Developing a transcriptomic approach dedicated to autotrophs in periphytic biofilms

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Developing a transcriptomic approach dedicated to autotrophs in periphytic biofilms

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Applying transcriptomics at community level

Periphyton are complex and dynamic microbial communities playing an essential role in river processes such as primary production, nutrient recycling… Since the last decade, -omics tools have been developed to characterize the functional groups of heterotrophic and fungal communities while -omics application dedicated to microalgae and diatoms were mostly restrained to diversity assessment (e.g. based on 18S rRNA or rbcL genes). To characterize autotrophs functions in periphyton and to understand better contaminant effect on microalgae and diatoms, we aimed to **design a microarray targeting functional genes of diatoms and microalgae in periphyton**.

Objectives

- **Optimize a method for the extraction of clean RNA from periphytic communities**
- **Design probes based on homologous genetic sequences of microalgae/diatoms for a RNA microarray**
- **Proof of concept : validate the hybridization of RNA from periphyton on the microarray**

RNA extraction & labelling

Periphytic biofilm
1.5 cm X 20 ~870 mg ~1079 mg

↓ Centrifugation
10 000 g, 5 min, 16°C
+ 1mL Trizol + glass beads

↓ Cell disruption
Bead beating
5 min incubation room T°C

↓ Chloroform RNA extraction

↓ RNA precipitation
+ polyethylene glycol solution
2h incubation room T°C

↓ Centrifugation
max. speed, 10 min, 4°C

↓ Washing : RNA pellet
+ cold ethanol

↓ Air drying

↓ + 50µL water

↓ DNAse treatment

↓ RNA purification
Rneasy Mini Plant kit (Qiagen)
+ β-mercaptoethanol

↓ RNA from biofilm
~3.8 µg
~13.7 µg

Periphytic biofilms contain humic acids and polysaccharides from extracellular polymeric substances (EPS) which are likely to interfere with RNA extraction, purification or labelling.

Specific steps were added to ensure RNA extraction from this complex matrix.

RNA from biofilm
~900 ng

↓ Labelling with cyanine 3
Quick Amp Labelling kit (Agilent Technologies)

↓ RNA purification
Rneasy Mini Plant kit (Qiagen)
+ β-mercaptoethanol

↓ Labelled RNA

Microarray design

Following the method used for the design of a Functional Gene Array (FGA) dedicated to bacteria and fungi (He et al. 2010), the design of the probes of the microarray was based on consensus sequences found in the genome of various microalgae. Thus, genome information from 6 autotroph species found in periphyton (4 chlorophytes: *Chlamydomonas reinhardtii, Scenedesmus vacuolatus, Oedogonium cardiacum, Stigeoclonium helveticum* and 2 diatoms : *Phaeodactylum tricornutum, Thalassiosira pseudonana*), was used to design probes targeting 83 genes coding for 57 enzymes involved in various metabolic pathways.

Probes design workflow

NCBI (National Center for Biotechnology Information)
NCBI database
Retrieve gene sequences available from the different species

↓ EMBL-EBI
Kalign software
Select homologous sequences : *more than 60 bp homology between at least 2 species*

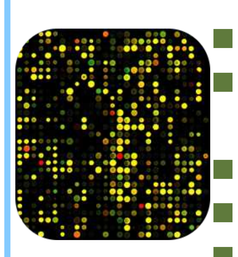
↓ Agilent Technologies
eArray software
For each gene 2 probes (60mer oligonucleotides) were designed based on homologous sequences

↓ Agilent Technologies
Microarray printing - *4 x 44K format, Agilent technologies*
Unique probes printed in duplicate

Pathways targeted by the designed probes in the microarray

Energy	Photosynthesis/respiration/energy	**N metabolism**	Transamination/glutamine biosynthesis
	chlorophyll synthetase, cytochrome c oxidase, ATP synthase…		*glutamate-1-semi aldehyde aminotransferase…*
C metabolism	Calvin cycle		Glutathione biosynthesis
	phosphoglycerate kinase, fructose 1,6 bisphosphatase…	**Stress Response**	Carotenoids biosynthesis
	TCA cycle		*phytoene desaturase/synthase, beta-carotene ketolase…*
	pyruvate dehydrogenase, succinate dehydrogenase…		Antioxidant system
	Pentosephosphate pathway		*catalase, ascorbate peroxidase…*
	glucose 6 phosphate dehydrogenase, ribose 5 phosphate isomerase…		Protecton/protein folding
	Glycolysis		*chaperonin, heat shock protein…*
	hexokinase, phosphofructokinase…	**Others**	*cytochrome p450, translation elongation factor,uroporphyrinogen, decarboxylase, actin, alpha/beta tubulin, enoyl-[acyl-carrier-protein] reductase (fabi)*
	Glyoxylate cycle		
	isocitrate lyase, malate synthase…		

Proof of concept for a Functional Gene Array for autotrophs in periphyton



- Hybridisation at different temperatures (55, 60, 65 °C) of RNA from biofilms and from reference species : *C. reinhardtii, S. vacuolatus*
- Microarray scanning at 5 µm resolution (GenePix 4400A Scanner)
- Low impact of hybridisation temperature on signal distribution and intensities
- High variability in the percentage of probes detected per gene
- **RNA biofilm hybridized to most of the genes, even under high stringency conditions (65°C)**

Conclusion and perspectives

These methodological developments represent the first basis towards the application of transcriptomics in periphyton. They include a method of RNA extraction from biofilms, a list of oligonucleotides probes corresponding to a part of the periphyton metagenome and a validation of the principle of FGA for the autotrophic biofilm. **This first microarray could thus be used in a simple microcosm study to validate its potential in detecting subtle contaminants effects on periphyton**.

Further research is now needed to overcome the limitations of the present study and tackle remaining challenges :

- Methodological developments are still required to obtain a reproducible and robust method of RNA extraction from periphyton.
- The specificity between probes and genes hybridized should also be further validated.
- Information from metagenome library of periphyton could be used to extend the number of genes on the microarray.

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