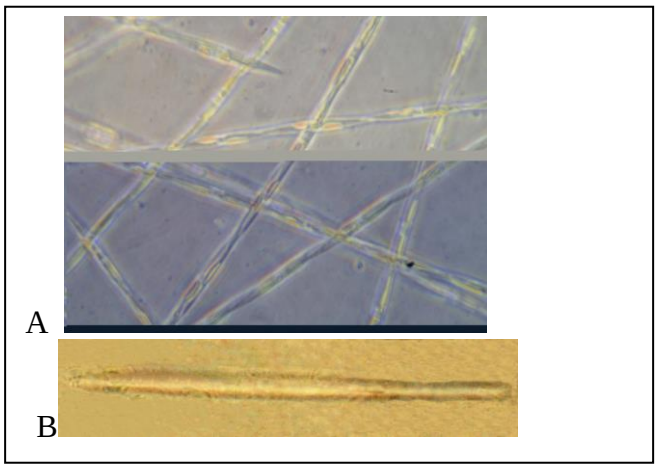

Figure 1. *Pseudo-nitzschia pseudodelicatissima*: A) chain cells; B) single cell appearance after removal of cytoplasma by acid cleaning

Effects of Salinities

P. pseudodelicatissima responded by different growth patterns at different salinities. Average growth rates were 0.77; 0.86, 0.97, 2.2; 1.46 and 0.92 cells/day respectively for *P. pseudodelicatissima* grown at 15, 20, 25, 30, 35, 40, and 45 PSU (Table 1.) Analysis of variance (ANOVA) followed by Duncan test for growth rates indicated that *P. pseudodelicatissima* grown at 30 PSU was significantly different ($P<0.05$) with that of 15, 20, 25, 40, 45 PSU but no significant differences with that of 35 PSU (Figure 2). ANOVA for cell density at day 7 indicated the cell density of *P. pseudodelicatissima* grown at 30 PSU was significantly different ($P<0.05$) with that of 15, 20, 40, 45 PSU but no significant differences with that of 25 and 35 PSU.

Results of salinitiy experiment indicated that this strain can tolerate a salinity range of 15 to 45 PSU or more, but they grow optimally at a narrower salinity range of 30 to 35 PSU. Growth test for *P. pseudodelicatissima* at lower salinity (5 and 10 PSU) has failed to grow when acclimatized. It was indicated that *P. pseudodelicatissima* was not tolerant to salinities lower than 15 PSU. This result was similar to the study of Thessen *et al.* (2005) who also reported the lowest salinity tolerance for the growth of three strains of *P. pseudodelicatissima* was 15 PSU. On the other hand, *P. pseudodelicatissima* are halotolerant species. In accordance with Thessen e*t al.*, (2005), *P. pseudodelicatissima* was able to grow at higher salinities up to 45 PSU or maybe more. This result also agrees with the natural condition in Louisiana coastal waters observed by Thessen *et al.* (2005), who showed that *Pseudonitzschia* spp. were present over a salinity range of 1 to >35 PSU, but they occurred more frequently at higher (between 22 to 28 PSU) rather than lower salinities.

Table 1. Growth rates (cells/day) of *P. pseudodelicatissima* at 15, 20, 25, 30, 35, 40, and 45 PSU, 23°C under 3000 lux at 12 hrs/day illumination

Replicates	Salinities (PSU)						
	15	**20**	**25**	**30**	**35**	**40**	**45**
A	1.15	0.88	0.68	2.11	1.33	1.40	0.88
B	0.55	0.78	1.03	2.15	1.49	1.40	0.91
C	0.61	0.93	1.21	2.34	1.57	1.06	0.99
Average	0.77	0.86	0.97	2.20**	1.46*	1.29	0.93
SD	0.33	0.08	0.27	0.12	0.12	0.20	0.06

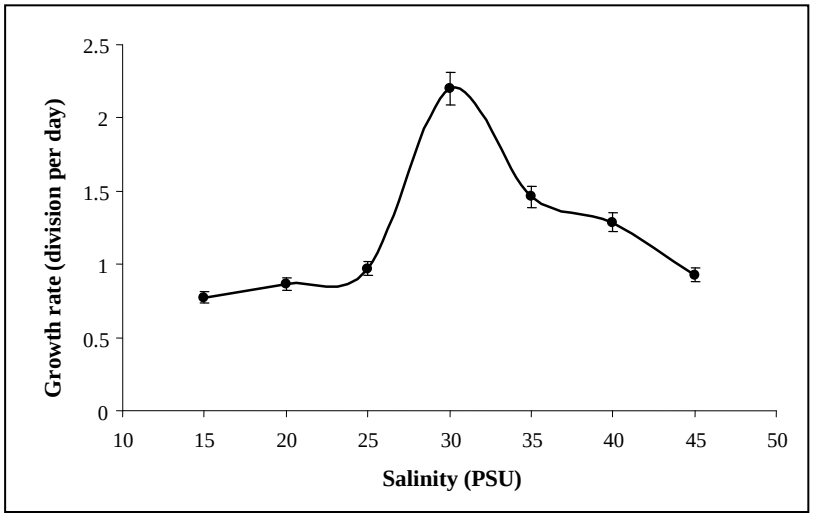

Figure 2. Growth rate of *Pseudo-nitzschia pseudodelicatissima* isolate from Lampung Bay cultured at different salinities under 23^{0}C room temperature and 3000 lux, at 12 hrs/day illummination

Comparing to other *Pseudonitzschia* spp., *P. pseudodelicatissima* was not tolerant to low salinities. According to Thessen *et al.* (2005), tolerance of low salinities decreased from *P. delicatissima* to *P. multiseries* to *P. pseudodelicatissima*. The limit of lower salinity for *P. delicatissima* is 6.25 PSU; *P. multiseries* is 10 PSU and *P. pseudodelicatissima* is 15 PSU respectively.

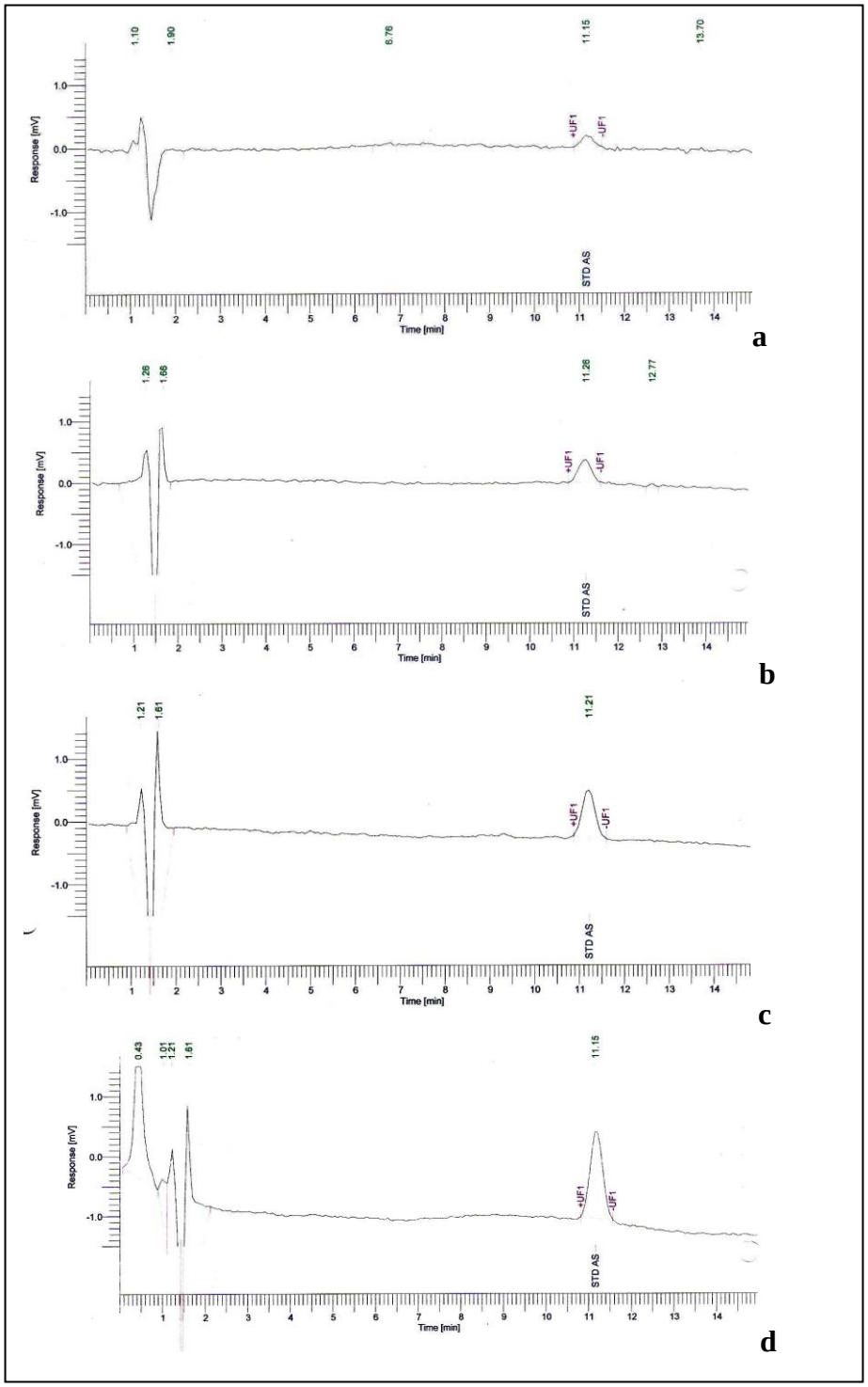

Figure 3. HPLC-UV chromatogram Domoic acid standard: a) 0,1 ppm; b) 0,2 ppm; c) 0,4 ppm; d) 0,8 ppm

Domoic acid analysis

Figure 5 shows HPLC-UV chromatograms of the extract of *P. pseudodelicatissima* culture at day 9 after inoculation. In contrast with chromatogram of standard for ASP (0.1-0.8 ppm domoic acid) and blank samples (Figure 3, 4), the extract of *P. pseudodelicatissima* showed no peak at all indicating that the strain was not producing domoic acid. However this result must be considered preliminary. Chromatogram of the 9 day culture was not reliable. It has been reported so far (Kotaki, 2002) that levels of DA production were varied among *Pseudo-nitzschia* spp. High level of DA content was observed in *Pseudo-nitzschia multiseries*, (Hasle) *P. australis* Frenguelli and *P. seriata* (Cleve) H. Peragallo, while *P. pseudodelicatissima* and four other *Pseudo-nitzschia* spp. were included in the diatoms producing low level of DA.

According to Kotaki (Pers. Communication, 2005), the sampling time (day 9 after inoculation) for domoic acid analysis was been early. At this time, at this stage at domoic acid might have not produced yet or just started to be produced by *P. pseudodelicatissima*. The inexperienced author has sampled randomly and assumed that at any stationary phase the diatom cells were already producing domoic acid. This assumption was based on the study of domoic acid production for *Nitzschia* sp. which was observed by Kotaki *et al.*, (2000). Kotaki suggested (Pers. Communication, 2005) that appropriate sampling time for *Pseudo-nitzschia* should be at the late stationary phase when cells of *Pseudo-nitzschia* ceased their division and domoic acid per cell became maximum. Low content of domoic acid in the culture of *Pseudo-nitzschia* might probably be detected at later stationary phase at least after 21 days culturing.

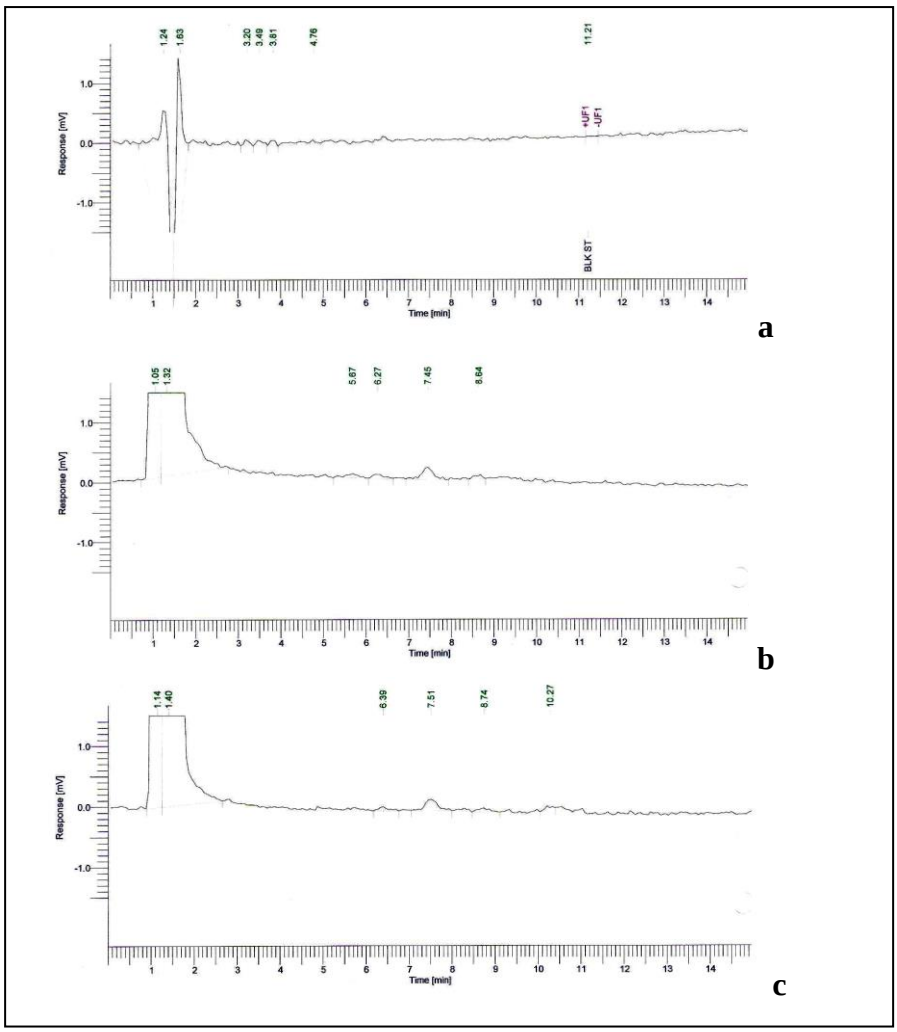

Figure 4. HPLC-UV chromatogram of the a) blank, b) blank reagen for domoic Acid 01 and c) 02

Conclusions

The diatom strain *Pseudo-nitzschia pseudodelicatissima* from Lampung Bay was tolerant to higher (15 to 45 PSU or more) rather than lower salinities (no growth under 10 PSU or below) but they grow optimally at 30 to 35 PSU. The halotolerant character of this species agreed with other studies (Thessen et al., 2005). The strain was considered non producing domoic acid, however, result of this study must be considered preliminary. Non toxigenic character of this strain should be reconfirmed by future works. Isolate was identified as *Pseudo-nitzschia pseudodelicatissima*. Proper description of the *Pseudo-nitzschia* species will be published elsewhere.

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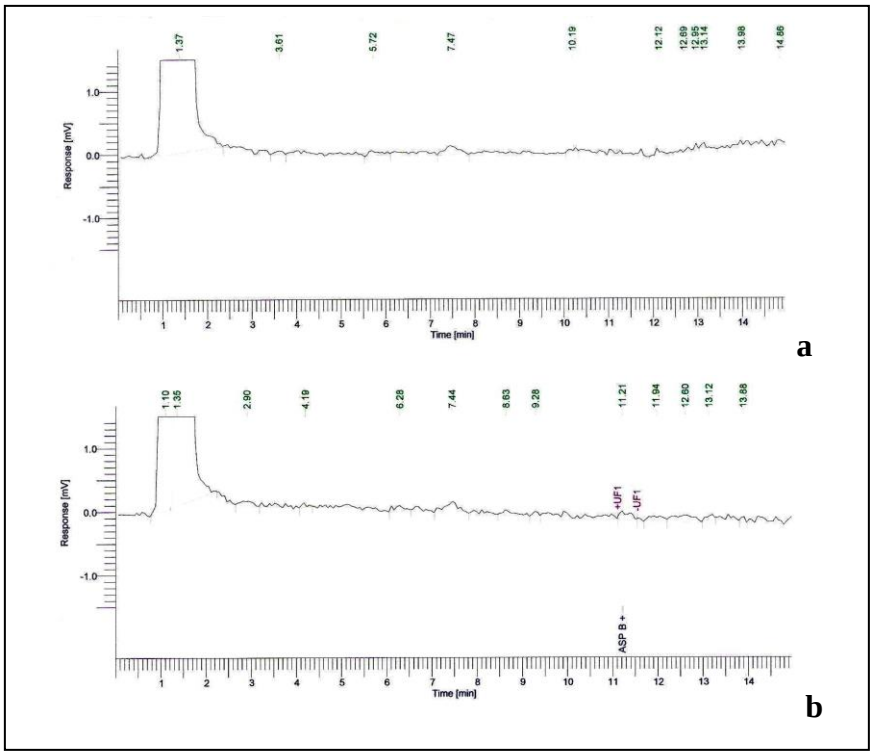

Figure 5. HPLC-UV chromatogram of the a) sample A; b) sample B added by 0.8 ppm domoic acid

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