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Application of FITC-conjugated lectin probes for the recognition and differentiation of some Korean coastal red tide microalgae

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Harmful microalgae isolated from Korean coastal waters, were tested with FITC-conjugated lectins and observed by epifluorescent microscopy to distinguish each other. Strain-specific sugar composition at the cell surface was suggested by the affinity of lectins to different microalgae. The microalgae *Cochlodinium polykrikoides* (CP-1) and *Gymnodinium* A_3 (GA_3-1), are morphologically similar, but exhibited different binding activity with the lectins ECA, HPA and WGA. In Peridiniales, the microalga *Alexandrium tamarense* (AT) bound HPA and WGA, but *Scrippsiella trochoidea* (ST-1) did not bind those lectins. Three species of *Prorocentrum* also exhibited different binding specificity with HPA, PHA and SBA. A non-toxic Korean isolate of *Heterosigma akashiwo* (HA-2) bound ConA, PEA and UEA. These results suggest that lectins are useful in discriminating morphologically similar species, as well as different species or strains within the same genus.

Key words: epifluorescent microscopy, FITC-conjugated lectin, Korean coastal water, marine harmful microalgae, red tide, toxic phytoplankton

Introduction

With an increasing infrastrucure, aquaculture industry, and population, Korean coastal waters are becoming eutrophic, which could be a cause of unicellular algal blooms. Algal blooms can be harmful with about 57 species capable of producing potent toxins and causing serious problems globally (Costas et al., 1995). At present, there is little mitigation for prevention of harmful microalgae (Boesch et al., 1997), and monitoring programmes are relied on to prevent fish kills and predict shellfish biotoxin events (Emsholm et al., 1996). Moreover, it is difficult to distinguish morphologically similar species and differentiate non-toxic phytoplankton from toxic phytoplankton in certain species. Scanning electron microscopy (SEM) and HPLC is often required to identify different strains, which is expensive and time consuming (Miller and Scholin 1996). To overcome the need for SEM and HPLC, new technologies have been applied to differentiate between non-toxic and toxic microalgae (Rhodes et al., 1995; Scholin et al., 1997). For example, the genus *Pseudo-nitzschia* consists of many species with a global distribution. Some species produce domoic acid, a neuroexcitatory amino acid which can cause amnesic shellfish poisoning in humans, but toxic *Pseudo-nitzschia* species are difficult to identify, requiring SEM and expert taxonomic skills (Scholin et al., 1994). Additionally, Costas et al. (1995) suggested that the toxic species *Gymnodinium catenatum* and the non-toxic *Gymnodinum* A_3, which are morphologically similar, could be distinguished by an immunological technique, DNA sequence analysis and lectin probes. These novel techniques are particularly useful when morphological criteria can change depending on the environmental conditions and physiological state of the cells. Lectins in particular provide a simple and rapid method for the characterization of strains. Lectins are a highly specific carbohydrate-binding proteins or glycoproteins of non-immune origin, which can also agglutinate cells. Recently

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Fluorescein isothiocyanate (FITC)-conjugated lectins have been used to differentiate marine dinoflagellates (Costas et al., 1993; Costas and Rodas, 1994; Costas et al., 1995; Rhodes et al., 19 95; Andujar et al., 1997; Rodas and Costas, 1997). However, there have been few investigations of FITC-conjugated lectins for identifying harmful marine microalgae collected from Korean coastal waters. In this study we have assessed the use of lectin specificity to distinguish morphologically similar species and other red tide microalgae in Korea.

Materials and Methods

Microalgae

Microalgal samples were taken from red tide waters in Korea and their strains were isolated using capillary pipette under microscope. This isolation procedure was done at National Fisheries Research and Development Institute, Pusan, Republic of Korea during last two years (1996-1997). Twelve clonal isolates were used in this study (Table 1). The organisms were grown in f/2-Si medium (Guillard and Ryther, 1962) at 20℃ under 100 μmol m^{-2} s^{-1} light intensity with 12:12 h light: dark cycle and maintained in exponential growth phase by serial transfers.

Application of fluorescent probes

Each strain for this study was cultured for 7 days. Then 100 $\mu\ell$ percoll was added to the 1 mℓ culture for cell aggregation, and the cells harvested by centrifugation at 3000 rpm for 10 min. After centrifugation, the supernatant was removed and aliquots of 10^3~10^5 cells/mℓ were mixed with 50 $\mu\ell$ FITC-conjugated lectins (Table 2; Sigma Chemical Co.), and then added to 950 $\mu\ell$ of 50 mM phosphate-buffered saline (PBS) for 1 hr incubation at 20℃. After incubation, 100 $\mu\ell$ percoll was added again and cells were centrifuged at 3000 rpm for 10 min. The supernatant was removed, and 1 mℓ PBS was added to resuspend the pellet. The treated cells were examined for binding activity under an epifluorescent microscope, Olympus BX 40 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 by direct observation and recorded as: + (clear fluorescent outline of the cell) or − (no fluorescence or autofluorescence).

Table 1. Isolates of harmful algal species used in FITC-conjugated lectin binding experiment

Strains	Isolated time	Location
Alexandrium tamarense (AT)	Feb 1997	Chinhae
Cochlodinium polykrikoides (CP-1)	Sep 1997	Chungmu
Eutreptiella gymnastica (EG-1)	Aug 1997	Namhae
Gymnodinum A_3 (GA_3-1)	Aug 1997	Chungmu
G. mikimotoi (GM-1)	Sep 1997	Chungmu
G. sanguineum (GS-1)	Nov 1996	Masan
Heterosigma akashiwo (HA-2)	Jun 1997	Jindong
Prorocentrum micans (PM-1)	Oct 1996	Masan
P. minimum (Pmini-1)	Aug 1997	Chungmu
P. triestinum (PT-2)	Jul 1997	Namhae
Pyraminonas sp. (Pyramin-1)	Aug 1997	Pusan
Scrippsiella trochoidea (ST-1)	Aug 1997	Yosu

Results

Lectin binding to Gymnodiniales

We have tested FITC-conjugated lectins on the four dinoflagellates, *Cochlodinium polykrikoides*, *Gymnodinium* A_3, *G. mikimotoi* and *G. sanguineum* belonging to the order Gymnodiniales. The microalgae *C. polykrikoides* and *Gymnodinium* A_3 are morphologically similar. The binding of FITC-conjugated lectins to these two strains of *C. polykrikoides* and *Gymnodinum* A_3 differed markedly, in that *C. polykrikoides* bound strongly to ConA (Fig. 1b), PEA, PNA, SBA, UEA and WGA, whereas *Gymnodinum* A_3 bound ConA, HPA, PEA, PNA, SBA and UEA (Table 3). From Table 3, the lectins ECA, HPA and WGA allowed differentiation of *C. polykrikoides* from *Gymnodinum* A_3, as *C. polykrikoides* bound ECA and WGA, but not HPA, whereas *Gymnodinum* A_3 bound HPA, but not ECA and WGA. On the other hand, *G. mikimotoi* and *G. sanguineum* showed the same positive binding for the FITC-conjugated lectins tested (Fig. 1c). *Gymnodinum* A_3 differed from *G. mikimotoi* and *G. sanguineum*, in that it did not fluorescent with ECA or WGA.

Lectin binding to Peridiniales

The microalgae *Alexandrium tamarense* and *Scrippsiella trochoidea* are armoured dinoflagellates with cellulosic thecal plates. Both *A. tamarense* and *S. trochoidea* appeared to bind ConA, ECA, PEA, PNA and SBA, with a fine fluorescent outline of the cell being observed (Table 3 and Fig. 1a). *A. tamarense* also bound HPA and WGA, but *S. trochoidea* did not bind to these lectins.

Table 2. FITC-conjugated lectins used as probes

Lectins	Sources	Specificity
ConA	*Canavalia ensiformis*	Methyl α-D-mannopyranoside; D-mannose; D-glucose
ECA	*Erythrina cristagalli* (coral tree)	α-Lactose; N-acetyl-D-galactosamine; D-galactose
HPA	*Helix pomatia* (snail)	N-acetyl-D-glucosamine; N-acetyl-D-galactosamine; D-galactose
PEA	*Pisum sativum* (pea)	Methly α-D-mannopyranoside; D-mannose; D-glucose
PHA	*Phaseolus limensis* (lima bean)	N-acetyl-D-galactosamine
PNA	*Arachis hypogaea* (peanut)	α-Lactose; D-galactose
PWM	*Phytolacca americana* (pokeweed)	N-acetyl-D-glucosamine
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

Lectin binding to Prorocentrales

The microalgae *Prorocentrum micans, P. minimum* and *P. triestinum* all bound ConA, PEA, PNA, UEA and WGA. However, there were variations in the binding of HPA, PHA, SBA; *P. micans* bound to HPA, PHA and SBA, *P. minimum* bound HPA and SBA, and *P. triestinum* bound only PHA (Table 3 and Fig. 1d). Thus the variation in lectin binding to HPA, PHA and SBA can be used to distinguish the three species of Prorocentrales tested.

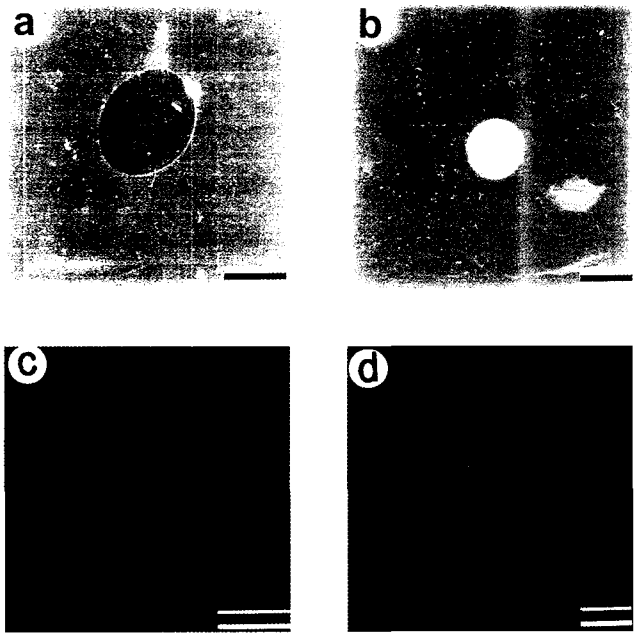

Fig. 1. Some representative labellings of red tide algae using FITC-conjugated lectin. A. FITC-ConA labelling of *Alexandrium tamarense* (sacle bar=20 μm). B. FITC-ConA labelling of *Cochlodinium polykrikoides* (scale bar=20 μm). C. FITC-ConA labelling of *Gymnodinum sanguineum* (scale bar=30 μm). D. FITC-ConA labelling of *Prorocentrum triestinum* (scale bar=20 μm).

Lectin binding to other microalgae

Both of the microalgae *Eutreptiella gymnastica* and *Pyraminonas* sp. bound ConA, HPA, PEA, PNA and SBA. The strain of *E. gymnastica* bound UEA, but *Pyraminonas* sp. did not (Table 3). However, *Heterosigma akashiwo* differed from *E. gymnastica* and *Pyraminonas* sp., as it only bound ConA, PEA and UEA.

Discussion

Our results showed the possibility that lectins are able to discriminate harmful marine microalgae collected from Korean coastal waters. Recently *Gymnodinium* A_3 was isolated from Chungmu, Korea. This species is morphologically similar to *Cochlodinium polykrikoides*, and is thus difficult to identify under the light microscope. There has been no report of toxin production from either species, but these dinoflagellates are associated with damage to and mortality of fish, and are thus regarded as harmful dinoflagellates in Korea (Kim et al., 1997). From Table 3, ECA, HPA and WGA showed highly different binding to *C. polykrikoides* and *Gymnodinium* A_3, indicating that this technique is useful for the differentiation of these morphologically similar species. Both of the microalgae *C. polykrikoides* and *Gymnodinium* A_3 bound ConA, PEA, PNA, SBA and UEA, suggesting that mannose, glucose and fucose residues were

Table 3. Binding response of Korean microalgal isolates to different lectins used as fluorescent probes

Microalgae	ConA	ECA	HPA	PEA	PHA	PNA	PWM	SBA	UEA	WGA
Gymnodiniales										
Cochlodinium polykrikoides	+	+	−	+	−	+	−	+	+	+
Gymnodinium A_3	+	−	+	+	−	+	−	+	+	−
G. mikimotoi	+	+	+	+	−	+	−	+	+	+
G. sanguineum	+	+	+	+	−	+	−	+	+	+
Peridiniales										
Alexandrium tamarense	+	+	+	+	−	+	−	+	−	+
Scrippsiella trochoidea	+	+	−	+	−	+	−	+	−	−
Prorocentrales										
Prorocentrum micans	+	−	+	+	+	+	−	+	+	+
P. minimum	+	−	+	+	−	+	−	+	+	+
P. triestinum	+	−	−	+	+	+	−	−	+	+
Others										
Eutreptiella gymnastica	+	−	+	+	−	+	−	+	+	−
Pyraminonas sp.	+	−	+	+	−	+	−	+	−	−
Heterosigma akashiwo	+	−	−	+	−	−	−	−	+	−

present at the cell surface (Table 2). The alga *C. polykrikoides* did not bind HPA, and *Gymnodinium* A_3 did not bind ECA and WGA, so those species are distinguishable from each other.

Different sugar compositions at the cell surface led to differential binding activity for most of the different species tested, although *G. mikimotoi* and *G. sanguineum* exhibited the same lectin-binding profiles (Table 3). Lack of PHA and PWM binding suggested that galactosamine and glucosamine residues were lacking at the cell surface of *C. polykrikoides*, *Gymnodinium* A_3, *G. mikimotoi* and *G. sanguineum*. Interestingly, *P. micans* and *P. triestinum* had positive binding activity with PHA (Table 3), whereas no other microalgae were bound, suggesting that the 10 tested Korean harmful algal bloom species have the common characteristics of lack of N-acetyl D-galactosamine residues at the cell surface. The microalga *A. tamarense*, which causes paralytic shellfish poisoning (PSP), and the non-toxic *S. trochoidea* were not bound at all by PHA, PWM and UEA, suggesting that these armoured dinoflagellates lacked galactosamine, glucosamine and fucose moieties on the cell surface, but were rich in mannose, glucose, galactose residues and/or sialic acid (Table 3). There was common lectin binding activity for *A. tamarense* and *S. trochoidea*, suggesting that the two strains have similar sugars at the cell surface. Meanwhile *A. tamarense* bound HPA and WGA, but *S. trochoidea* did not, so it is possible to discriminate them using FITC-conjugated lectins.

Blooms caused by *H. akashiwo* have occurred annually in June and July in Korean coastal waters since 1980, with no reported fish kills (Kim et al., 1996), whereas dense blooms of the species resulted in the death of cultured young salmon in New Zealand in 1989 (Chang et al., 1990; Mackenzie, 1991). *Heterosigma* is also regarded as a fish killer in Japan, Canada, USA, Great Britain and Philippines (Tyrrell et al., 1996). Differences between non-toxic and toxic strains are not clear. It is therefore desirable to discriminate the non-toxic from the toxic phytoplankton. From the results in this study, it appears that a non-toxic *H. akashiwo* strain was responsible for binding ConA, PEA and UEA, but the toxic *H. akashiwo* from New Zealand showed no binding of lectins (Cho et al., 1997). It is possible that accessible sugars are lacking at the cell surface of the New Zealand strain, whereas *H. akashiwo* from Korea contains mannose, glucose and/or fucose moieties at the cell surface. It is therefore possible to differentiate between those toxic and non-toxic strains of *H. akashiwo* using FITC-conjugated lectins.

The Korean shellfish industry has a long history, and has increased over times, but PSP contaminated mussels and oysters, caused by *Alexandrium*, have affected exports to other countries with associated losses for shellfish harvesters. Therefore, a marine biotoxin monitoring system is needed to rapidly differentiate toxic from non-toxic *Alexandrium* species. *Alexandrium* is difficult to identify under the light microscope without observing thecal plate features (for example, with the stain calcofluor white or by using sodium hypochlorite solution to separate the plates). These methods are time consuming and require taxonomic expertise, which is an impediment for rapid

phytoplankton monitoring. So far, four species of *Alexandrium* (*A. affine, A. fraterculus, A. tamarense and A. catenella*) have occurred in Chinhae Bay, Republic of Korea, with *A. tamarense* and *A. catenella* producing a potent PSP toxin, and *A. affine* and *A. fraterculus* being non-toxic (Lee, 19 91). However, a convenient identification system has not been developed for these species yet. It appears that lectins are promising for the development of quick procedures for the precise monitoring and differentiation of closely related toxic and non-toxic *Alexandrium* species. Further study needs to be done on the binding of other different FITC-conjugated lectins to these species, as well as to different clones of the same species of *A. tamarense*, the main PSP-toxin producing species. Overall, lectins show promise as monitoring tools in the Korean context.

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