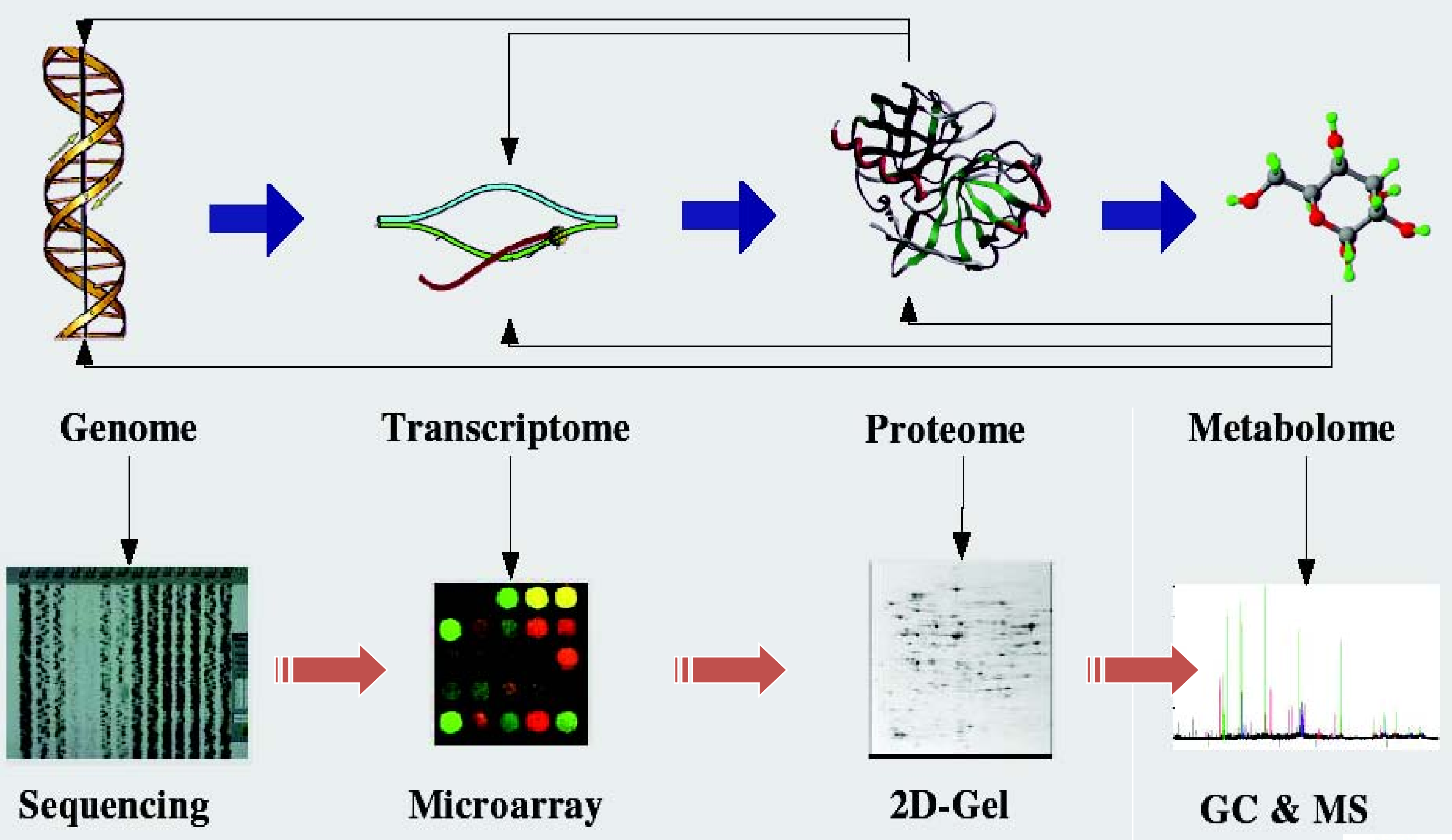
"Algal genomics may offer new insights into seaweed uses "

Philippe POTIN
& Team Algal Defenses
CNRS-UPMC UMR 7139
Marine Plants & Biomolecules

Genomics uses transdisciplinary HT approaches



Genome

Transcriptome

Proteome

Metabolome

Sequencing

Microarray

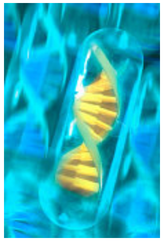
2D-Gel

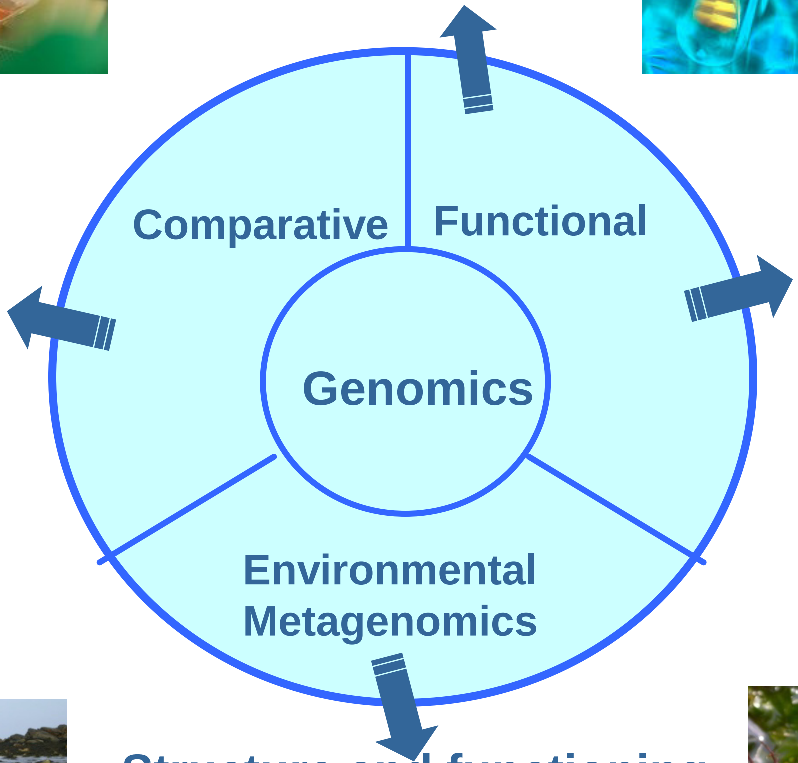
GC & MS

to understand the complexity of life

The applications of genomics to marine sciences

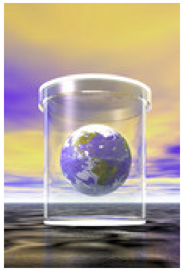
Gene mining for Health and Biotech





Environment and Climate change, Adaptation

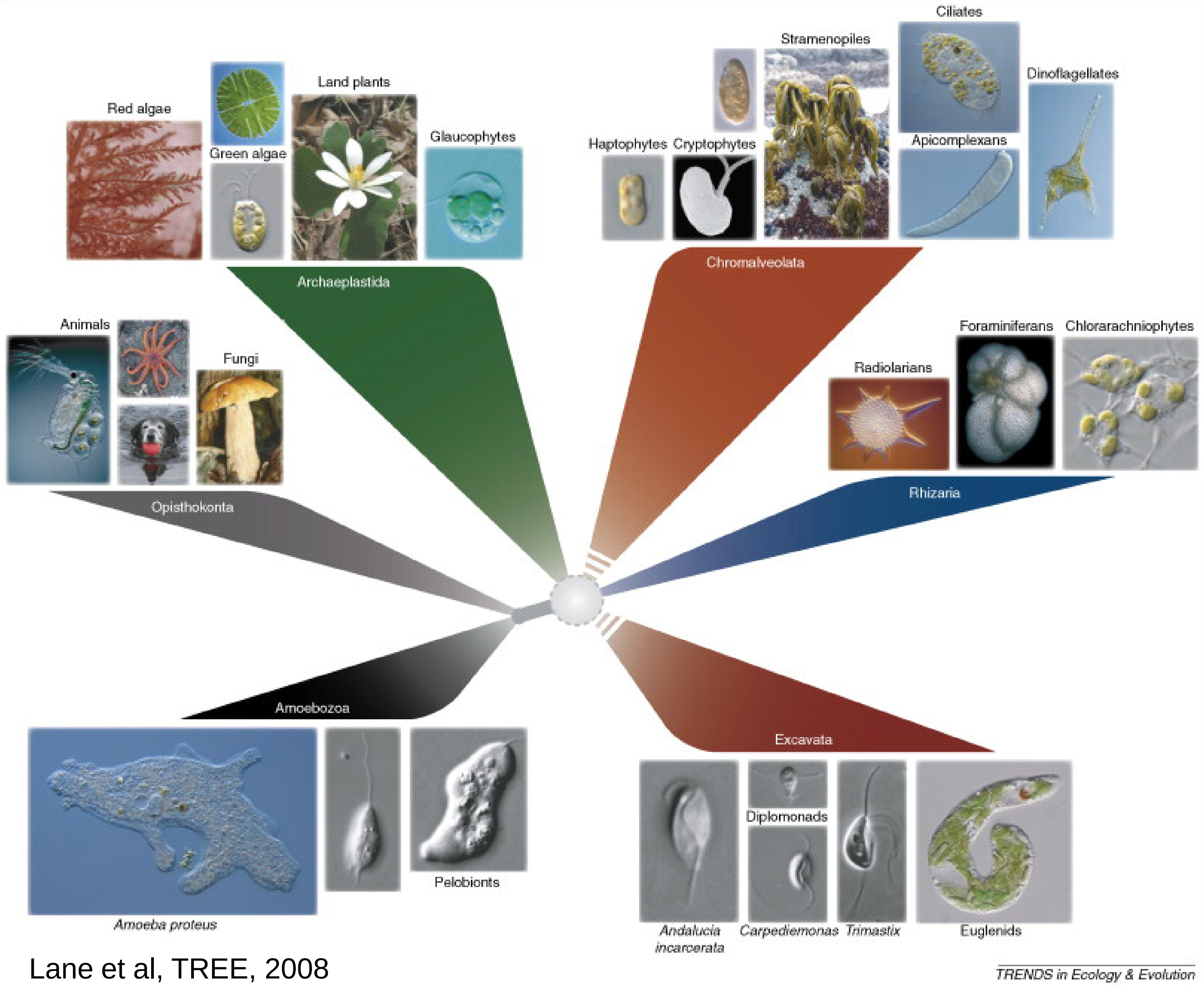
Management of Marine Resources



Structure and functioning of marine diversity

Marine genomics allows to draw **global conclusions** about our surrounding environment and to take a **holistic approach** to ocean management

Seaweeds in the eukaryotic tree



Lane et al, TREE, 2008

An industrial reality

An industrial reality

Chondrus crispus **raceway cultivation tank (Innovalg)**

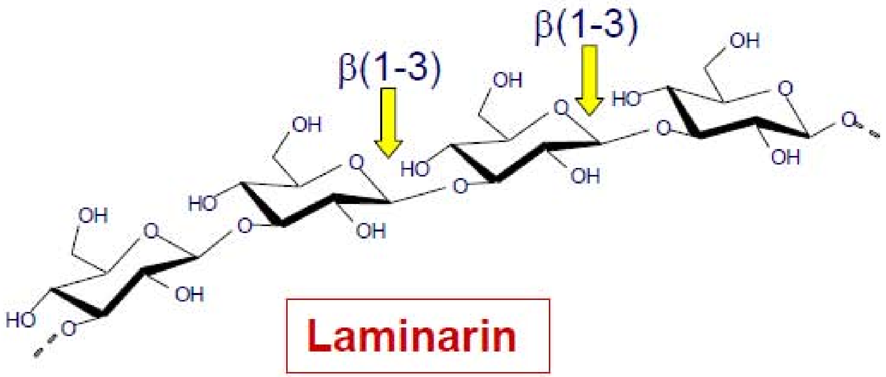
An industrial reality

©2002 Yannick LE BRIS

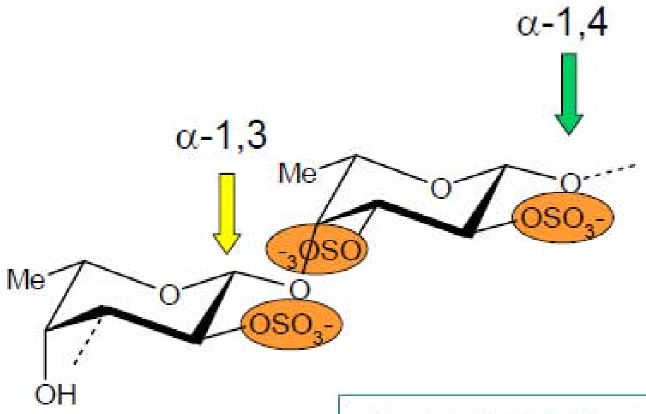
Various poly/oligosaccharides

An industrial reality

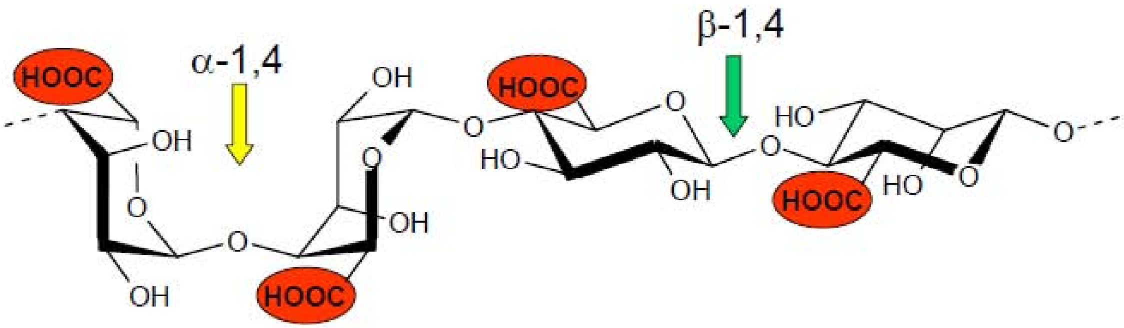
Polysaccharides unique to brown algae



Laminarin



Sulfated Fucans



Alginates

L-Guluronic acid

D-Mannuronic acid

An industrial reality

Iodus 40®,

The first natural immunomodulator of wheat disease resistance

Marketed : Spring 2003

Active principle = LAMINARIN
(storage polysaccharide of *Laminaria digitata*)

Les réponses que nous cherchons sur terre Goëmar les trouve dans la mer.

An industrial reality

Bioessays of Iodus 40

Control:

Induced resistance conferred by Iodus 40 :

GOËMAR
LE LABORATOIRE DE LA MER

CNRS UPMC INSU
Station Biologique
Roscoff

Current trends in seaweed biotech.

Novel bioproducts in cosmetic, health and nanotechnologies

oxylipins
poly- and oligosaccharides
halogenated compounds
....

Bioprocesses

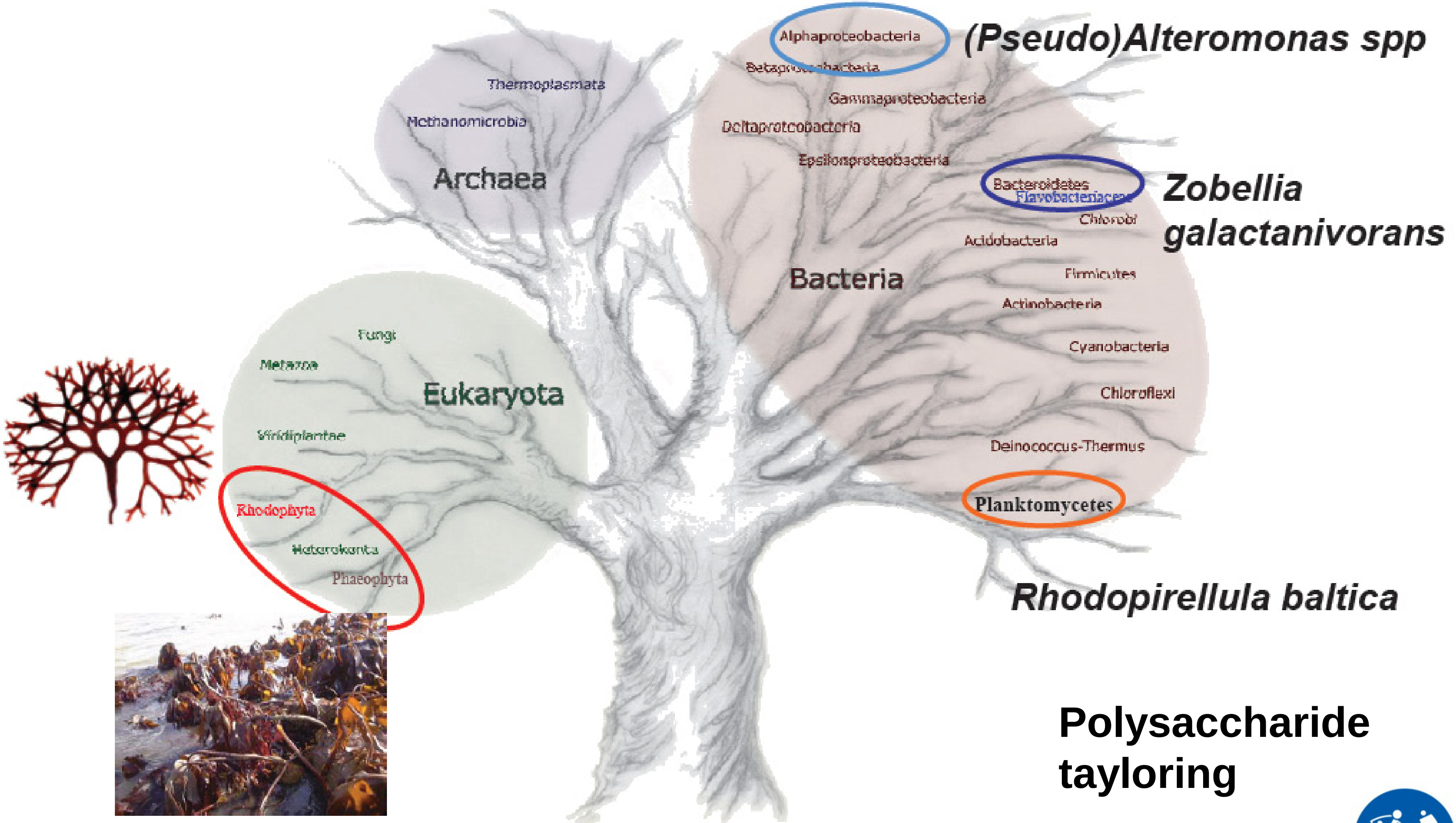
polysaccharide tayloring
(de)halogenation
new adhesion catalysts
...

Knowledge and methods for genetic improvement

decipher the biogenesis of natural products
decipher the molecular bases of disease resistance
identify genetic markers for helping in genotype selection
....

Current trends in seaweed biotech.

Genomic resources for deciphering the biology of macroalgae



Polysaccharide tayloring

Biodegradation

Biosynthesis

Brown algal genomics

The *Ectocarpus* genome (200 Mb) project J.-M. Cock

- **GENOME SEQUENCING : 3,000,000 reads (10X, shotgun)**

cDNA SEQUENCING :   100,000 reads (full-length cDNAs)

J. M. Cock, Roscoff

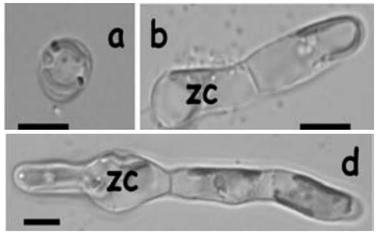
P. Rouzé / Y. van der Peer

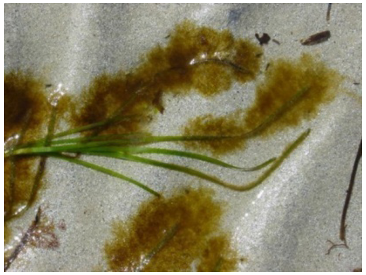
+ *Es* Genome Consortium

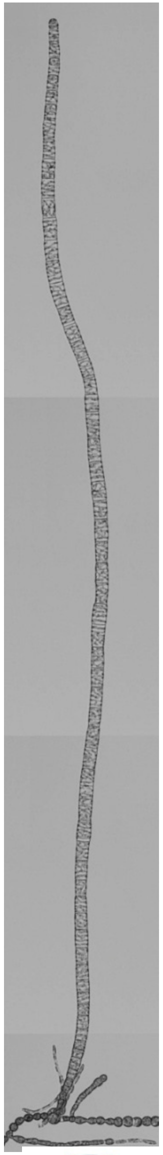
- STRAIN SELECTION
- BIOLOGICAL MATERIAL
- LIBRARIES

- LIBRARIES
- SEQUENCING
- ASSEMBLY

- AUTOMATIC ANNOTATION
- EXPERT ANNOTATION







Cock JM et al. (74 authors)
Nature. 2010 Jun 3; 465:617-21.

Brown algal genomics

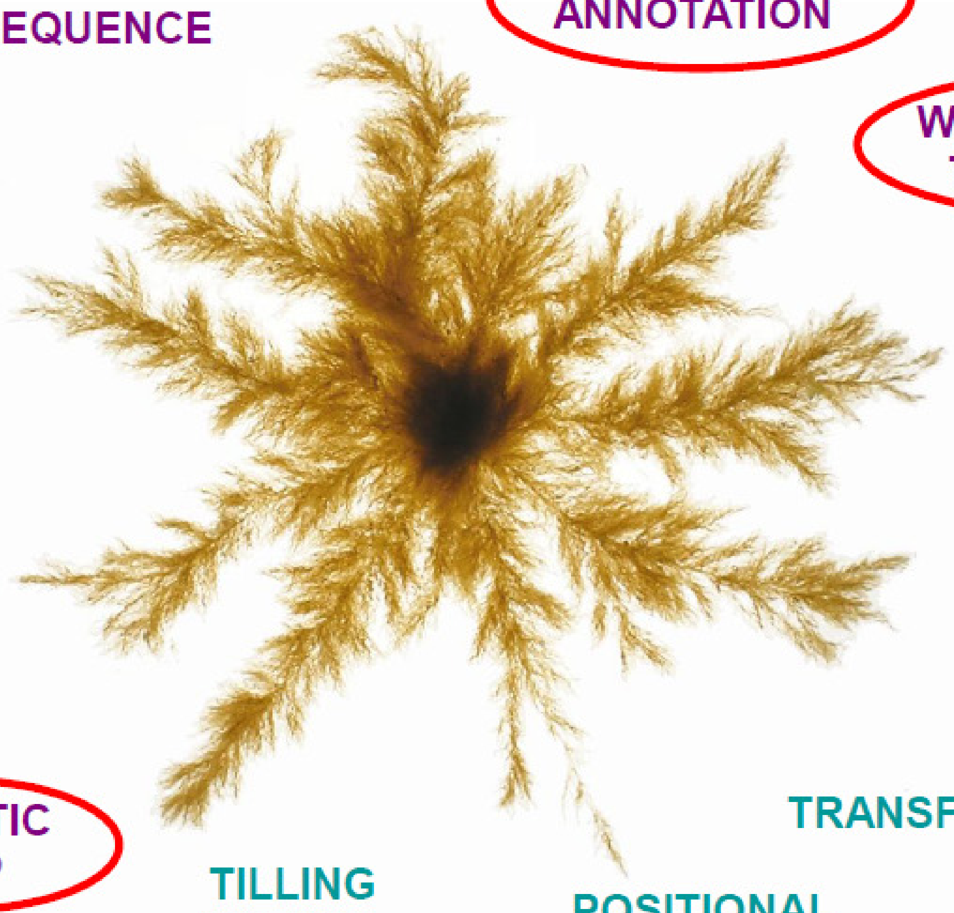
Ectocarpus siliculosus – a model organism for the brown algae

- Small size
  - compared with *Fucus, Laminaria*
- Ease of culture
  - in Petri dishes
- Life cycle in laboratory
  - 3 months for complete cycle
- Highly fertile
- Many ecotypes available
- Genetic crosses are possible
- Small genome
  - 200 Mbp (1000 Mbp for *Fucus*)
- EsV-I integrative DNA virus
- Well described in the literature
- Related to commercial seaweeds
  - eg. *Laminaria spp.*

Cock JM et al. (74 authors)
Nature. 2010 Jun 3; 465:617-21.

Brown algal genomics

Development of genetic and genomic tools for *Ectocarpus*



COMPLETE GENOME SEQUENCE

ESTs

SMALL RNA SEQUENCES

CLASSICAL GENETICS

MUTANT COLLECTION

GENETIC MAP

TILLING MUTANT SCREENING

POSITIONAL CLONING

TRANSFORMATION

RNAi

GENOME DATABASE

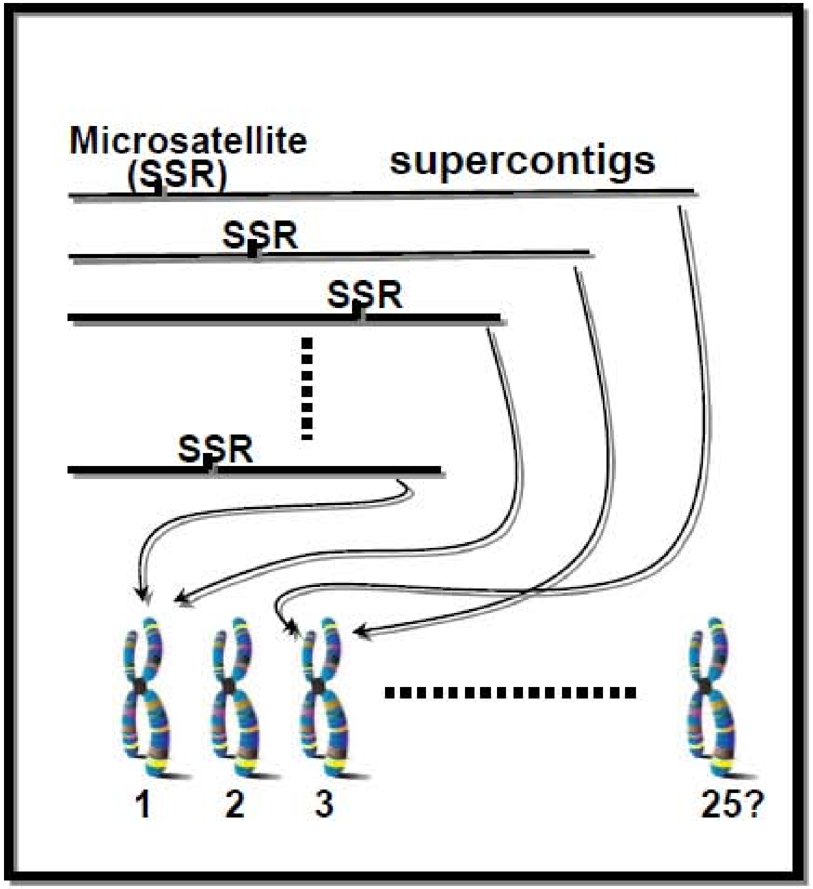
MICROARRAY ANALYSIS OF GENE EXPRESSION

WHOLE GENOME TILING ARRAY

GENE ANNOTATION

Europole Mer project

Large-scale genome assembly using a genetic map



1152 SSR loci tested

406 SSR markers mapped

325 of the longest supercontigs mapped
(70% of genome)

34 linkage groups
(25 large + 9 small)

Assembly of the genome into pseudochromosomes

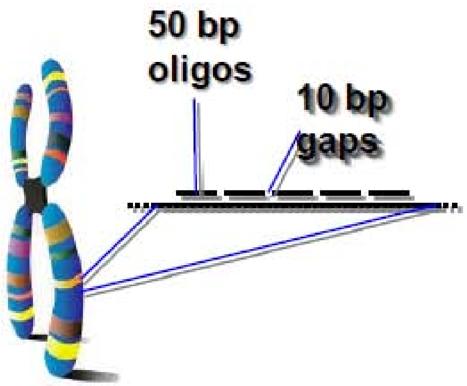
Svenja Heesch, Roscoff
Ga Youn Cho, Roscoff

New Phytologist (2010)
doi: 10.1111/j.1469-8137.2010.03273.x

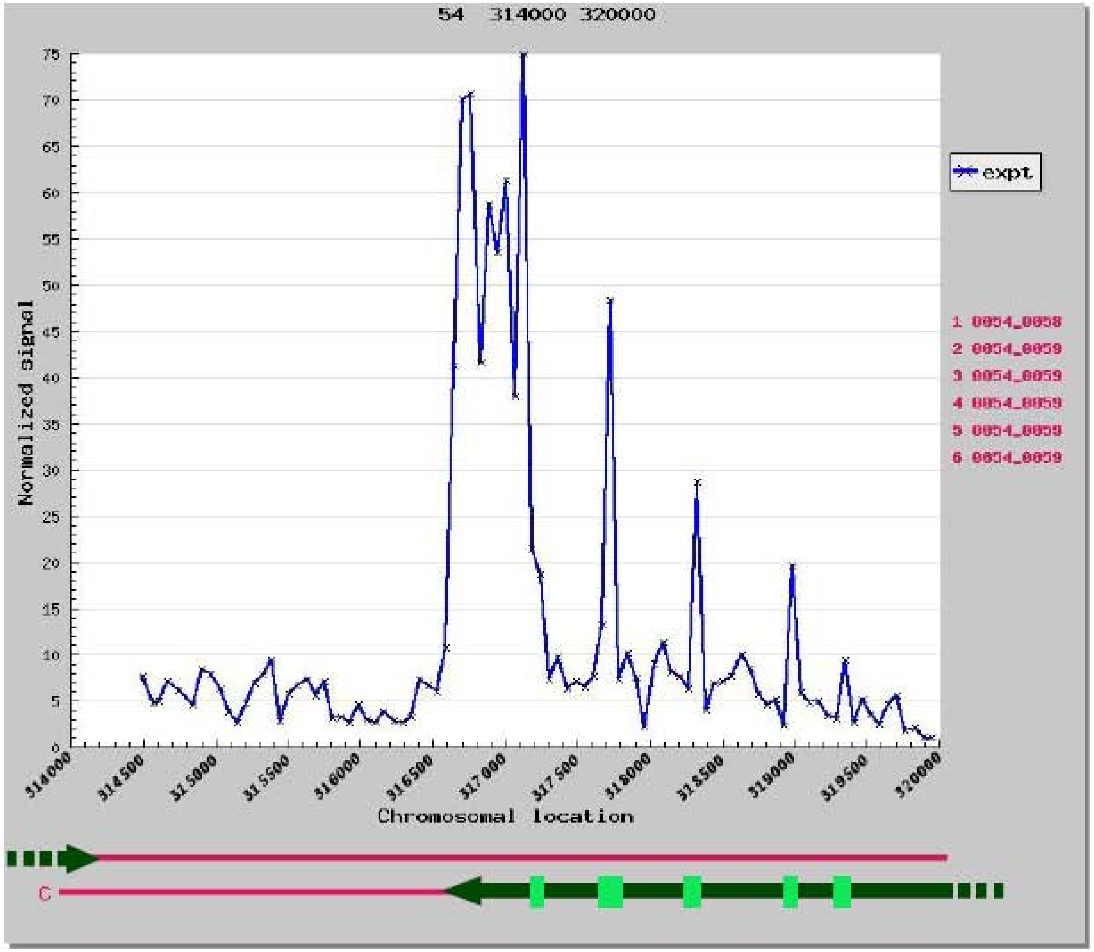
Brown algal genomics

Whole genome tiling array

8 Nimblegen arrays
(3,065,615 oligos)



Hybridisation with
mixed cDNA
(different life cycle
stages and
conditions)



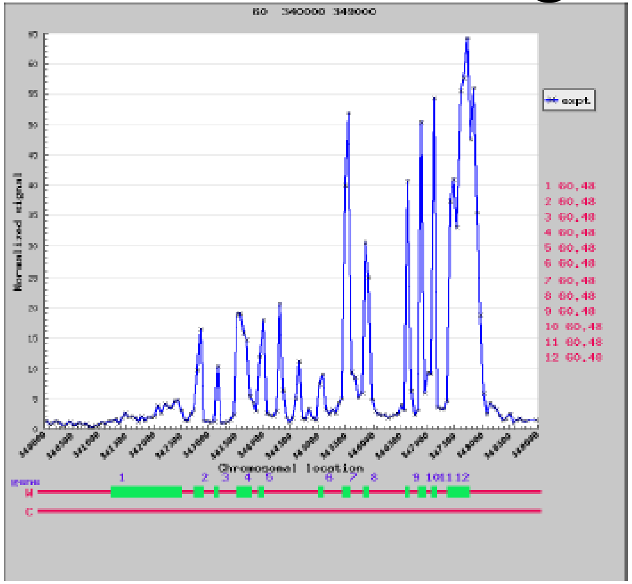
Esi0054_0059 - Ubiquitin-conjugating enzyme
(last 5 exons shown)

Manoj Samanta
Delphine Scornet

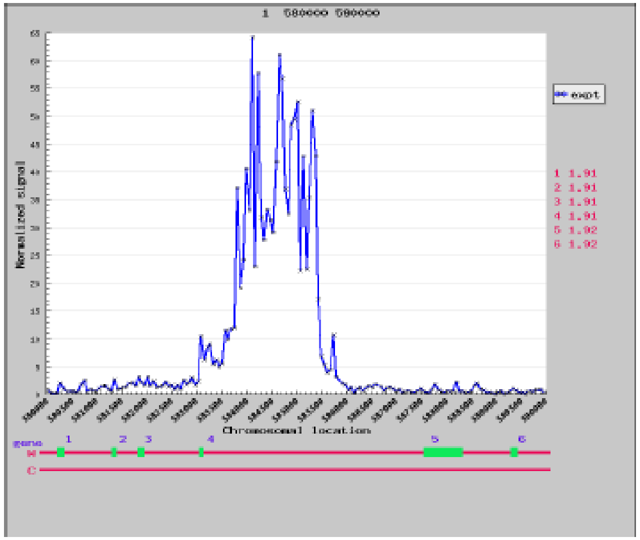
Cock JM et al. **Nature**. 2010 Jun 3; 465:617-21.

Brown algal genomics

Whole genome tiling array-results



- **Transcription confirmation** for 6,474 gene models (39.8% of proteome)
- 2100 of the 6,474 genes **did not have EST support**
- **Confirmation of exon structure** for many genes
- **8,741 new expressed regions** outside existing gene models



Transcriptomic support for gene models

Cock JM et al. **Nature**. 2010 Jun 3; 465:617-21.

Brown algal genomics

Expert annotation of the genome

- 5,439 genes have been manually annotated

33% of the 16,256 gene models
50% of the 10,859 gene models with database matches

- Gene families annotated:

Carbon storage and cell wall metabolism
Photosynthesis genes
Biosynthesis of tetrapyrroles, carotenoids and sterols
Nitrogen metabolism
Amino acid biosynthesis
Thiamine pyrophosphate (vitamin B1) biosynthesis
Lipid and fatty acid metabolism
P450 oxidoreductases
Secondary metabolism
Halogen metabolism
Mechanisms for alleviating oxidative and metal stress.
Iron uptake and storage
Selenoproteome

Transcription associated proteins
Protein kinases
Cell cycle genes
TOR kinase pathway
Putative membrane-localised receptors
Photoreceptors
P-loop GTPases
Defence signaling and apoptosis
Ion channels and Ca signalling
mRNA maturation
mRNA translation
Meiosis
Integrins
Cytoskeleton
Vesicle trafficking
Flagella
Viral genes
Organellar genomes

➡ **Gene function information added to genome**

The *Ectocarpus* genome consortium

Cock JM et al. **Nature**. 2010 Jun 3; 465:617-21.

Brown algal genomics

Main conclusions on brown algal carbohydrate metabolism (G. Michel, SB Roscoff)

- Carbohydrate central metabolism and protein glycosylation is well conserved with other Eukaryotes

- No remnants of glycogen / starch metabolism from the secondary endosymbiont. Instead, a complex laminarin metabolism for carbon storage.

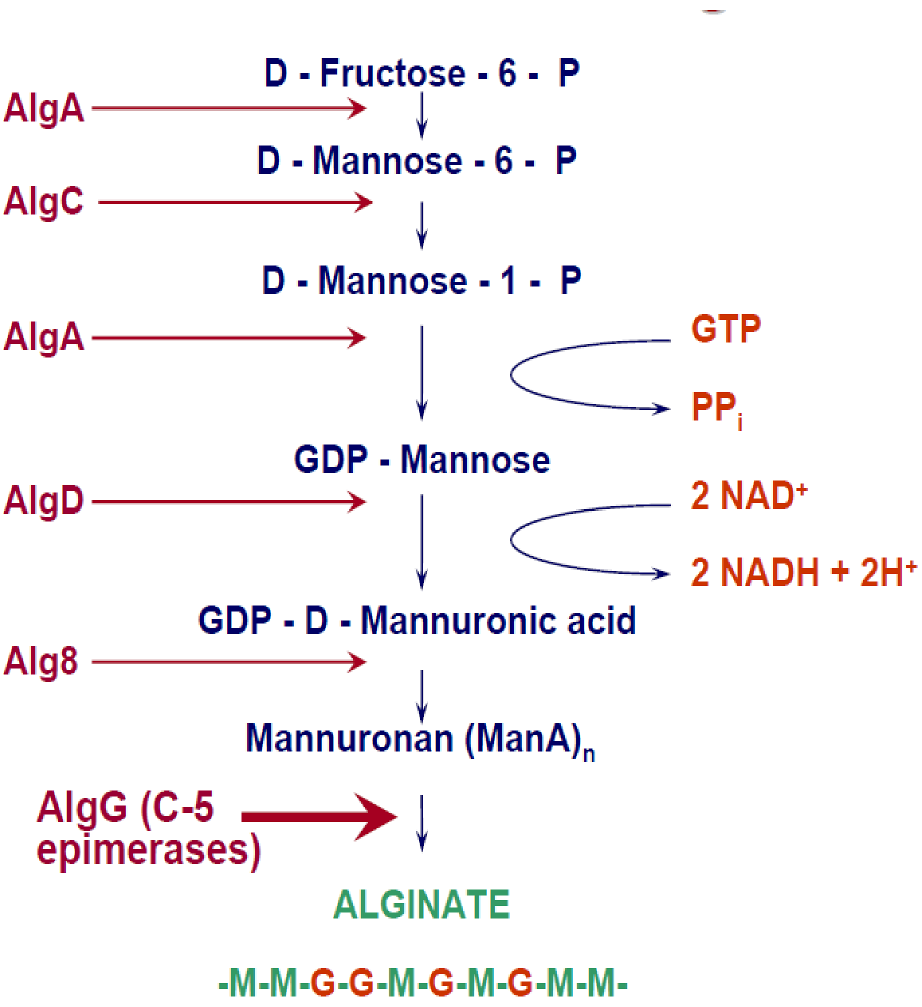
- The cell wall machinery (synthesis and remodelling of cellulose, alginates and fucans) is either poorly conserved or makes use of very genuine brown algal genes

→ numerous novel CAZyme families are expected to be identified in brown algae.

Michel et al. **New Phytol.** 2010 PMID: 20618908
Michel et al. **New Phytol.** 2010 PMID: 20618907

Brown algal genomics

The origin of alginate synthesis route in brown algae is likely the result of an horizontal gene transfer from Actinobacteria



D - Fructose - 6 - P

AlgA

D - Mannose - 6 - P

AlgC

D - Mannose - 1 - P

AlgA

GTP

PP_i

GDP - Mannose

AlgD

2 NAD^+

2 NADH + $2H^+$

GDP - D - Mannuronic acid

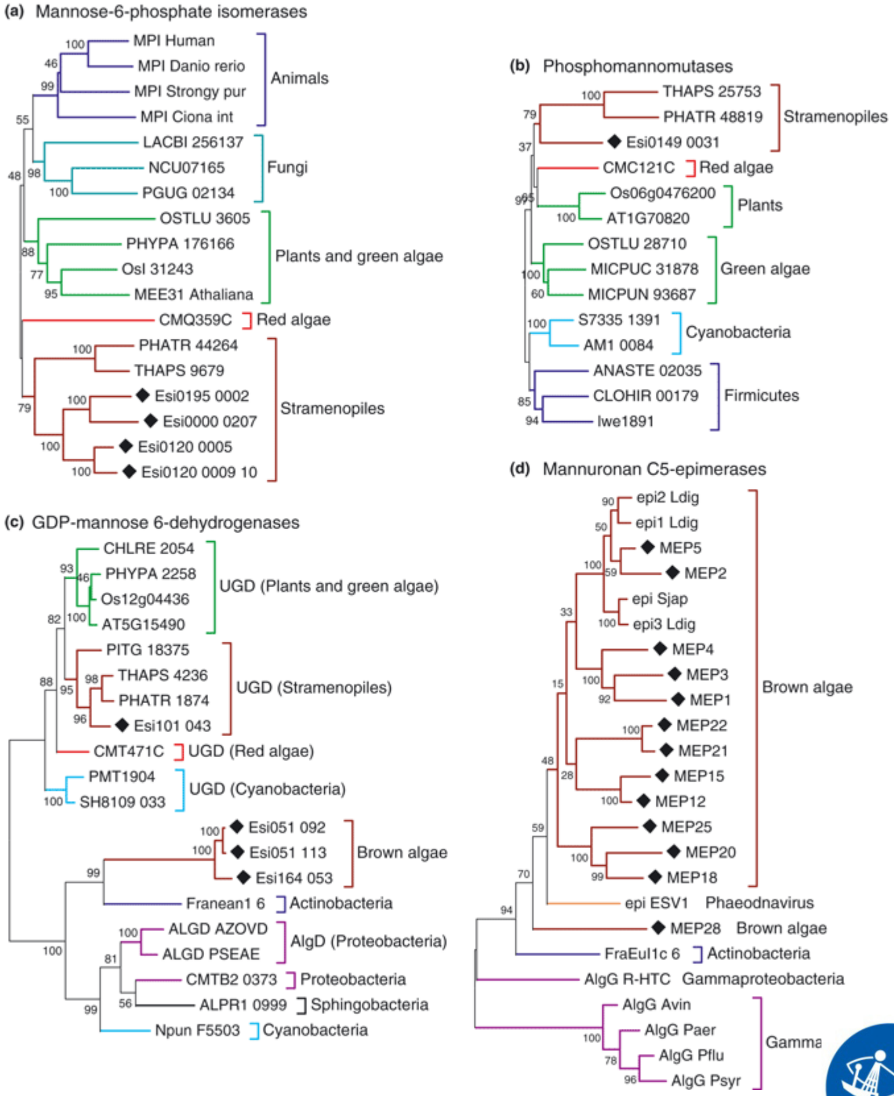
Alg8

Mannuronan $(ManA)_n$

AlgG (C-5 epimerases)

ALGINATE

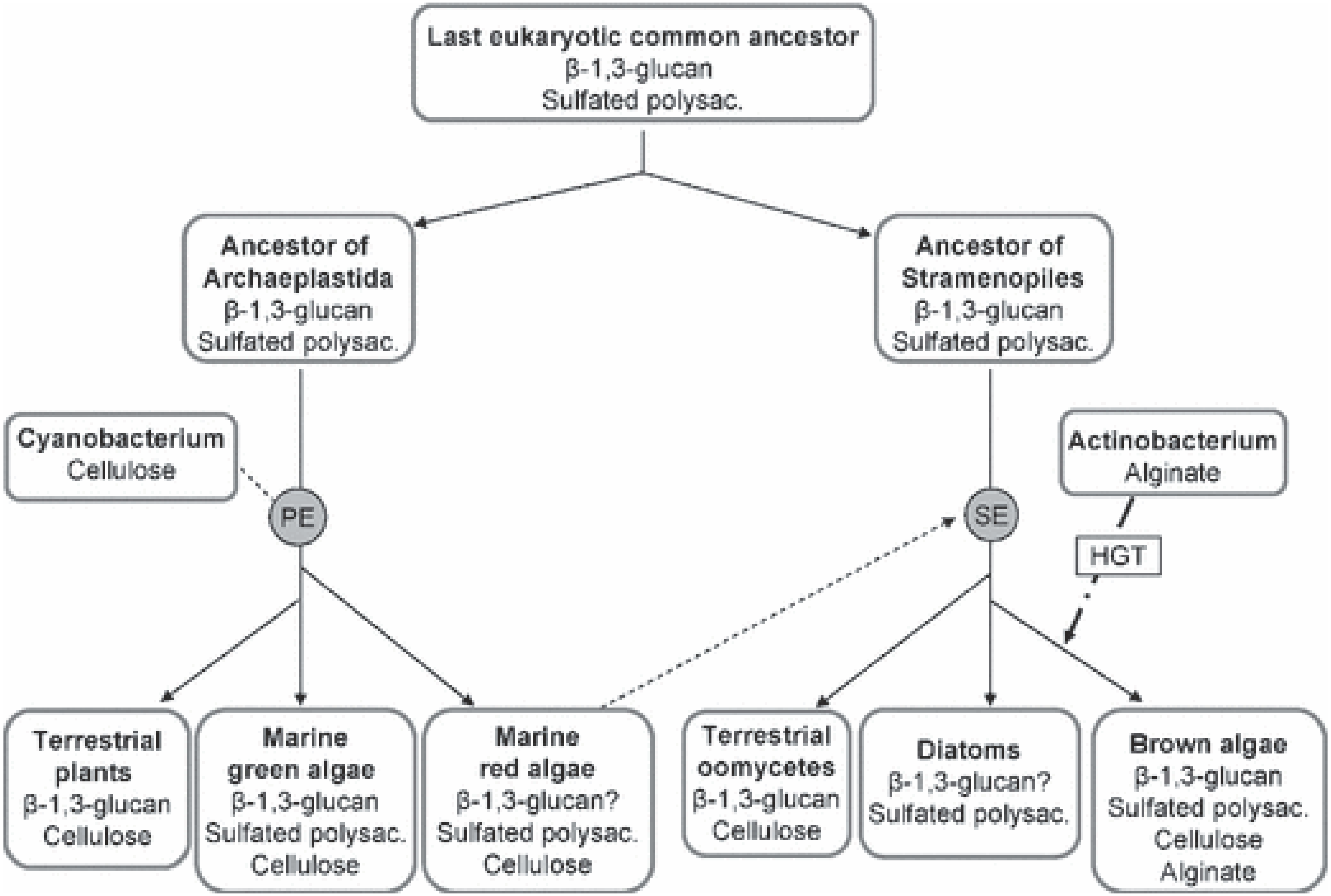
-M-M-G-G-M-G-M-G-M-M-



Michel et al. **New Phytol.** 2010 PMID: 20618907

Brown algal genomics

The origin of alginate synthesis route in brown algae is likely the result of an horizontal gene transfer (HGT) from Actinobacteria



Last eukaryotic common ancestor
β-1,3-glucan
Sulfated polysac.

Ancestor of Archaeplastida
β-1,3-glucan
Sulfated polysac.

Ancestor of Stramenopiles
β-1,3-glucan
Sulfated polysac.

Cyanobacterium
Cellulose

PE

SE

Actinobacterium
Alginate

HGT

Terrestrial plants
β-1,3-glucan
Cellulose

Marine green algae
β-1,3-glucan
Sulfated polysac.
Cellulose

Marine red algae
β-1,3-glucan?
Sulfated polysac.
Cellulose

Terrestrial oomycetes
β-1,3-glucan
Cellulose

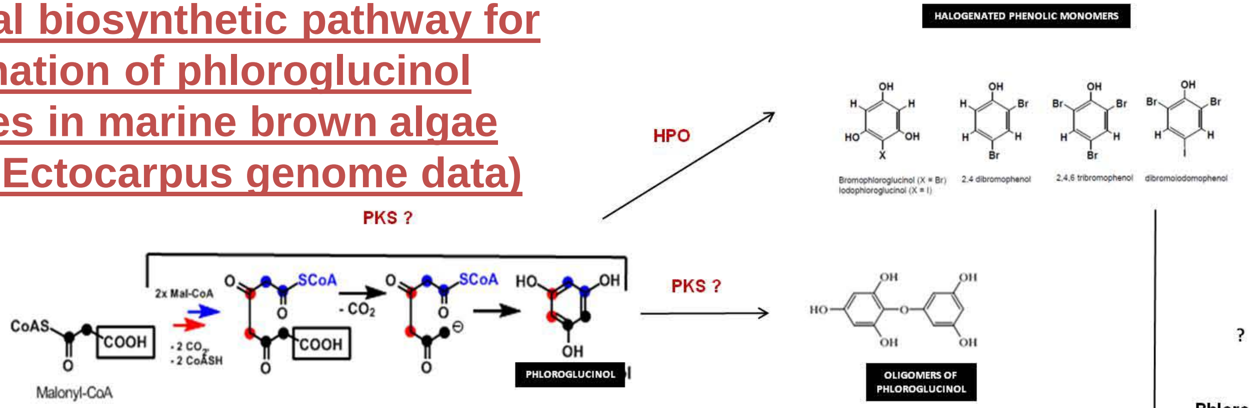
Diatoms
β-1,3-glucan?
Sulfated polysac.

Brown algae
β-1,3-glucan
Sulfated polysac.
Cellulose
Alginate

Michel et al. **New Phytol.** 2010 PMID: 20618907

Brown algal genomics

Hypothetical biosynthetic pathway for the formation of phloroglucinol derivatives in marine brown algae (based on Ectocarpus genome data)



- PKS: Polyketide Synthase III (3 genes CHS-like)
- HPO: Vanadium-Haloperoxydase (1 gene)
- PKI: Polyketide Isomerase (2 genes CHI-like)
- PKR: Polyketide Reductase (5 genes CHR-like)
- PKH: Polyketide Hydroxylase (4 genes FSII and IFS-like)
- DOX: Dioxygenase (2 genes LDOX-like)
- LOX: Lipoxygenase (4 genes)
- GT: Glucosyltransferase (1 gene)
- ST: Phenol Sulfotranferase (5 genes)
- OMT: O-Methyltransferase (1 gene)

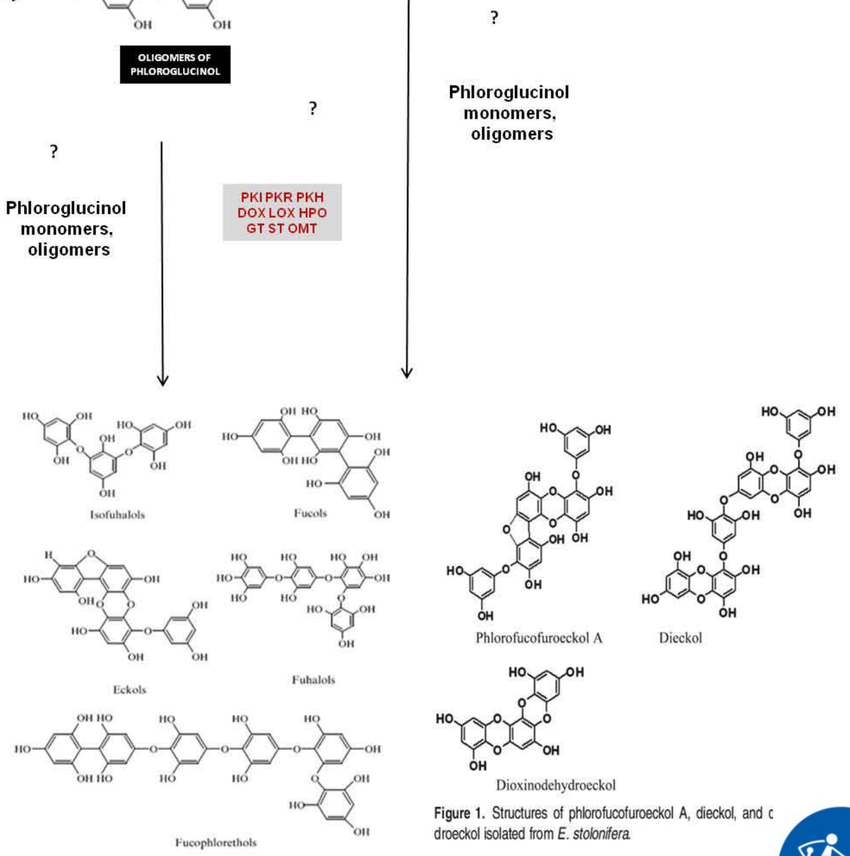
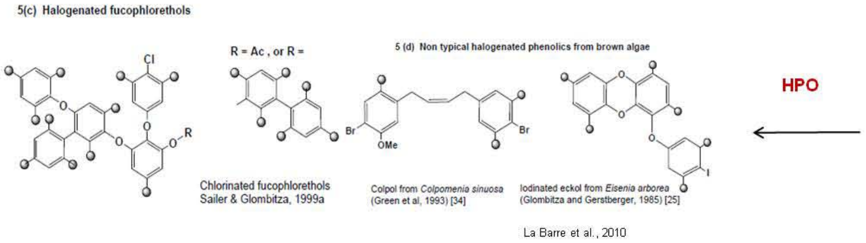


Figure 1. Structures of phlorofucofuroeckol A, dieckol, and d droeckol isolated from *E. stolonifera*.

PHLOROTANNINS AND POLYPHENOLS



Chlorinated fucophlorethols Sailer & Glombitza, 1999a

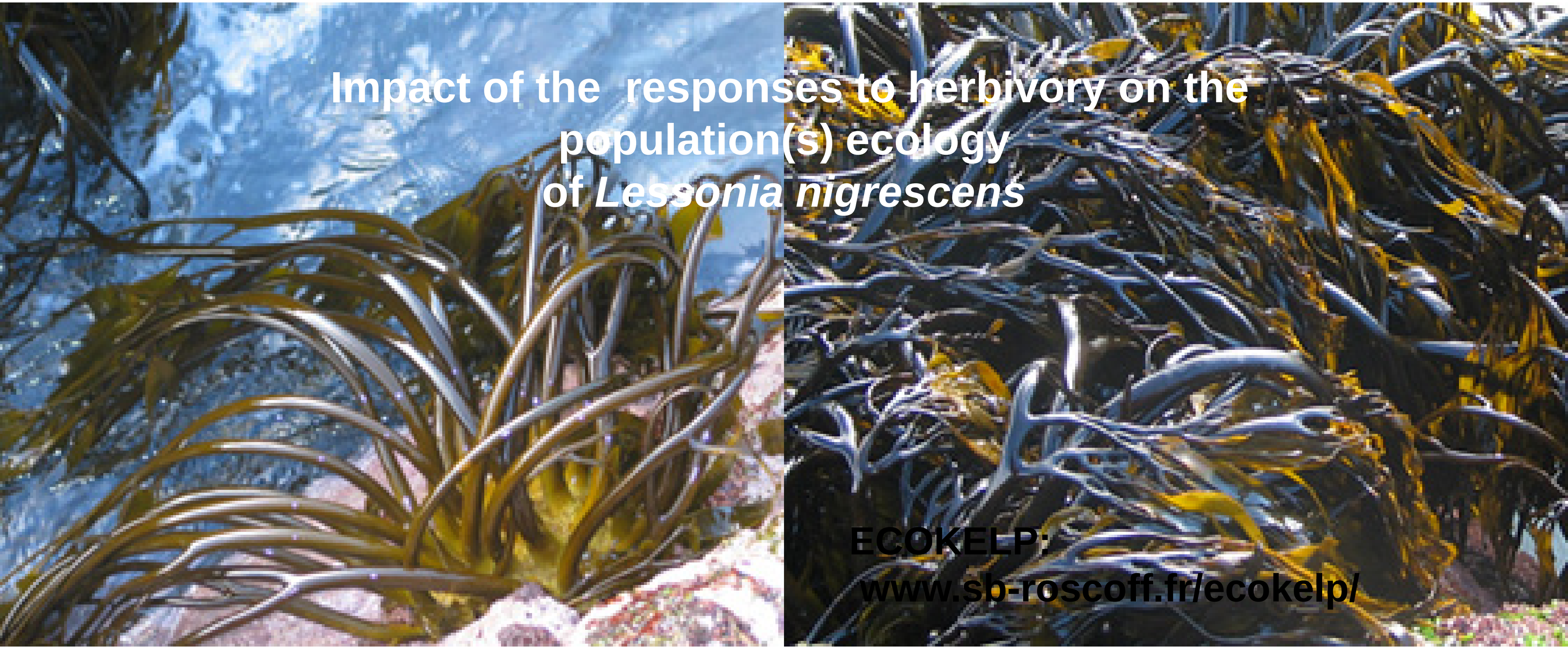
Colpol from *Colpomenia sinuosa* (Green et al, 1993) [34]

Iodinated eckol from *Eisenia arborea* (Glombitza and Gerstberger, 1985) [25]

La Barre et al., 2010

HALOGENATED PHLOROTANNINS AND POLYPHENOLS

Brown algal genomics

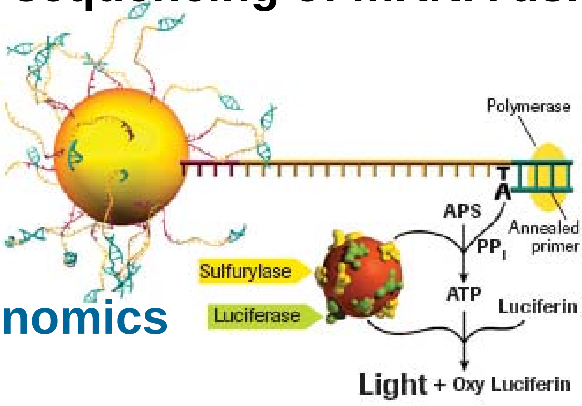


Impact of the responses to herbivory on the population(s) ecology of *Lessonia nigrescens*

ECOKELP:
www.sb-roscoff.fr/ecokelp/

Experimental grazing

Massive sequencing of mRNA using 454



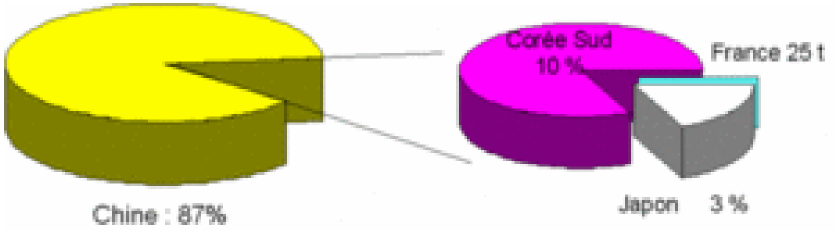
www.marine-genomics-europe.org

Brown algal genomics

- **A maricultured kelp**
- **An invasive species**

- **A proposal for a pilot project between Japan & France**
- **1,5X coverage**
- **100 to 200,000 ESTs**

WAKAME 2.7 Mt



Undaria pinnatifida

Sources: Ifremer

Brown algal genomics

- A maricultured kelp

- Project Chile/USA

- about 1,000,000 ESTs

- A proposal for a pilot project between Chile, USA & France

-1,5X coverage?

Red algal genomics

CNRS UPMC INSU
Station Biologique
Roscoff

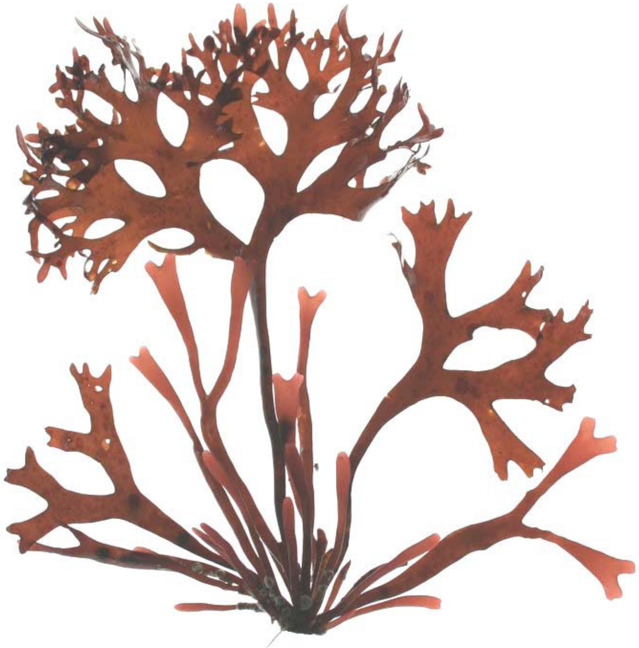
GENOSCOPE
Centre National de Séquençage

NSF

JGI
DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

The *Chondrus* genome project

The *Porphyra* genome projects



Kazusa DNA Res. Inst.
かずさDNA

Red algal genomics

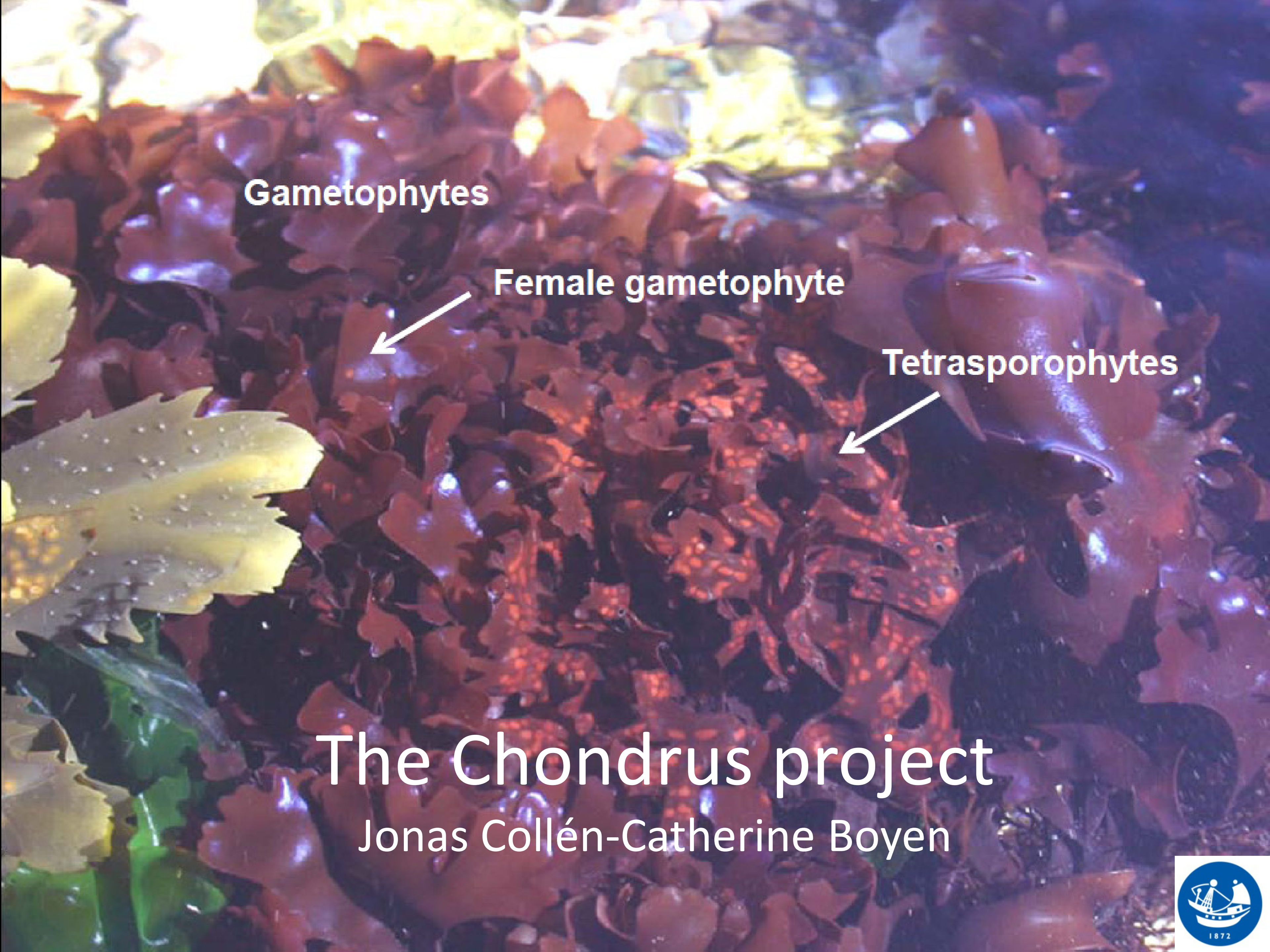
Which red seaweed to sequence?

- **A Florideophyte**
  - *Cyanidioschyzon* is already sequenced
  - complex multicellularity
  - the largest extant group
  - avoid competition with *Porphyra*
- **Economically important cell wall**
  - carrageenan or agar
- **Relatively small genome**
- **Preferably with a research history**
- **Amenable to laboratory culture**

Red algal genomics

Chondrus crispus was chosen

- Probably the best studied florideophyte species
- Common along Northern Atlantic rocky shores
- Related species in the Pacific Ocean
- Cellwall of carrageenan
- Related to commercially important species
- Morphologically variable
- “Typical” red algal life cycle



The Chondrus project

Jonas Collén-Catherine Boyen

The *Chondrus* genome

- Sequenced by Genoscope
- Sequencing 14.3 X3 kb ⇒10.8 X
- 10 kb ⇒3.2 X
- 20 kb ⇒0.3 X

- 925 scaffolds(1,172 to 2 kbp)

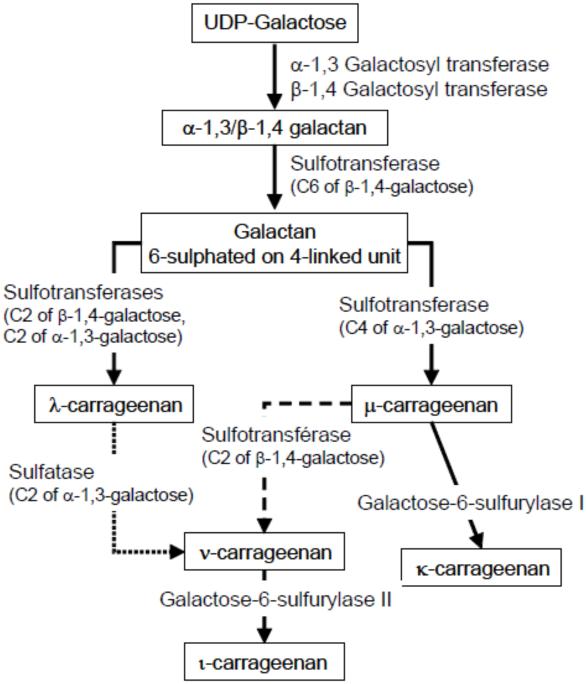
  (hypothetical chromosomes 3 Mbp)
- N50= 243 kb

The *Chondrus* genome

- Automatic annotation supported by:
  - 300,000 ESTs (454), (95% matching)
  - *Abinitio prediction*
  - GenBank All proteins
  - Public red/brown ESTs
  - *Cyanidioschyzon genome data*

The *Chondrus* genome objectives

- Clarify the position of the red algae
- Check green –brown hypothesis
- Better understanding of multicellularity
- Finding cell wall genes
- Unexpected discoveries



UDP-Galactose

α-1,3 Galactosyl transferase
β-1,4 Galactosyl transferase

α-1,3/β-1,4 galactan

Sulfotransferase
(C6 of β-1,4-galactose)

Galactan
6-sulphated on 4-linked unit

Sulfotransferases
(C2 of β-1,4-galactose,
C2 of α-1,3-galactose)

Sulfotransferase
(C4 of α-1,3-galactose)

λ-carrageenan

μ-carrageenan

Sulfotransférase
(C2 of β-1,4-galactose)

Sulfatase
(C2 of α-1,3-galactose)

Galactose-6-sulfurylase I

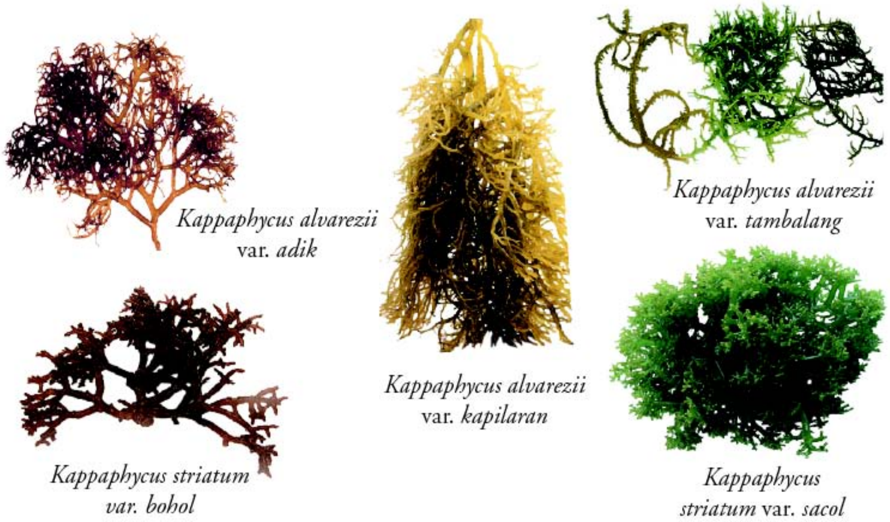
ν-carrageenan

κ-carrageenan

Galactose-6-sulfurylase II

ι-carrageenan

Trends in seaweed biotechnology



- **genetic markers for breeding elite varieties**
- **transcriptomics for phenotyping at the genome level**

Merging genomics into algal biotechnology

Business need

Desired traits

Population and quantitative genetics

Biochemistry, molecular biology

Selected plants

Molecular genetics

Isolated gene(s)

Traditional and molecular genetics

~~Genetic engineering GMOs~~

Elite varieties

Cellular biology - Reproduction biology

Propagation

Agronomy, physiology

Production

Towards the systems biology of maricultured algae

Conclusions

- **Merging population genomics and classical and quantitative genetics in seaweed aquaculture.**

- **Mining genomics resources and developing post-genomics approaches.**

- **Developing biotechnological tools to exploit the metabolic diversity of seaweeds, including protein expression systems to taylor precursors of bioactives**

- **Testing alternative processes in seaweed industry, including filtration and fermentation.**

J.M. Cock

J. Collén

C. Boyen

G. Michel

M. Czjzek

B. Kloareg

Thank you !