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The Rapid Differentiation of Toxic *Alexandrium* and *Pseudo-nitzschia* Species Using Fluorescent Lectin Probes

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Since toxic *Alexandrium catenella* and non-toxic *A. fraterculus* are morphologically similar, they are difficult to discriminate under the light microscope. However, a novel technology, such as fluorescein isothiocyanate (FITC)-conjugated lectin probes enables easy and rapid differentiation. Toxic *A. catenella* bound seven different lectins, whereas the non-toxic *A. fraterculus* did not bind *Arachis hypogaea* (PNA) lectin. In addition, *Pseudo-nitzschia* species in this study were also difficult to identify to species level with light microscope techniques, but it was possible to classify them using fluorescent lectins. *Pseudo-nitzschia multistriata*, *P. subfraudulenta* and *P. pungens* bound *Canavalia ensiformis* (ConA), whereas *P. subpacifica* did not, and *P. pungens* also bound *Ricinus communis* (RCA). These results imply that lectin could be used as a critical tool in the differentiation of *P. multistriata*, *P. subfraudulenta* and *P. pungens*. However, *P. subpacifica* was not differentiated by the lectins tested. Therefore, it isconcluded that lectin probes are useful for discriminating toxic *A. catenella* from non-toxic *A. fraterculus*, and for the identification of some *Pseudo-nitzschia* species. In addition, this method has a great potential to speed and detection between non-toxic and toxic harmful algal blooms (HABs) in Korean biotoxin monitoring systems.

INTRODUCTION

Recently, toxic marine microalgae have gained attention due to the socio-economic impacts on sea-farming, tourism and shellfish industries in Korea (Kim *et al.*, 1997). The differentiation of toxic and non toxic species is essential for biotoxin risk assessment based on microalgae (Scholin and Anderson, 1994; Scholin *et al.*, 1996a, b). However, the differentiation between toxic and non-toxic but morphologically indistinguishable species often requires electron microscopy, which is time consuming and expensive (Scholin *et al.*, 1997). To overcome the need for electron microscopy, new technologies, such as immunological techniques (Vrieling and Anderson, 1996), genetic techniques using rRNA-targeted DNA probes (Miller and Scholin, 1996; Rhodes *et al.*, 1997; Scholin *et al.*, 1996a, b, 1997) and lectins probes (Costas and Rodas, 1994; Costas *et al.*, 1995; Rhodes *et al.*, 1995), have been tested to complement phytoplankton monitoring systems, in particular to differentiate toxic from non-toxic marine phytoplankton. These novel technologies have the advantage of being practical and simple to use in the discrimination of morphologically similar species. However, there has been few investigation of these techniques for identifying harmful microalgae isolated from Korean coastal waters (Cho *et al.*, 1998).

In a previous report, we showed that lectins were useful in discriminating morphologically similar species, as well as different species (Cho *et al.*, 1998). In this study, we applied fluorescently tagged lectins, carbohydrate-binding protein or non-enzymatic secretary proteins which detect cell surface sugars, to differentiate between toxic *Alexandrium catenella* associated with paralytic shellfish poisoning (PSP) and non-toxic *A. fraterculus*, and to discriminate between species of pennate diatoms, *Pseudo-nitzschia* spp.

MATERIALS AND METHODS

Target species

A toxic dinoflagellate, *Alexandrium catenella* which is associated with paralytic shellfish poisoning (PSP), was obtained from Inje University, Kimhae,

Korea. The non-toxic *A. fraterculus* isolate was obtained from Pukyong National University, Pusan, Republic of Korea. *Pseudo-nitzschia* species (*P. multistriata*, *P. subpacifica* and *P. subfraudulenta*) for this study were isolated for the first time in Korea coastal waters by Dr. Jong Gyu Park. *Pseudo-nitzschia* complexes were collected by picking cells out indiviually under the light microscope with a micropipette. After a clonal culture was established, it has been maintained in f/2+Si medium (Guillard and Ryther, 1962) containing an antibiotic mixture (Hasui *et al.*, 1995). A temperature controlled room was maintained at 20°C, light intensity of 100 μmol m^{-2} s^{-1} and continuos light period with cool white fluorescent lamp. All algae maintained in exponential growth phase by serial transfers in 100 mL plastic containers every 2–3 week and have been kept in the Harmful Algae Biology Division, NFRDI.

Application of fluorescent probes

Fresh solutions of FITC-conjugated lectins (Table 1; Vector Lectin Kit, fluorescein FLK-2100, Vector Laboratories Inc., Burlingame, CA 94010) were prepared as described by Kim *et al.* (1995). Fluorescein isothiocyanate (FITC)-labeled lectins (Table 1) were added to slide glasses containing 10 μL aliquots of *ca.* 10^3 cells, and were incubated for 40 min at room temperature. Slide glasses were sealed gently with a solution of 3-aminopropyltriethoxy-saline (3%). During the incubation, distilled water was addedgently via filter paper to reduce possibe evaporation of lectin. The treated cells were mounted on siliconised glass slides and examined for binding activity under an epifluorescence microscope, inverted Carl Zeiss MC-80 attached with FITC filter set using UV (excitation, 330-385 nm; emission, >420 nm) and blue light (excitation, 450-480 nm; emission, 515 nm). Binding activity was determined as described by Cho *et al.* (1998).

RESULTS

The potentially toxic dinoflagellate species, *Alexandrium catenella*, showed the binding activity to all tested lectin probes, whereas the non-toxic *A. fraterculus* bound ConA, SBA, UEA, WGA, DBA and RCA except PNA (Table 2). We have tested FITC-conjugated lectins on the four non-toxic *Pseudo-nitzschia* species, *P. multistriata*, *P. subpacifica*, *P. subfraudulenta* and *P. pungens* (Table 2). *P. multistriata*, *P. subfraudulenta* and *P. pungens* had the same binding of ConA with a fine fluorescent outline of the cell being observed (Fig. 1), whereas *P. subpacifica* showed no binding with any tested lectins. The RCA probe bound only with *P. pungens* (Table 2).

DISCUSSION

Lectins exhibit specific carbohydrate conjugation

Table 1. Fluorescein isothiocyanate -conjugated lectins used as probes during this study

Lectins	Sources	Specificity
ConA	*Canavalia ensiformis*	Methyl α-D-mannopyranoside; D-mannose; D-glucose
RCA	*Ricinus communis*	β-D-galactose
DBA	Dolchis biflorus	N-acetyl-D-galactosamine
PNA	*Arachis hypogaea* (peanut)	α-lactose; D-galactose
SBA	*Glycine maxima* (soy bean)	N-acetyl-D-galactosamine; D-galactose; methyl α-D-galactopyranoside
UEA	*Ulex europaeus* (gorse)	L-fucose
WGA	*Triticum vulgaris* (wheat germ)	N-triacetylchitotriose; N-diacetylchitobiose; sialic acid

Table 2. Binding response of *Alexandrium* and *Pseudo-nitzschia* species to FITC-labelled lectins. The symbols '+' and '–' represent binding and non-binding to lectins tested, respectively

Spcecies	ConA	PNA	SBA	UEA	WGA	DBA	RCA
Alexandrium fraterculus	+	–	+	+	+	+	+
A. catenella	+	+	+	+	+	+	+
Pseudo-nitzschia multistriata	+	–	–	–	–	–	–
P. subpacifica	–	–	–	–	–	–	–
P. subfraudulenta	+	–	–	–	–	–	–
P. pungens	+	–	–	–	–	–	+

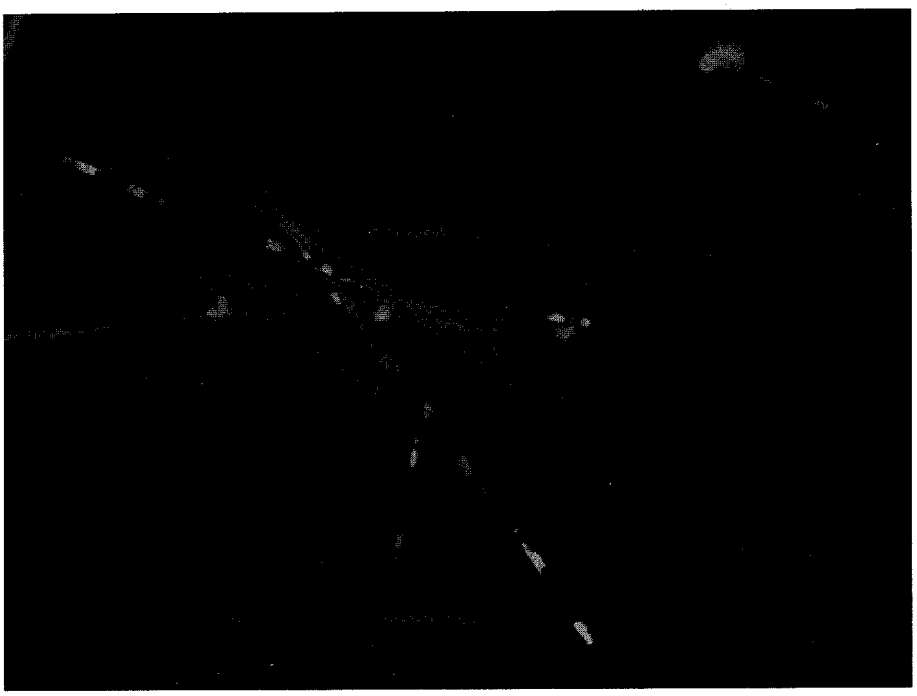

Fig. 1. *Pseudo-nitzschia pungens* with FITC-conjugated ConA labelling (scale bar is 30).

and are accordingly classified in five groups; glucose/mannose; galactose/N-acetyl-D-galctosamine; N-acetyl-glucosamine; fucose; and sialic acids (Slifkin and Doyle, 1990). Because of their specificity, lectins have been used in various applications, eg. clinical microbiology, and recently, even to microalgae to detect toxic phytoplankton from non-toxic phytoplankton, and to identify clones within the same species (Slifkin and Doyle, 1990; Rhodes *et al.*, 1995). Fluorescently tagged lectins used to test the binding response of some red tide microlage in Korean coastal waters easily distinguished between the harmful dinoflagellate, *Cochlodinium polykrikoides*, and the morphologically similar *Gyrodinium impudicum* (Cho *et al.*, 1998). With regard to this result, it is assumed that FITC-conjugated lectins could play an important role in the identification of unicellular microalgae and the detection of biochemical structure at the cell surface.

Paralytic shellfish poisoning (PSP) contaminated mussels and oysters, caused by *Alexandrium,* cause a serious problems to regional shellfish industries. It is desirable to make a monitoring tools in Korean context to rapid differentiate toxic from non-toxic *Alexandrium*. However, the identification of *Alexandrium* to species level remained confusing because of the great variation in morphological features depending on environmental conditions (Sako *et al.*, 1990; Adachi *et al.*, 1993). Four species of *Alexandrium* have been reported from Chinhae Bay. Both *Alexandrium tamarense* and *A. catenella* considered toxic phytoplankton and *A. affine* and *A. fraterculus* considered non-toxic (Lee, 1991). The toxic dinoflagellate, *A. catenella*, bound seven different lectins (Table 2), indicating that it had the same binding activity as *A. tamerense* (Cho *et al.*, 1998) which also binds the lectins DBA, RCA (unpublished data). It is thought that this method is unsuitable for differentiating between *A. catenella* and *A. tamarense*. It has been suggested that DNA probe technology will discrimate between these two species and is expected to be useful for identifying cells in both and field samples (Adachi *et al.*, 1996). In the future it is proposed that these new technologies will be available for identifying harmful phytoplankon blooms in Korea. Meanwhile, although lectin probes in this study were unsuccessful for distinguishing between *A. catenella* and *A. tamarense*, PNA was able to differentiate the non-toxic *A. fraterculus* from the toxic *A. catenella* (Table 2) and *A. tamarense* (Cho *et al.*, 1998).

Pseudo-nitzschia pungens isolated from New Zealand coastal waters showed domoic acid by HPLC screen (Rhodes, 1998), but no toxin fraction was found in *P. pungens* collected from Korean coastal waters (Han Seok Pyun, Sanitation and Processing Division, National Fisheries Research and Development Institute, personal communication). It is difficult to identify the diatoms *P. multistriata, P. subpacifica, P. subfraudulenta* and *P. pungens* under the light microscope, but FITC-conjugated lectin

Table 3. The comparative characteristics of three strains of *Pseudo-nitzschia pungens* isolated from Korean (KR), New Zealand (NZ) and Spanish (SP) coastal waters using fluorescent lectins binding of *Canavalia ensiformis* (ConA) and *Triticum vulgaris* (WGA)

Specimen	ConA (KR)	ConA (NZ[1])	ConA (SP[2])	WGA (KR)	WGA (NZ)	WGA (SP)
Pseudo-nitzschia pungens	+	+	-	-	-	-

Note: [1]Refered to Rhodes (1998) as identification of potentially toxic *Pseudo-nitzschia* (Bacillariophyceae) in New Zealand coastal waters, using lectins. [2]Refered to Fraga *et al.* (1998) as toxicity, DNA content and lectin binding assay.

probes allowed some differentiation of them in this study. *Pseudo-nitzschia multistriata*, *P. subfraudulenta* and *P. pungens* bound lectin ConA, with a fine fluorescent outline of the cell being observed, whereas *P. subfacifica* did not bind ConA (Table 2 and Fig. 1). ConA is suitable for differentiating *P. subfacifica* from the other tested *Pseudo-nitzschia*, but *P. multistriata* and *P. subfraudulenta* were not differentiated by fluorescent lectins. Meanwhile, *P. pungens* was responsible with binding activity of ConA and RCA (Table 2), suggesting that RCA could be used to easily discriminate *P. pungens* from the other tested *Pseudo-nitzschia* species. *Pseudo-nitzschia multistriata*, *P. subfraudulenta* and *P. pungens* therefore have glucose and mannose like sugar moieties at the cell surface (Table 2), indicating similar results to a previous report (Cho *et al.*, 1998), but the lack of glucosamine, galacosamine, compared to some others Korean coastal red tide microalgae (Cho *et al.*, 1998). To identify *P. multistriata* from *P. subfraudulenta*, other lectins probe will be assessed in the future. Some studies have demonstrated that the production of surface sugars by *Pseudo-nitzschia* varies depending on geographical separation and environmental conditions (Rhodes, 1998), and in this study the Korean *P. pungens* appeared close in this regard to New Zealand *P. pungens* isolates than to Spanish *P. pungens* isolates (Table 3).

Consequently, our results show that it is possible to discriminate toxic *Alexandrium catenella* from non-toxic *A. fraterculus* based on the PNA lectin binding response. In addition, some *Pseudo-nitzschia* species, indistinguishable under the light microscope, can be identified. This new technique is a rapid means of detecting phytoplankon within an hour and is an easy and prospective procedure for Korean upcoming monitoring system.

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