

Practical Work 1 : Algae

Introduction

Photosynthetic organisms include those capable of performing photosynthesis, that is, the light-driven synthesis of carbon-based organic compounds from mineral matter (refer to Chapter 3 on metabolism). Among them are prokaryotic organisms, notably Cyanobacteria (see Chapter 1 and especially Biotechnology), but above all, eukaryotic organisms, which are the focus of this practical session.

Comprising numerous and diverse groups, we will attempt to understand the organization, reproduction modes, and some evolutionary traits of eukaryotic photosynthetic organisms. Our study will be limited to the well-defined aspects outlined in the curriculum while occasionally extending to related taxa.

I. Photosynthetic organisms, a polyphyletic group of organisms

Required capacities	<ul style="list-style-type: none"> ✓ Compare the organization of a green algae and an embryophyte on a macroscopic scale. ✓ Establish the overall diversity and discuss the polyphyletism of all photosynthetic organisms.
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A. Phylogenetic position of chlorophyllous "plant" organisms

1. Concept of << vegetable >>, << plant >> or << fungus >>

a. Concept of "plant"

- The definition of what is meant by a plant organism has varied according to the times and authors. Three trends can be identified:
 1. Traditionally, following LINNAEUS in particular, what existed was divided into 3 kingdoms: animal, vegetable, mineral. A vegetable was anything that was alive but was not an animal.
 2. Another definition, more recent and still often in use, excludes 'prokaryotes': plants then include algae (uni- and multicellular), terrestrial 'plants' and 'fungi'.

Some authors continue to include Cyanobacteria or "Blue Algae." This is still the Code of Nomenclature, botany which governs their systematics.

- 3. Finally, a last definition corresponds to all eukaryotic photosynthetic organisms (= 'plants'): uni- and multicellular 'algae', terrestrial 'plants'.

b. Concept of << plant >>

- The term 'plant' covers:
 1. All eukaryotic photosynthetic organisms (uni- and multicellular 'algae', land plants).
 2. All multicellular eukaryotic photosynthetic organisms (multicellular 'algae' and terrestrial 'plants').
 - NB In this case, the unicellular 'algae' were placed in the old (now obsolete) kingdom of 'protists'.

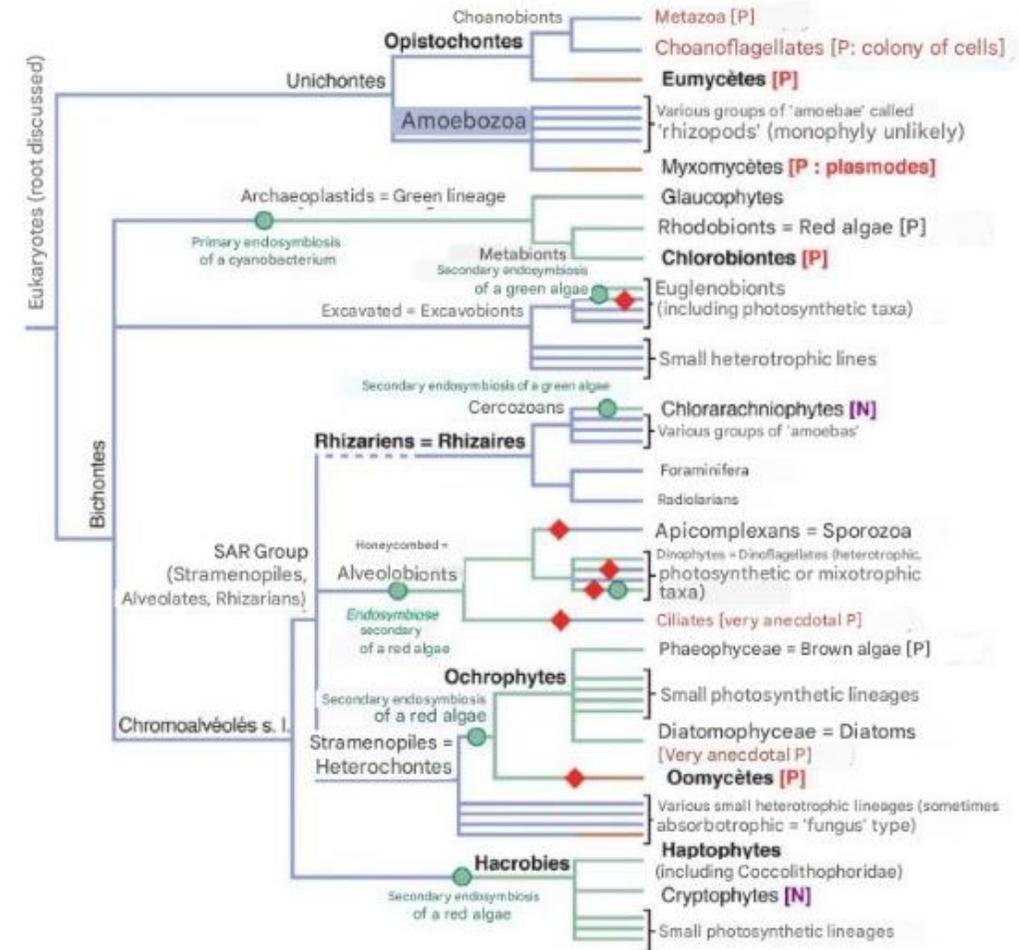
c. Concept of "mushroom"

See TP 4.2. (Fungi in ecosystems)

- 'Fungi' are single- or multi-cellular organisms:
 1. Heterotrophic eukaryotic organisms feeding by absorption: Eumycetes, Microsporidia, Oomycetes, Mycetozoa (Myxomycetes and minor groups)...
 2. Restricted definition: Fungi = (Eu)mycetes (monophyletic).

2. Phylogeny of eukaryotes showing the position of photosynthetic organisms and the convergent acquisition of plastids

- Photosynthetic capacity was acquired many times and sometimes secondarily lost (Figure 1).



Bloodlines

- Heterotrophic eukaryotic lineage [dotted: highly debated position]
- Photosynthetic lineage
- Heterotrophic lineage clearly of the absorbtrophic type ('fungus' type in the broad sense: the program is limited to Eumycetes and Oomycetes)

[P] Multicellularity of at least part of the group (characteristic acquired many times, sometimes lost: it is difficult or even impossible to precisely position the events of loss or acquisition of this characteristic, the very definition of which poses a problem)

Plastics

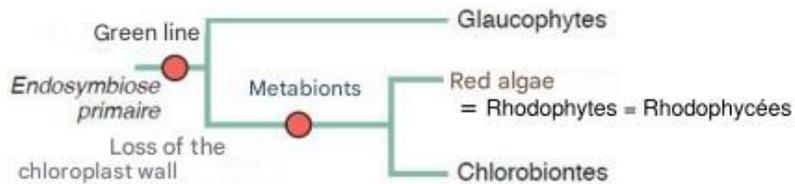
- Supposed acquisition of the plastid (with precision of its endosymbiotic origin) [N]
- ◆ Alleged loss of plastid (or regression)
The modalities and stages of plastid acquisition are discussed in the text and are not unanimous among scientists. For some authors, the plastid from a red Alga in Chromoalveolates would have been acquired only once, sometimes by their ancestor and then lost secondarily many times. The plastid history of Alveolates is also particularly complex and fiercely debated, with multiple loss and reacquisition events (sometimes by tertiary endosymbiosis in Dinophytes).

Not taxa
Some nodes have not been named (sometimes because the name has not yet been proposed).

FIGURE 1. Phylogenetic tree of eukaryotes. According to SEGARRA et al. (2015) [Document that I had produced from numerous bibliographic references].

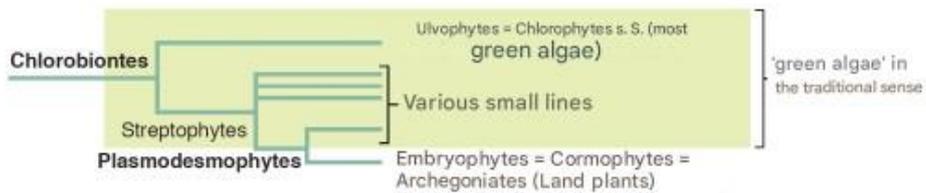
The 'algae' of the Green Lineage (Red Algae or Rhodophytes + green 'algae'): phylogenetic position

According to SEGARRA et al. (2015)



Green line
Molecular arguments
Two-membrane plastids (many derived characteristics) Presence of starch reserves outside the chloroplast

▲ Green Lineage Tree.



▲ Simplified tree of Chlorobiontes.

Rhodophytes [Rhodophyta] = Rhodobiontes

- Particular starch grains
- Persistence of the nuclear membrane during mitosis
- Gametes are protoplasts (no cell wall)
- Own pigments (phycobilisomes)

Chlorobiontes

- Loss of phycobilins
- Chlorophyll b
- Organization of the thylakoids
- RuBisCO gene transferred from chloroplast DNA to the nucleus

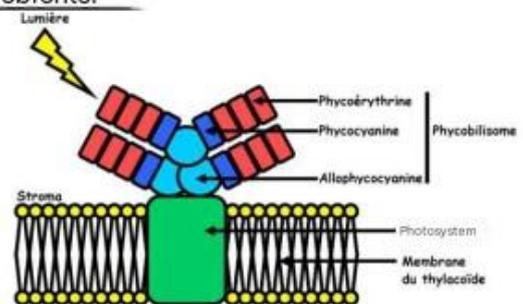
Chlorophytes [Chlorophyta] = Ulvophytes

- Essentially molecular arguments
- Cytoskeletal organization of the flagellum base

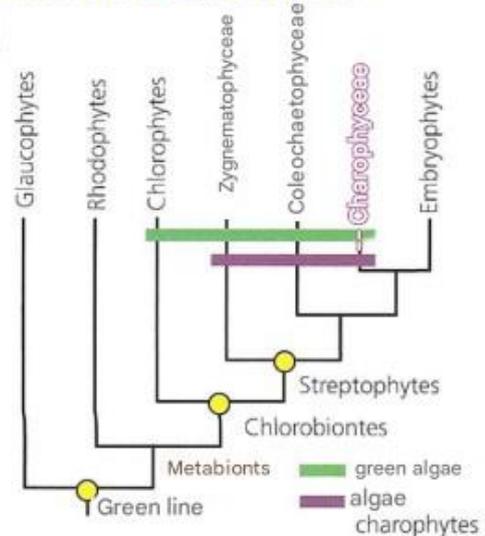
'charophytes': paraphyletic group of algae mainly present in fresh water



Chara baltica, one of the rare 'charophytes' marines
<http://www.norfolkbiodiversity.org/actionplans/speciesactionplans/stonewort.aspx>



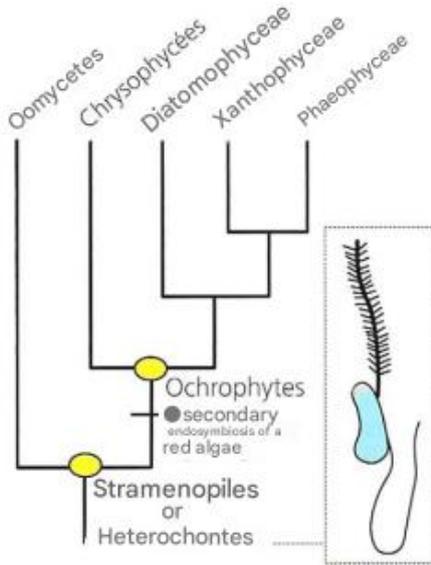
Phycobilisome
M. RAYMOND & F. JAUZEIN
<http://www.felb-botanica.org/sites/botanica/uf/fr/documents/biblio/article%20en%20ligne/phylogenetic%20classification.pdf>



Simplified phylogeny of the Green Lineage

According to MEYER et al. (2008)

Ochrophytes (including in particular Phaeophyceae or brown algae)



phylogenetic tree of Stramenopiles (after Andersen, 2004) Inset, diagram of a heterochont cell.

Simplified phylogeny of Stramenopiles

According to MEYER et al. (2008)

Stramenopiles = Heterochontes
 Heterochontous flagella (differ morpho-anatomically)
 Presence of a transition helix at the base of the flagella

Ochrophytes
 Four-membrane plastids (endosymbiosis of a red alga)

Phaeophyceae = Brown algae
 Presence of plasmodesmata
 Structure of the reproductive system



Thalle
 Wikipedia



Receptacles
http://www.algaebase.org/search/species/detail?species_id=67&sk=10

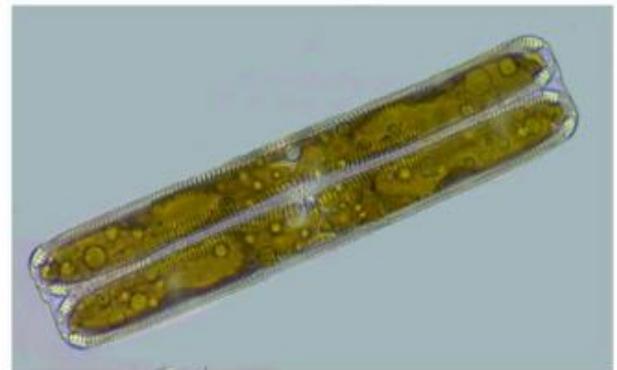
Fucus vesiculosus, a typical Phaeophyceae (brown algae)

▲ Overview of Ochrophytes and positioning within Stramenopiles.



Chrysophyceae (Golden Algae)

Especially in fresh waters.
 According to Wikipedia



Diatoms

Planktonic unicellular algae (2 μm to 1 mm) present in all aquatic environments (with a preference for cold waters) and enveloped by an external siliceous skeleton (frustule of two thecae).

Cliché W. DIONI (2004)

<http://forum.mikroskopia.com/index.php?showtopic=1199>



Xanthophyceae (Yellow Algae)

Unicellular, green-yellow algae, usually freshwater, moving by two unequal flagella or by emitting pseudopodia.

Cliché André? (2004)

<http://forum.mikroskopia.com/index.php?showtopic=2018>

Practical Work to be Done:

E. Study of a Chlorophyte: Ulva Ulva sp.

Activity 3. Macroscopic and microscopic study of Ulva

How does the study of Ulva allow us to understand its organization?

Knowledge to build		Macroscopic and microscopic organization of an Ulva	
Know-how requested	Targeted ability or attitude		Assessment
	Manipulate, master a tool, a technical gesture, software		
	> Optical microscope > Microscopic preparation		
		Analysis, interpretation, reasoning, relating data	

Work to be done

1. Carry out a macroscopic study of the Ulva thallus.
2. Make a cross-section of the thallus in the water and observe it in the MO.
3. Complete the figures and simplify the reproduction cycle.

1. Study of the vegetative apparatus (thallus)

- See Figures 9-10.

Ulva (*Ulva lactuca*) is an edible seaweed that lives in the tidal zone. It is therefore only found in salt water, but it tolerates variations in salinity due to its position in the intertidal zone.

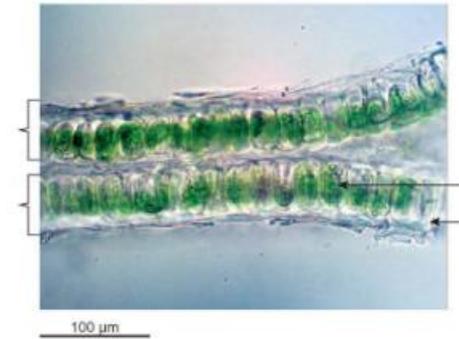
The vegetative apparatus is a thin green blade, 10 to 40 cm long, with a fixing disc at its base (figure). It allows it to attach to a rock. No organ is differentiated: it is therefore a thallus.



Morphological organization of a thallus of *Ulva lactuca*

▲ FIGURE 9. Thalle d'Ulva. D'après SEGARRA *et al.* (2015).

A cross-section of the thallus grown in salt water shows that it is made up of only two cell layers (figure): the thallus is of the biseriate nematohalial type. The walls are poor in cellulose fibrils and rich in hydrophilic polysaccharides (agar in particular), which makes the thallus particularly flexible.



Histological organization of an *Ulva lactuca* thallus

No cellular differentiation is observed: the cells are polygonal, containing a single chloroplast that often obscures the nucleus. The chloroplast appears green because the thylakoid membranes contain chlorophylls a and b, as well as carotenoids. This lack of differentiation may be related to the growth pattern: each cell of the thallus can divide, so it is a diffuse growth. This characteristic is visible thanks to the existence of paired cells, half the size of the others and located in different places on the thallus when viewed from the front under a microscope.

Staining with iodine water reveals the presence of starch grains in the chloroplast. These grains surround a structure made mostly of Rubisco: the pyrenoid. Each chloroplast contains one or more pyrenoids.

There is one exception to the lack of cell differentiation: cells located on the margin of the thallus are involved in reproduction and form gametocytes or sporocysts.

▲ FIGURE 10. Ulva thallus studied in the MO. From SEGARRA *et al.* (2015).

2. Study of the reproductive cycle

- See Figures 11-12.

1. Microscopic Observation of a Drop of Natural Freshwater (Oued, Lake, River, etc.)

1.1 Identification of Microscopic Unicellular Green Algae

- Identify species such as *Euglenan spp.* and *Chlamydomonas spp.*.

1.2 Drawing of Observed Green Algae at Two Magnifications

- **10x10 magnification:** General view showing all organisms present in the water drop.
- **10x40 or 10x100 magnification:** Detailed view of the studied algae.
 - The **legend** must include all cellular components, with a particular focus on the **pyrenoid**, which is highly visible in algae.

2. Microscopic Observation of a Filamentous Freshwater Alga (*Spirogyra spp.*)

2.1 Drawing of Observed Filamentous Alga at Two Magnifications

- **10x10 magnification:** General view of the multicellular organism.
- **10x40 or 10x100 magnification:** Focus on two to three cells.
 - The **legend** must include all cellular components, with a particular emphasis on the **pyrenoid**, which is clearly visible in these algae.