

UNESCO GSC Chair:

1st International Conference on Biomass Valorization for Sustainable Applications

2nd - 3rd November 2023, Morocco



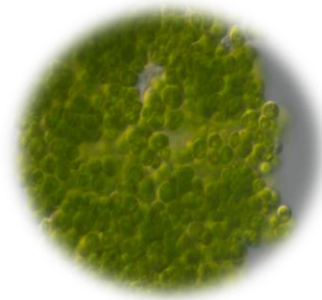
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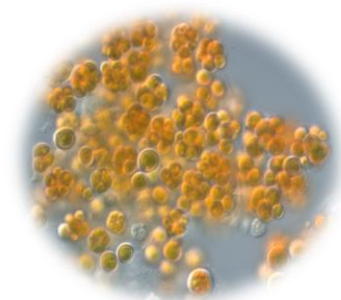
Oral Presentation

Carotenoids Bioproduction by two Psychrophilic Microalgae: *Sphaerocystis sp.* and *Pleurastreum sp.*



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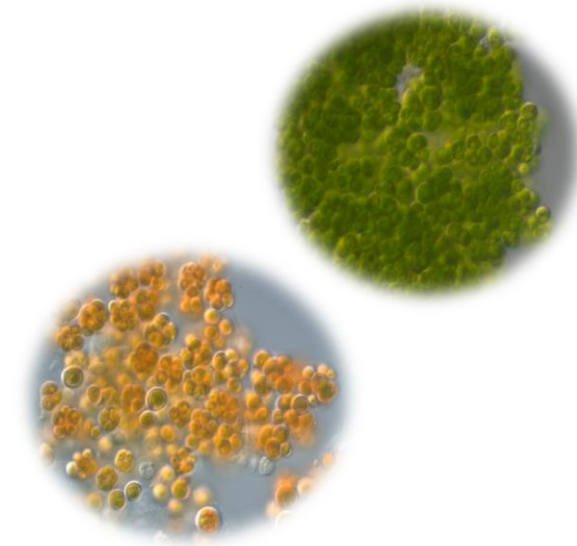
Supervisors

Pr. Nabil Grimi, Dr. Julien Lemaire, Madeleine Charbonnier, Fanny Duval

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Plan

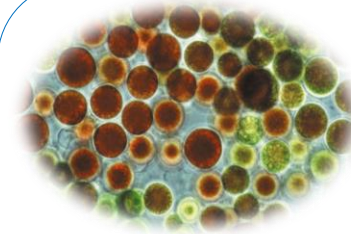
- I. Introduction & Context
- II. Materials & methods
- III. Results
- IV. Conclusion



Carotenoids: Astaxanthin

- Super-vitamin E
- High value-added molecule + Commercial value
- High antioxidant activity
- Novel Food

Natural sources



Ex: **Microalgae**, Yeast, Phytoplankton consumers like Flamingo bird



Health benefits



Cardiovascular Support

Skin Health anti-aging and UV-Protection

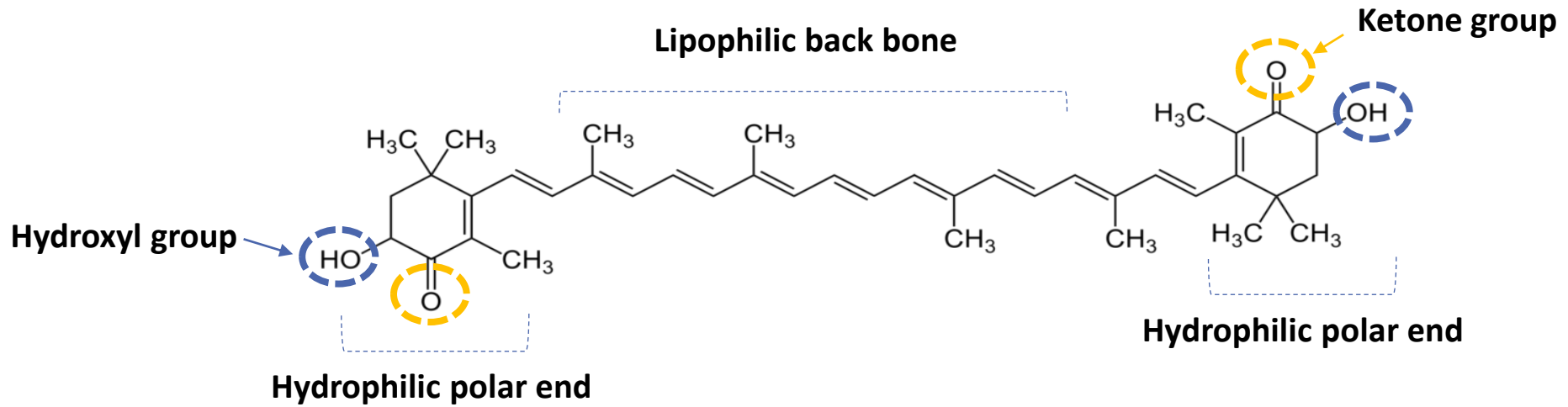
Athletic Performance & Energy Levels

Price (USD/kg)

Natural Astaxanthin	Synthetic Astaxanthin
2500 to 10,000	1000 to 2000
Recently approved by Food & Drug administration (FDA, USA)	95% of the market-available Astaxanthin

Chemistry and synthesis pathways

Chemical structure of free Astaxanthin, Molecular formula $C_{40}H_{52}O_4$



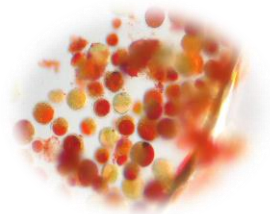
-OH can be bonded with **fatty acids** to form **esterified-Astaxanthin**

Synthesis pathways

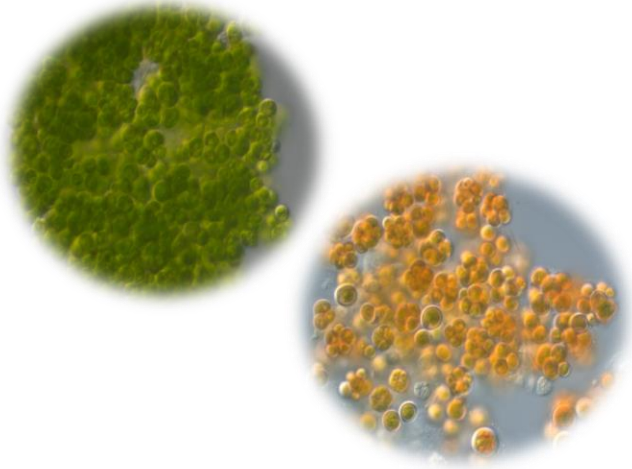
Chemical

- The **Wittig reaction** involves the use of two salts of phosphonium (C15) and a dialdehyde (C10)
- The process of **hydroxylating** the carotenoid **Canthaxanthin**

Natural via *Haematococcus pluvialis* (reference microalgae)



Psychrophilic microalgae



- “Red Snow Algae” appear during the summer melt season
- Polar and Arctic regions
- Chlorophyceae, unicellular, and photo-trophic
- Psychrophilic: Cell growth between 0°C to 15°C

Green Stage	→ ←	Red Stage
Natural growth conditions <ul style="list-style-type: none"> • Temperature < 5°C • Low light intensity • Low nutrients availability 		Abiotic stresses <ul style="list-style-type: none"> • Temperature < 15°C • High light intensity • High salinity • Nutrient deficiency (N/P)

N: Nitrate; P: Phosphate



Watermelon snow below the toe of the Lava Glacier. Mt. Garibaldi. BC. Photo: Casey Engstrom.

✓ An alternative source of Astaxanthin and secondary carotenoids

✓ Northern Europe climate compatible for living

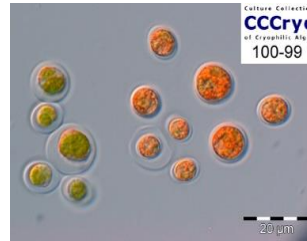
✓ Thick cell wall, robust cells

Psychrophilic microalgae: Selected strains and experimental conditions

Pleurastrum sp.



Sphaerocystis sp.



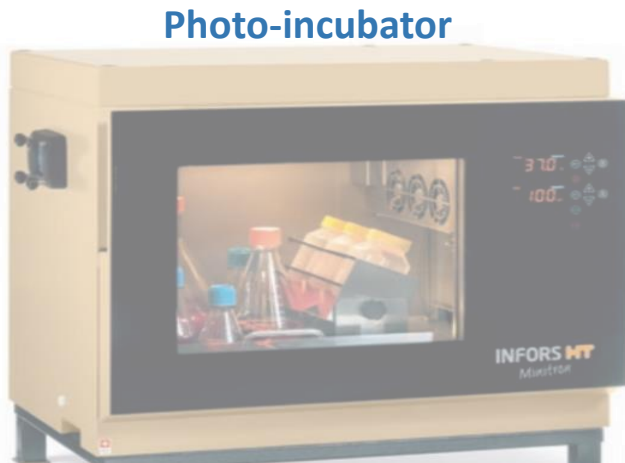
Monitoring of cultures by measuring:

- Optical density at 800 nm
- Dry weight
- Concentration of nutrients
- Pigments

Properties	T°max = 18 °C T°opt = 8 °C	T°max = 18 °C T°opt = 14 °C
Growth rate μ (day⁻¹)	0,2	0,5

T°: temperature, opt: optimal

➤ The experimental conditions of culture



Experimental conditions	<i>Pleurastrum sp.</i>	<i>Sphaerocystis sp.</i>
Green biomass	12 °C BBM-Medium 1 g/L NaHCO ₃	14 °C BBM-Medium 1 % CO ₂
Red biomass	• 12 °C • Luminous stress : 500-600 $\mu\text{mol photons/m}^2/\text{s}$	

BBM (Bold Basal Medium): a nutrient-rich growth medium that contains nitrate and phosphate

Acclimatisation

Biomass
production

Harvesting

Stress induction

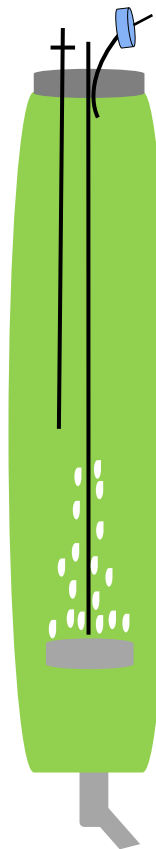


Photo-bioreactor

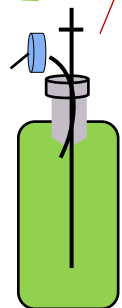
Rich medium

BBM

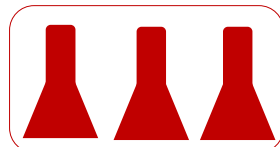
12°C

Low light

Biomass harvesting



**Transferring biomass
and washing it using
stress mediums in flasks**



Nutritionally-deprived mediums

BBM + S - N - P BBM - N - P

BBM + S - N BBM - N

12 °C, 225 – 325 $\mu\text{mol}/\text{m}^2/\text{s}$

Biomass harvesting

Freeze-drying

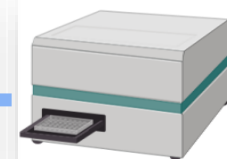
**Cells disruption,
pigments extraction, and analysis**



**Bead-
beating
EtOH 96%**



**HPLC-UV
DAD**



**Absorbance
spectrum 350 –
800 nm**



--: without; +: with; N: Nitrate; P: Phosphate; S: Salinity

Green-phase

Favorable conditions for growth

Scaling up in Photobioreactors

Pleurastrum sp.

- $\sim 70 \mu\text{mol}/\text{m}^2/\text{s}$
- BBM medium
- $1 \text{ g}/\text{L NaHCO}_3$
- 12°C

DW $\sim 1.2 \text{ g}/\text{L}$
 $\mu \text{ max} = 0.20 \text{ day}^{-1}$

Sphaerocystis sp.

- $\sim 70 \mu\text{mol}/\text{m}^2/\text{s}$
- BBM medium
- $1\% \text{ CO}_2$
- 14°C

- Both psychrophilic strains have successfully acclimated and adapted to our laboratory conditions, from the flask to the photobioreactor scale
- The specific growth rates obtained ($\mu \text{ max}$) are close to the reference (Leya, 2009)

Red-phase

Unfavorable conditions for growth
(stress adaptation)

Culture pigmentation and cells observation

Sphaerocystis sp.

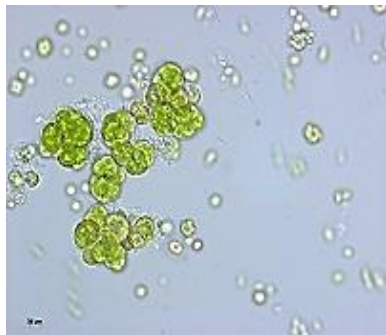
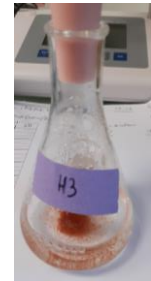
Pleurastrum sp.



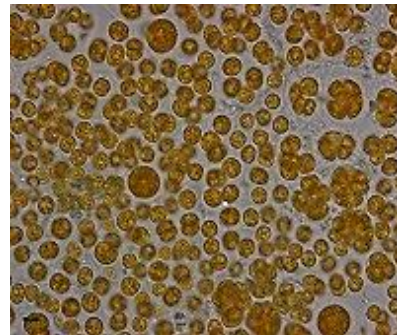
After 14 days



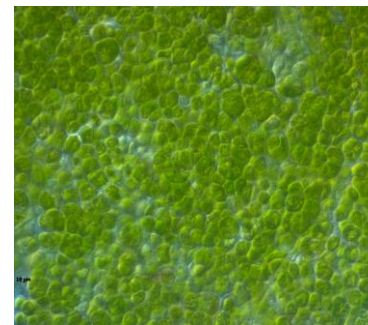
After 21 days



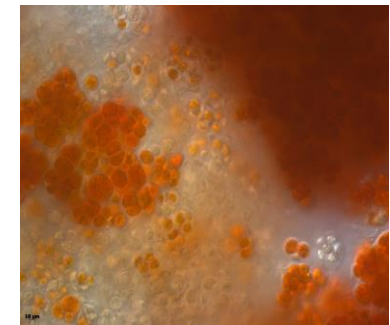
BBM



BBM - N - P + Luminous stress



BBM

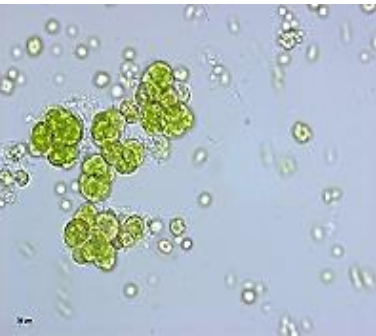


BBM - N - P + Luminous stress

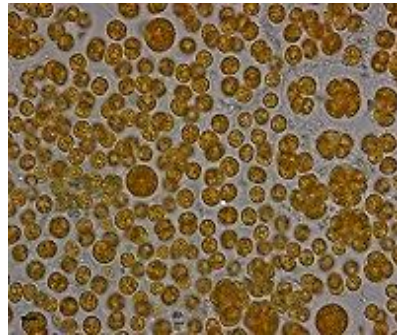
➤ Microlgae strains *Sphaerocystis sp.* and *Pleurastrum sp.* in flasks, and optical microscope pictures (400X)

Sphaerocystis sp.

After 14 days



BBM



BBM – N –P + Luminous stress

➤ Quantification of pigments by spectrophotometry

Phase	Pigments	Content (mg/g DW)
Green	Chlorophyll <i>a</i>	10
	Chlorophyll <i>b</i>	6,9
	Total carotenoids	1,5
Red	Chlorophyll <i>a</i>	0,3
	Chlorophyll <i>b</i>	0,8
	Total carotenoids	2,4

DW: Dry weight

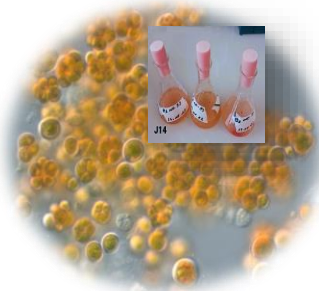
➤ Quantification of pigments by HPLC-UV DAD

Interesting peaks observed at 450 nm attributed to the presence of **Canthaxanthin**, **Astaxanthin**, and **Lutein**

The selected psychrophilic strains, *Sphaerocystis sp.* and *Pleurastrum sp.*, were successfully **acclimated** to our laboratory conditions for the **growth** and **accumulation of carotenoids**.

The results showed that both microalgae can accumulate carotenoids of interest such as **Astaxanthin**, **Canthaxanthin**, and **Lutein**

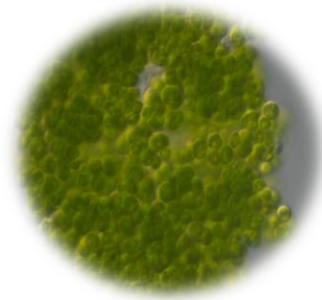
Work is still underway on biomass production, **pretreatment**, and **pigment extraction** to optimize their productivity and recovery yield.



*Thanks For Your
Attention*

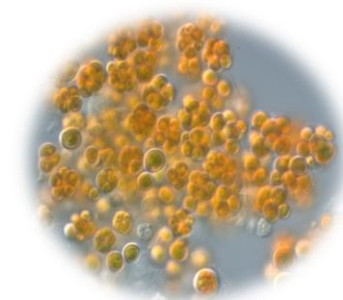
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