



UNIVERSITÀ
di **VERONA**

Dipartimento
di **BIOTECNOLOGIE**



European Research Council

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Produzione di astaxantina in microalghe: limiti e possibili soluzioni biotecnologiche

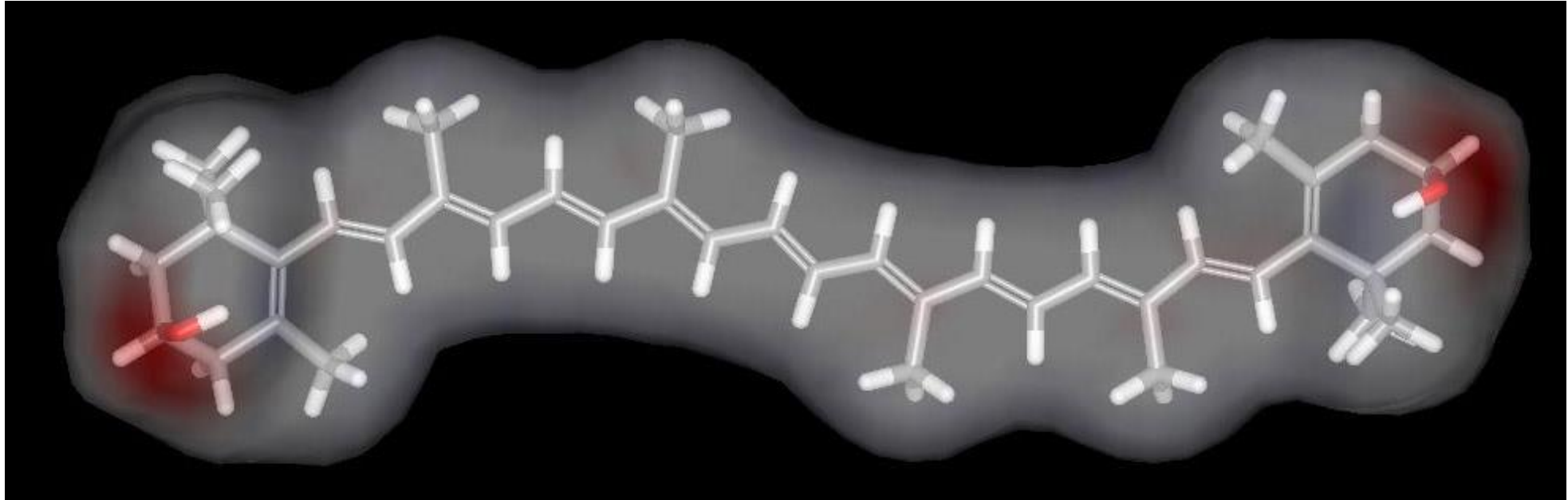
Prof. Matteo Ballottari

University of Verona,
Department of Biotechnology



I carotenoidi sono una classe di più di 600 pigmenti naturali sintetizzati da piante, alghe e batteri fotosintetici. Queste molecole riccamente colorate sono le fonti dei colori gialli, arancioni e rossi





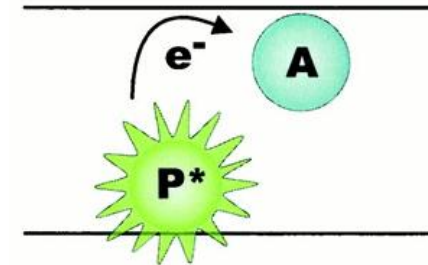
sono terpenoidi polienici, quasi sempre a 40 atomi di carbonio, costituiti da una lunga catena centrale idrocarburica a 22 atomi, con diversi doppi legami coniugati, e da due unità terminali cicliche

Fotosintesi

La Fotosintesi è il processo biologico tramite cui l'energia solare viene utilizzata per fissare CO₂ in biomassa



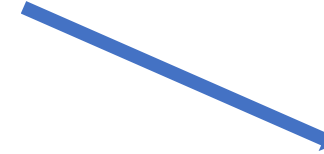
Photosynthesis



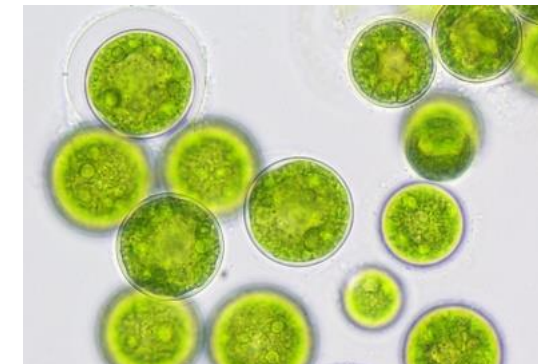
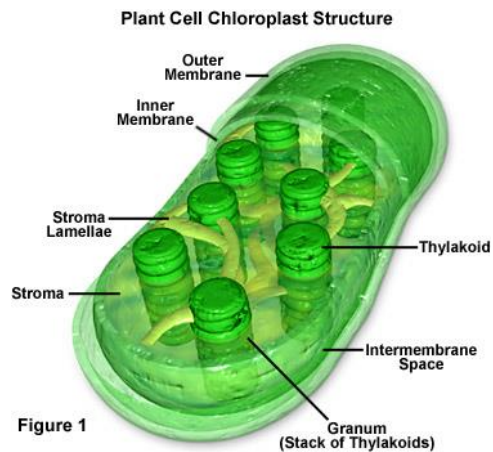
LIGHT ENERGY



CHEMICAL ENERGY



BIOMASS



Fotosintesi

Quando la luce è in eccesso la Fotosintesi è saturata:
formazione di ROS

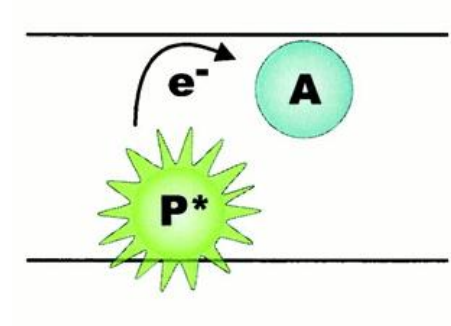


HIGH LIGHT

LIGHT ENERGY



CHEMICAL ENERGY



Photosynthesis

O₂

ROS

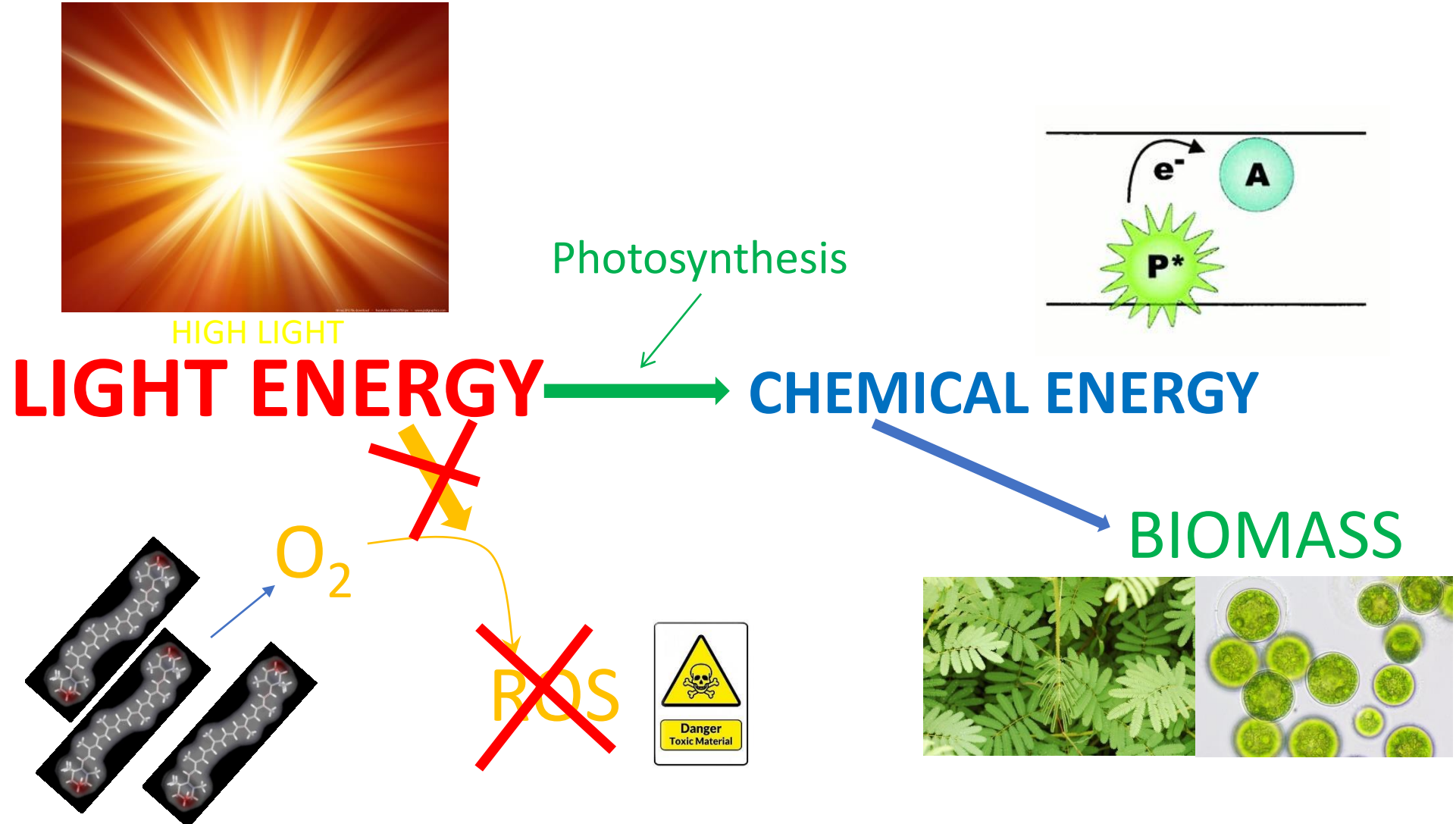


BIOMASS

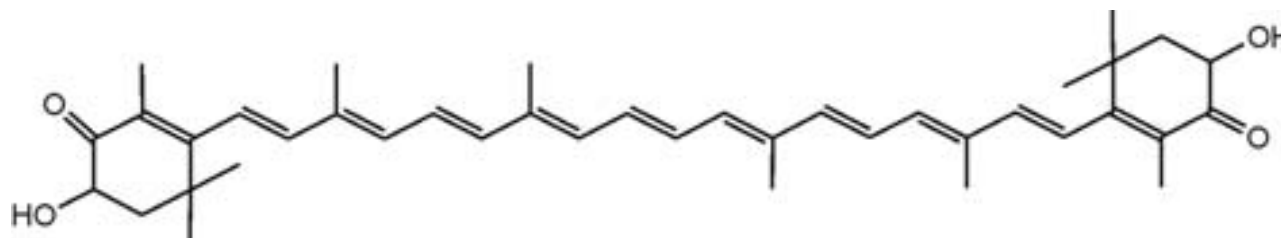


Fotosintesi

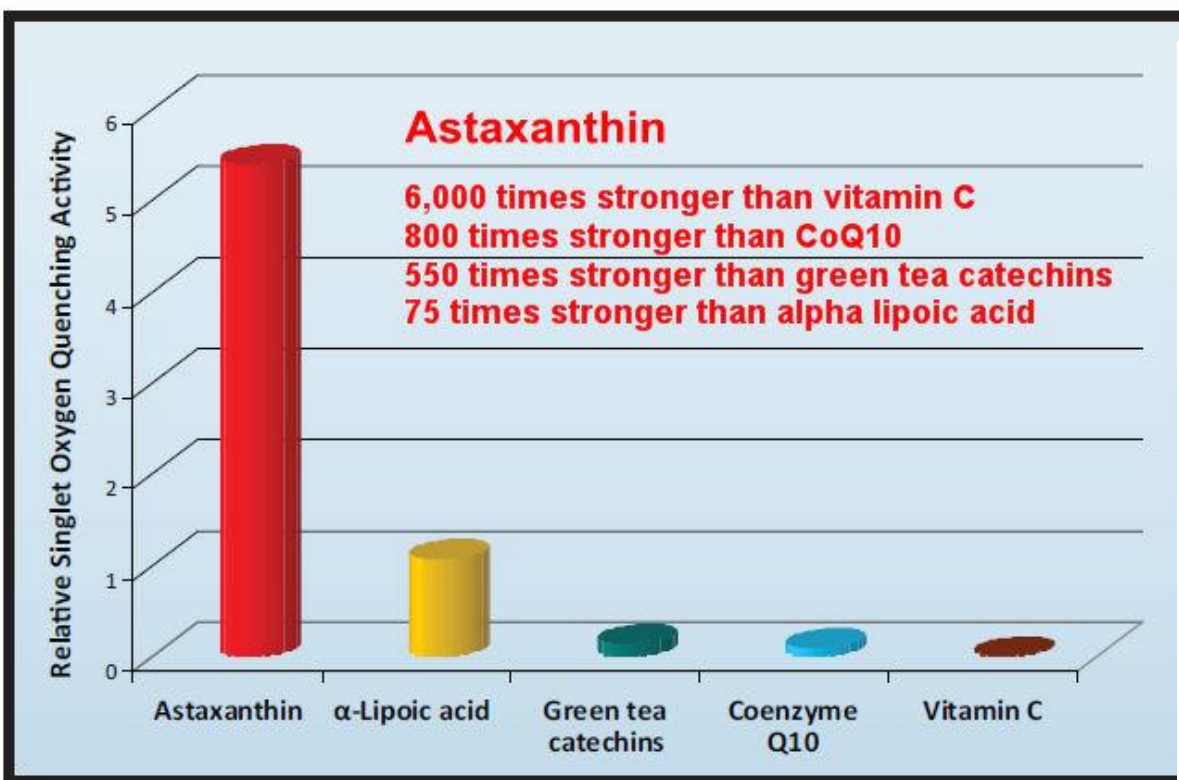
Quando la luce è in eccesso la Fotosintesi è saturata:
formazione di ROS



Astaxanthin



The ketocarotenoid astaxanthin has a great commercial interest owing to its high price (approximately US\$ 2,500 kg). It is used as a food coloring agent, natural feed additive for the poultry industry and for aquaculture



Y. Nishida, E. Yamashita and W. Miki. 2007

SOLE Lab

Solar Energy Bio-exploitation Lab

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Revised from Nishida Y, et al., (2007). Carot. Sci. 11: 16-20

Comparing Astaxanthin In Different Sources



Salmon
5 ppm



Krill
120 ppm



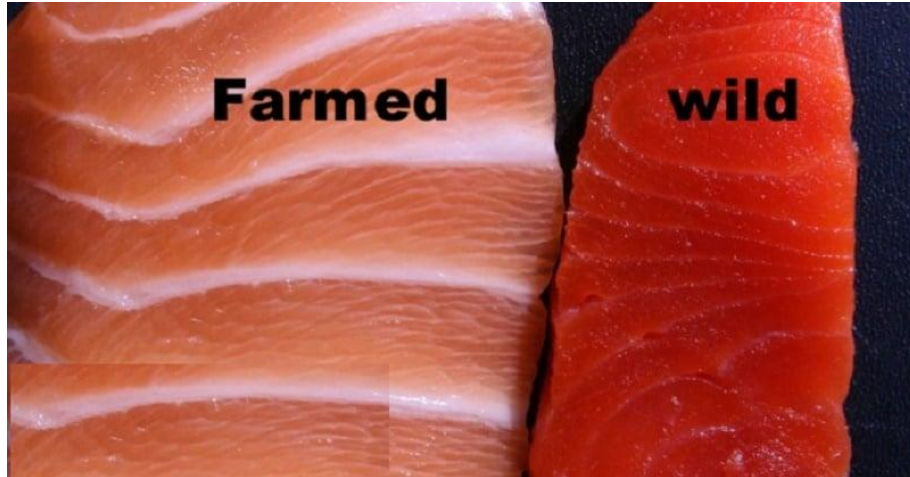
Artic Shrimp
(*Pandalus Borealis*)
1200 ppm



Green Algae
(*Haematococcus pluvialis*)
40000 ppm

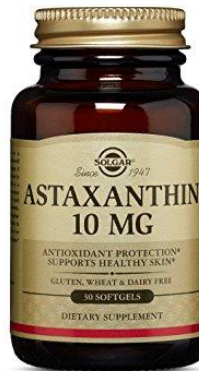


*Astaxanthin Concentration (ppm)

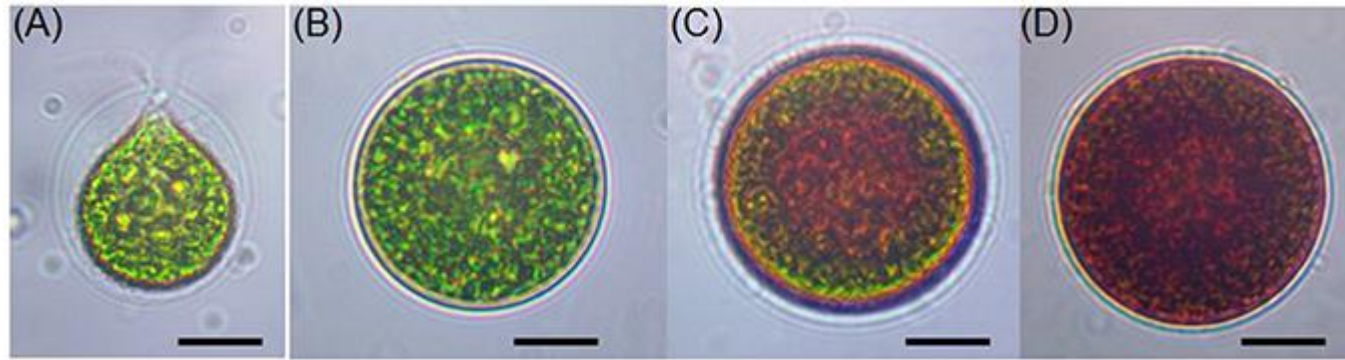


Farmed

wild



Haematococcus pluvialis



Alga verde in grado di accumulare elevati livelli di astaxantina in condizioni di stress fino al **5% del suo peso secco**

Fattori in grado di indurre la sintesi di astaxantina:

- Alta luce
- Carenza di nutrienti
- Alta/bassa temperatura
- Stress osmotico



Produzione di astaxantina tramite coltivazione in due fasi

Green Stage

- Indoor cultivation with a single colony of Haematococcus followed by outdoor cultivation in PBRs.
- For the maximal production of biomass

Red Stage

- Cells subjected to stress conditions (heavy doses of irradiation and changes in growth media)
- Cells form thick walled cysts
- Synthesize and accumulate astaxanthin in the esterified form.

Product Recovery

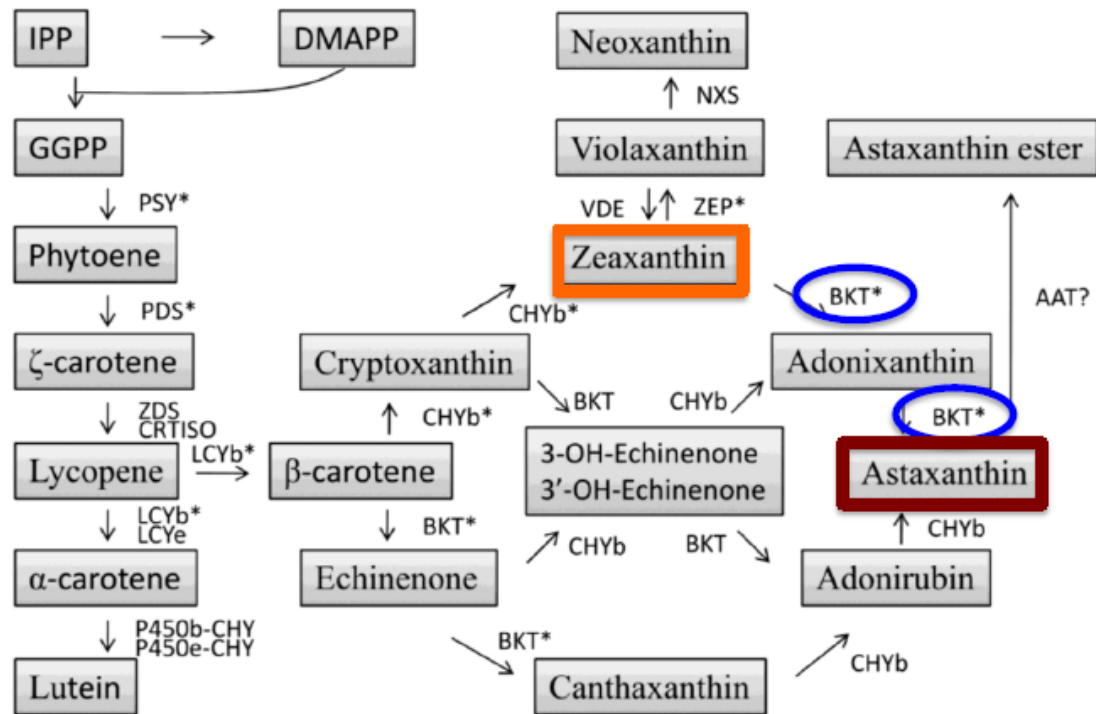
- Cell disruption and biomass extraction (Organic solvent mediated extraction)
- Purification



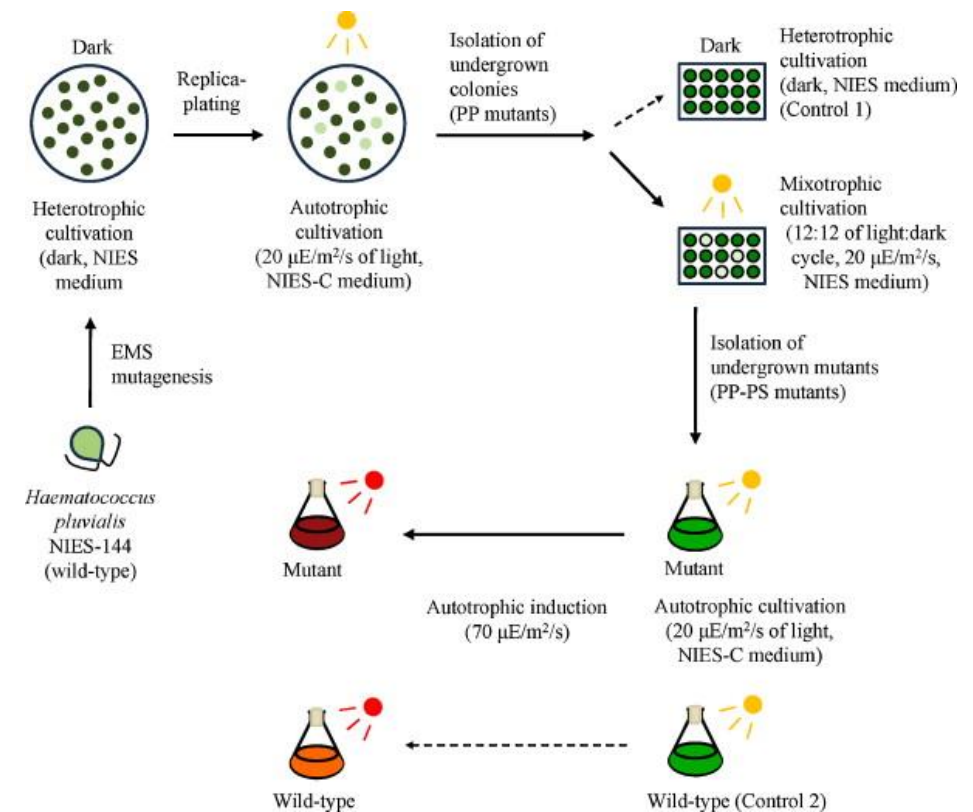
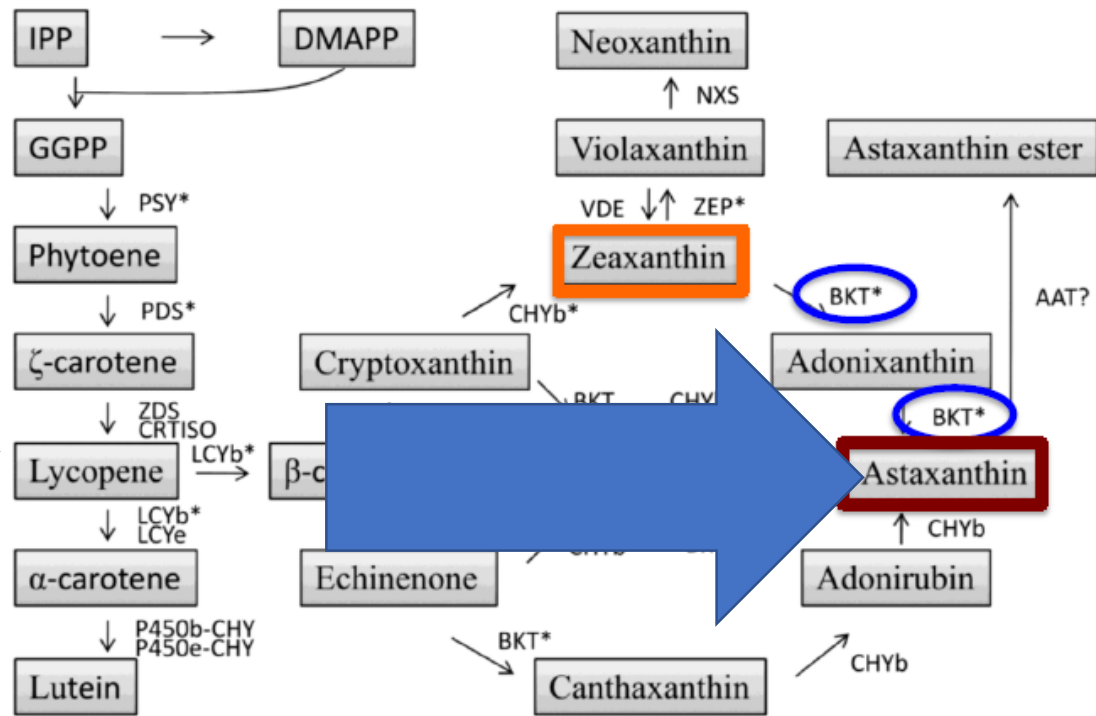
Limitazione per la produzione di astaxantina

- Bassa resa in biomassa di *H. pluvialis*
- Necessità di coltivazione in due fasi
- Parete cellulare molto resistente: bassa biodisponibilità

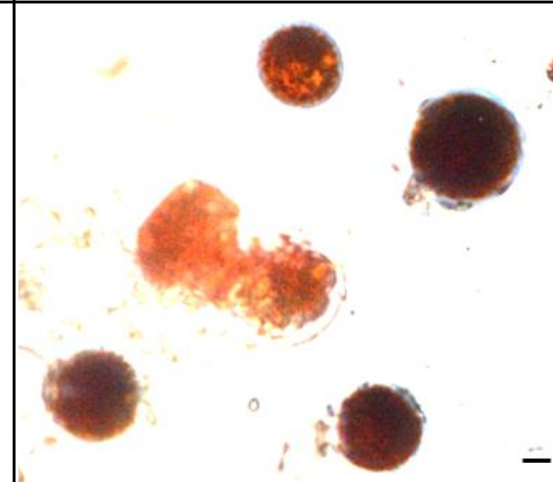
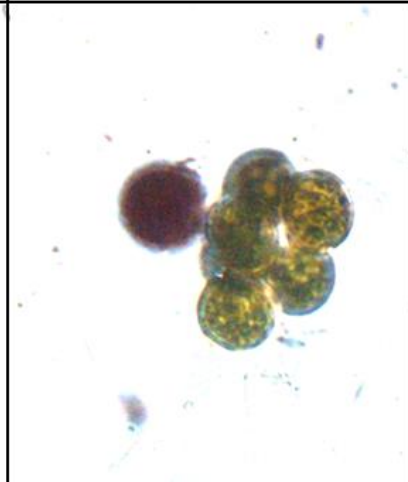
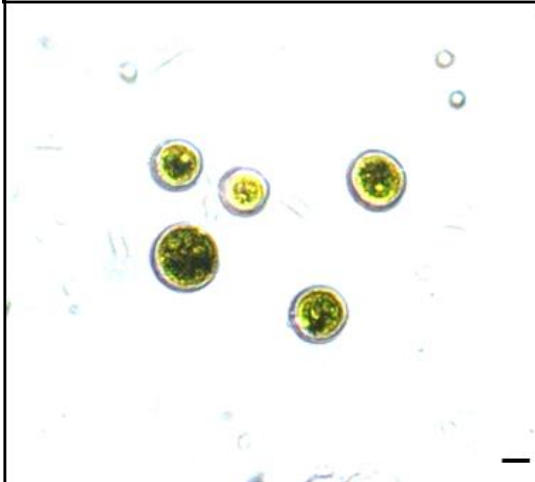
Soluzione biotecnologica #1: aumento sintesi carotenoidi



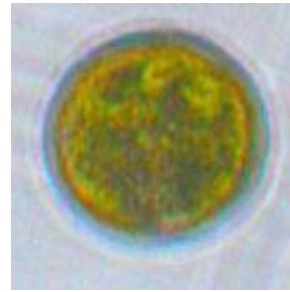
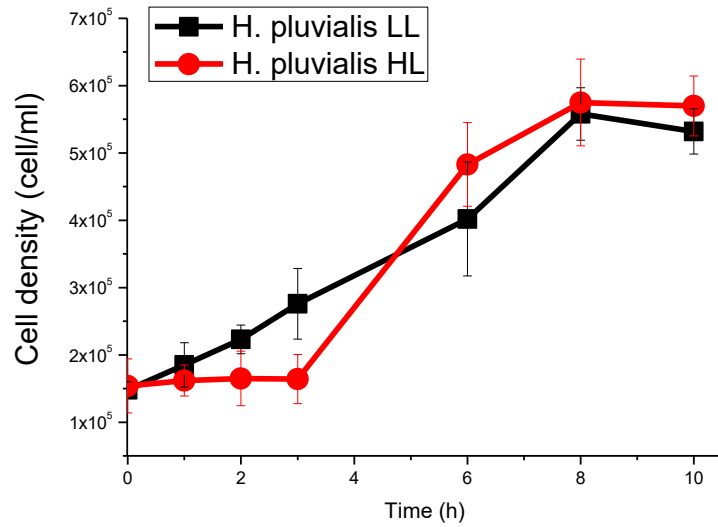
Soluzione biotecnologica #1: aumento sintesi carotenoidi



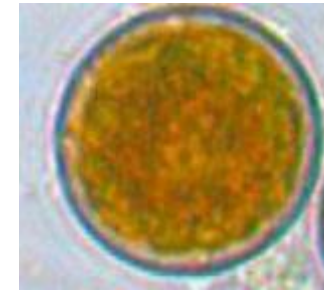
Soluzione biotecnologica #2 migliorare l'utilizzo della luce in *H. pluvialis*



H. pluvialis in condizioni di alta luce

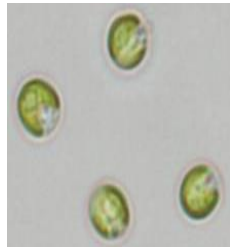
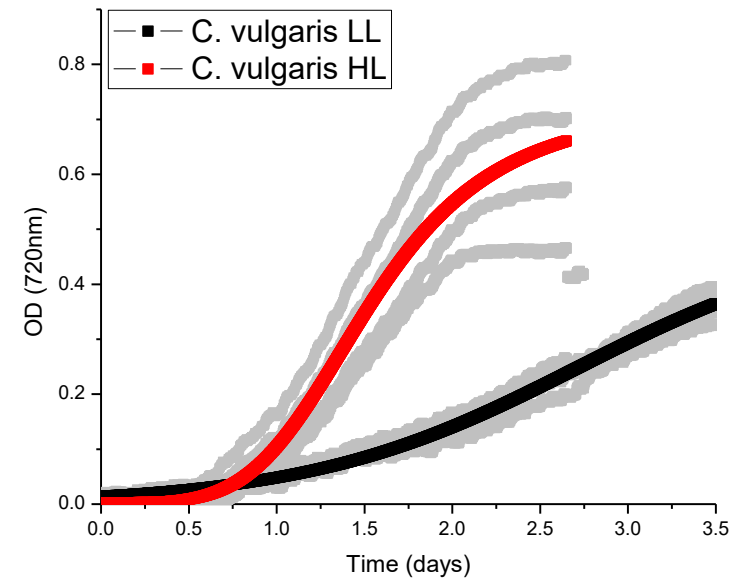
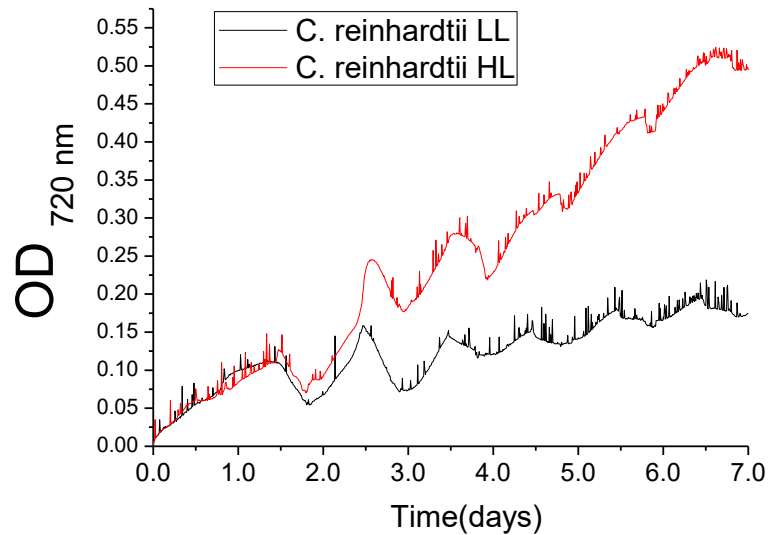


LL

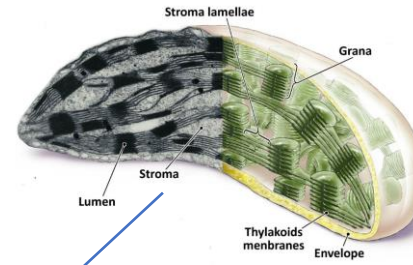
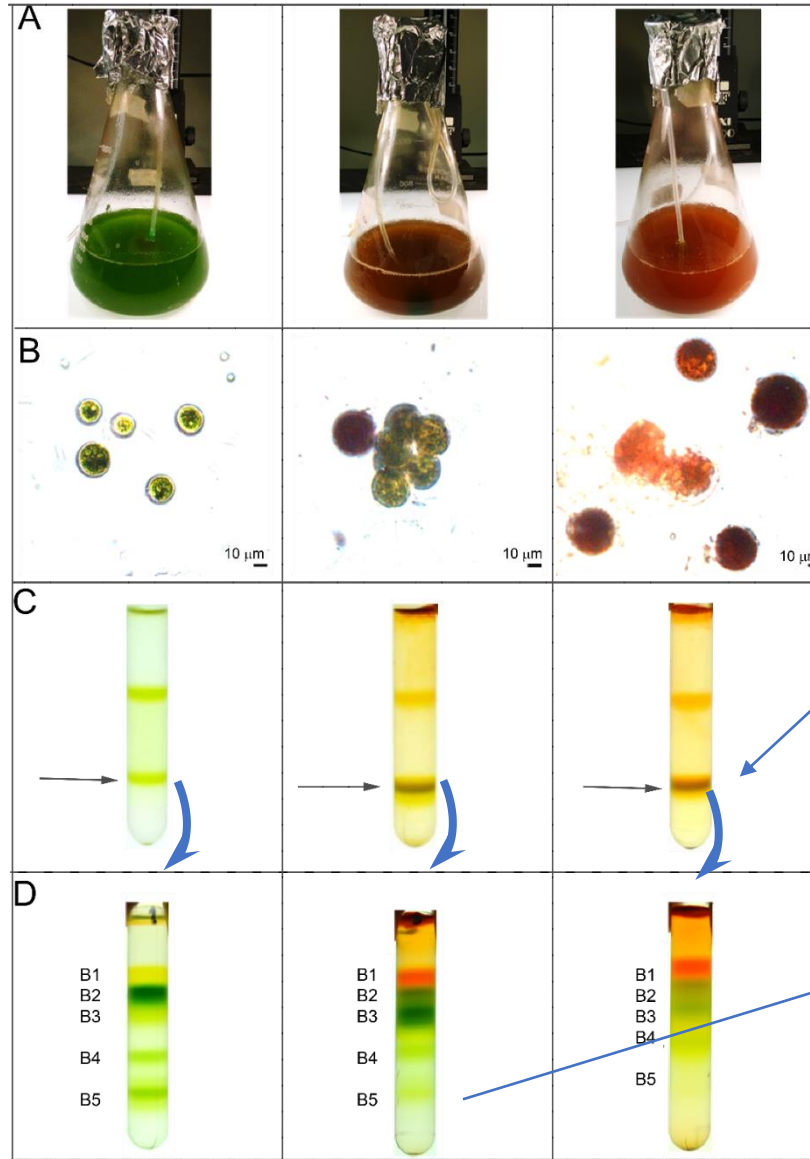


HL

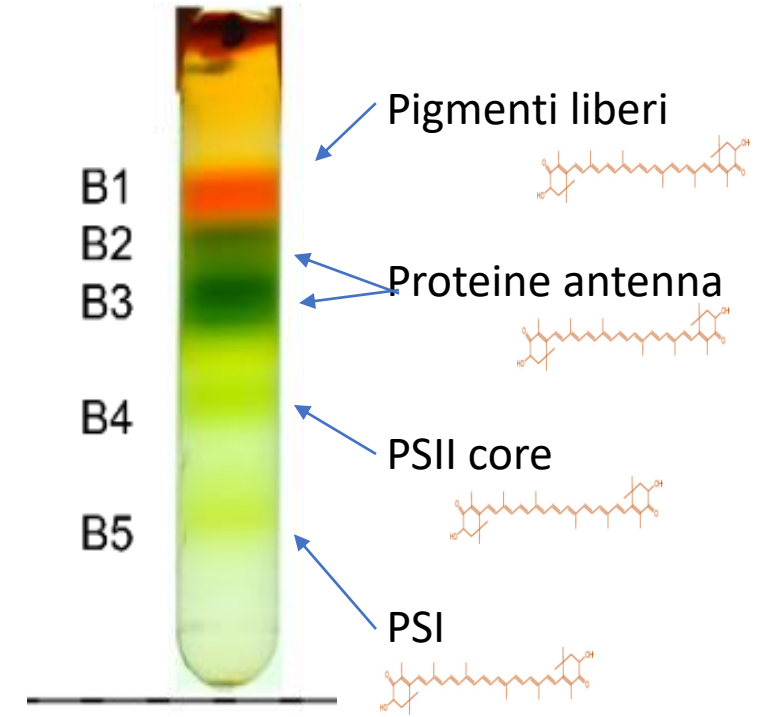
***Haematococcus pluvialis* non utilizza efficientemente la luce in condizione di HL (alta luce)**



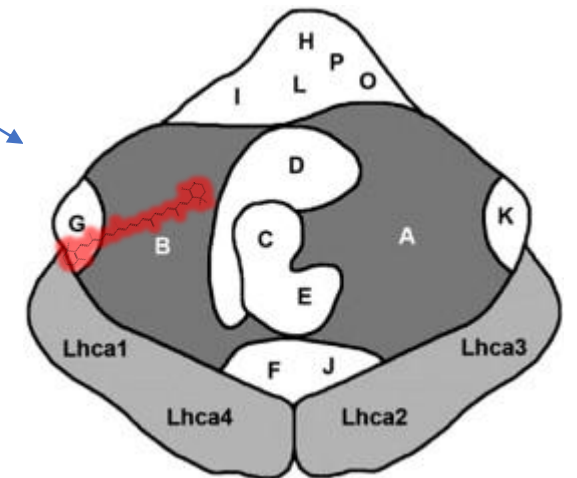
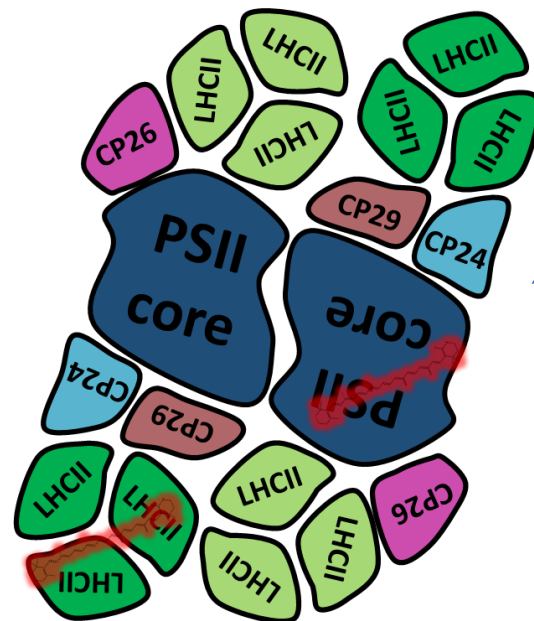
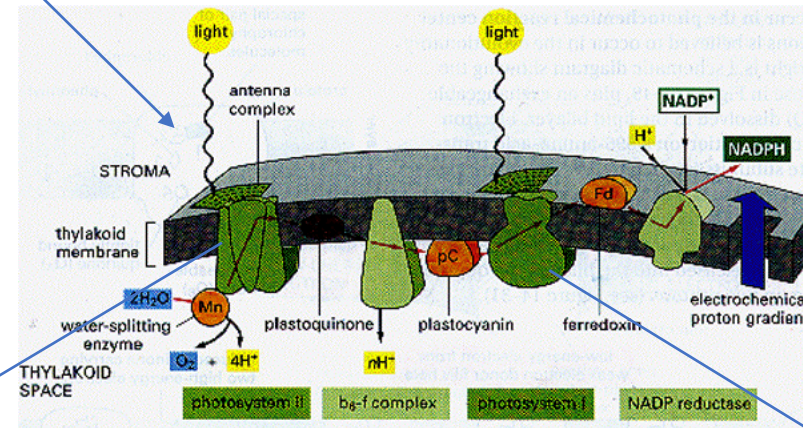
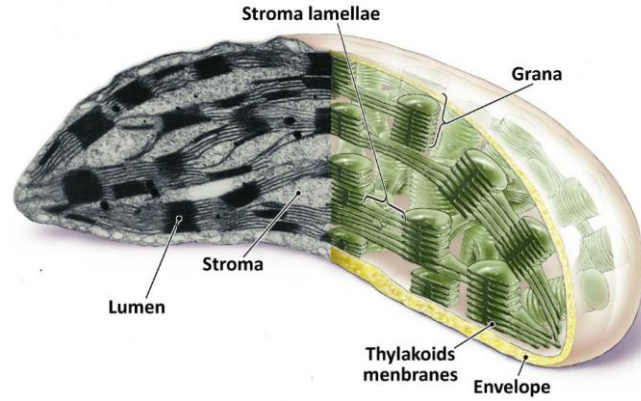
Astaxantina nei complessi fotosintetici



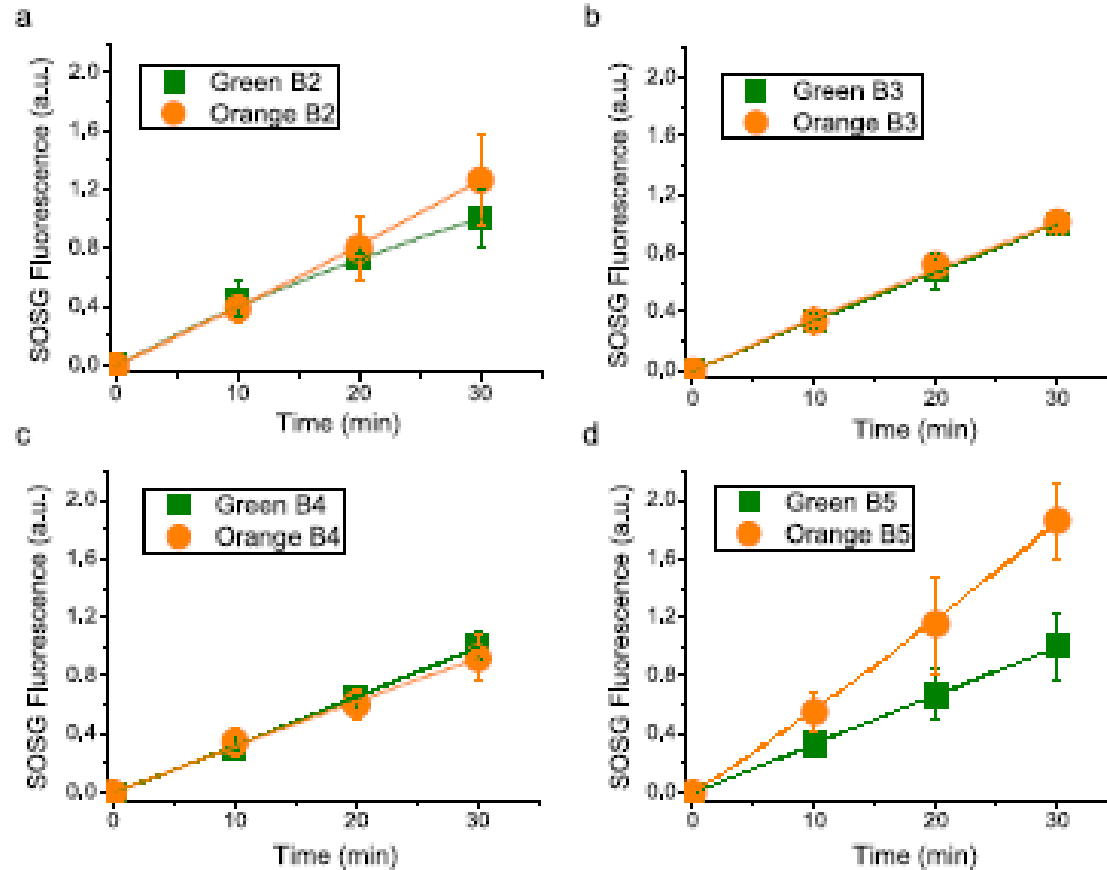
Membrane interne al cloroplasto



Astaxantina nei complessi fotosintetici

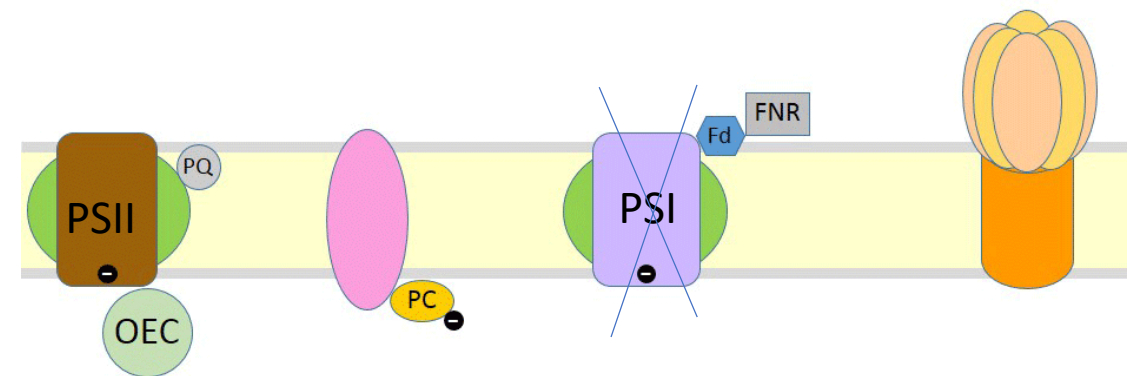


Astaxantina nei complessi fotosintetici

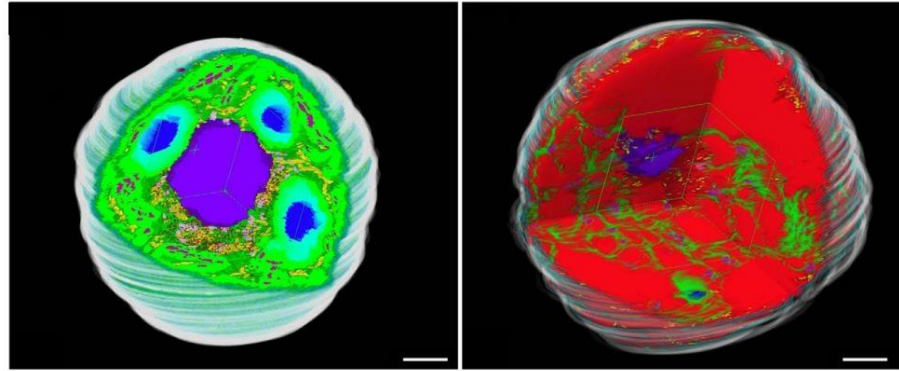


Il fotosistema I è parzialmente destabilizzato dalla presenza di astaxantina

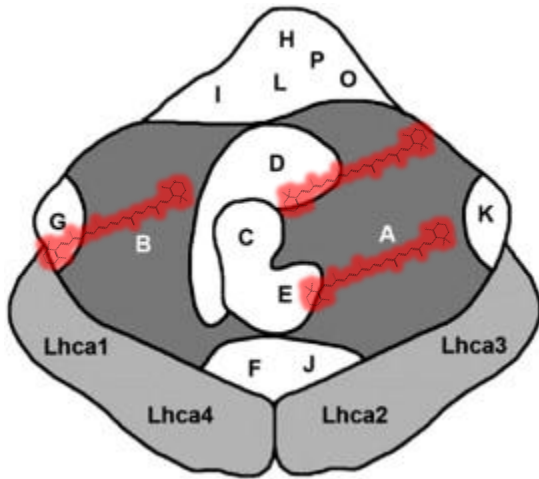
L'astaxantina nei complessi fotosintetici non ne aumenta la fotoprotezione



Astaxantina e fotoprotezione

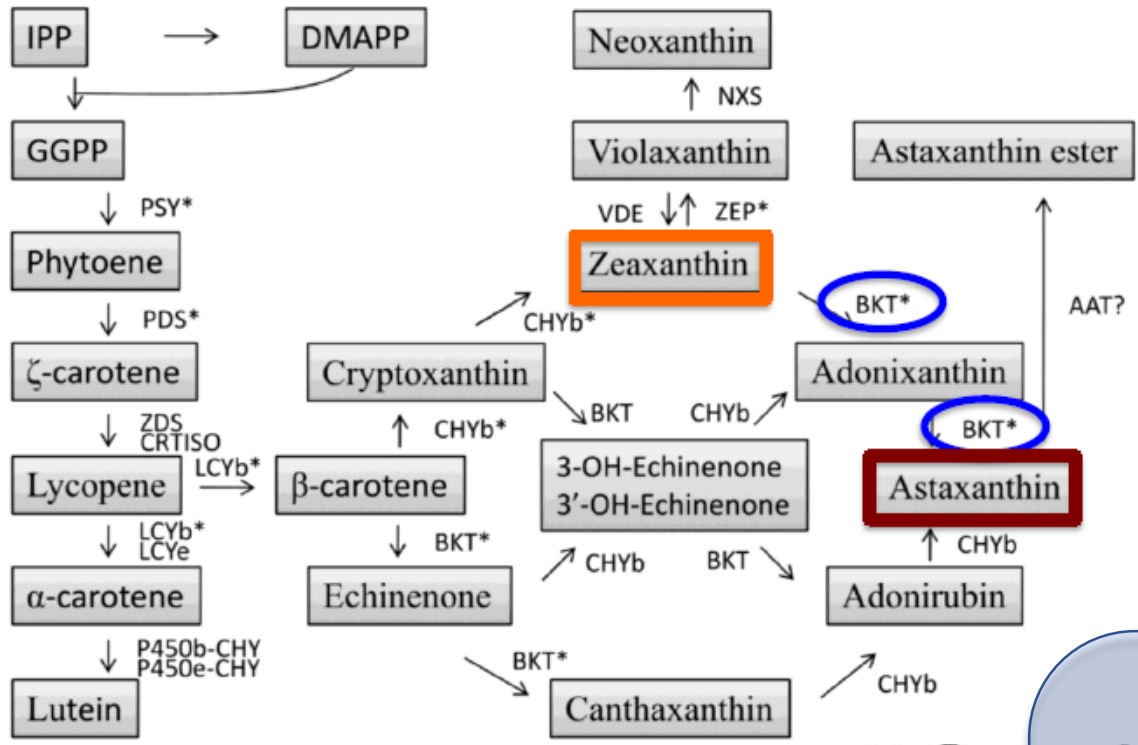


Astaxantina si accumula in condizioni di stress nel citoplasma per proteggere il nucleo da raggi UV

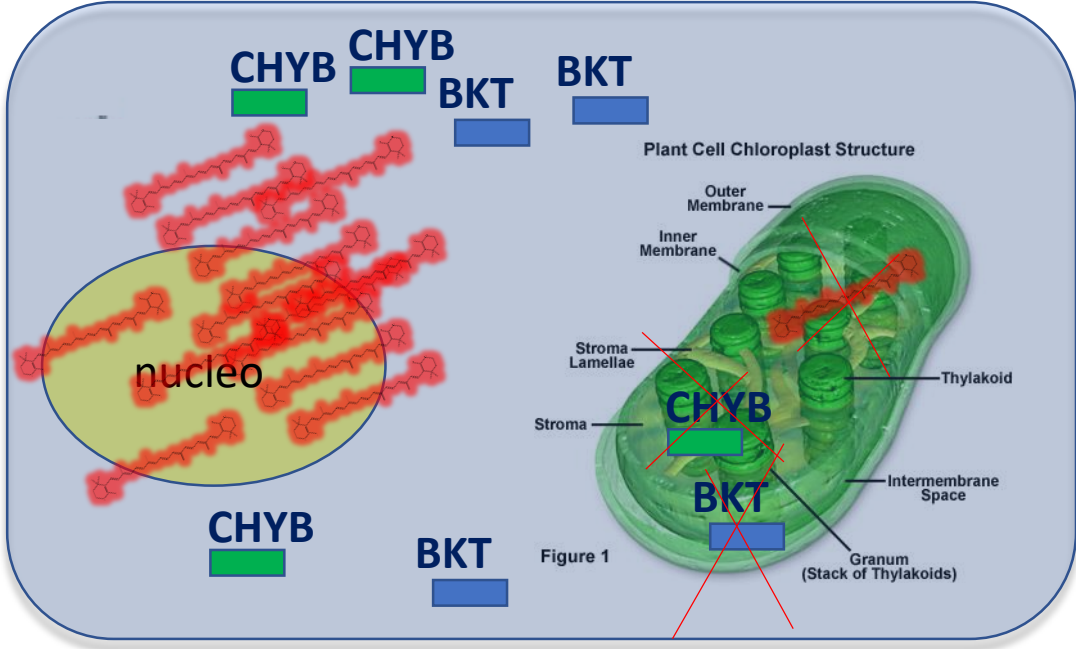


La cellula in condizioni di stress rallenta l'attività fotosintetica e una parte di astaxantina destabilizza i complessi fotosintetici: riduzione della produzione di biomassa

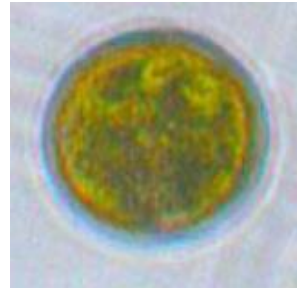
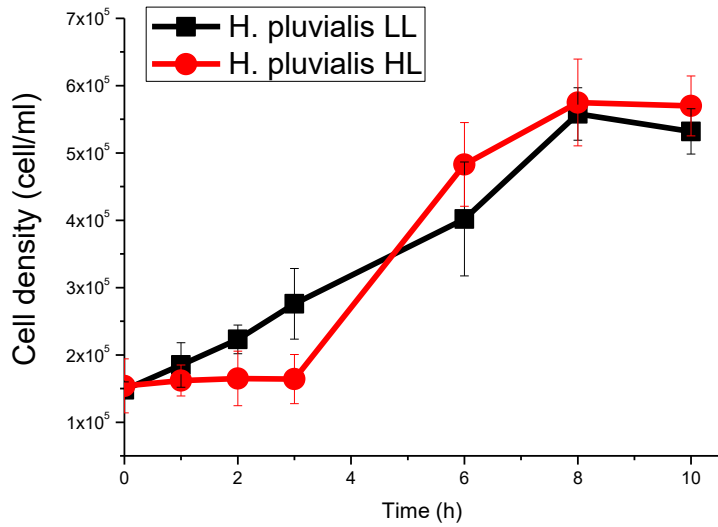
**POSSIBILE SOLUZIONE BIOTECNOLOGICA:
ELIMINARE LA SINTESI DI ASTAXANTINA NEL
CLOROPLASTO**



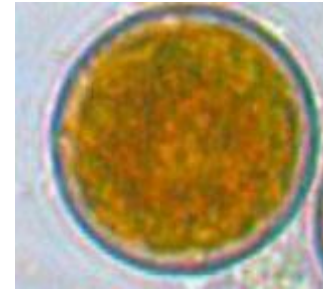
**POSSIBILE SOLUZIONE BIOTECNOLOGICA:
ELIMINARE LA SINTESI DI ASTAXANTINA NEL
CLOROPLASTO**



H. pluvialis in condizioni di alta luce

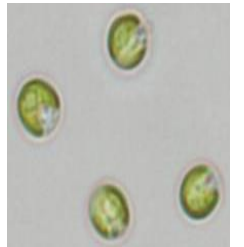
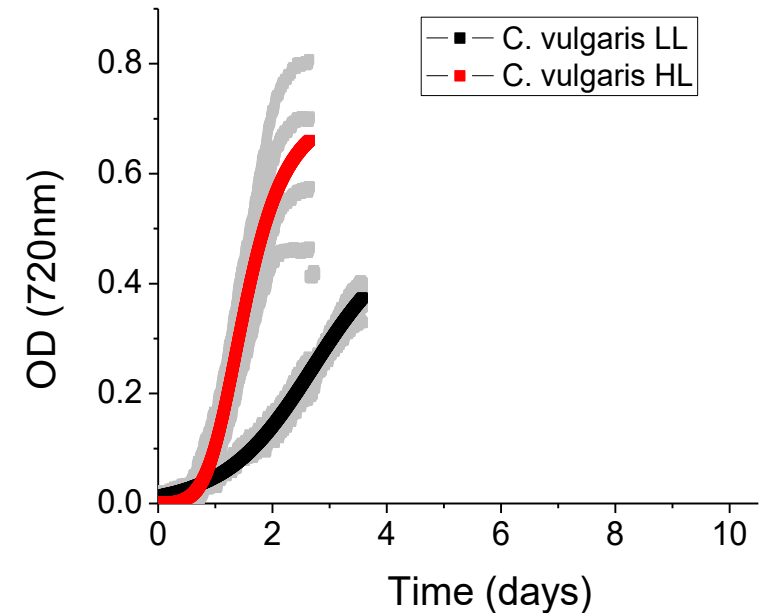
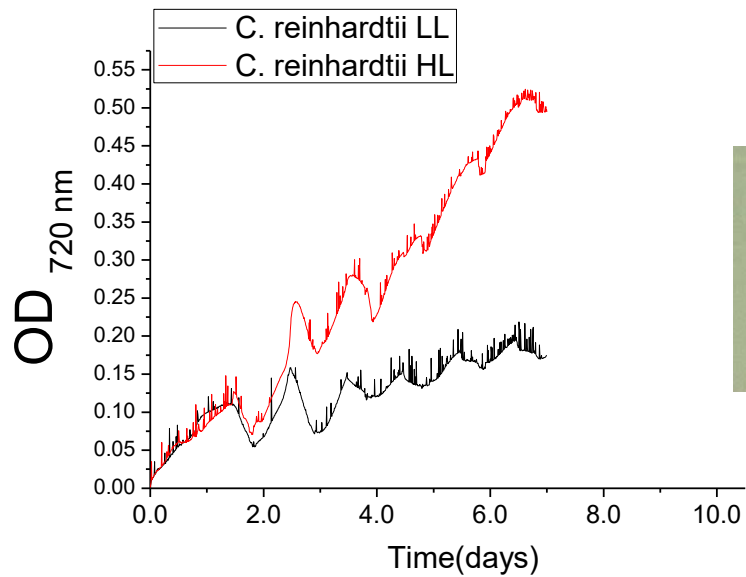


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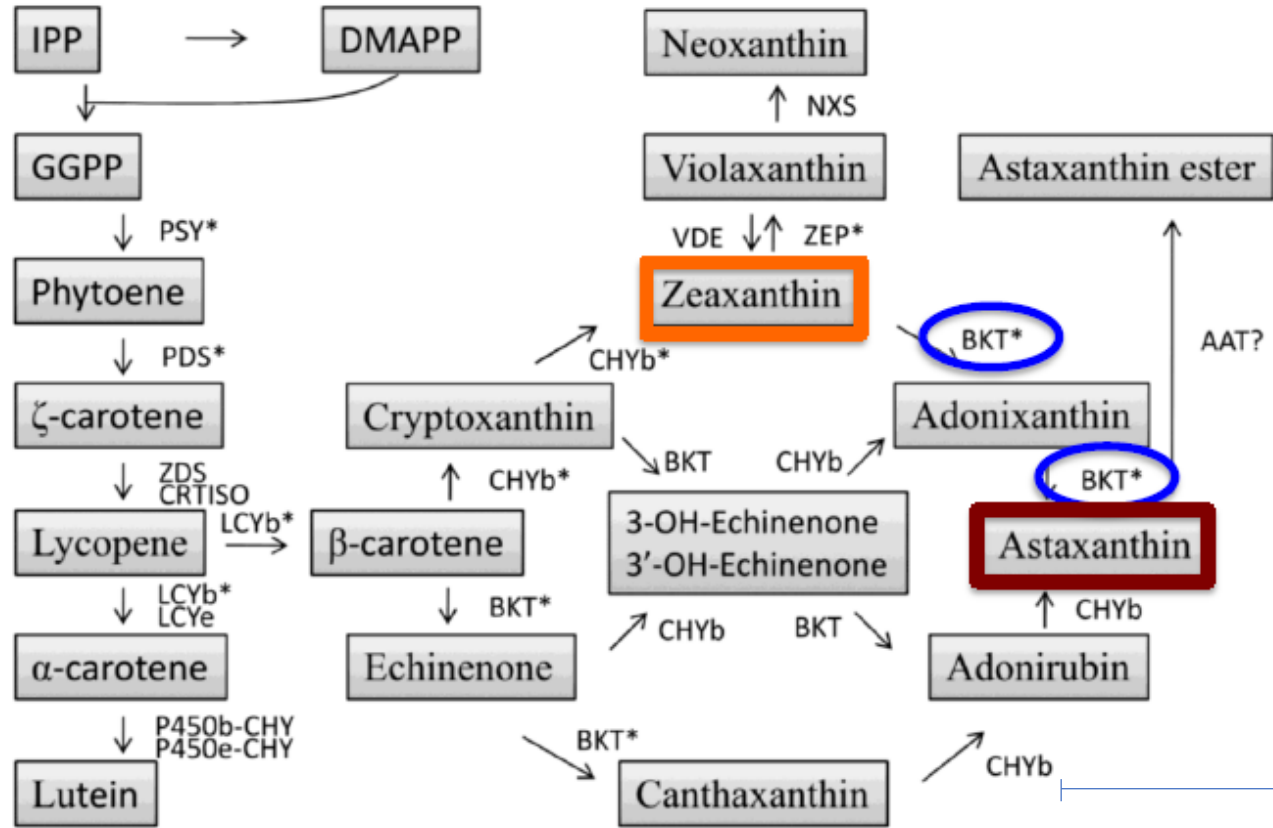


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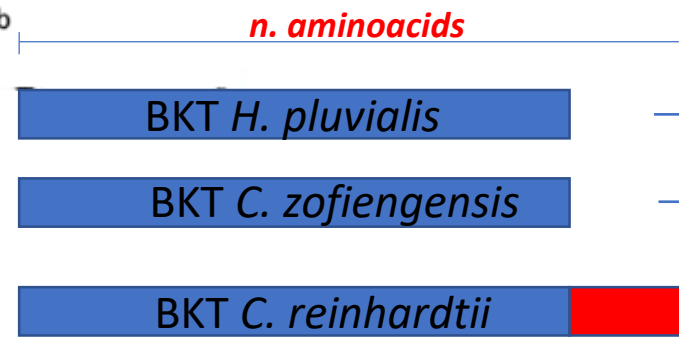
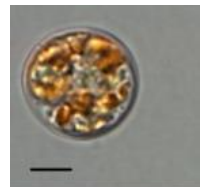
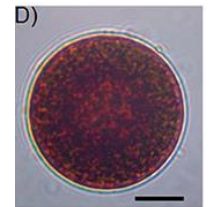
***Haematococcus pluvialis* cresce molto lentamente**



Produzione di astaxantina in altre microalghe

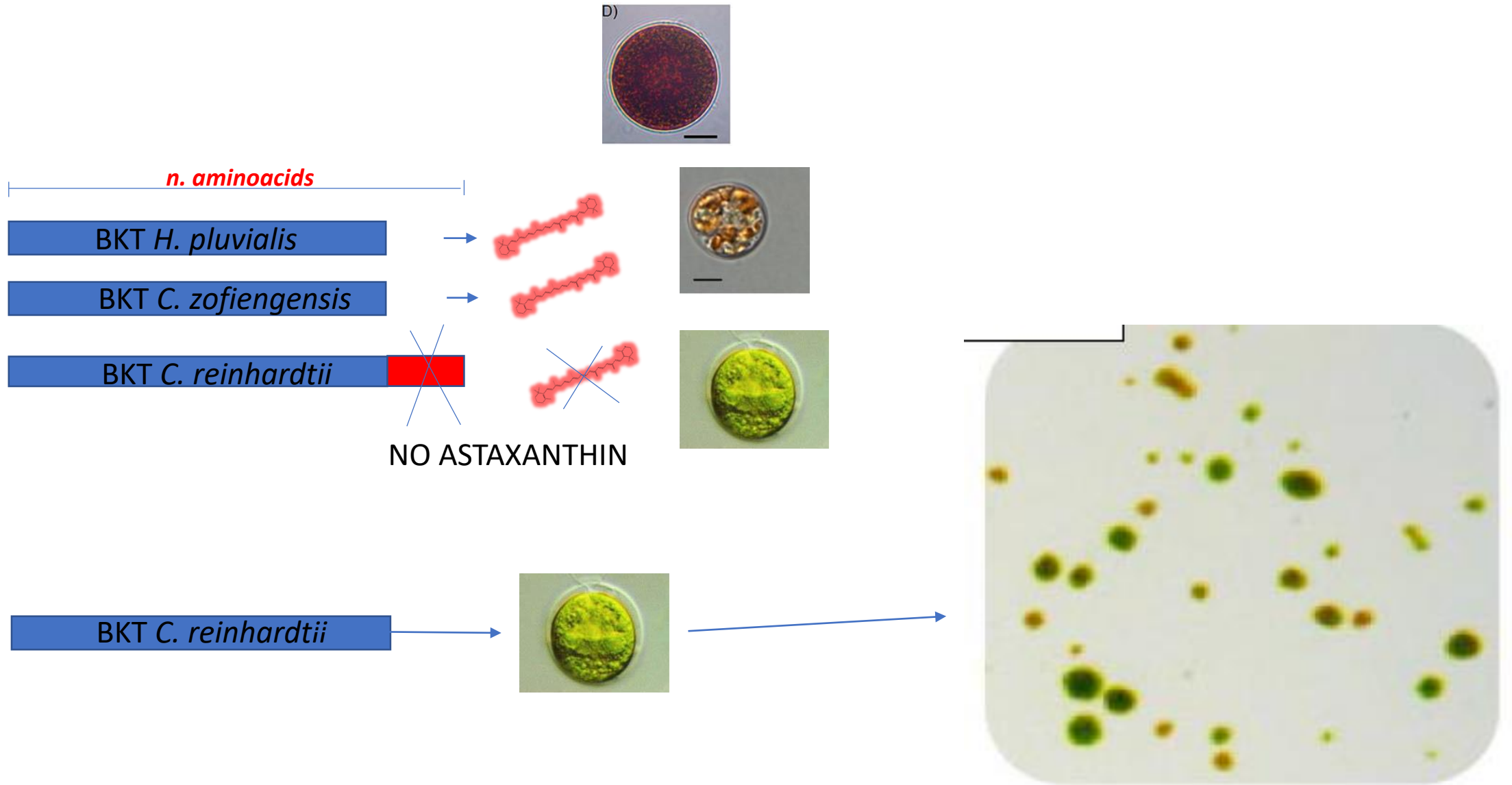


L'enzima chiave per la produzione di astaxantina (BKT) è presente anche in *Chlamydomonas* ma non è attivo

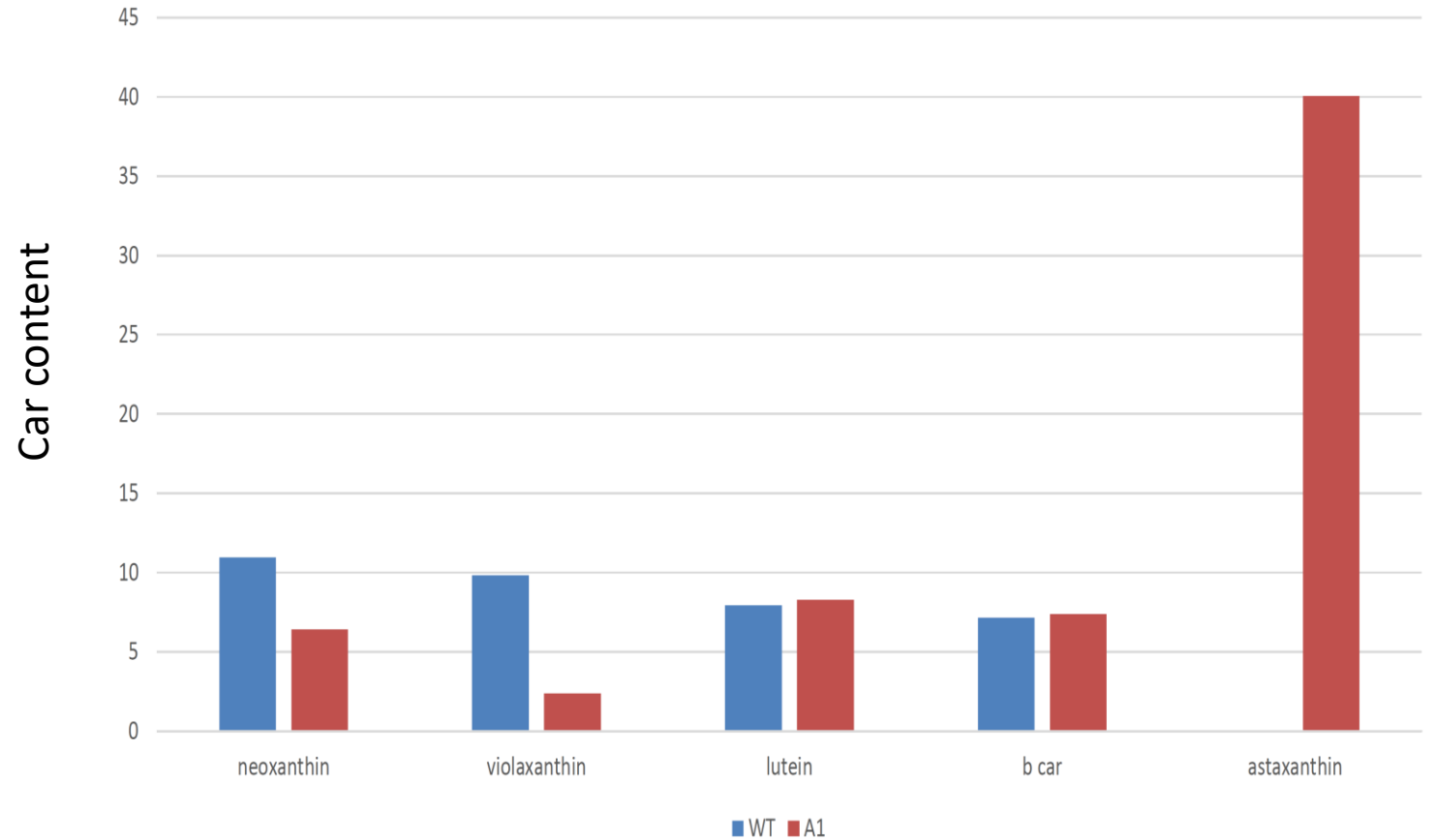
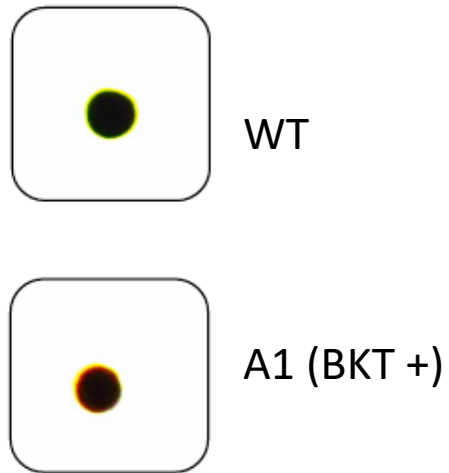


NO ASTAXANTHIN

Astaxantina in Chlamydomonas reinhardtii

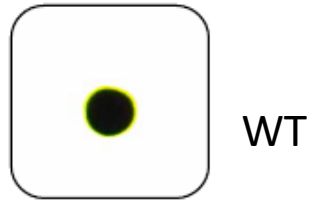


Astaxantina in Chlamydomonas reinhardtii



Più del 70% dei carotenoidi di *C. reinhardtii* sono astaxantina in presenza di BKT troncata

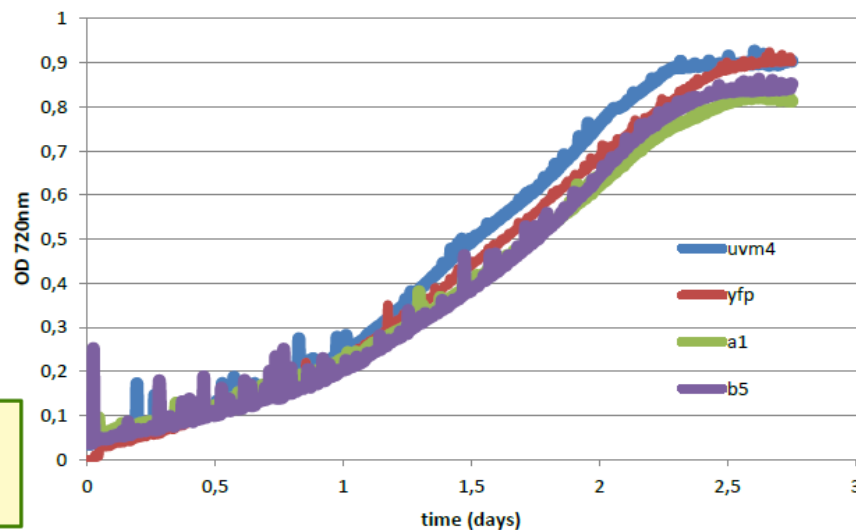
Astaxantina in Chlamydomonas reinhardtii



WT

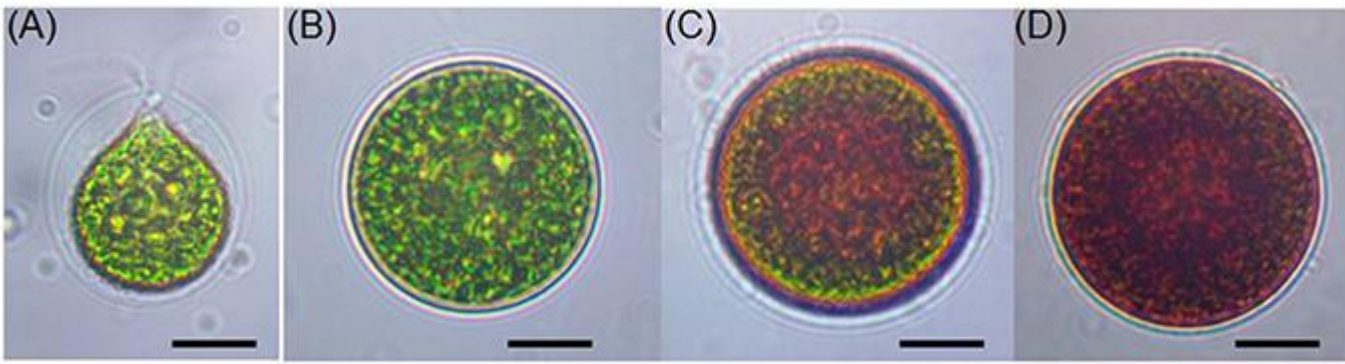


A1 (BKT +)



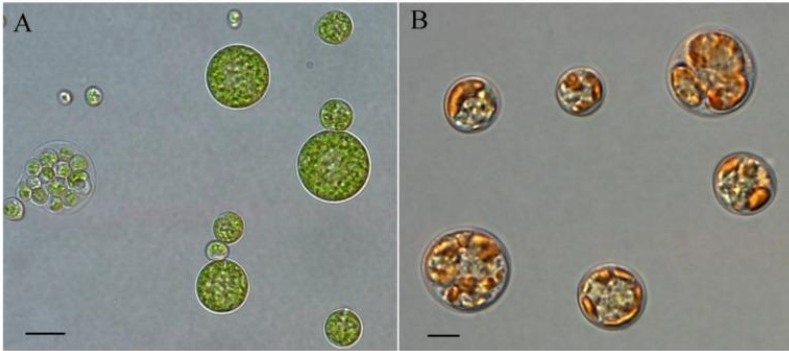
L'accumulo di astaxantina non ha solo un piccolo effetto sulle cinetiche di crescita

Crescita in condizioni non stressanti: 0.4% astaxantina



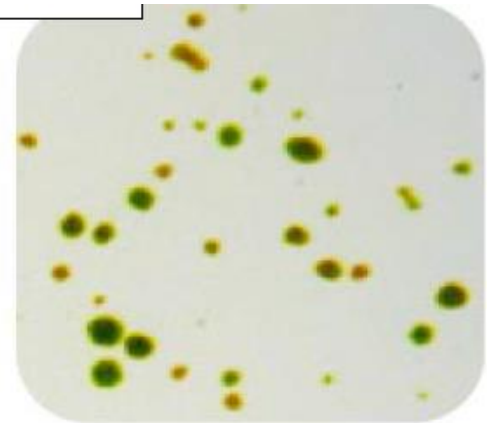
H. pluvialis

- Bassa produttività (0.1-0.2 gr/L/day)
- Alto contenuto in astaxanthin (5%)
- STRESS per sintesi Astaxantina
- PARETE CELLULARE RESISTENTE



C. zofingensis

- Alta produttività (1.2 gr/L/day)
- Contenuto di astaxantina 0.15-0.7%
- STRESS per sintesi Astaxantina
- PARETE CELLULARE RESISTENTE



C. reinhardtii

- Produttività media (0,3-0,6 gr/L/day)
- Contenuto di astaxantina 0.4%
- NO STRESS
- PARETE CELLULARE DEBOLE

**MAGGIORE
BIODISPONIBILITA' DI
ASTAXANTINA NEL
PRODOTTO FINALE,
ESTRAZIONE PIU'
SEMPLICE**





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