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Comparative lectin binding patterns of *Cochlodinium polykrikoides* Margalef

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Four different FITC-conjugated lectins were used to visually evaluate lectin binding activity by optical staining quality using confocal laser scanning microscopy (CLSM) of *Cochlodinium polykrikoides* in nature (wild type) and culture (cultured type). Cells from the field and cultures treated with ConA fluoresced only at the outer cell wall, and the abundance and distribution of the fluorescent signal were similar. Treatment with PWM and HPA did not elicit fluorescence at the cell surface, but the wild type exposed to HPA showed greater binding than did the cultured cells, possibly due to greater concentrations of glucosamine. The wild type cells treated with LBL lectin showed a strong green fluorescence on the cell surface, whereas cultured cells did not. Signal intensity and abundance were greater than for any other lectins tested in this study. These results suggest that wild type and cultured type are significantly different based on surface sugar production. In particular, the wild type cells apear richer in galactosamine-like moieties. Neither glucose nor mannose-like moieties were present in either wild types or cultured cells.

INTRODUCTION

In Korea, the first outbreak of *Cochlodinium polykrikoides* Margalef bloom was observed in 1982 (Kim *et al.*, 1997). Since then, blooms caused by *C. polykrikoides* have occurred every year, associated with massive mortalities of caged fish. As this species is regarded as a potentially harmful microalgae, it has attracted public attention in Korea (Kim *et al.*, 1997).

It was reported that lectins could provide a simple and rapid method for the characterization of microalgae in Korean coastal waters (Cho *et al.*, 1998). Subsequently, we have applied FITC- tagged lectins to differentiate *C. polykrikoides*, *Gyrodinium impudicum* Fraga et Bravo (Cho *et al.*, 1998), *Gymnodinium catenatum* Graham (Cho *et al.*, 2000a), and four morphologically similar *Pseudo-nitzschia* species (Cho *et al.*, 1999a), and suggested that this method could be used in phytoplankton monitoring in Korea. We have also investigated lectin probe binding patterns under different environmental conditions and at different growth stages of *C. polykrikoides*, to ascertain that the lectin binding patterns from cultures could be applied to natural samples for qualitative and quantitative determination of *C. polykrikoides* (Cho *et al.*, 2000b).

In natural samples many chain-forming cells are found, but few occur in culture. In addition, the morphological features of *C. polykrikoides* can differ between natural and cultured cells. In order to understand the molecular architecture and structure of the algal cell surfaces, fluorescent lectin probes have been applied to determine structural differences in the cell membranes (Kim and Fritz, 1993). Previously, we have observed sugar accumulation points in vegetative and long chain-forming cells of *G. impudicum* stained with ConA lectin, analyzed using confocal microscopy (Cho *et al.*, 1999b). We have also investigated the effect of several types of yellow loess on sugar composition at the cell surface using fluorescent lectin probes (Cho *et al.*, 1999c), and concluded that lectins are an important tool for analysing cell surfaces and cell components in *C. polykrikoides*, in both wild and cultured cells.

In this work, we examined the presence of glycan moieties and their distribution on the cell surface of *C. polykrikoides* in the nature and in cultures by fluorescein isothiocyanate (FITC) lectin labelling, and compared our results with previously published data.

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MATERIALS AND METHODS

Cultures of C. polykrikoides

Cochlodinium polykrikoides cells (wild type) was isolated from red tide waters off Chodo in 1999. *C. polykrikoides* (CP-1), maintained in culture, was isolated from Tongyong in 1997, and grown 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 mmol m^{-2} s^{-1} cool white fluorescent lamp (Cho *et al.*, 1998). The strain was maintained in exponential growth phase by serial transfers and is kept in the Harmful Algal Research Division, NFRDI.

Confocal laser scanning microscopy (CLSM)

Confocal imaging microscopy was performed on a Zeiss LSM 510 confocal laser scanning microscope with a Zeiss Plan-Apochromat magnification ×63° or ×100° (oil immersion objective (NA=1.4), using excitation wavelengths of 488 and 543 nm, from Argon and HeNe laser, respectively. Fluorescence was detected using a 505-530 bandpass (BP) filter for FITC or 560 long pass (LP) filter. Pairs of images were collected simultaneously in the green and red channels. The degree of colocalization was displayed with colocalization mode and line scan supported by LSM 510 software (Carl Zeiss, 1999).

Application of fluorescent probes

Fresh solutions of FITC-conjugated lectins (100 μg mL^{-1}; Sigma Chemical Co.) were made with filtered sea water. Microalgal cell cultures were harvested by centrifugation (300 g; 10 min, 15°C), and 50 μl volumes of different lectins (Table 1) were added to aliquots of cell suspension (10^3–10^5) cells on glass slides, and coated with a solution 3-aminopropyltriethoxy-saline (3%) for 40 min at room temperature. Unbound lectin was removed by washing the slides in filtered sea water. The treated cells were mounted on siliconised glass slides and examined for binding activity under CLSM. Binding activity was determined as described by Cho *et al.* (1998).

Signal intensity

Signal brightness is as follows: red > yellow > blue.

RESULTS AND DISCUSSION

Binding of the lectin from *Canavalia ensiformis* (ConA) to the surface of wild type cells (Fig. 1a) and cultured type (Fig. 1b) of *C. polykrikoides* was observed under CLSM, and signal intensity and distribution of ConA fluorescence was similar (Fig. 2a, b). Lectin treatment of *C. polykrikoides* using *Phytolacca americana* lectin (PWM) and *Helix pomatia* lectin (HPA) elicited a fine fluorescent outline for both types (Fig. 1c, e, d, f), although with PWM lectin, the fluorescence signal was not detectable in either wild type (Fig. 2c) or culture type (Fig. 2d). When cells were treated with HPA lectin, the wild type (Fig. 2e) exhibited a yellow fluorescence signal on the cell surface, whereas no signal was detected in cultured cells (Fig. 2f). Treatment of *C. polykrikoides* wild type cells with the *Phaseolus limensis* (LBL) lectin not only elicited a fine fluorescent outline of the cell (Fig. 1g), but also a strong red signal intensity. LBL appeared to bind to a greater degree than any other lectins in this study (Fig. 2g). In contrast to the wild type, the cultured cells were not seen to bind to LBL under CLSM (Fig. 1h, 2h).

Generally, the visual evaluation of lectin binding activity by optical staining quality is made by direct observation with a fluorescent microscope. However, some researchers used a spectrofluorometer to obtain a precise quantitative measurement of binding (Costas and Rodas, 1994). Recently, CLSM has been introduced for chromosome counting in cell cycle studies, the detection of changes in the chloroplast morphology of individual cells and the localization of cell organelles (Vrieling and Anderson, 1996). However, this technology has not yet been exploited to any great extent in phytoplankton research. Since CLSM allows high-resolution of fluorescence imaging, it can be used

Table 1. FITC-conjugated lectins used as probes

Lectin	Source	Specificity
ConA	Canavalia ensiformis (jack bean)	Methyl α-D-mannopyranoside; D-mannose; D-glucose
HPA	Helix pomatia (snail)	N-acetyl-D-glucosamine; N-acetyl-D-galactosamine; D-galactose
LBL	*Phaseolus limensis* (lima bean)	N-acetyl-D-galactosamine
PWM	*Phytolacca americana* (pokeweed)	N-acetyl-D-glucosamine

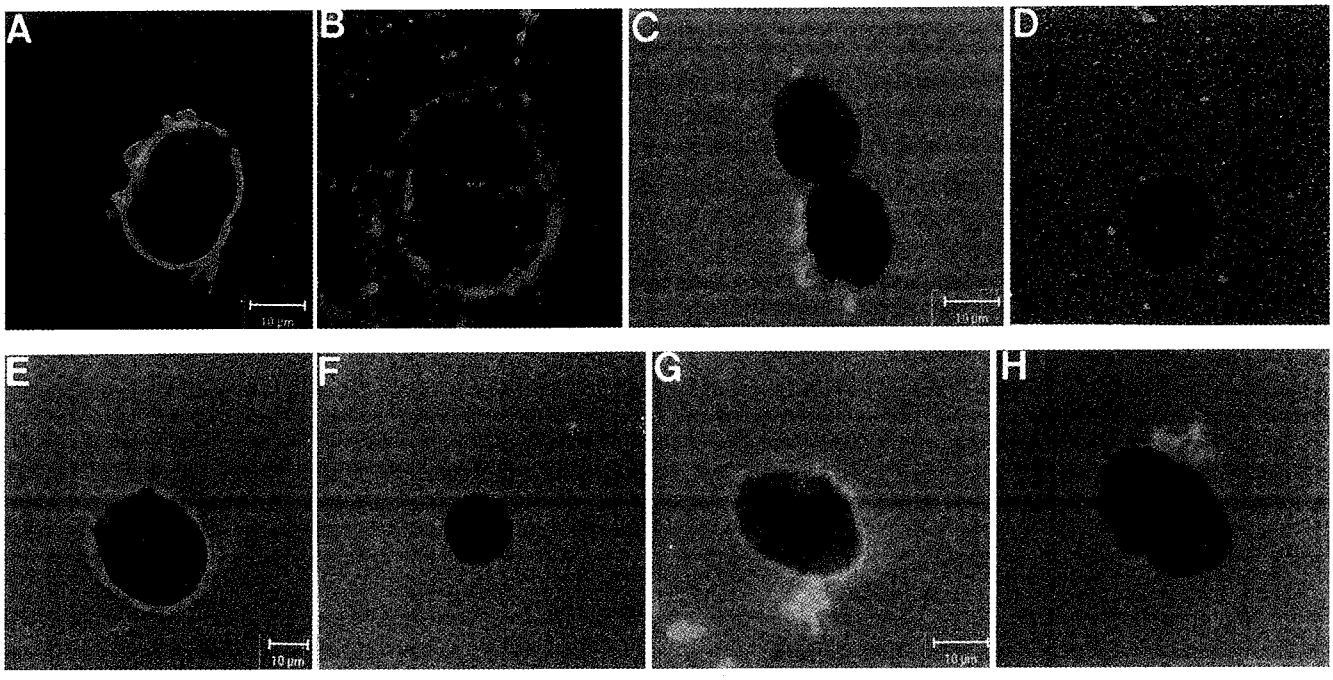

Fig. 1. *Cochlodinium polykrikoides* with FITC-conjugated lectins. A: Natural populations treated with ConA lectin, B: Laboratory cultures treated with ConA lectin, C: Cells from the field treated with PWM lectin, D: Cultured cells treated with PWM lectin, E: Cells from the field treated with HPA lectin, F: Cultured cells treated with HPA lectin, G: Cells from the field treated with LBL lectin, H: Cultured cells treated with LBL lectin.

to examine the target cells and to guarantee the detection of even low abundances and fluorescence intensities of the bound FITC-lectins (Vrieling *et al.*, 1993).

Although FITC-conjugated lectin probes were applied to red tide phytoplankton in Korean coastal waters, only epifluorescence microscopy has been used to examine binding activities (Cho *et al.*, 1998, 1999a, c, 2000a, b, c). When we compare ConA binding response in laboratory cultured of *C. polykrikoides*, using either CLSM or epifluorescence microscopy, ConA lectin was found to bind to the cell surface in both instances (Cho *et al.*, 1998). Treatment of *C. polykrikoides* with PWM, HPA and LBL lectins resulted in no binding response when analyzed under both CLSM (present result) and epifluorescence micrroscopy (Cho *et al.*, 1998). These result indicate that the analysis of lectin binding using the conventional epifluorescence microscopes give equivalent results to those obtained by CLSM.

The most unique features of the CLSM are laser excitation, auxiliary image intensification, and computer-assisted analysis technology so that even the weakest fluorescence emission can be quantified (Vrieling and Anderson, 1996). As can be seen in Fig. 2, ConA lectin treatments of wild type and cultured type in *C. polykrikoides* showed similar fluorescent signals (red, yellow) and distribution of signal intensity, suggesting that the composition and residues of mannose and glucose-like moieties on the cell surface were similar for both wild type and culture type. Treatment of *C. polykrikoides* (wild type and culture type) with PWM, did not result in a fluorescent signal (Fig. 2c, d), indicating that glucosamine-like moieties were lacking at the cell surface. In contrast to PWM, wild type of *C. polykrikoides* did appear green signal at the cell surface when HPA lectin was treated (Fig. 1e), a yellow signal was deleted around the cell surface (Fig. 2e). This signal was not present in cultured cells (Fig. 2f). The presence of yellow signal for the wild type indicates that the components of galactose-like are higher in the wild type than in the cultured type, although residues were not present in sufficient concentrations to elicit green fluorescence on the outer cell wall. Surprisingly, treatment of the wild type with LBL lectin, which specifically binds to galactosamine, resulted in a red fluorescence signal rather than a yellow signal (Fig. 2g), presumably due to the presence of galactosamine moieties on the cell surface. However, the cultured type lacked galactosamine (Fig. 2h). In particular, the red fluorescence signal was greater than the one elicited after treatment with ConA. Probably, surface sugar production in wild type and

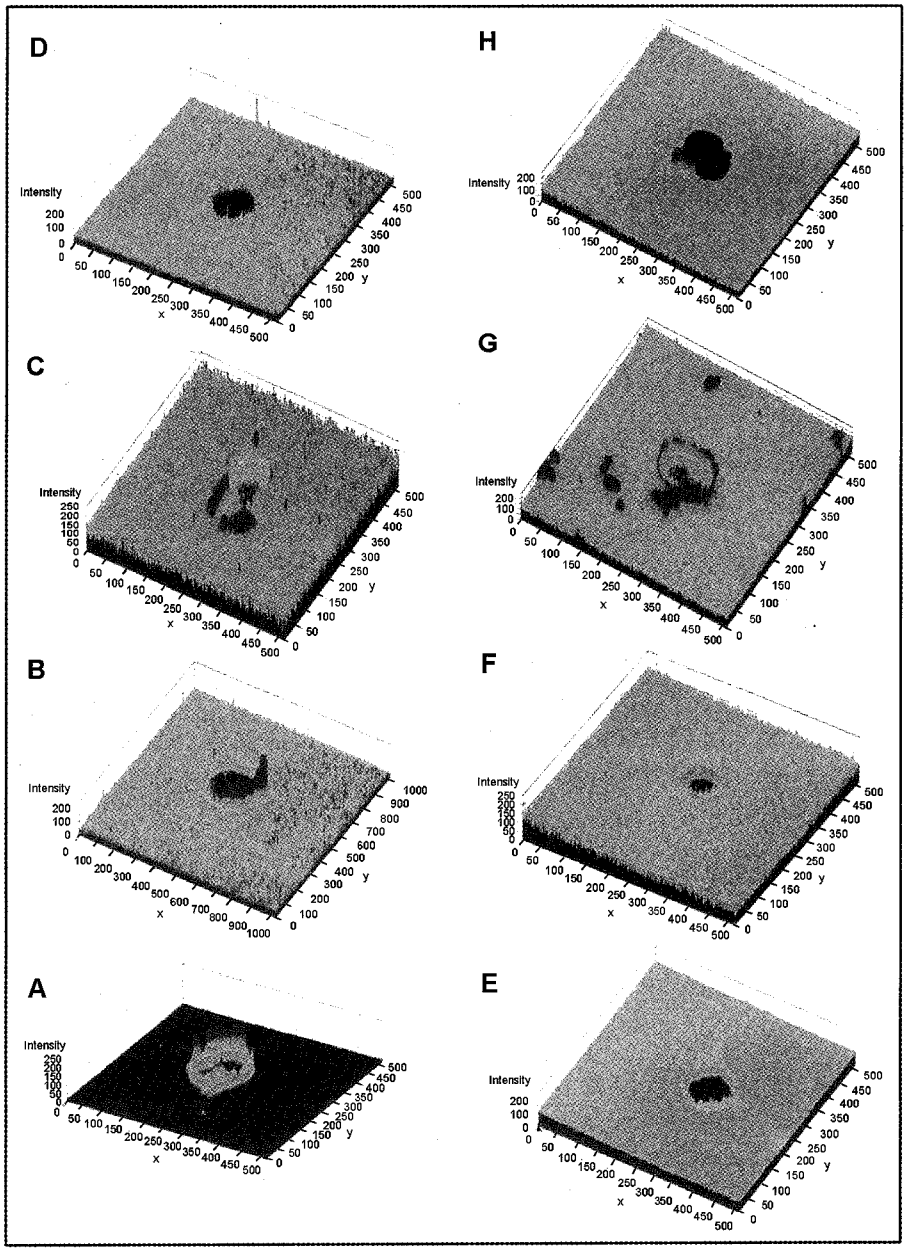

Fig. 2. The determination of labeling intensities by confocal laser scanning microscopy. A: Cells from the field treated with ConA lectin, B: Cultured cells treated with ConA lectin, C: Cells from the field treated with PWM lectin, D: Cultured cells treated with PWM lectin, E: Cells from the field treated with HPA lectin, F: Cultured cells treated with HPA lectin, G: Cells from the field treated with LBL lectin, H: Cultured cells treated with LBL lectin.

culture type shoud be significantly different, particularly in galactosamine-moieties. We intend to further investigate into this possibility further with biochemical techniques.

According to Rhodes (1998), FITC- tagged lectin binding associated with the production of surface sugars varies depending on geographical separation and environmental conditions. From our earlier works, it is clear that extracellular polysaccharides were produced by *C. polykrikoides* (cultured type), and which were stained strongly blue around the cell surface with Alcian blue (Cho *et al.*, 1999d). Total polysaccharides contents found in *C. polykrikoides* were also higher than those in any other tested red tide microalgae (Cho *et al.*, 1999). Hence, massive red tides of *C. polykrikoides* in the nature cause increases in sea water viscosity, especially through the excretion of large amounts of extracellular polysaccharides on the cell surface. It is likely that during exponential growth phase surface sugar productions are excreted more galactosamine than glucose and mannose-like compounds in wild cells, whereas cultured cells do not produce galactosamine and are composed mostly of glucose and mannose-like moieties at their cell surface. These differences in surface sugar composition may be associated with different growth conditions.

CONCLUSION

Lectin ConA treatment of *C. polykrikoides* cells both in nature and in cultures resulted in similar binding profiles (signal distribution and abundance). PWM lectin did not bind to either wild type or cultured cells. On the contrary, wild and cultured cells treated with HPA lectin showed different signal intensities, the wild type appearing to have more galactose-like moieties, compared with cultured cells. The wild type also had a greater amount of galactosamine-like moieties, suggesting that the differences in surface sugar compositions are associated with different environmental conditions for *C. polykrikoides* cells.

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