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FLUORESCENT LECTINS: THE WAY FORWARD IN RAPID ALGAL IDENTIFICATION

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Abstract

The identification of microalgal species is an area that requires reorganization and advancement. Whilst there is no doubt that conventional morphological criteria are sufficient in the generic identification of microalgae, their species-level identification is far from satisfactory. On one hand, fine-scale morphology is necessary to solve complex taxonomic problems between morphologically similar species. Many modern molecular techniques have been used to improve the characterization of microalgal species and strains in recent times, none however have so far achieved a rapid and sensitive procedure to clarify this problem. In this sense, fluorescent lectins fulfill these primary objectives. Their potential to differentiate between morphologically closely related species represents an important advancement in the pace of the identification of microalgae. For example, the toxic PSP-producing *G. catenatum* is readily discriminated from the morphologically similar non PSP-producing *G. impudicum* using the lectin WGA. This presentation will outline how fluorescent lectins are excellent discriminant characters in the rapid and simple identification of microalgal species. This is especially pertinent when the unequivocal and rapid separation of toxic and non-toxic species is now of mounting importance in routine monitoring programmes.

Introduction

Unicellular algae were classified in species based on morphological criteria. As a rule, morphology is generally sufficient to species determination. But in some cases morphology is insufficient. For example, the PSP toxic dinoflagellate *Gymnodinium catenatum* can not be distinguished of *Gyrodinium impudicum* in lugol´s fixed samples using light microscopy. However is known that while the first is the most toxic dinoflagellate specie, the second is completely inofensive. In that way is neccesary the development of molecular procedures to identify morphologically similar species and to separate different strains and clones of the same specie. The relevance of this kind of laboratory technics to identify red tides and/or toxic dinoflagellates and other harmful algae species is the increasing attention due to the human health and the economic impact they have on the aquaculture, fisheries and tourism.

Recent advances in technology allow nowadays the use of antibodies to cell surface proteins to label species, and nucleic acid-based probes that bind to target sequences of rRNA or rRNA genes (ANDERSON, 1995; COSTAS & LÓPEZ-RODAS, 1994; COSTAS & al., 1995).

Another item is the use of molecular procedures based on lectins. There are most examples of the application of lectins to taxonomic characterization of protists (SCHOTTELIUS & MULLER, 1984; GHOSH & al., 1987). To date, lectins have been

Clones	ConA	PEA	ECA	DBA	SBA	HPA	PNA	PWN	WGA	LPA	UEA-I	PHA
Cm1V	++	-	-	-	-		+		++	-	-	-
Cm3V	+	-	-	-	-		++		-	-	-	-
Cm4V	++	-	-	-	-		-		-	-	-	-
Cm5V	-	-	-	-	+		-		+	-	+	+
Cm2V	++	-	-	-	-		-		-	-	-	-
GLEN	++	+	-	-	-		-		-	-	-	-
AMPH	-	-	-	-	-		-		-	-	-	-
S2V	++	-	-	-	-		-		-	-	-	-
S3V	++	+	-	-	+		-		+	-	+	-
Am1V	++	-	-	+	-	-	-	-	-	++		-
Am2V	++	-	-	+	-	-	-	++	-	+		++
Al18V	++	-	-	+++	-	-	-	++	-	++		++
Aa1V	+	-	-	-	-	-	+++	+	-			+
Aa2V	+	-	-	-	-	-	+++	+	-			+
Aa3V	+	-	-	-	-	-	+++	+	-			+
Aa4V	+	-	-	-	-	-	+++	+	-			+
Ae1V	+	+	-	-	+	+	+	++	-			++
Ae2V	+	-	-	-	-	+	+	+	-			+
Pl5V	+	+	-		+		+		-			+++
Pl6V	+	-	-		-		+		-			+++
Pl7V	+	-	-		-		-		-			+++
Pl8V	+	-	-		-		+		-			+++
Pt3V	+++	-	+		+		-		-			-
Pt5V	+++	-	-		+		+		-			-
Pmin	++	-	-	-	-		-		-	-	-	-
Pr1V	+	-	-	-	-		-		-	-	-	-
Gc7V	+	-	-	++	-	-	-	-	+++	-		-
Gc9V	+	-	-	++	-	-	-	-	+++	-		-
Gc19V	+	-	-	++	-	-	-	-	+++	-		-
Gc21V	+	-	-	++	-	-	-	-	+++	-		-
Gg7P	-	-	-	-	-	-	-	-	-	-		-
Gg8P	-	-	-	-	-	-	-	-	-	-		-
Gg9P	-	-	-	-	-	-	-	-	-	-		-
Gg1P	-	-	-	-	-	-	-	-	-	-		-
CHAET	-	-	-	-			-		++	-	-	+++
THp	+++	++	-	-			-		+	-	-	-
THw	+++	+	-	+			+		++	-	+	+++
NAV	-	-	-	-			-		+	-	+	+
PS2V	+++	-	-	+			-		+	-	-	++
PS3V	+++	+	-	-			-		+	-	-	++
PSaIV	+++	-	-	-			-		++	-	+++	++
NIe	+++	-	-	+	-		++		++	-	-	-
NIc	+++	-	-	-	-		-		+	-	-	-
NIl	+++	+	-	-	-		-		-	-	-	+
ISO	++	-	-	-	-		-		-	-	-	-
PAU	-	-	-	-	-		-		+	-	-	-

Clones	ConA	PEA	ECA	DBA	SBA	HPA	PNA	PWN	WGA	LPA	UEA-I	PHA
PRYM	+	-	-	-	-		-		-	-	-	-
HET	-	-	-	-	-		-		-	-	-	-
Ha1V	-	-	-	-	-		-		-	-	-	-
Ha2V	-	-	-	-	-		-		-	-	-	-
Chaton	+++	++	-	-	-		-		-	-	-	-
DUNt	-	-	-	-	++		+++		-	-	-	-
DUNm	-	-	-	-	-		-		-	-	-	-
DUNs	-	-	-	-	-		-		-	-	-	-
TETR	-	-	-	-	-		+		-	-	-	-
HEM	+++	-	-	-	-		-		+++	+++	++	-
CHLO	-	-	-	-	-		-		-	-	-	-

Table 1. Lectin binding patterns. Lectins used: *Canavalia ensiformis* (ConA); *Pisum sativum* (PEA); *Erythrina cristagalli* (ECA); *Dolichos biflorus* (DBA); *Glycine maxima* (SBA); *Helix pomatia* (HPA); *Arachis hipogaea* (PNA); *Phytolacca americana* (PWN); *Triticum vulgaris* (WGA); *Limulus polyphemus* (LPA); *Ulex europaeus* (UEA-I); *Phaseolus vulgaris* (PHA). Clones of algae species: *Colia monotis* (Cm1V, Cm2V, Cm3v, Cm4V, Cm5V); *Glenodinium foliaceum* (GLEN); *Amphydinium carterae* (AMPH); *Scripsiella sp.* (S2V, S3V); *Alexandrium minutum* (Am1V, Am2V); *Alexandrium lusitanicum* (Al18V); *Alexandrium affine* (Aa1V, Aa2V, Aa3V, Aa4V); *Alexandrium excavatum* (Ae1V, Ae2V); *Prorocentrum lima* (Pl5V, Pl6V, Pl7V, Pl8V); *Prorocentrum triestinum* (Pt3V, Pt5V); *Prorocentrum minimun* (Pmin); *Prorocentrum rostratum* (Pr1V); *Gymnodinium catenatum* (Gc 9V, Gc19V, Gc21V); *Gyrodinium impudicum* (Gg7P, Gg8P, Gg9P, Gg1P); *Chaetoceros calcitrans* (CHAET); *Thalassiosera pseudonana* (THp); *Thalassiosera weisfloggii* (THw); *Navicula sp.* (NAV); *Pseudonitschia fraudulenta* (Ps2V, Ps3V); *Pseudonitschia subpacifica* (Psa1V); *Nitschia epithermoides* (NIe); *Nitschia closterium* (NIc); *Nitschia longiformis* (NIl); *Isochrysis galbana* (ISO); *Paulova lutheri* (PAU); *Prymnesium parvum* (PRYM); *Heterosigma akashiwo* (HET, Ha1V, Ha2V, Chaton); *Dunaliella tertiolecta* (DUNt); *Dunaliella minuta* (DUNm); *Dunaliella salina* (DUNs); *Tetraselmis sp.* (TETR); *Hemiselmis rufecans* (HEM); *Chlorella sp.* (CHLO).

employed in a small number of studies of algae (FRITZ, 1992). In a recent paper, COSTAS & al. (1993) were able to recognize and differentiate species and strains of unicellular algae using specie-specific and clone-specific lectins. In this work species of toxic and nontoxic algae had been studied at different levels like class level, genus level, species level and clones of the same specie using fluorescent lectins, and measuring the fluorescence with a epifluorescence microscopy.

Material and methods

Cultures used in this study were grown axenically in f/2 medium (Sigma) at 20º C and 50 micromol. photons. $m^{-2.}$ s^{-1} from day-light tubes (Phillips daylight) under 12:12 h LD cycles as previously described. These cultures were mantained in exponential

growth by serial transfers once every 15 days and periodically checked using epifluorescence procedures to show the absence of bacteria. More details are given in COSTAS & LÓPEZ-RODAS (1994).

Every exponentially growing clone was treated with ten fluorescein isothiocyanate-labeled lectins. Cells were collected by centrifuging (140 x g, 10 min), and aliquots of $10^5 \pm 10^3$ cells were treated with each fluorescent lectin (100 µg ml^{-1} for 1 h at 20º C) as previously described (COSTAS & LÓPEZ-RODAS, 1994). Afterward, cells were washed three times in f/2 medium (Sigma).

The binding activity of lectins was measured as previously described (COSTAS & LÓPEZ-RODAS, 1994). In short: Washed cells were observed in a Zeiss Axiovert microscope with an FITC Filter set (450-490 nm excitation) for epifluorescence. Quality of staining was estimated using the following scale: (3+) bright stain, 100% of cells stained; (2+) less bright stained; (+) low-intensity stain but obviously different from controls; (-) nondetectable reaction. All tests were read "blind", i.e. the person reading the test did not know the identity of the tested material.

Four replicates from different culture flasks of each clone were measured. Statistical comparisons were performed using the Mann-Whitney U-test.

Results and discussion

The visual evaluation of lectin binding activity by optical staining quality did allow for the study of isolated cells by direct observation with a fluorescent microscope. Among different classes, various trends in lectin binding can be easily recognised. In general, virtually every species within each of the genera analyzed was positively labelled by the lectin ConA, which indicates the almost homogeneous occurrence of glucose and manose based surface sugars in the microalgal cell membranes.

At the genus level, specific lectin reactions were more aparent, when different species of the same genus could be analyzed. For example, in the class Dinophyceae, virtually every individual genus showed consistant and intrinsic lectin staining. Certain genera could only be distinguished by ConA, the lectin with the highest frecuency of reaction among the members of the phytoplankton classes analyzed, as *Prorocentrum, Alexandrium* and *Gymnodinium*. In the latter case, the toxic PSP-producing genus *Gymnodinium* can be rapidly separated from the nontoxic but morphologically similar genus *Gyrodinium*, using WGA or DBA.

At the species level, lectin staining started to display very high specificity. For instance, different species of the same genus could be separated with considerable ease. Moreover, lectins could be easily distinguish between different species that are morphologically very similar. That is the case of the toxic DSP-producing *Prorocentrum lima*, that can be rapidly distinguished from the other morphologically discrete species of *Prorocentrum* using the lectin PHA.

Where different clones of the same species could be analyzed, considerable variation was seen in lectin staining for all phytoplankton classes, indicating the existance of distinct cell surface sugars among clones. Lectin binding appears constant and independent of culture medium, temperature and light intensity. All results are shown in Table 1.

This results show that lectins are an adequate tool to distinguish morphologically similar species as well as different strains of same species. In this way, and in agreement with COSTAS & LÓPEZ-RODAS (1994), lectins can complement other molecular techniques such as nucleic acid-based or inmunological probes, although more work is necessary to obtain batteries for a complete characterization of genoespecies in dinoflagellates and other algae (COSTAS & LÓPEZ-RODAS, 1994).

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