Phytochip: a new tool to study the diversity of toxic phytoplankton in the Bay of Seine

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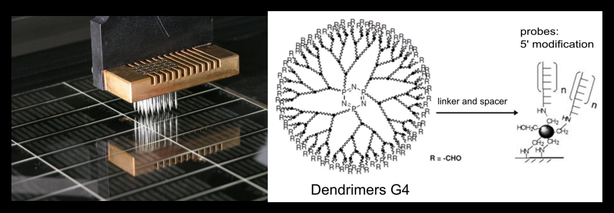
Introduction

Detection of harmful algal blooms (HABs), also called red tides, has become a challenging concern due to the direct impacts on public health and economy. Current methods consist in microscopic identification and enumeration of the cells of interests. However it is time-consuming, tedious and requires expert taxonomists. Advances in molecular biology allow the development of new tools. In this context, we aimed at developing a new DNA microarray: the phytochip. It should be a rapid and accurate method dedicated to the identification of the main toxic phytoplankton species in French waters.

Materials and Methods

1. Probe design : ARB software

2. Spotting on dendrimeric coating glass slides



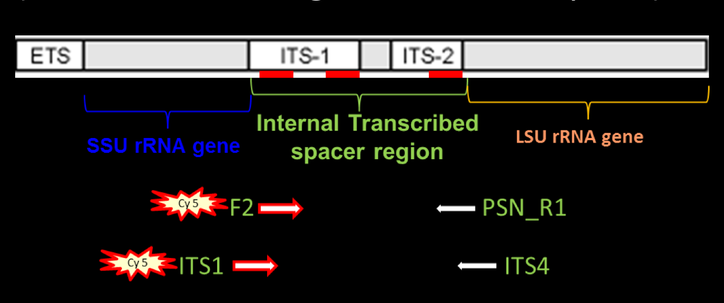
3. DNA extraction

(CTAB procedure or the DNAeasy Plant Mini Kit -Qiagen) :

-microalgae cultures
-seawater spiked with cells
-environmental samples

4. Asymetric PCR with Cy5 labelled forward primer

(*Pseudo-nitzschia* genus and Eukaryotes)



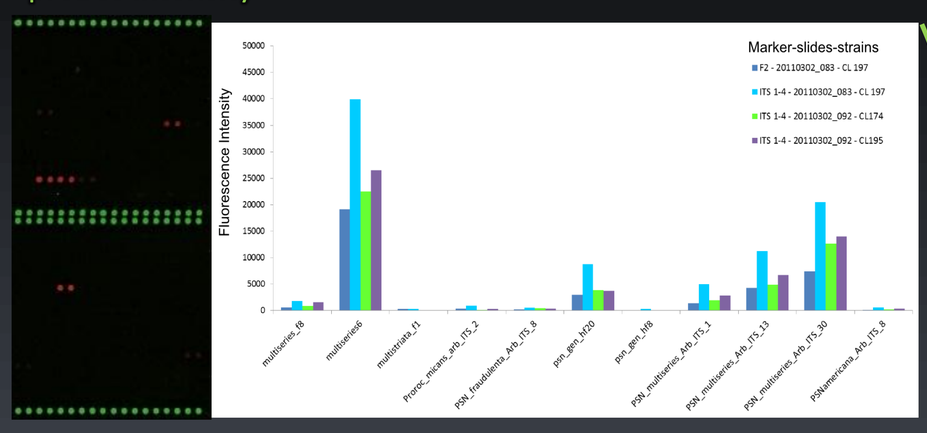
5. Hybridization : 65° C for 30 min on 8 zone custom slides

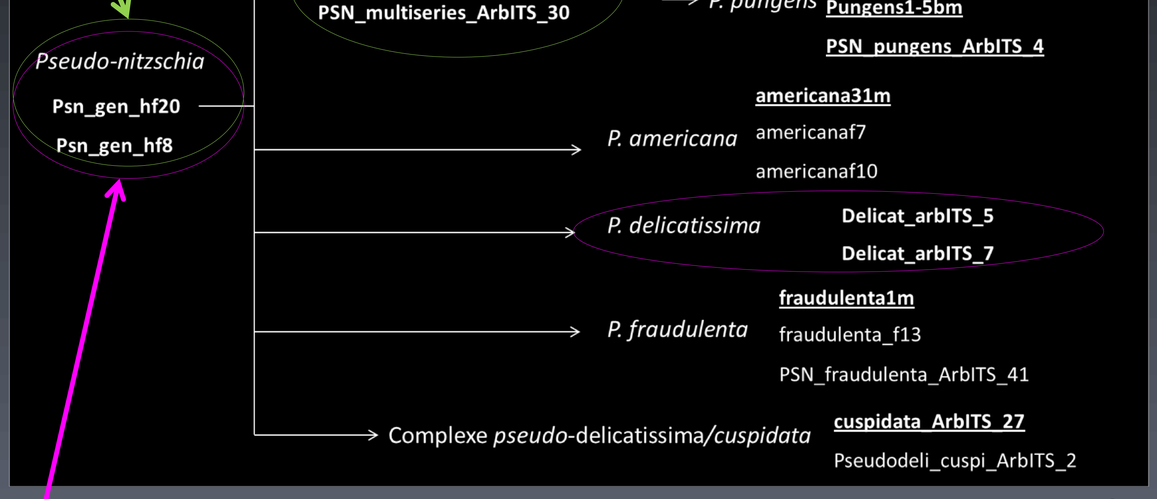
6. Scan: Innoscan 900

7. Data analyses: MAPIX (Innopsys)

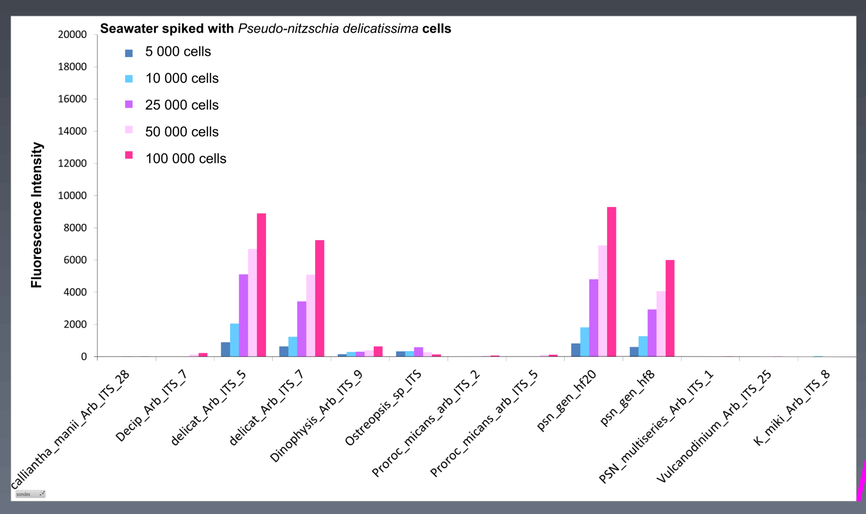
Results

P. multiseries hybridization: exemple of a microarray scan and probes intensity





Hybridization of seawater spiked with an increasing number of *P. delicatissima* cells



Specificity of the probes was tested with various monoclonal cultures, mixtures of DNA from cultures, and natural seawater spiked with *P. delicatissima* cells. 8 species of the *Pseudo-nitzschia* genus can be detected and accurately identified by specific probes of the phytochip. We propose the following hierarchical probe-set detemination key to identify *Pseudo-nitzschia* species in environmental samples.

After amplification with the Eukaryotes primers, *Pseudo-nitzschia* were detected in Cabourg (Bay of Seine) in samples collected the 04/07/12 and 06/08/12. *P. americana* was detected weeks 27 and 32, *P. pungens* only week 32 and *P. australis* was detected week 27.

	CABOURG M1	
	04/07/2012	06/08/2012
Spot Intensity (ITS1-ITS4_primers)	Week 27	Week 32
psn_gen_hf20	4952	7216
psn_gen_hf8	6610	13683
americana31m	2381	5082
americana_f10	678	2658
americana_f7	980	2127
seriata_f10	157	
seriata_f4	232	
Seriata_australis_Arb_ITS_25	393	
australis_Arb_ITS_13	133	
paustr5-2m	617	
PSN_multiseries_Arb_ITS_30		40
PSN_pungens_Arb_ITS_4		3832
pungens_p1		2110
pungens1-5bm		4058
Microscopic counts		
Total abundance of Pseudo-nitzschia cells	1,02E+05	8,28E+04
Abundance of the seriata complex	1,02E+05	8,40E+03
Abundance of the delicatissima complex		
Abundance of *P. americana*		7,44E+04
[Domoic Acid] ng.L-1	**1740,97**	**3,24**

Probes were designed for the following species:

Pseudo-nitzschia

P. americana
P. australis
P. brasiliana
P. caciantha
P. calliantha
P. decipiens
P. delicatissima
P. fraudulenta
P. galaxiae
P. Pseudodelicatissima / cuspidata
P. multiseries
P. pungens
P. seriata
P. subpacifica

Alexandrium

A. catenella
A. minutum
A. tamarense
A. tamutum

Karenia

K. brevis
K. mikimitoi
K. selliformis

Dinophysis

D. acuta
D. acuminuta
D. norvegica

and

Lingulodinium polyedrum
Ostreopsis ovata
Vulcanodinium rugosum

Perspectives

Specific probes were designed for several *Pseudo-nitzschia* species responsible for the ASP (Amnesic Shellfish Poisoning), species inducing PSP (Paralytic shellfish Poisoning) e.g. *Alexandrium sp.* and DSP (Diarrheic Shellfish Poisoning) e.g. *Dinosphysis sp*. syndromes. We are currently testing the specificity and sensitivity of the phytochip using DNA samples from our collection. A series of environmental samples collected in the Bay of Seine will be analyzed and results will be compared with microscopic cell counts. The phytochip seems to be a promising method for the identification of toxic phytoplankton and could be a useful tool to help environmental monitoring.

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Trevisiol E, *et al.* (2003) Dendrislides, dendrichips: a simple chemical functionalization of glass slides with phosphorus dendrimers as an effective means for the preparation of biochips. New Journal of Chemistry 27

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