CYAnobacterial platform Optimised for bioproduction

# Cianobatteri: fabbriche intelligenti di molecole preziose

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Meeting Inaugurale Progetto CYAO

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Consiglio Nazionale delle Ricerche ALGATECH fondazione cariplo

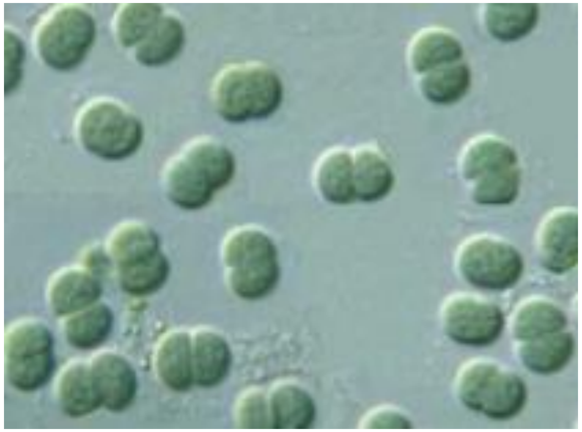
**MICROALGAE**

Are a huge group of **photosynthetic microorganisms** from freshwater, and brackish and marine systems, typically unicellular and eukaryotic, comprising:
- green algae (Chlorophyceae),
- red algae (Rhodophyceae),
- diatoms (Bacillariophyceae),
- brown algae (Phaeophyceae)

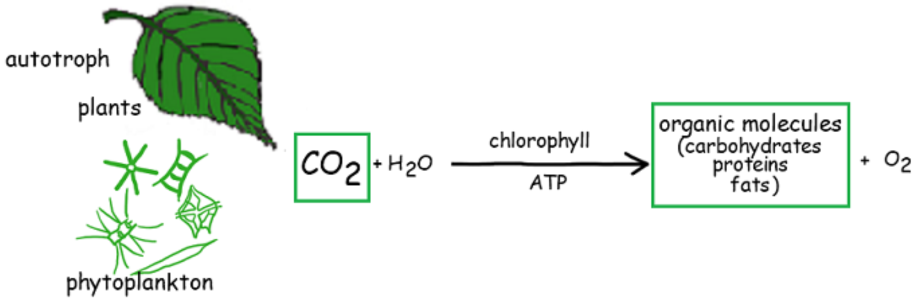
and

- **CYANOBACTERIA** (blue green algae), photosynthetic prokaryotes.

*Arthrospira platensis* (Spirulina)



*Synechocystis* PCC6803




**Most MICROALGAE are autotrophs:** they produces complex organic compounds (such as carbohydrates, fats, and proteins) from simple substances present in their surroundings (**inorganic compounds** like $CO_2$, N, S, P) and use **light as energy source**.

Some are **heterotrophic** instead, since they cannot fix carbon and therefore need organic carbon for their growth.

**Mixotrophic** microalgae can use both sunlight or organic carbon. They can simultaneously perform photosynthesis and heterotrophic metabolisms or are able to switch between the two metabolisms, depending on environmental conditions.

MICROALGAE convert captured solar energy into biomass with an **efficiency** that generally exceeds those of terrestrial plants (3% reported for marine microalgae against 0.2–2% for terrestrial plants).

Moreover, they **can be grown on lands not destined to agriculture and animal farming** (arid or low quality land), thus **avoiding competition** with these activities.

Even if MICROALGAE cultivation is carried out in aquatic environment, they **use less water than terrestrial crops,** so the freshwater consumption is strongly reduced.

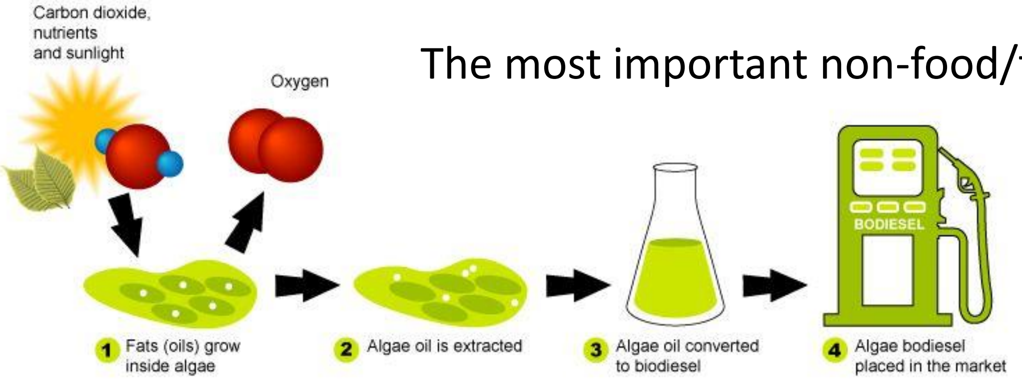
MICROALGAE may be cultivated in brackish and sea water **avoiding herbicide or pesticide application,** and **reducing the need of external nutrients** ($NH_4$, $NO_3$ and P).

With these **simple growth requirements**, MICROALGAE can **sustainably** generate lipids, proteins, and carbohydrates at a large scale, offering promising **environmentally friendly alternatives** to the current consumer products.

**Microalgal biomass** is an excellent source of **oils** (including high amounts of long chain polyunsaturated fatty acids (PUFAs), **proteins**, **polysaccharides** (such as starch, xylans, pectins, glucans, extracellular polysaccharides (EPS)) and **other high-added value compounds** (carotenoids, pigments, antioxidants, sterols and minerals).

All these molecules are **synthesised to different levels depending** on the particular microalgal **species**. In addition, their accumulation level can be increased by **tuning** microalgae growth conditions.

Therefore by an appropriate selection of algal species and cultivation parameters, it is possible to obtain **a wide range of commercially attractive biomolecules**, that can be used in **feed, food, nutraceutical, cosmetic and pharmaceutical industries**.




The most important non-food/feed application of **microalgae** is the production of **biofuels**. Current limitations exist mainly in the harvesting process and in the supply of $CO_2$ for high efficiency production.

## Cultivation systems

**Open**
(ponds and raceways)

autotrophic conditions

**Closed**
(photobioreactors, **PBR**, and fermenters)

autotrophic or heterotrophic conditions

In Open systems, the **environmental parameters vary** according to the season (unstable light supply and weather) and the **risk of contamination** of bacteria or unwanted algae is high. The selection of **good strains** able to tolerate environmental changes and becoming predominant in the culture, should reducing contamination problems.

In Closed systems, cultivation conditions are controlled, but the plants are **more expensive**, due to **capital and operating costs.**

**MICROALGAE biomass production** is still in a **developing phase** and a lot of work is necessary to **enhance the productivity** and to **reduce the production cost**.

**Commercial large-scale production** of MICROALGAE started in the early 1960s in **Japan** with the culture of *Chlorella* used as a food additive, followed by the cyanobacterium *Arthrospira*. Only after 1980 large-scale algae production facilities were established in **Asia, India, USA, Israel and Australia**.

Commercial **MICROALGAE farms for value-added products** are usually conducted in open ponds in locations having relatively warm temperature all the year or in fermenters under heterotrophic conditions.
(Cyanotech, Hawaii)

**In Europe the number of producers is continuously increasing**, even if the production volumes still remain relatively small, principally due to suboptimal climatic conditions in most countries. Therefore several companies prefer closed cultivation system (PBR) thus incurring in higher installation and operation costs.

| Microalgae production systems | Functional ingredients | Microalgae species | Commercial products |
|---|---|---|---|
| Ponds and raceways | **Proteins**<br>Phycobiliproteins<br>CAROTENOIDS<br>PUFAs | ***Arthrospira maxima***<br>***Arthrospira platensis***<br>*Chlorella* spp.<br>*Dunaliella salina*<br>*Dunaliella bardawil* | **Nutraceutical products**: tablets, capsules, energetic drinks.<br>Natural dyes to human foods. |
| PBR | ASTAXANTHIN | *Haematococcus pluvialis* | High antioxidant nutraceutical products, colorants to salmon, trout and poultry feed. |
| Fermenters | Lipids<br>PUFAs | *Crypthecodinium cohnii*<br>*Schizochytrium* sp.<br>*Nitzschia laevis* | Nutritional supplements, additive for infant formula, vegetarian products. |






**Microalgal PROTEINS**

Already during the 1950s some species of microalgae were proposed as innovative sources of proteins.
Microalgal biomass is particularly interesting due to both the high percentage of proteins and their **favorable amino acid profile**.

| | | % on total protein content | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Protein content g/Kg | Leu | Val | Lys | Phe | Met | Try | Thr | His |
| WHO/FAO reference | | 7.0 | 5.0 | 5.5 | 6.0 | 3.5 | 1.0 | | |
| Egg | 132 | 8.8 | 7.2 | 5.3 | 5.8 | 3.2 | 1.7 | 5.0 | 2.4 |
| Soybean | 370 | 7.7 | 5.3 | 6.4 | 5.0 | 1.3 | 1.4 | 4.0 | 2.6 |
| ***Arthrospira maxima*** | **600–710** | **8.0** | **6.5** | **4.6** | **4.9** | **1.4** | **1.4** | **4.6** | **1.8** |
| ***Arthrospira platensis*** | **600–710** | **9.8** | **7.1** | **4.8** | **5.3** | **2.5** | **0.3** | **6.2** | **2.2** |

**Spirulina proteins** are of **good qualitiy and quantity** (60–70% of dry weight), are rich in essential amino acids and have **good digestibility** (widely used as a protein supplement and to manufacture healthy foods).

*Nannochloropsis* spp. and *P. tricornutum* show a higher amount of hydrophobic and hydrophilic amino acids than soybean flour.

| Microalgae production systems | Functional ingredients | Microalgae species | Commercial products |
|---|---|---|---|
| Ponds and raceways | Proteins<br>**Phycobiliproteins**<br>CAROTENOIDS<br>PUFAs | ***Arthrospira maxima***<br>***Arthrospira platensis***<br>*Chlorella* spp.<br>*Dunaliella salina*<br>*Dunaliella bardawil* | **Nutraceutical products**: tablets, capsules, energetic drinks.<br>**Natural dyes** to human foods. |
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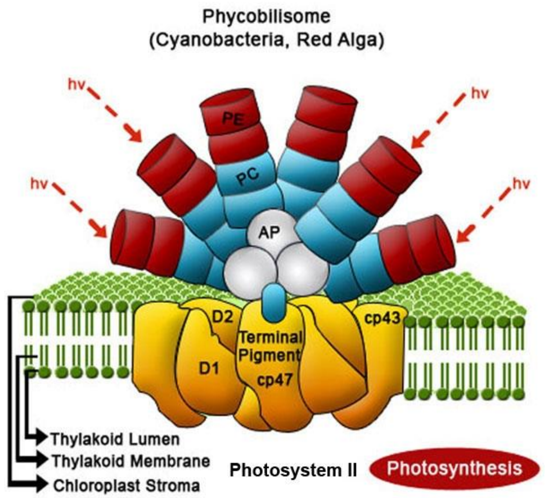
**PHYCOBILIPROTEINS** are a family of light-harvesting pigment protein complexes found in certain cyanobacteria and algae. These include **phycoerythrin**, **phycocyanin**, **allophycocyanin** and phycoerythrocyanin.






**Phycocyanin** from *Spirulina* spp. and **phycoerythrin** from *Porphyridium cruentum* are two of the most well-known phycobiliproteins that are produced commercially.

**Phycobiliproteins** have been used as **natural colorants** in foods (chewing gums, dairy products, ice creams and candies) and have been marketed in a variety of nutraceutical products such as tablets, capsules, etc. showing a variety of functional activities, such as antioxidant, neuroprotective, anti-inflammatory, hepatoprotective, hypocholesterolemic and anticancer.

| Microalgae production systems | Functional ingredients | Microalgae species | Commercial products |
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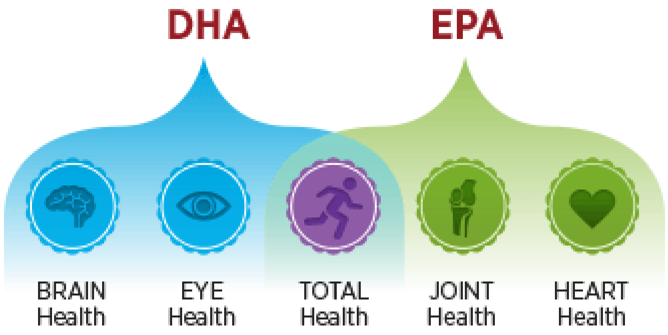
**Microalgal LIPIDS**

Microalgae fatty acids are a **reliable substitute for vegetable oils**, since the percentages of linoleic (C18:2, ω-6) and alpha/gamma-linolenic acids (C18:3, ω-3/ω-6) are usually higher than in rape seed, soy or sunflower oils.

High percentages of palmitic acid (C16:0) (useful for its food structuring properties) can be obtained in some cases.

The most interesting molecules as functional ingredients are the **long chain polyunsaturated fatty acids** (PUFAs) such as eicosapentaenoic acid (EPA, 20:5, ω-3) and docosahexaenoic acid (DHA, 22:6, ω-3), which can be obtained at very high concentrations.




The consumption of EPA and DHA supplements prevents cardiovascular diseases and inflammation, and improves brain function and development of nervous system in infants.

The main source of EPA and DHA for human nutrition comes now from **marine fish** such as mackerel, cod, salmon and mullet. However, fish stocks are more and more limited and the presence of some chemical contaminants such as mercury pushed companies to search for **alternative sources**.

| Organism | Amount of long chain ω-3 (%) | Type of ω-3 FA |
|---|---|---|
| **Fish** | | |
| *Merluccius productus*<br>*Saridnops sagax*<br>… | 34.99<br>40.08 | EPA + DHA<br>EPA + DHA |
| **Microalgae** | | |
| *Nannochloropsis salina*<br>*Isocrysis galbana*<br>*Crypthecodinium cohnii*<br>*Schizochytrium* sp.<br>… | ~28<br>~28<br>31.3<br>32.5 | EPA<br>EPA + DHA<br>DHA<br>DHA |

| Microalgae production systems | Functional ingredients | Microalgae species | Commercial products |
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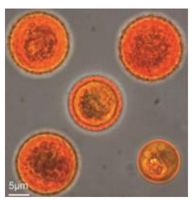
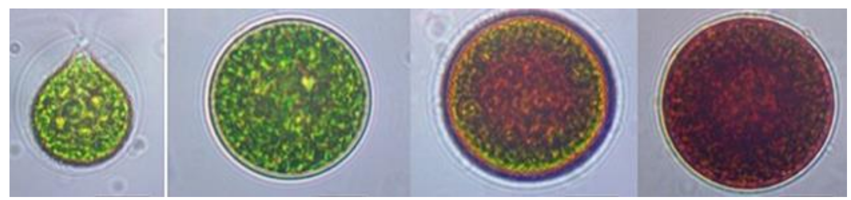
**Microalgal CAROTENOIDS**

Over a hundred different carotenoids have been identified from microalgae and their composition is highly variable within taxonomic groups.

The main carotenoids produced by microalgae are **β-carotene** from *Dunaliella salina* (β-carotene rich *Dunaliella* powder has been marketed in many countries since the 1980s) and **astaxanthin** from *Haematococcus pluvialis*.



β-Carotene is routinely used in soft-drinks, cheeses and butter or margarines.

Carotenoids are important **natural dyes** at low concentration: canthaxanthin, astaxanthin and lutein from *Chlorella* have been widely used as pigments in particular added to salmon, trout and poultry feed to intensify the reddish color of meat and yolk.

Numerous benefits have been claimed for **astaxanthin**: it enhanced eye health, improved muscle strength and endurance and it protected the skin from premature ageing, inflammation and UV-A damage.

Many positive features such as growth, vision, reproduction, immune function, and regeneration were reported also in animal nutrition, therefore FDA approved astaxanthin as a **feed additive for use in the aquaculture industry** in 1987, and in 1999 astaxanthin was further approved for use as a **dietary supplement**.

The **natural sources** of astaxanthin are: microalgae, yeast, shrimp, krill and plankton.

Astaxanthin can be found in the microalgae *Haematococcus pluvialis*, *Chlorella zofingiensis* and *Chlorococcum* sp. *H. pluvialis* is a freshwater green algae that can synthesize and accumulate a large amount of astaxanthin (4–5% of cell dry weight) under **oxidative stress**. Now it is cultivated at a large scale by several companies using different approaches to synchronize the algae at the same cellular phases until the cysts are rich in astaxanthin.

Despite “algae represent an emerging biological resource of great importance for its potential applications in different fields, including food and feed” as everywhere stated in all EU Bioeconomy strategy and action plan documents, **the European domestic demand for these products is still very low**.

Moreover the procedure for obtaining **commercial authorisation** of algae-derived products (not to mention GM microalgae) in the EU market is difficult and long lasting.

It is therefore important to **improve the public awareness** regarding the benefits of biobased product and in particular microalgae-derived ones.

**workflow:**

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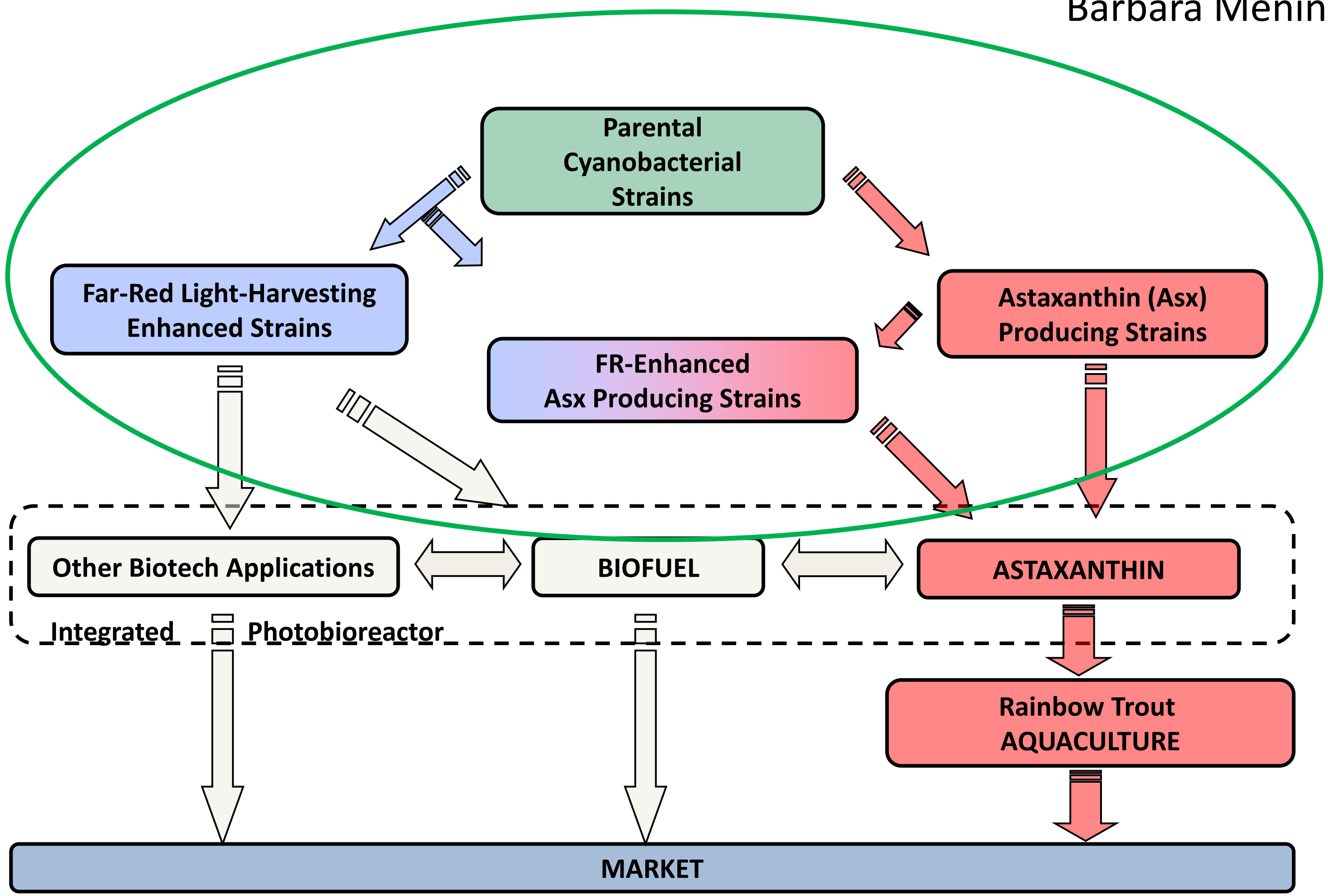
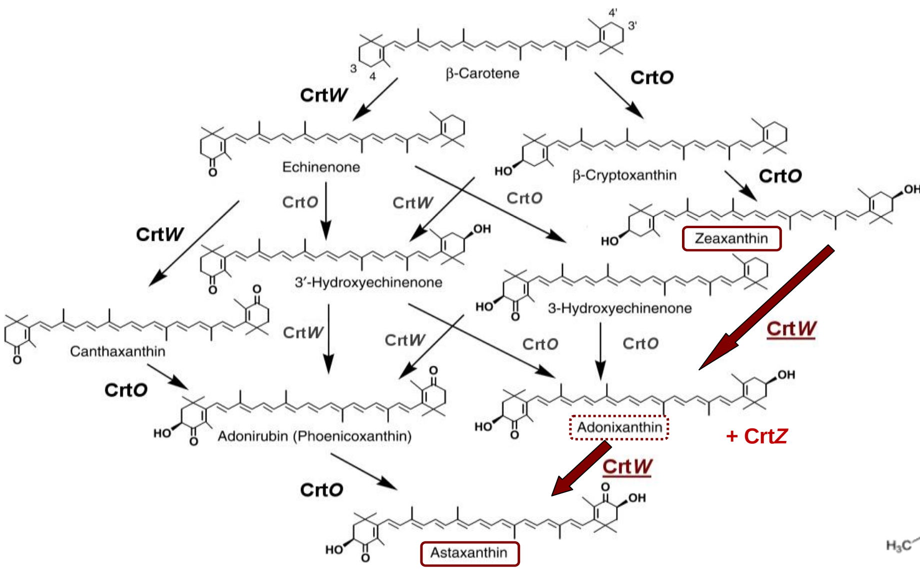
**IBBA/IBF team**

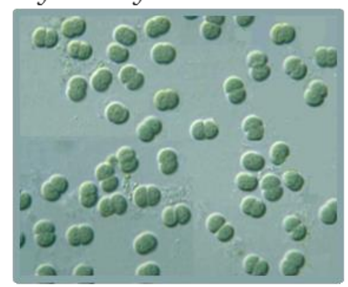



Consiglio Nazionale delle Ricerche ALGATECH fondazione cariplo

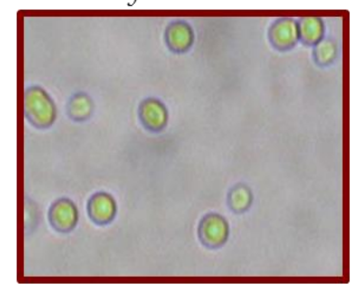
**Metabolic engineering** of the carotenoid and the chlorophyll biosynthetic pathway in *Synechocystis*, by introducing key enzymes from different organisms.

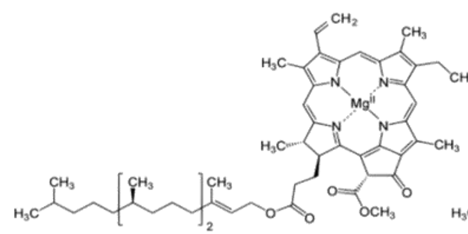



*Synechocystis* PCC6803

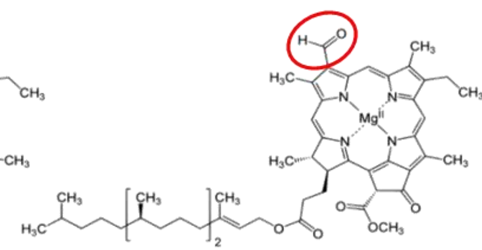


*Acaryochloris marina*

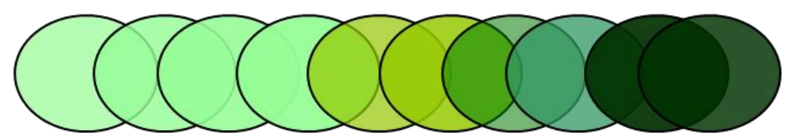




Chlorophyll *a*



Chlorophyll *d*



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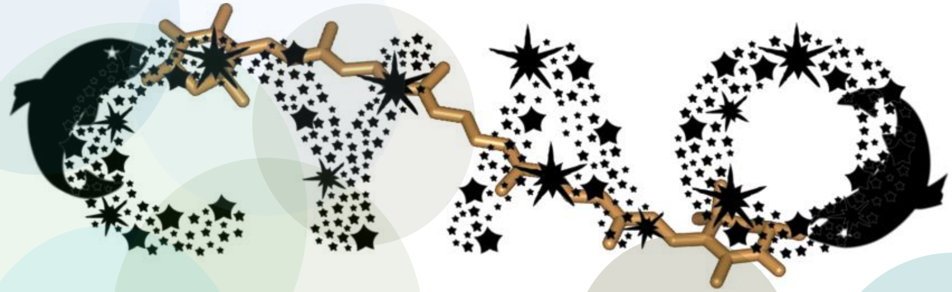

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