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# Identification of *Cochlodinium polykrikoides* against *Gyrodinium impudicum* and *Gymnodinium catenatum* in Field Samples using FITC Lectin Probes

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We have investigated lectin binding patterns in order to apply binding records of previous laboratory experiments to field settings before the first ourbreaks of harmful algal bloom (HAB). Although cells were grown under different conditions, the binding patterns were the same as in the control. In addition, culture days was not associated with the binding patterns, when compared with the control. In nature, this results suggest that ECA, HPA and WGA lectin are able to discriminate between *C. polykrikoides* and *G. impudicum*, as well as ECA and SBA have a capability as a tool for differentiating between *C. polyrikoides* and *G. catenatum*, although these species are closely similar under the light microscope fiexed with Lugol solution.

Key words: *Cochlodinium polykrikoides*, monitoring, lectin, environmental conditions

## Introduction

FITC-lectin binding assays initially looked promising for the rapid discrimination of harmful algal species. However in some cases the same species isolated from geographically separate sites exhibited different binding patterns (Lesley, 1998). A variability in lectin binding is not good evidence to assure as that it is a reliable taxonomic tool for detecting harmful algae (HA) in Korean events. In an attempt to solve these problems, we are responsible for selecting fluorescent lectin that should be used as a tool to monitor structural changes in cell membranes depending on various stimuli (Kim and Fritz, 1993). However, a little is known about the impact of environmental conditions to lectin binding patterns in *C. polykriikoides*. In this study, blooms caused by *C. polykrikoides*, associated with massive fish mortality considered to be the most ichthyotoxic dinoflagellate in Korea, have occurred simulataneously in the summer with non-toxic *Gyrodinium impudicum* and toxic *Gymnodinium catenatum*, which were morphologically similar and difficult to discriminate under the light microscope (Lee et al., 1999; Cho et al., 2000a). Lectin probes have been used to make useful identification tools to distinguish non-toxic from toxic phytoplankton in Korean coastal waters (Cho et al., 1998, 2000a). In order to ascertain a tool as discrimination of harmful *C. polykrikoides*, we investigated the application of fluorescent tagged lectins to binding patterns under different environmental conditions.

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## Material and Methods

### *Culture of C. polykrikoides*

*Cochlodinium polykrikoides* for this study were isolated from the waters off Tongyong, Korea in 1997. It was grown as described previously (Cho et al., 1998) in f/2-Si medium (Guillard and Ryther, 1962) containing an antibiotic mixture (Hasui et al., 1995) at 20℃ on a 12 : 12 h L : D cycle under 100 $\mu$mol $m^{-2}$ $s^{-1}$ with a cool white fluorescent lamp. This strain was maintained in exponential growth phase by serial transfers and has been kept in the Harmful Algal Research Divsion, National Fisheries Research and Development Institute.

### *Application of fluorescent probes*

Fresh solutions of FITC-conjugated lectins (100 $\mu g$ $mL^{-1}$; Sigma Chemical Co.) were made with filtered sea water. Microalgal cell culture was harvested by centrifugation (300 g; 10 min, 15℃) and different lectins (Table 1) were added to 50 $\mu\ell$ aliquots of $10^3$~$10^5$ cells on glass slides for 40 min at room temperature. Slide glasses were coated with a solution of 3-aminopropyltriethoxy-saline (3%). Unbound lectin was removed by washing in filtered sea water. 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 blue light (excitation, 450~480 nm; emission, 515 nm). Binding of the fluorescent probes was determined qulatitatively at the time of observation and recorded as described by Cho et al., (1998).

### *Effect of environmental parameters*

The culture experiment was carried out at three temperatures (15℃, 20℃ and 25℃), three salinity levels (20 psu, 30 psu and 40 psu) and three pH levels (7, 8 and 9). For the study of nutrient effects, cells were cultured without nitrogen ($NaNO_3$), phosphorus ($NaH_2PO_4$), trace metals ($MnCl_2$, $CoCl_2$, $CuSO_4$, $ZnSO_4$, $Na_2MoO_4$, $NaSeO_3$) and vitamins ($B_{12}$, biotin, thiamine) under the same culture conditions mentioned above. An initial cell density of 500~700 cells/mL was inoculated into the medium. The effects of the age of the culture on the fluorescence binding response was determined by harvesting cells from batch cultures at 5, 10, 20 and 25 days, respectively.

## Results and Discussion

In the present study, the test range of temperature showed the same lectin binding patterns as the control did (Table 2). Regardless of cells from early to late exponential growth (5~25 days) in batch culture tested, they exhibited consistent binding to control (Table 2). Temperature is an important environmental variable for understanding the physiological ecology of algae in nature, as it can affect key biological processes, including photosynthesis (Li and Morris, 1982), enzymatic activity (Kristiansen, 1983; Li et al., 1984) and respiration (Ahmed and Kenner, 1977). Also, temperature is one of the factors most frequently mentioned with respect to regulation of the timing in germination of overwintering resting cysts (Anderson and Keafer, 1987). It is thought that cells treated at different temperature are associated with different physiological activities, sugar moieties and composition of the cell surface. In *C. polykrikoides*, growth began at 15℃ and was well in the range of 20~25℃ (unpublished data). Thus, 15 ℃ played an important role in starting physiological activity for progressive growth. Blooms caused by *C. polykrikoides* occurred first between late August and early September has occurred first and persisted for around 30 days (Kim, 1998). In order to quickly predict the presence of *C. polykrikoides*, we used the lectin binding test before August. Because cells exposed to different temperatures and culture times showed the same lectin binding patterns as the control did (Table 2), it may be possible to apply previous binding response markers in the laboratory (Cho et al., 1998) to rapidly discriminate *C.*

Table 1. FITC-conjugated lectins used as probes

| Lectin | Source | Specificity |
|---|---|---|
| ConA | *Canavalia ensiformis* | Methyl α-D-mannopyranoside; D-mannose; D-glucose |
| ECA | *Erythtthrina cristagalli* (coral tree) | α-lactose; N-acetyl-D-galactosamine; D-glactose |
| HPA | *Helix pomatia* (snail) | N-acetyl-D-glucosamine; N-acetyl-D-galactosamine; D-glactose |
| PEA | *Pisum sativum* (pea) | Methyl α-D-mannopyranoside; D-mannose; D-glucose |
| LBL | *Phaseolu linensis* (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-galactopyranosde |
| UEA | *Ulex europaeus* (gorse) | L-fucose |
| WGA | *Triticum vulgaris* | N-triacetylchitotriose; N-diacetylchitobiose; sialic acid |

Table 2. Lectin binding patterns of *C. polykrikoides* at environmental regimes (temperature, salinity, pH and nutrients) and culture age. + (bright fluorescence), − (no fluorescence or autofluorescence)

| Condition | ConA | ECA | HPA | PEA | PHA | PNA | PWM | SBA | UEA | WGA |
|---|---|---|---|---|---|---|---|---|---|---|
| *Cochlodinium polykrikoides*[1] | + | + | − | + | − | + | − | + | + | + |
| **15℃** | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **20℃** | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **25℃** | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **20 psu** | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **30 psu** | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **40 psu** | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **pH 7** | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **pH 8** | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **pH 9** | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **$NaNO_3$**[2] | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **$NaH_2PO_4$**[2] | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **Minerals**[3] | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |
| **Vitamins**[4] | | | | | | | | | | |
| 5 days | + | + | − | + | − | + | − | + | + | + |
| 10 days | + | + | − | + | − | + | − | + | + | + |
| 20 days | + | + | − | + | − | + | − | + | + | + |
| 25 days | + | + | − | + | − | + | − | + | + | + |

[1]Species was reported in Cho et al., 1998 as application of FITC-conjugated lectin probes for the recognition and differentiation of some Korean coastal red tide microalgae. [2]Deletion of each nutrient, [3]Deletion of $MnCl_2$, $CoCl_2$, $CuSO_4$, $ZnSO_4$, $Na_2MoO_4$, $NaSeO_3$, [4]Deletion of $B_{12}$, biotin, thiamine.

*polykrikoides* from non-toxic *G. impudicum* and toxic *G. catenatum* in field samples taken before August.

In addition, when cells were cultured at different salinity and pH levels according to elapsed times, lectin binding patterns appeared to be a similar, compared with the control (Table 2). Lee et al., (1999) have observed morphological features in *C. polykrikoides, G. impudicum* and *G. catentum* by light microscope and scanning electron microscope. Cho et al., (2000a, b) and Kim et al., (2000) have tested biochemical analysis, gene sequencing and binding response by different lectin probes to provide a specific biomarker and discriminate between them. From our previous results, we realized that three dinoflagellate species with similar morphology are different in biochemical and molecular composition. In nature, from June to August, the range of temperature, salinity and pH level is 15~20℃, 30~35 psu, and 8~9, respecitively. The present data have been enough to differetiate *C. polykrikoides* from non-toxic *G. impudicum* and toxic *G. catenatum*, although the three species occurred concurrently. Thus, ECA, HPA and WGA lectin played a role in species differentiation between *C. polykrikoides* and *G. impudicum*, and ECA and SBA were useful tools to characterize and identify *C. polykrikoides* and *G. catenatum*.

Recently, Lee et al., (1999) demonstrated that the occurrence and abundance of *C. polykrikoides, G. impudicum* and *G. catenatum* were probably associated with eutrophication. Before blooms caused by *C. polykrikoides,* it appeared that essential nutrients (nitrogen and phosporus) were a minor component. Considering that, we investigated the effect of eleminating each nutrient on sugar moieties and the composition of the cell surface to determine the binding response of lectin. Although cells were treated with nutrient deleted solutions, lectin binding patterns showed the same record as that in the control (Table 2). Thus, during intensive field cruises for discriminate *C. polyrkrikoides* from June to August, based on minor components of essential nutrients, our present records will be applied to natural samples for qualtitive and quantitative analysis in *C. polykrikoides.*

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