



UNIVERSITÀ  
di VERONA

SOLE Lab

Solar Energy Bio-exploitation Lab



# Ingegnerizzazione biotecnologica della via biosintetica dei carotenoidi per indurre la produzione di astaxantina in *Chlamydomonas reinhardtii*.

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University of Verona  
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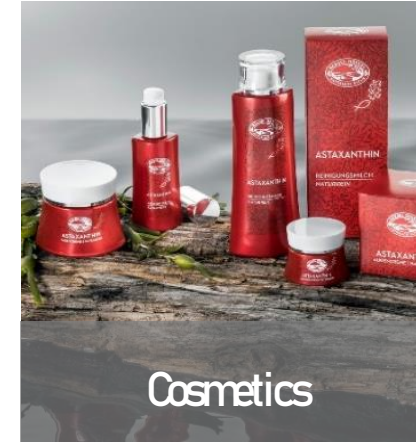
Pordenone 20/02/2020



Fish Feed



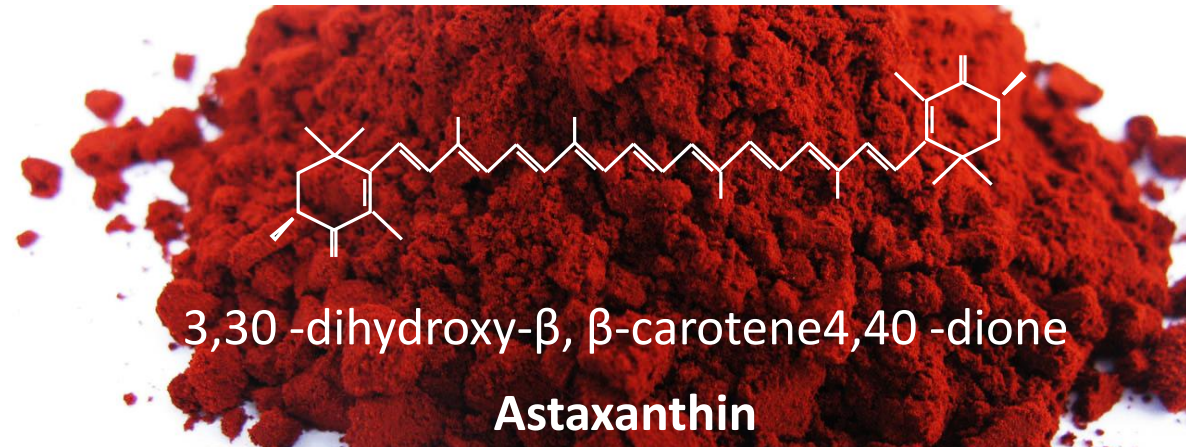
Food colouring agent



Cosmetics



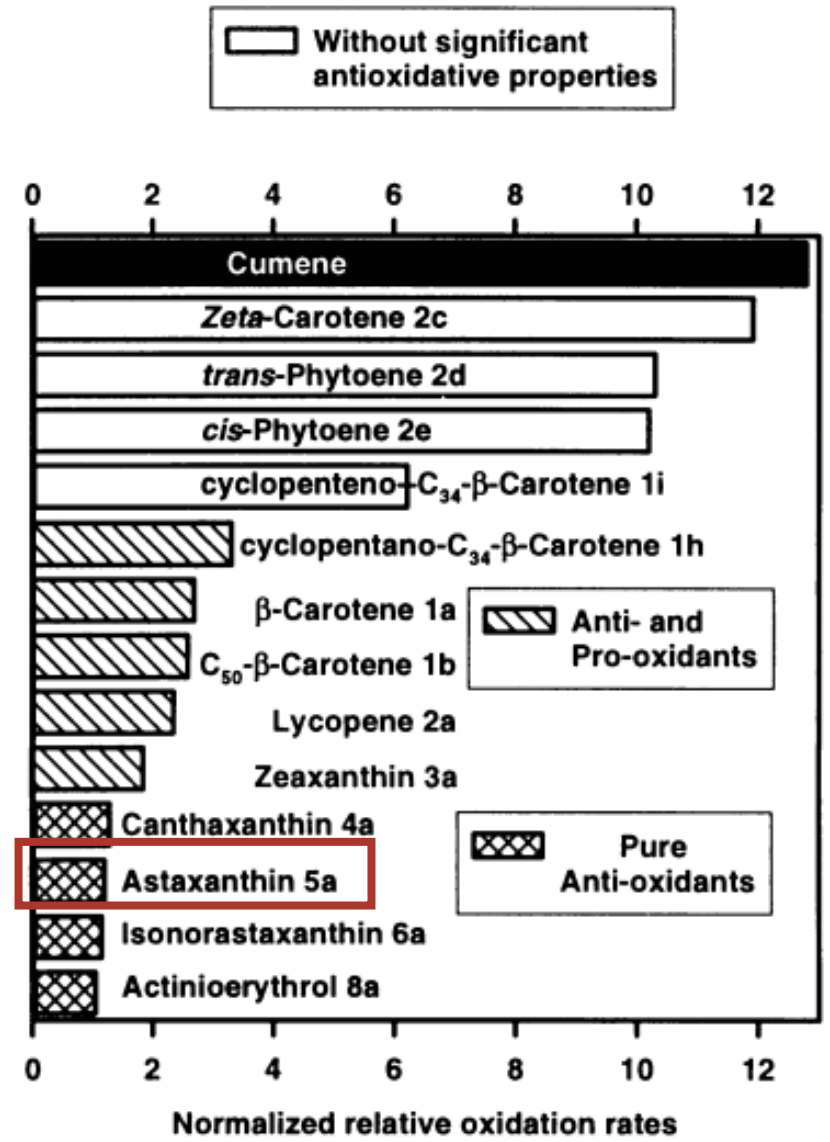
Poultry Feed





Nshada et al. 2007

Peroxide formation of pure methyl linoleate and in the presence of antioxidants



Martin et al. 1999

# ASTAXANTHIN

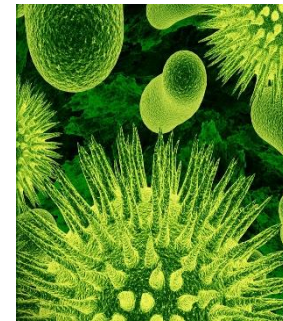
Astaxanthin is employed as healthy food and many clinical studies reveal its possible role in human disease treatment



Eye Health



Cardiovascular support



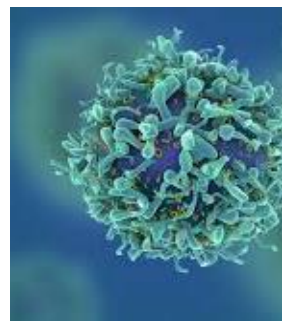
Immune system modulation



Brain Health



Male Fertility



Anti-Aging & Cellular health



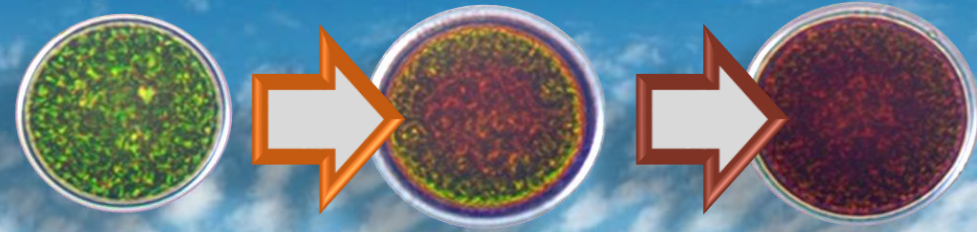
Joint, Tendon and muscle support



MAIN SOURCES OF NATURAL  
ASTAXANTHINIS *H. pluvialis*  
(now *H. lacustris*)

IT HAS THE CAPACITY TO  
ACCUMULATE 4-5% DRY WEIGHT  
UPON STRESS CONDITIONS

SEVERAL CONSTRAINTS IN  
CULTIVATION AND EXTRACTION



  
VERY SLOW  
GROWTH RATE

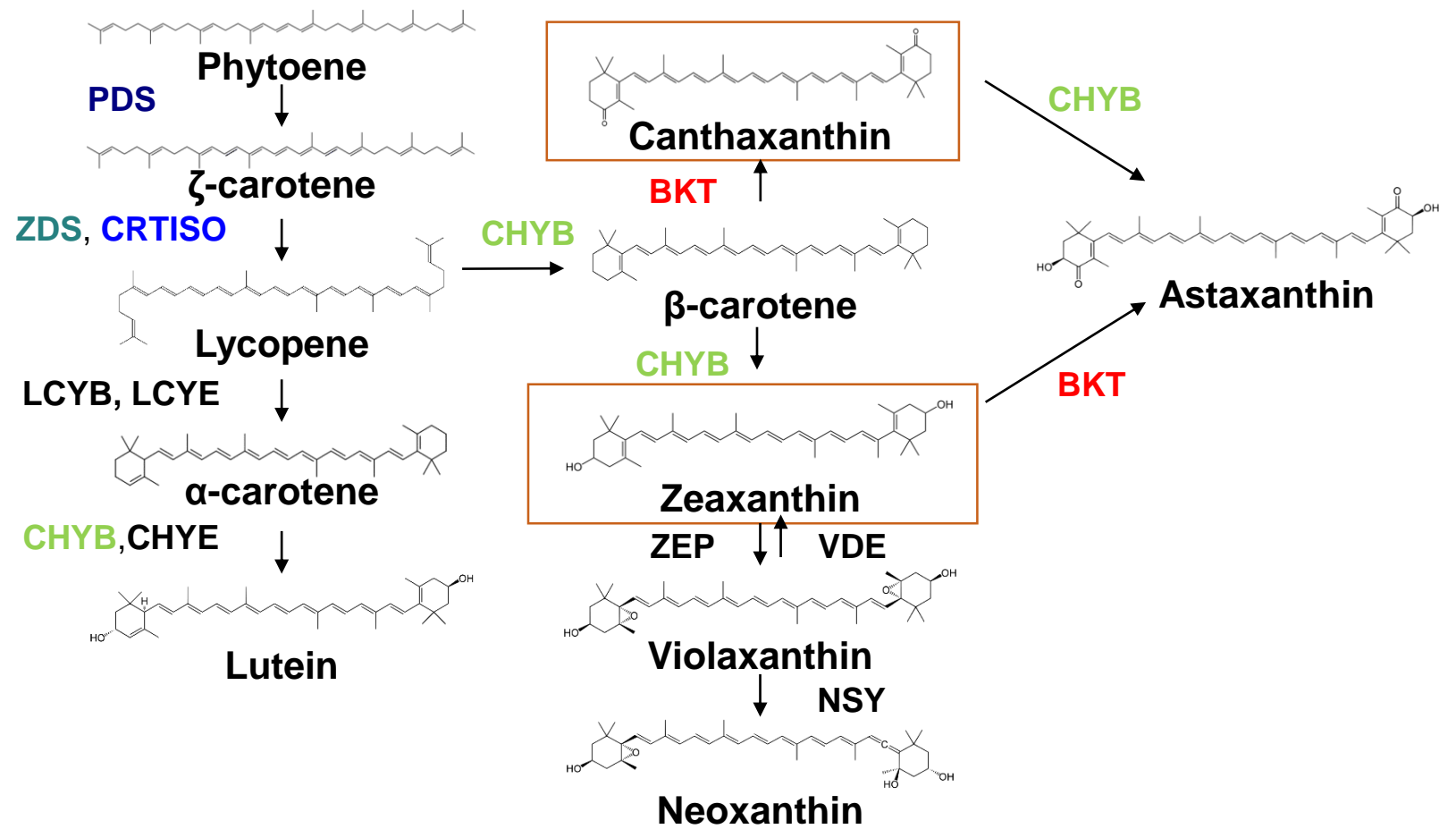
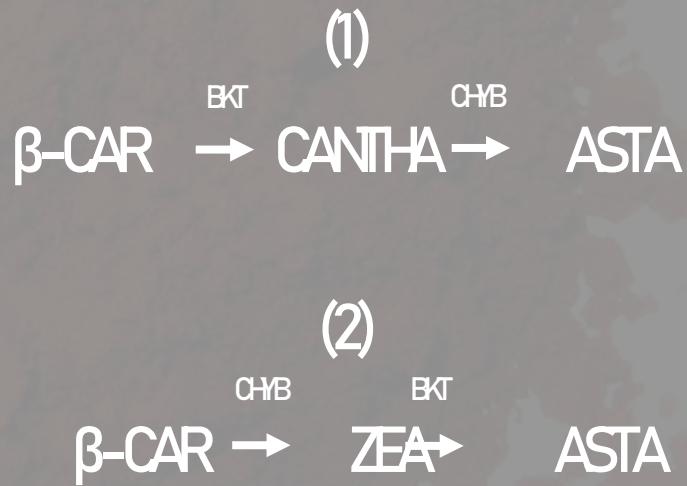


DIFFICULT TO  
CULTIVATE

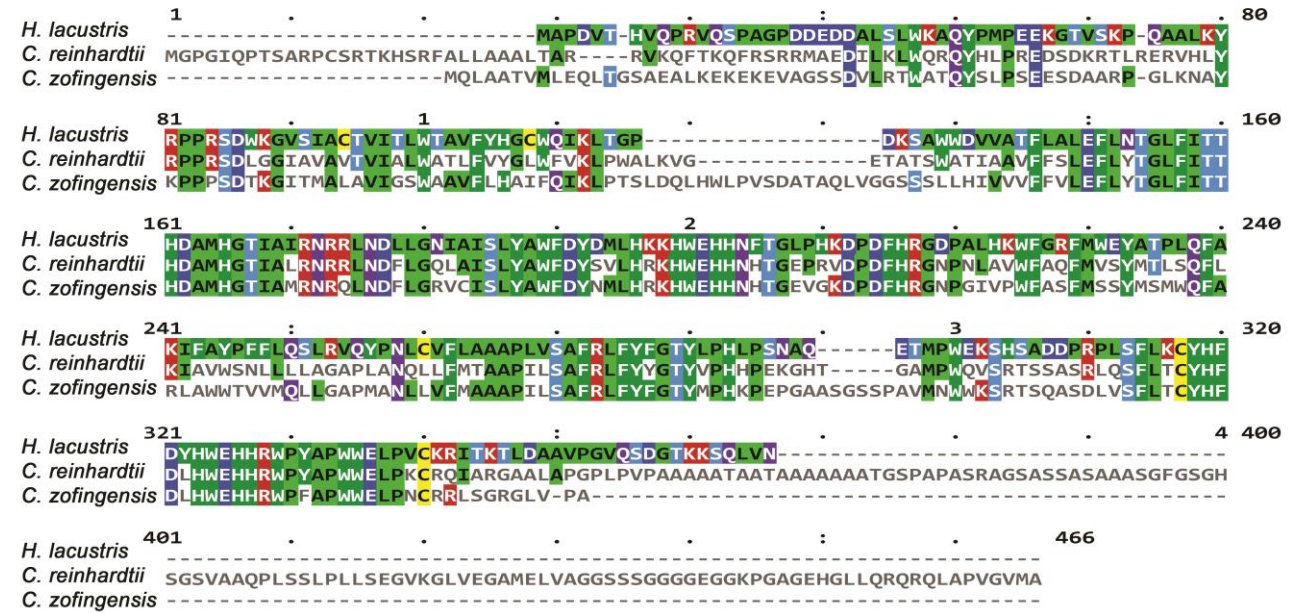


EXTRACTION  
VERY EXPENSIVE

*H. pluvialis* HAS TWO DIFFERENT PATHWAY FOR ASTAXANTHIN BIOSYNTHESIS



Present: BKT sequence is present in *C. reinhardtii*



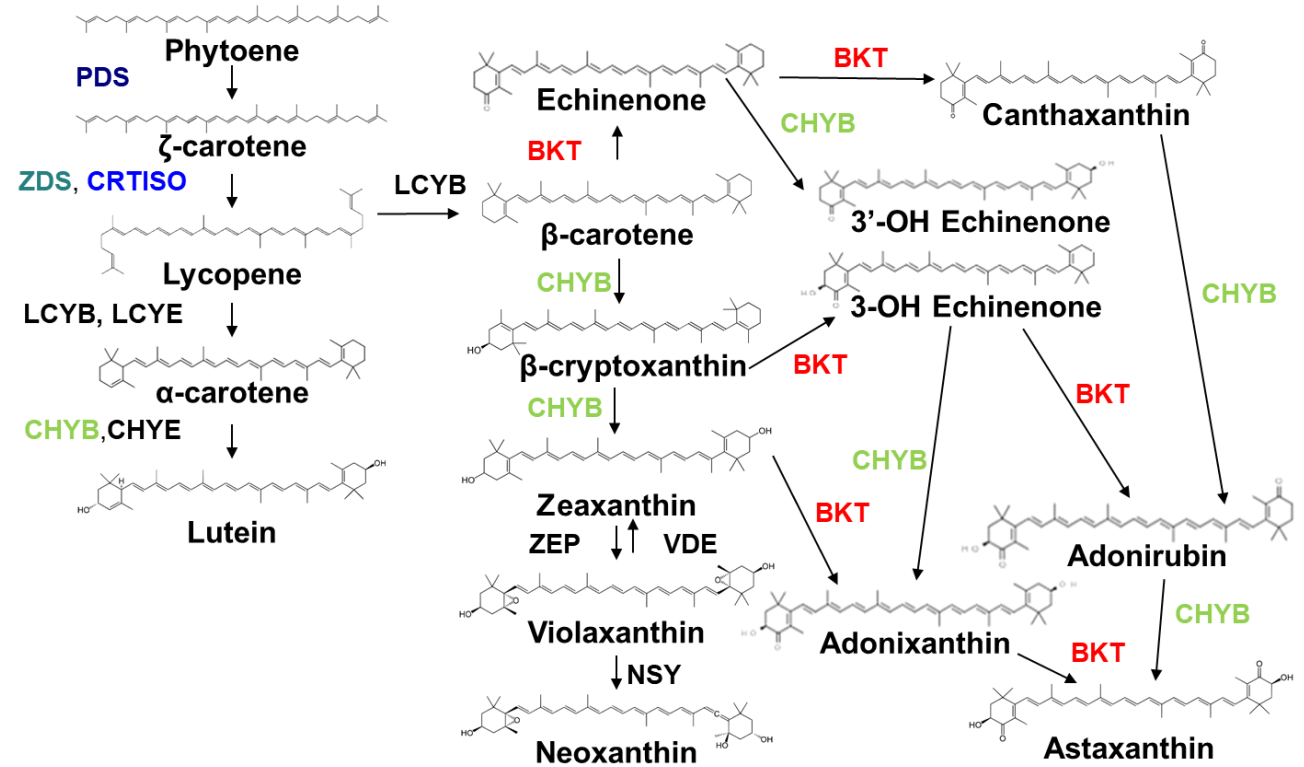
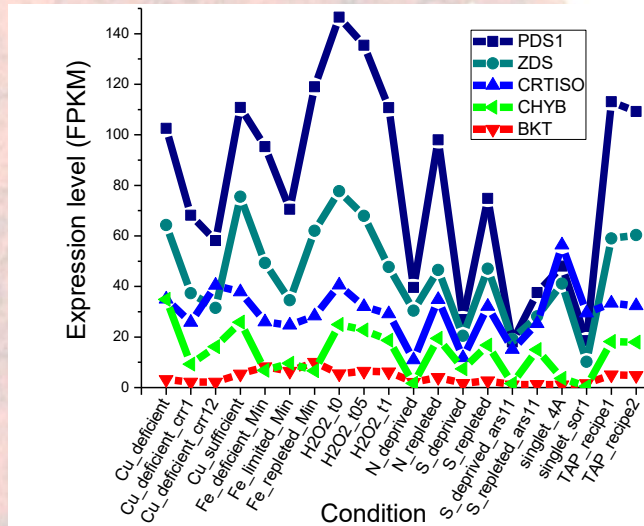




Present: BKT sequence is present in *C. reinhardtii*

Longer C-term 115aa extension absent in any ketolase

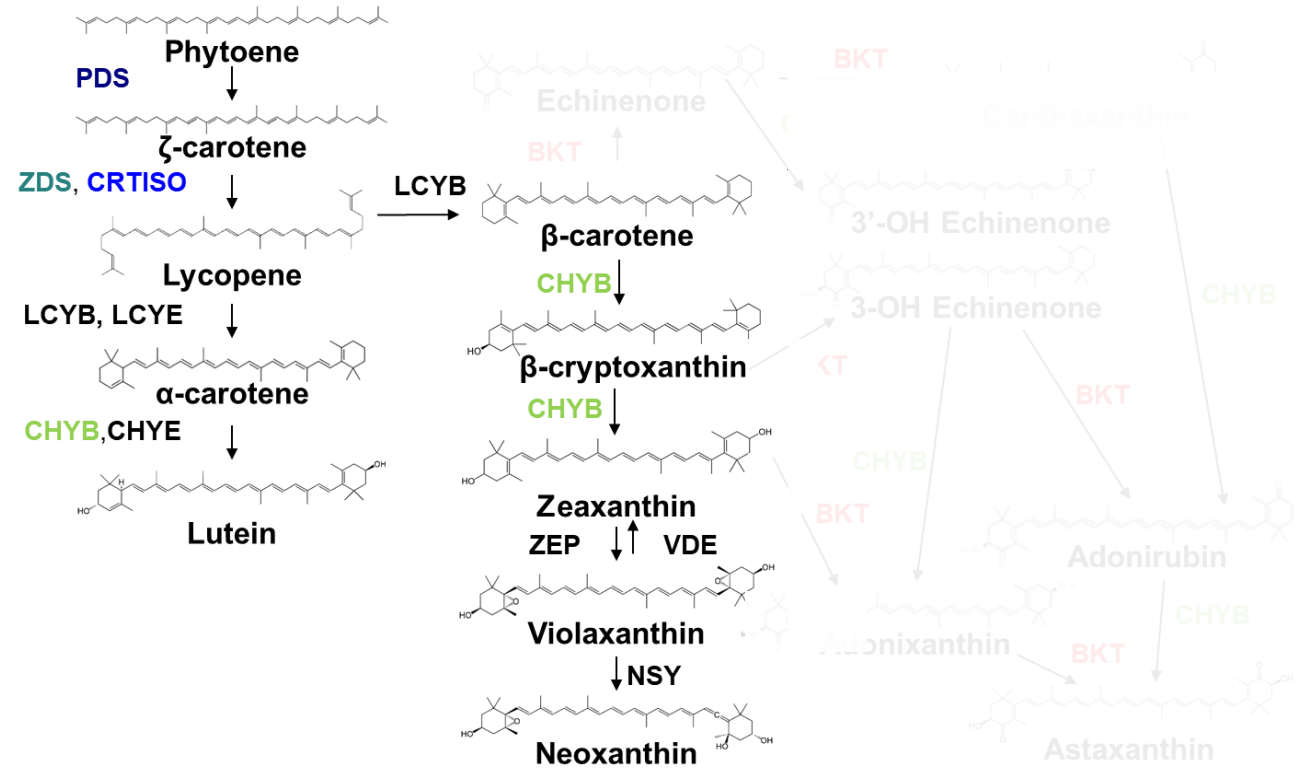
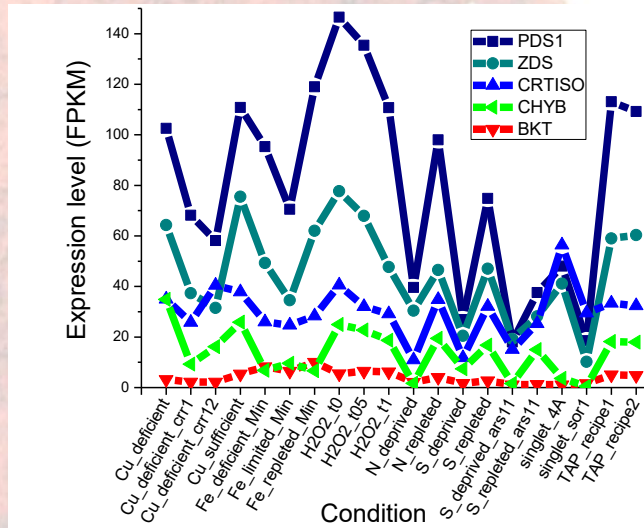
Expressed: BKT sequence is poorly expressed



Present: BKT sequence is present in *C. reinhardtii*

Longer C-term 115aa extension absent in any ketolase

Expressed: BKT sequence is poorly expressed



**Present:** BKT sequence is present in *C. reinhardtii*

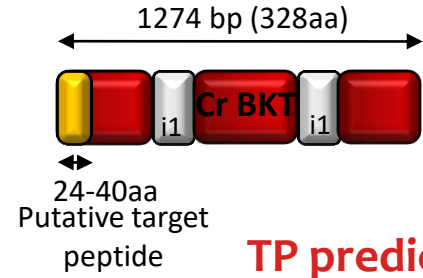
**Longer C-term:** 115aa extension absent in any ketolase

**Expressed:** BKT sequence is poorly expressed

**No astaxanthin:** was ever found in *C. reinhardtii*

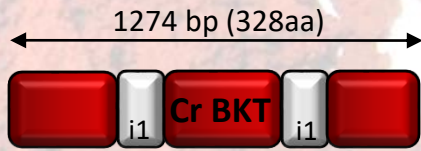
IS IT POSSIBLE TO PRODUCE ASTAXANTHIN IN CHLAMYDOMONAS?



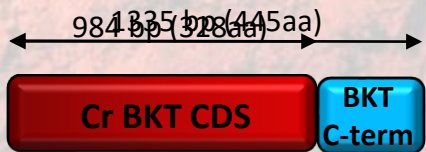


TP prediction

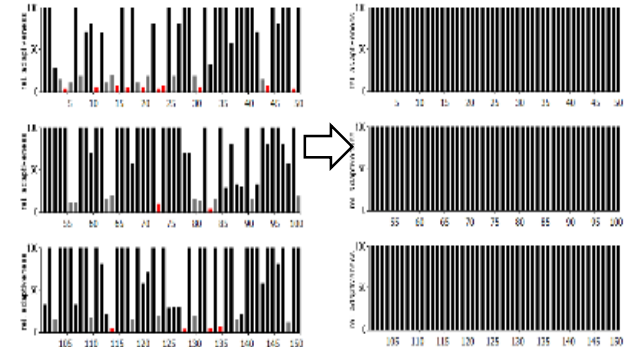
Introns insertion



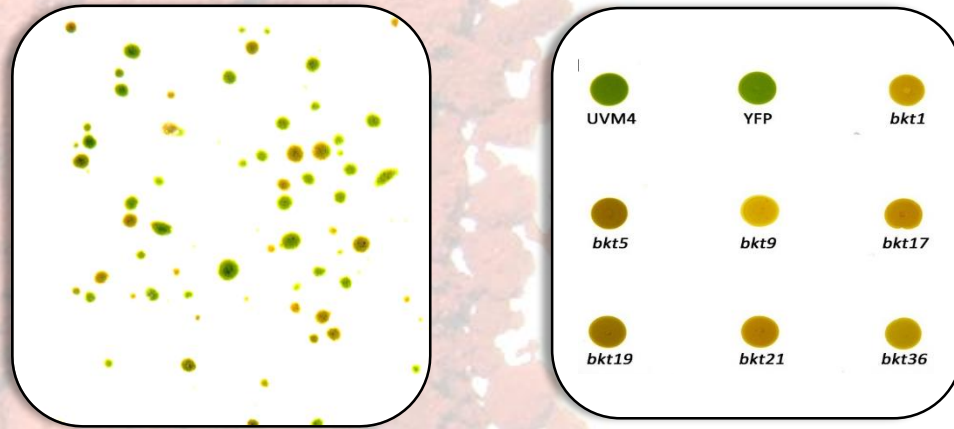
C-term removing



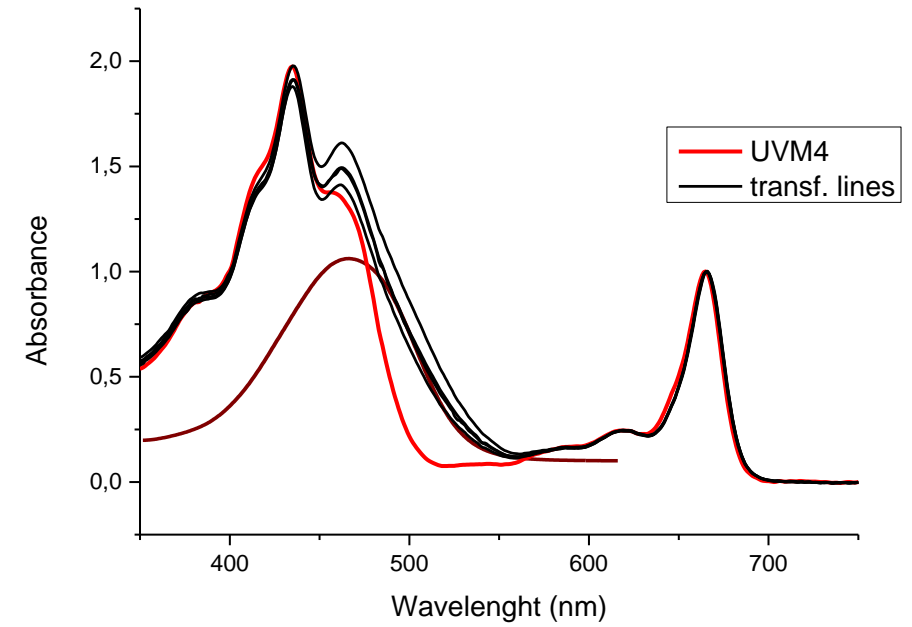
Codon usage optimization



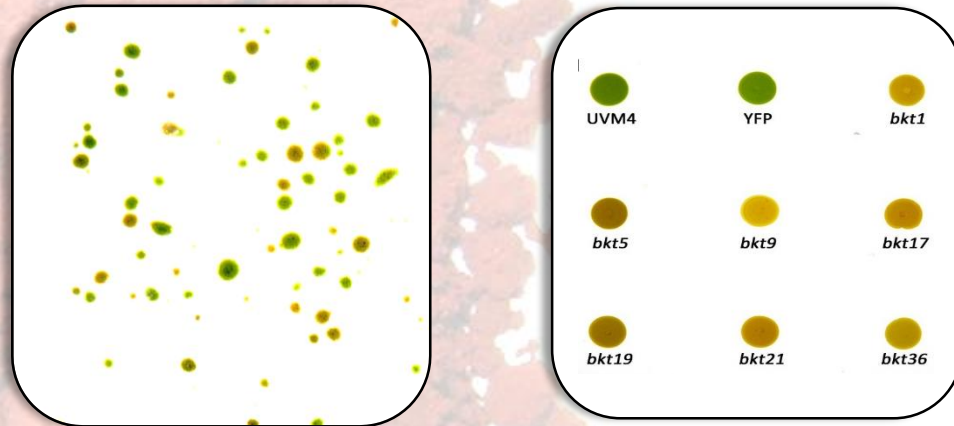




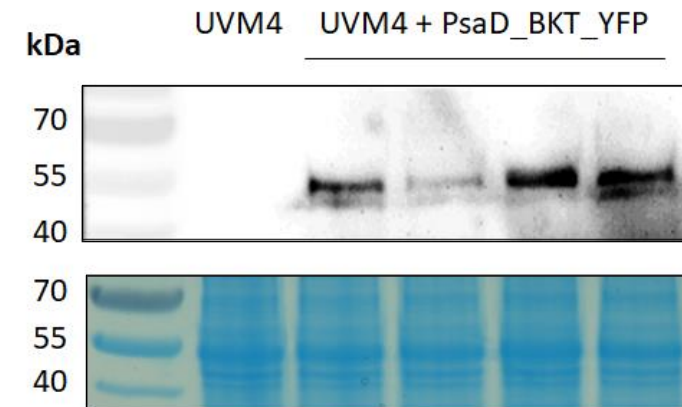
RED/ORANGE PHENOTYPE IS  
VISIBLE BY EYES



ABSORPTION SPECTRA REVEAL THE  
PRESENCE OF RED-SHIFTED CAROTENOIDS

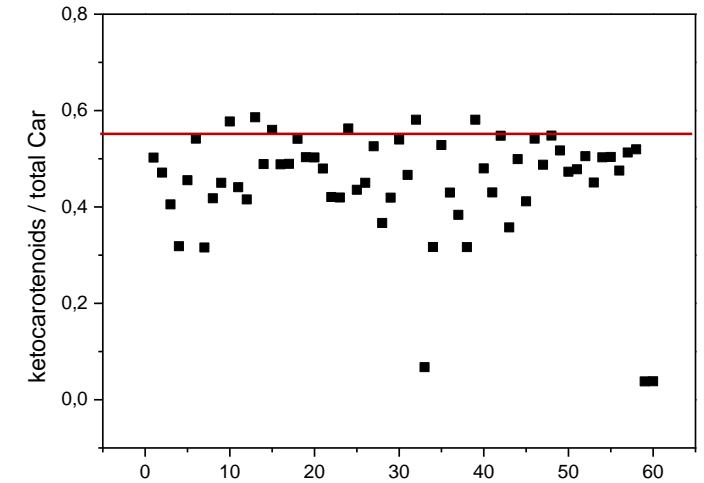
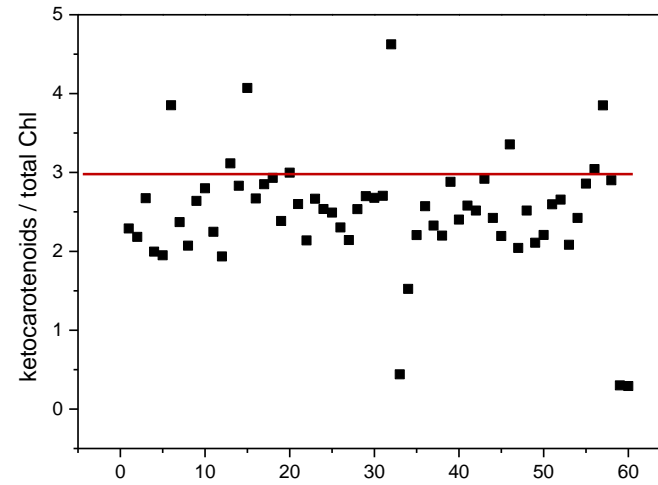
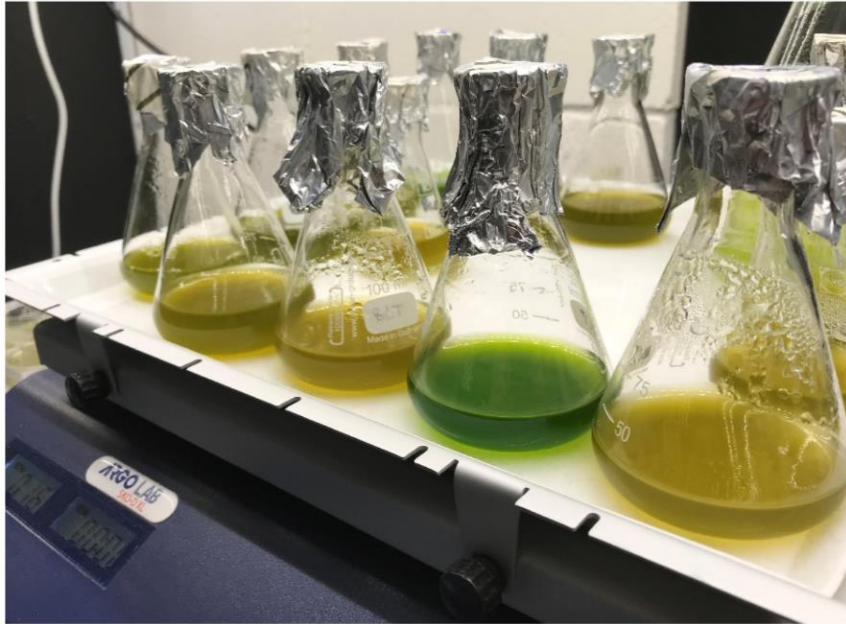


RED/ORANGE PHENOTYPE IS  
VISIBLE BY EYES

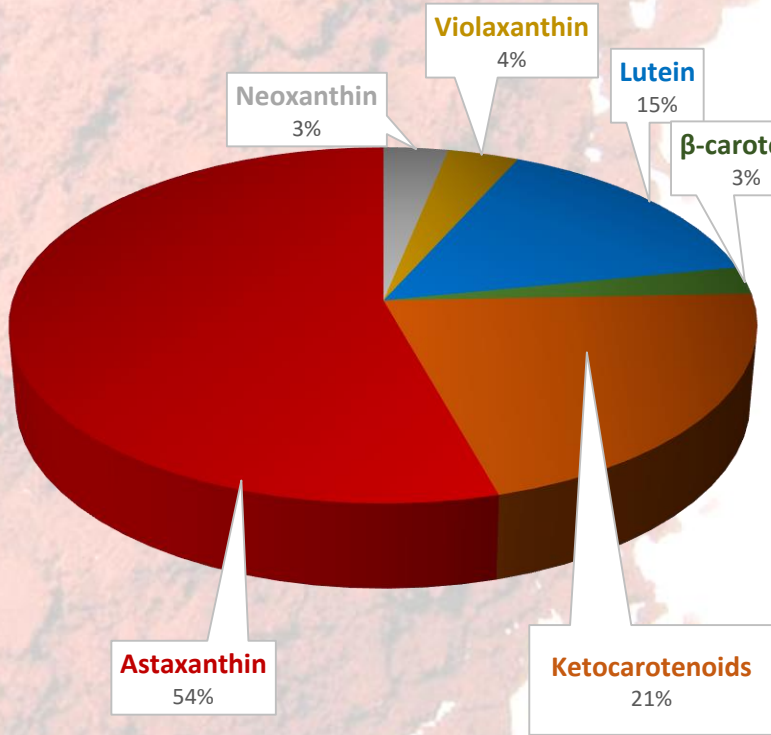


The accumulation of recombinant BKT-YFP protein in transformed cells was then verified by immunoblot developed using an antibody recognizing the fused YFP

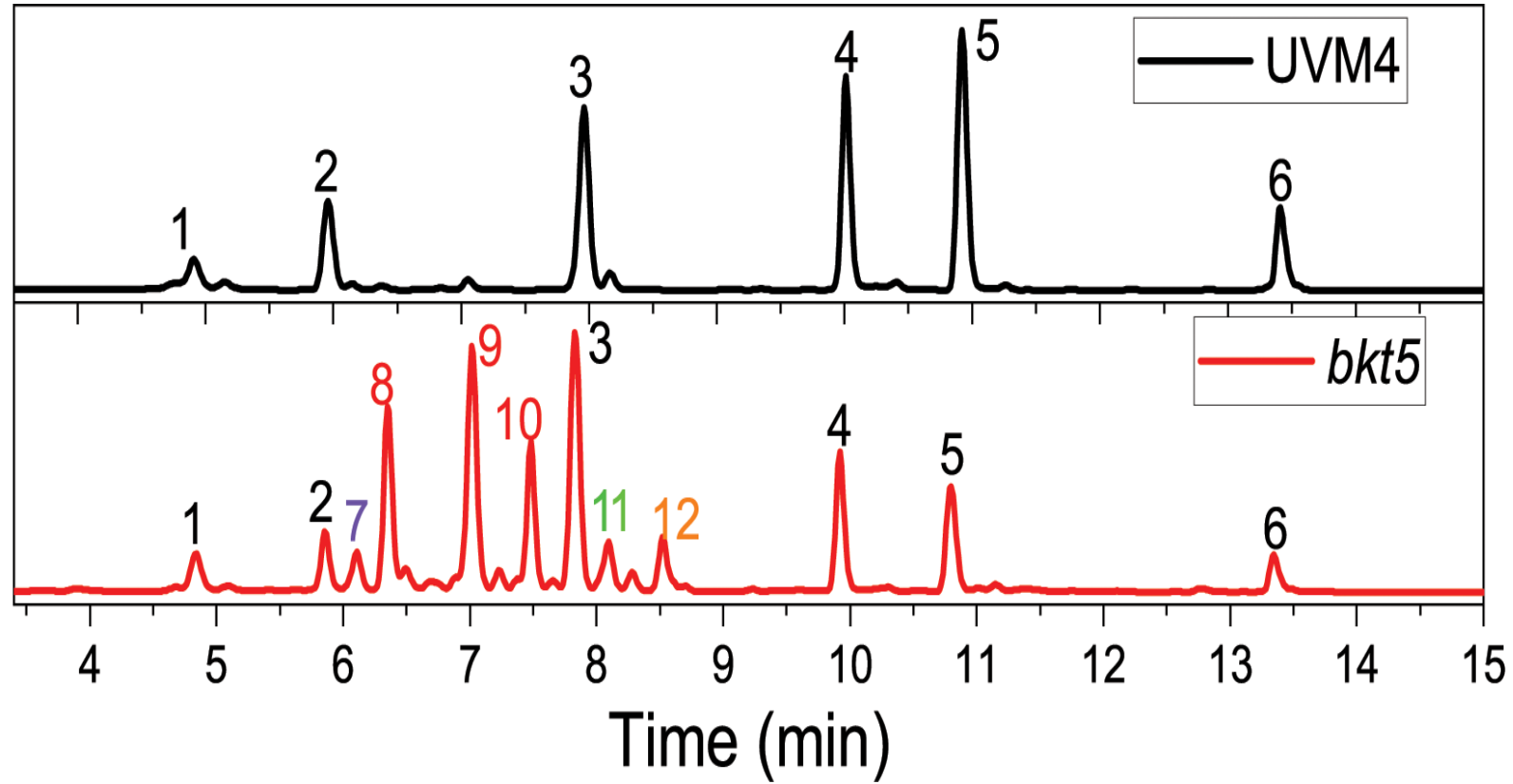




40 LINES WERE SCREENED IN ORDER TO SELECT BEST  
3 LINES WITH HIGHEST ACCUMULATION

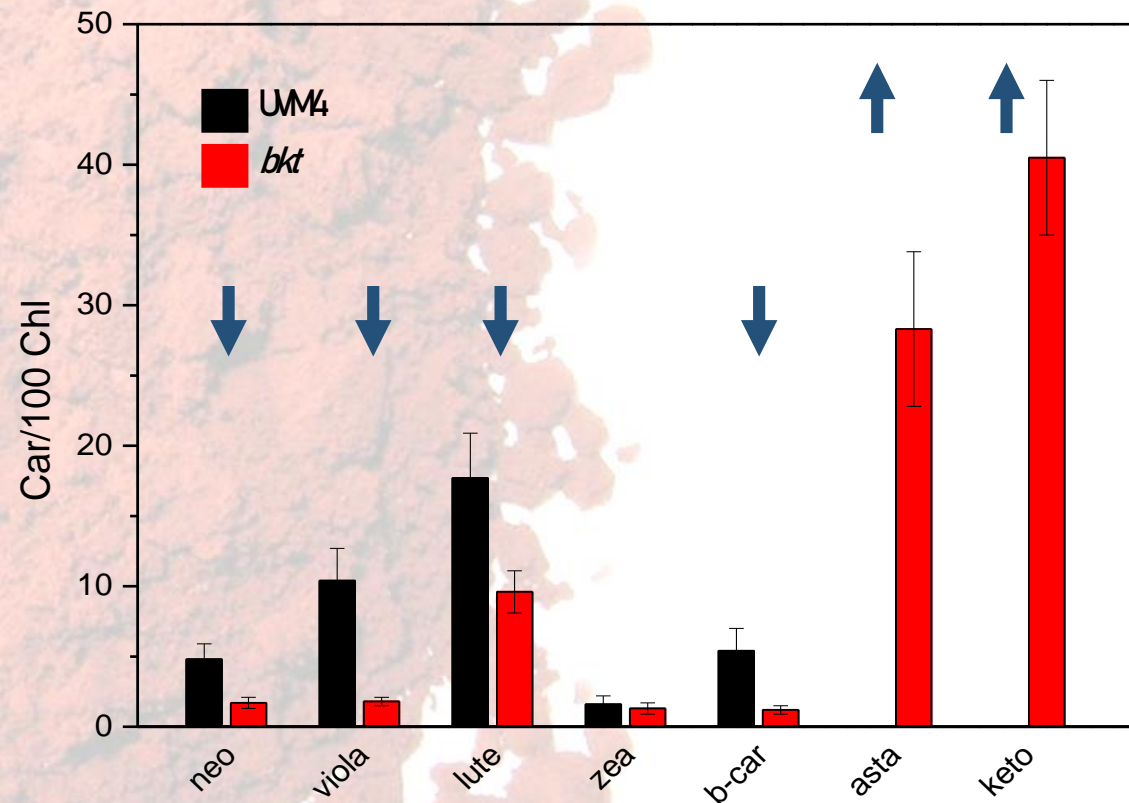


ABS (440 nm)



- Astaxanthin
- Canthaxanthin
- Adonirubin

HPLC REVEALS THE PRESENCE OF ADDITIONAL PICKS

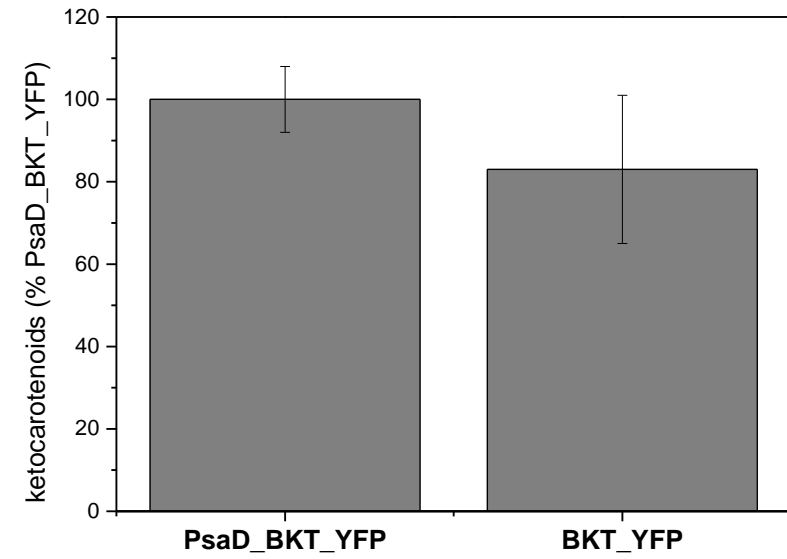
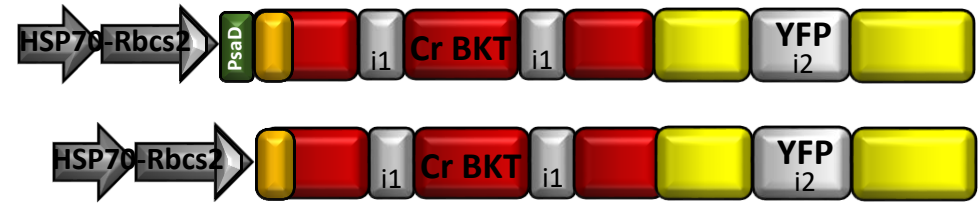
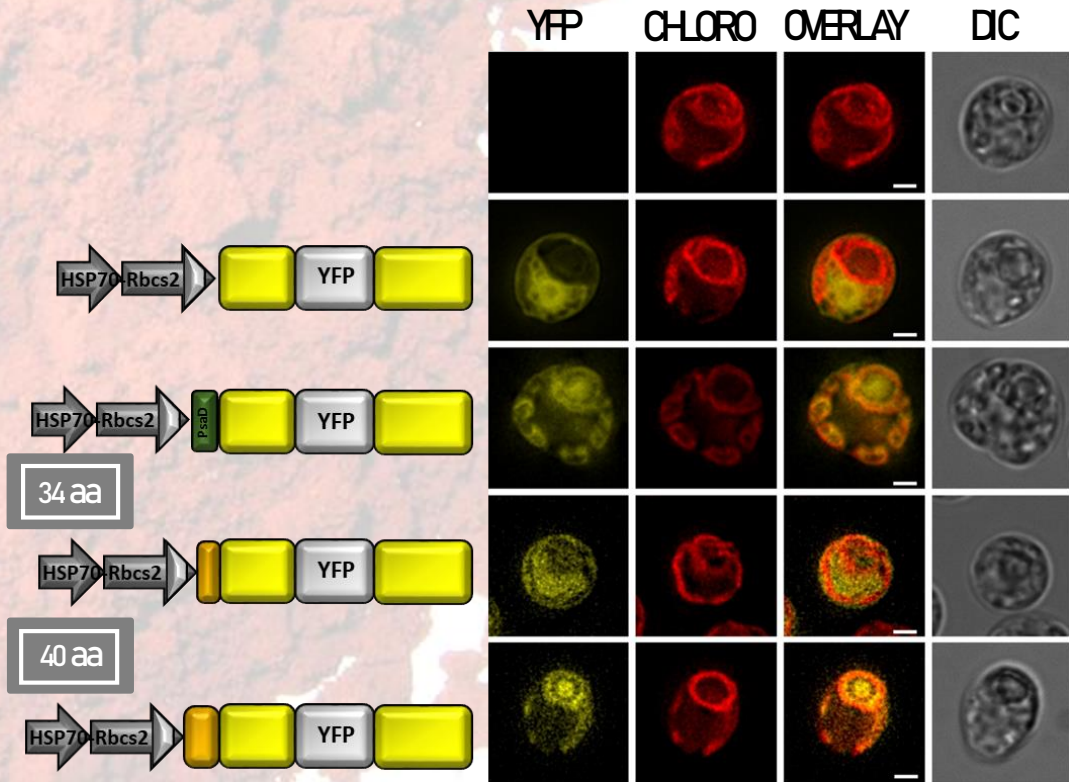


KETOCAROTENOIDS REPRESENT 69-75% OF TOTAL CAROTENOIDS

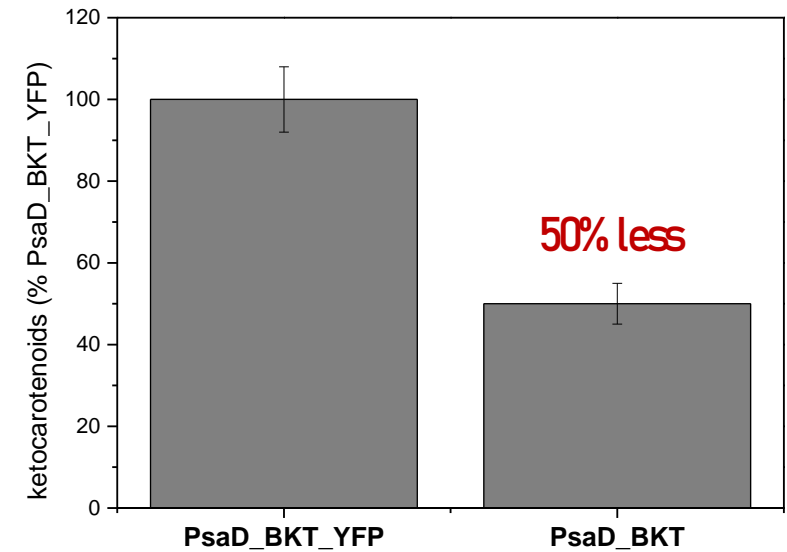
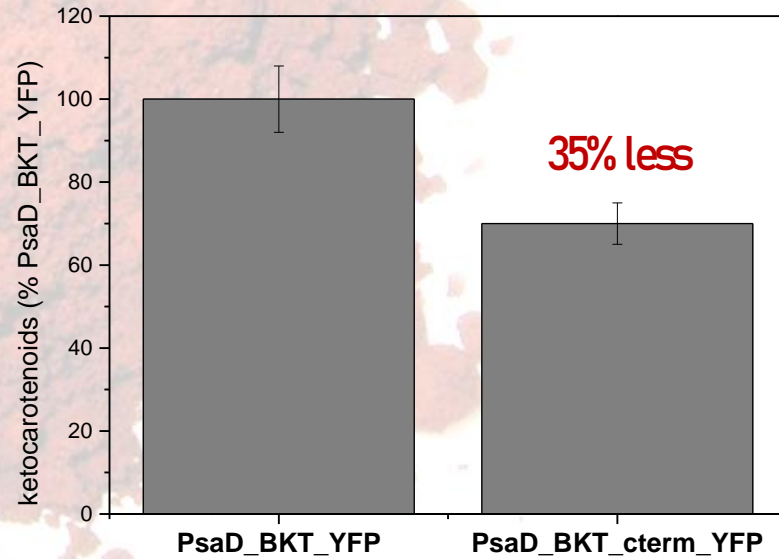
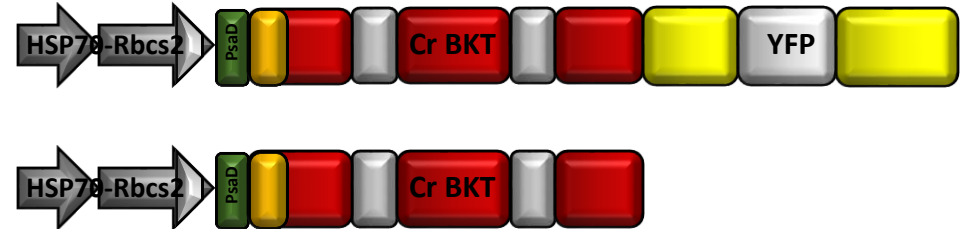
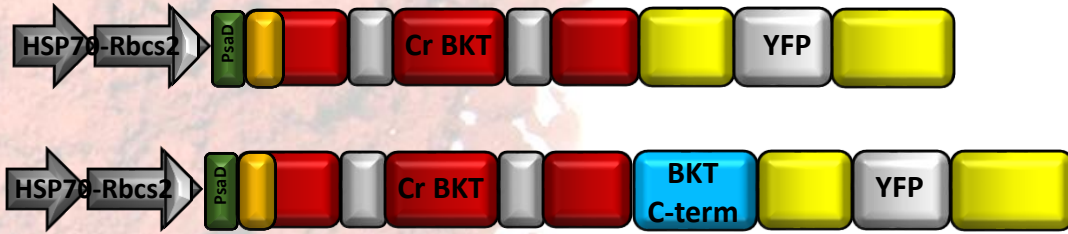
ASTAXANTHIN IS THE MOST ABUNDANT (37-49% of carotenoids)

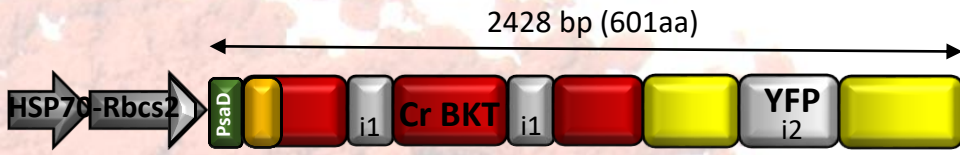


# N-TERMINAL CHARACTERIZATION




# C-TERMINAL CHARACTERIZATION





**Growth media:**

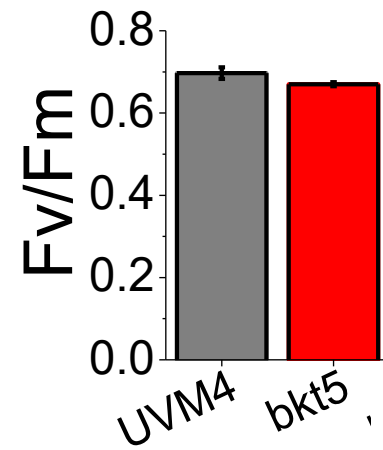
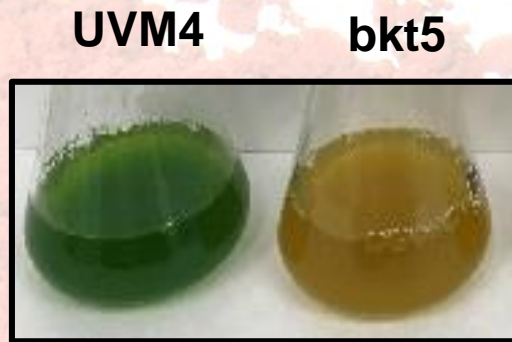
- ◆ TAP
- ◆ HSM



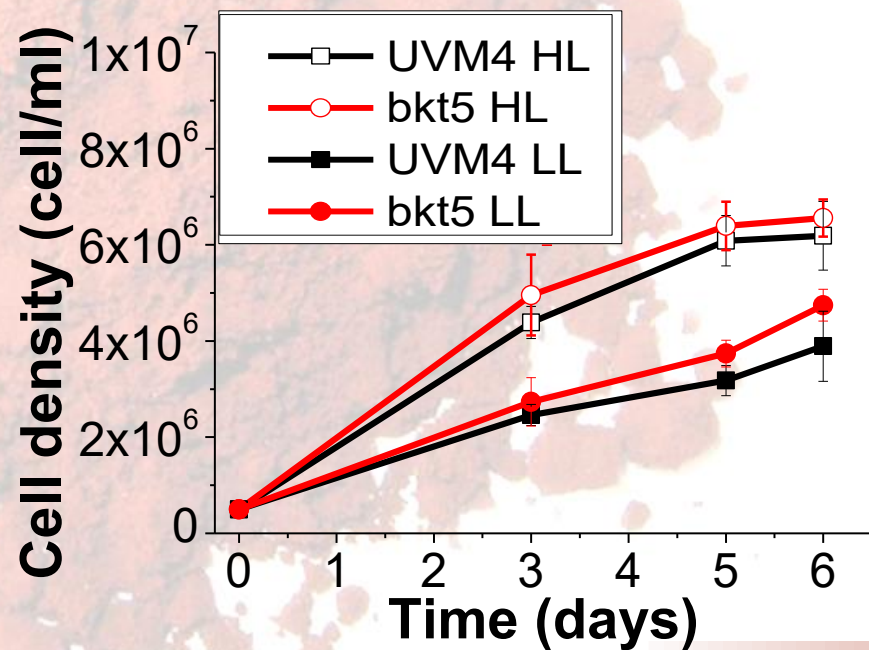
**Light intensity:**

- ◆ 100  $\mu$ E
- ◆ 500  $\mu$ E

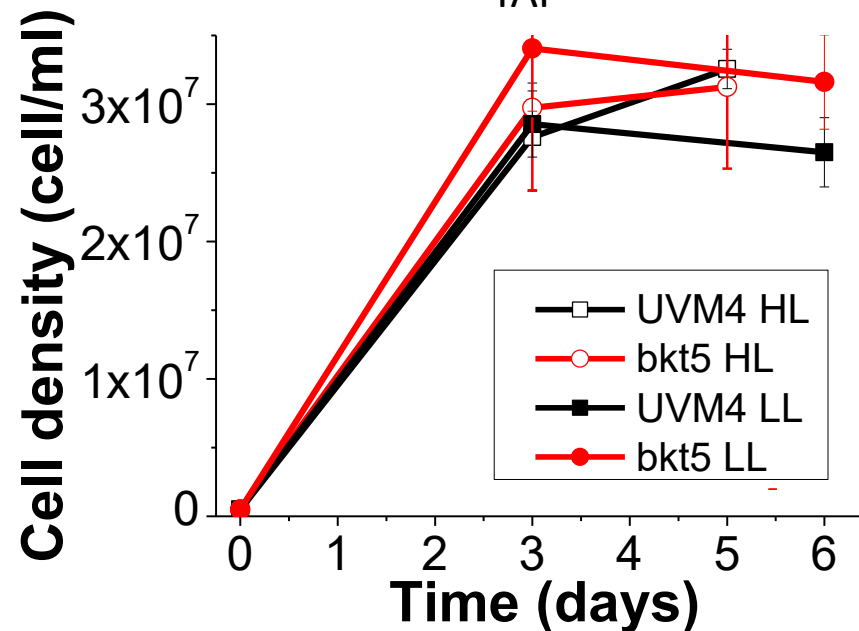




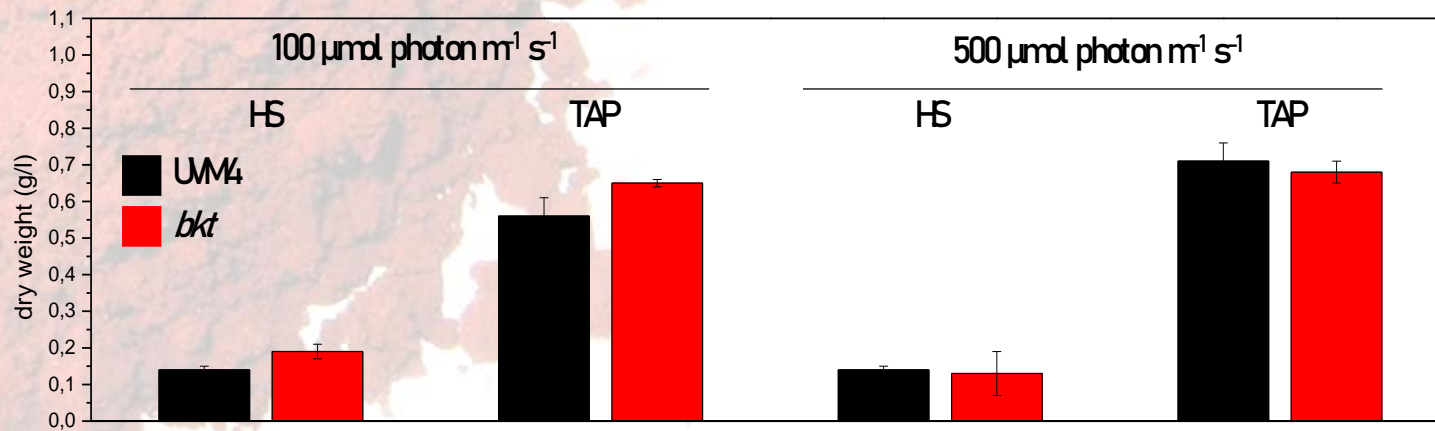
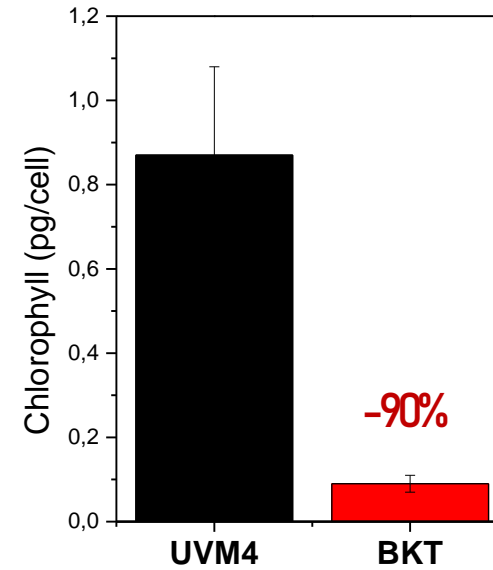
HSM



TAP



CHLOROPHYLL CONTENT  
IS STRONGLY REDUCED

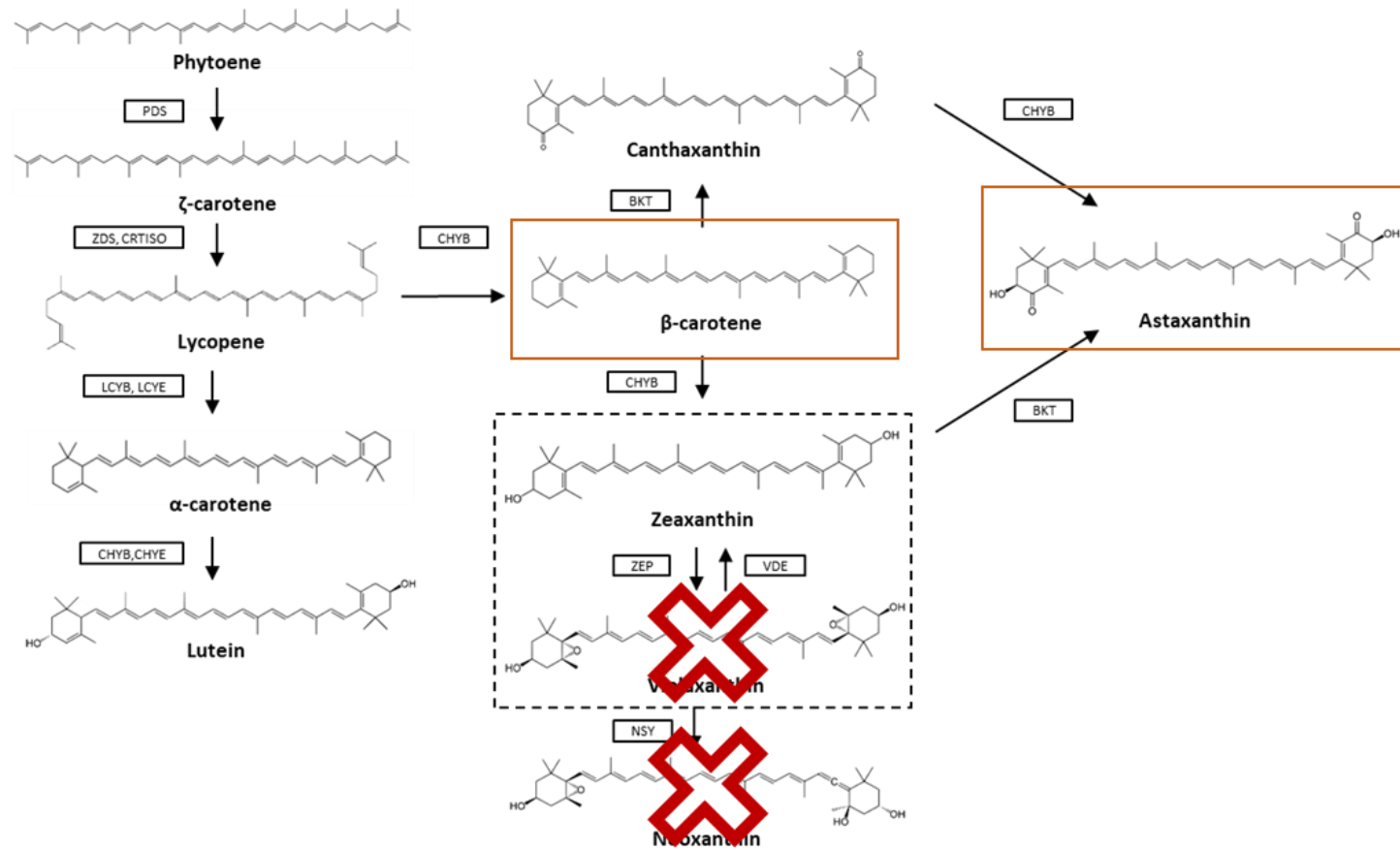


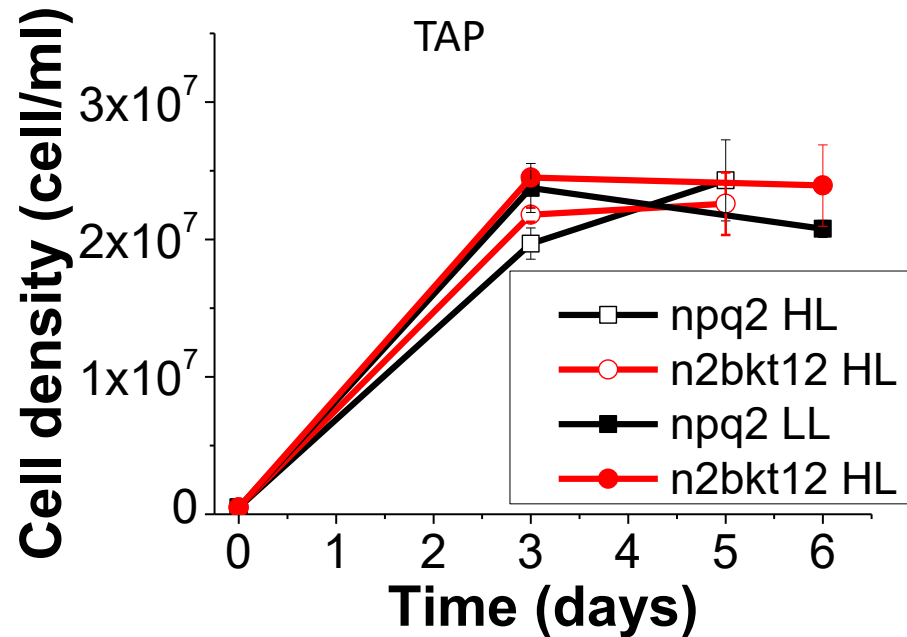
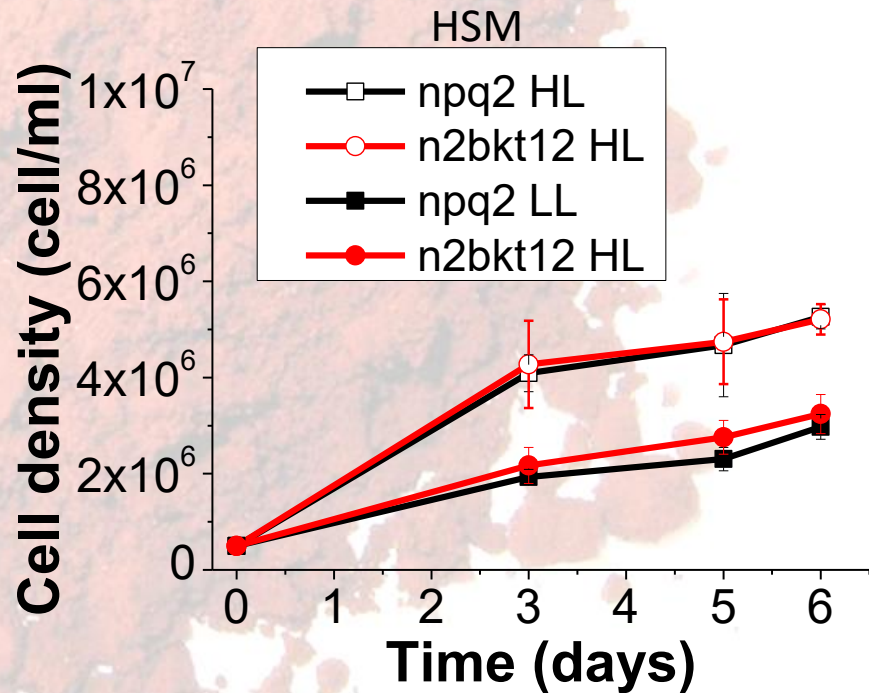
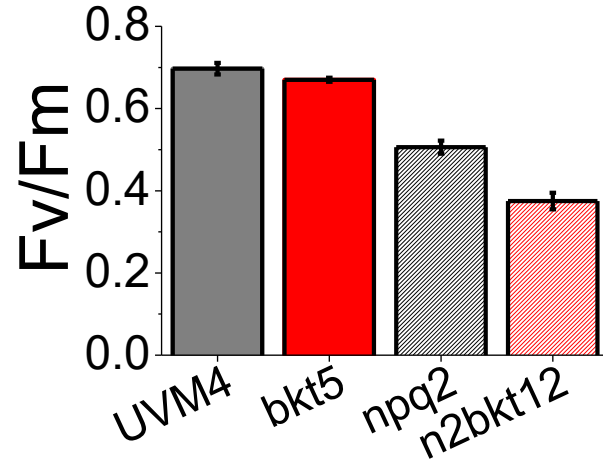
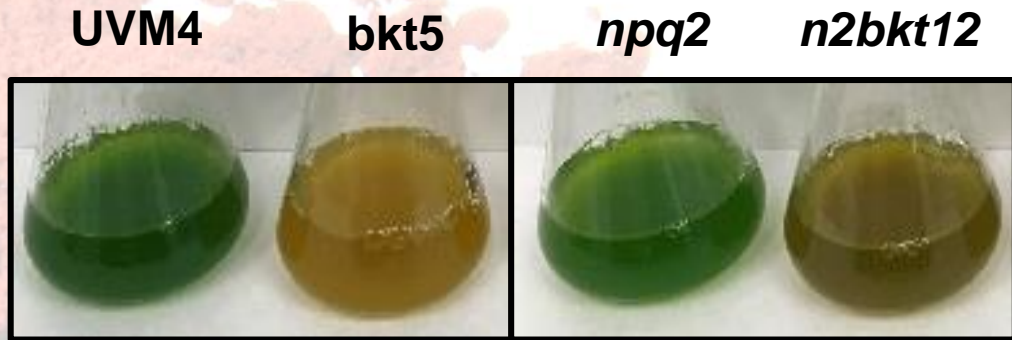
THIS REDUCTION DOES NOT  
INTERFERE WITH GROWTH



*npq2* MUTANT ACCUMULATE  
CONSTITUTIVELY ZEAXANTHIN

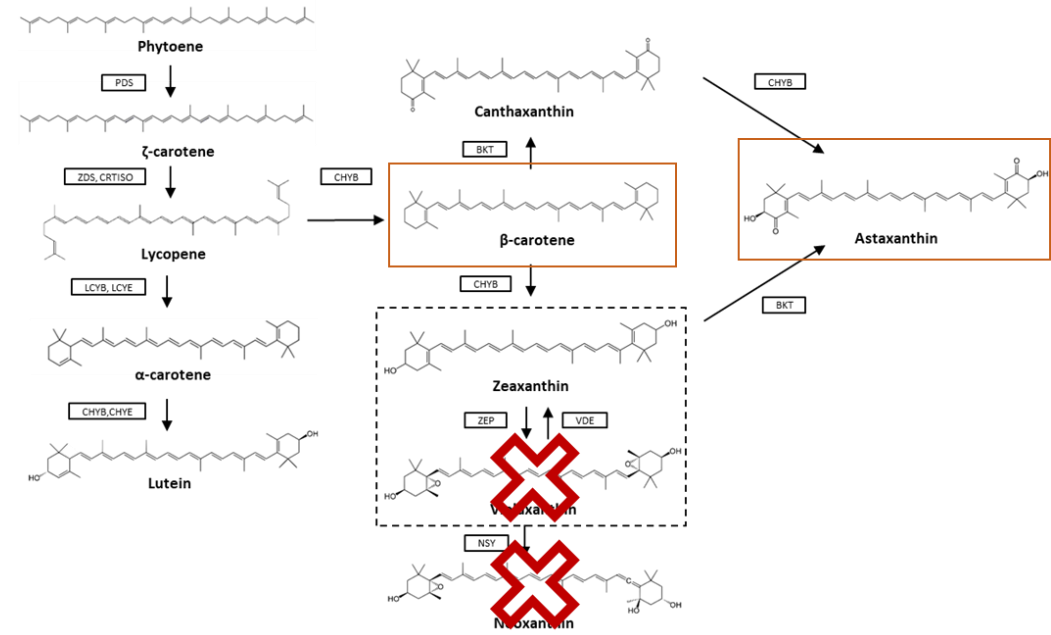
LINES WERE SELECTED AS UWM4 AND  
GROWN IN THE SAME CONDITIONS





*npq2* MUTANT ACCUMULATE  
CONSTITUTIVELY ZEAXANTHIN

LINES WERE SELECTED AS UMM4 AND  
GROWN IN THE SAME CONDITIONS

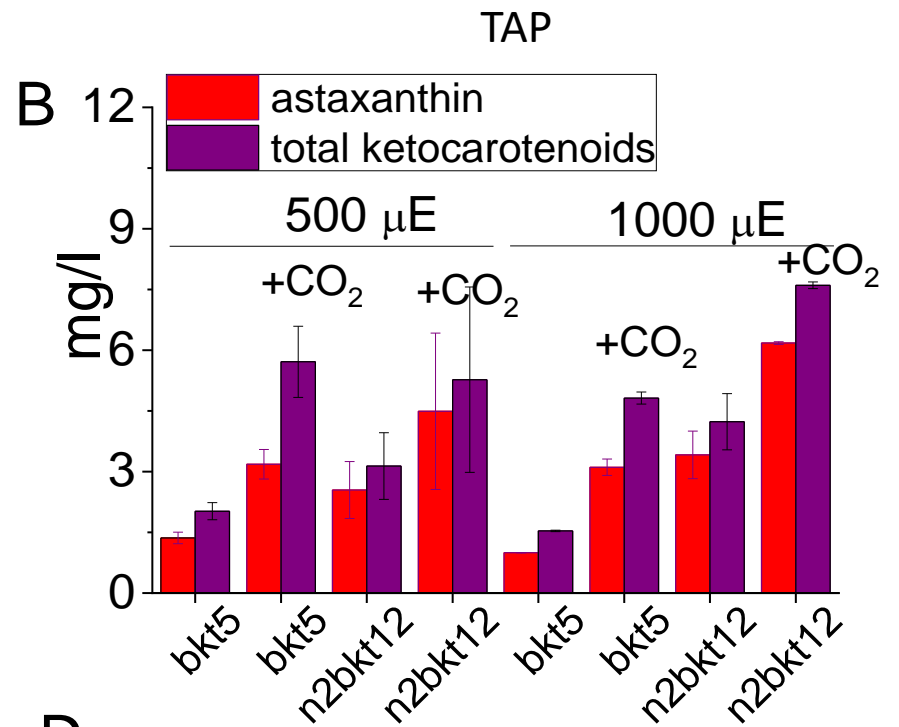
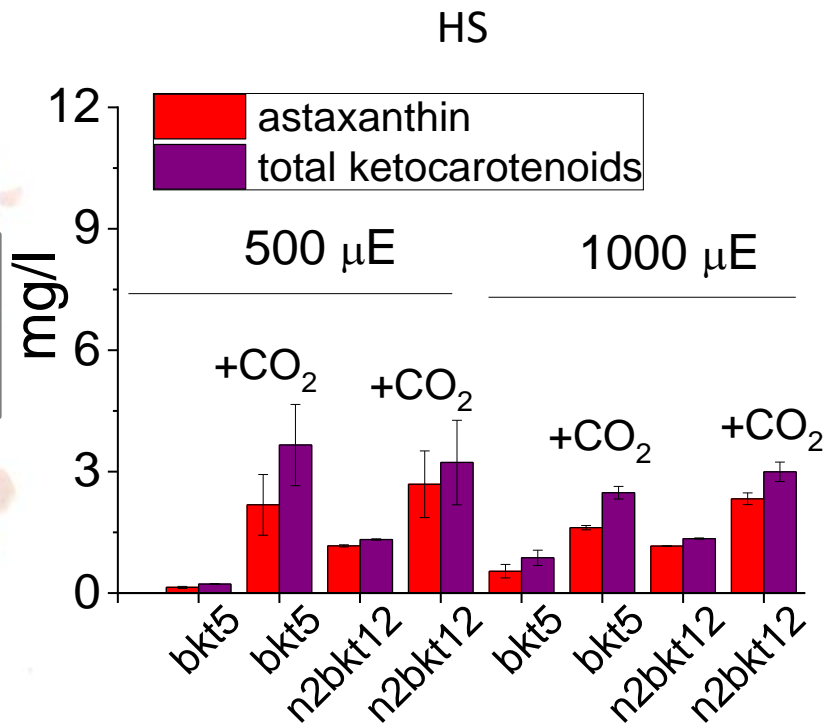




- Light: 500 or 1000  $\mu$ E
- Media: HS or TAP
- $\text{CO}_2$ : air or 3%  $\text{CO}_2$

**CO<sub>2</sub> INCREASES PRODUCTIVITY**

**ORGANIC CARBON POSITIVELY AFFECTS PRODUCTIVITY**

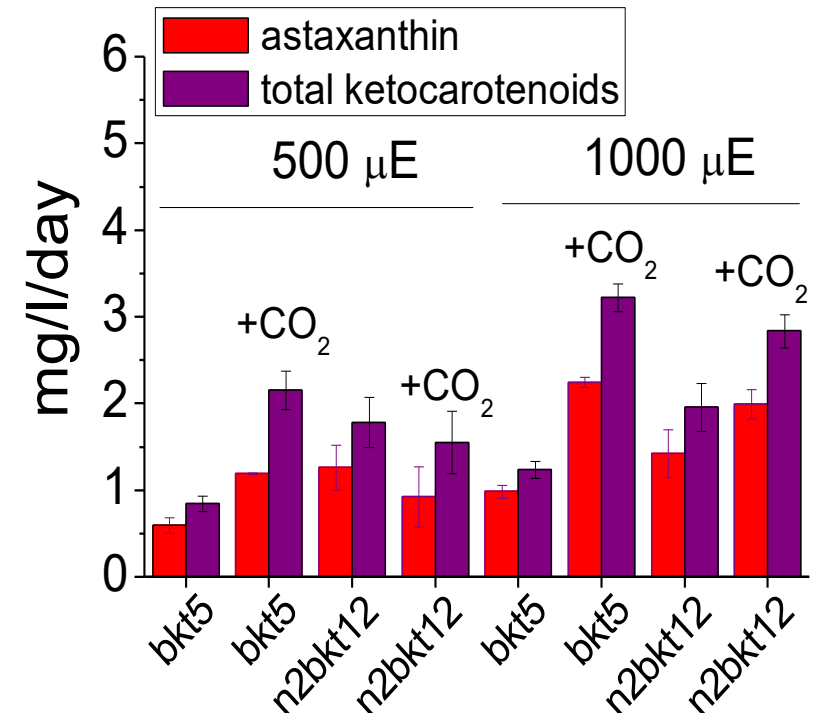
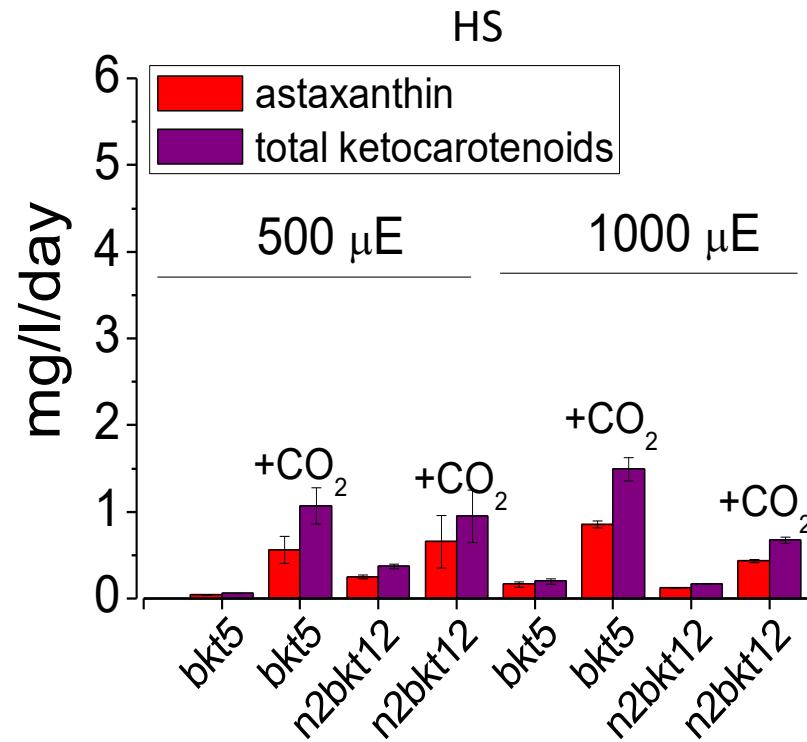




CO<sub>2</sub> INCREASES PRODUCTIVITY

ORGANIC CARBON POSITIVELY AFFECTS PRODUCTIVITY

- Light: 500 or 1000  $\mu$ E
- Media: HS or TAP
- CO<sub>2</sub>: air or 3%



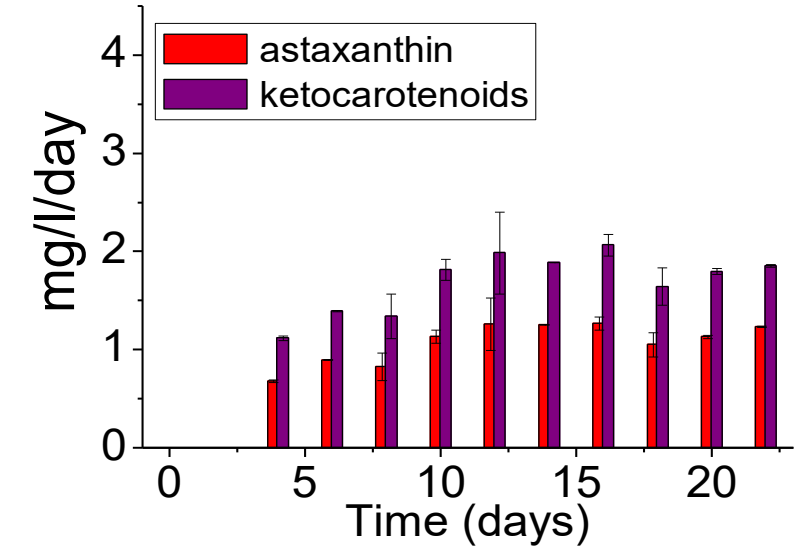
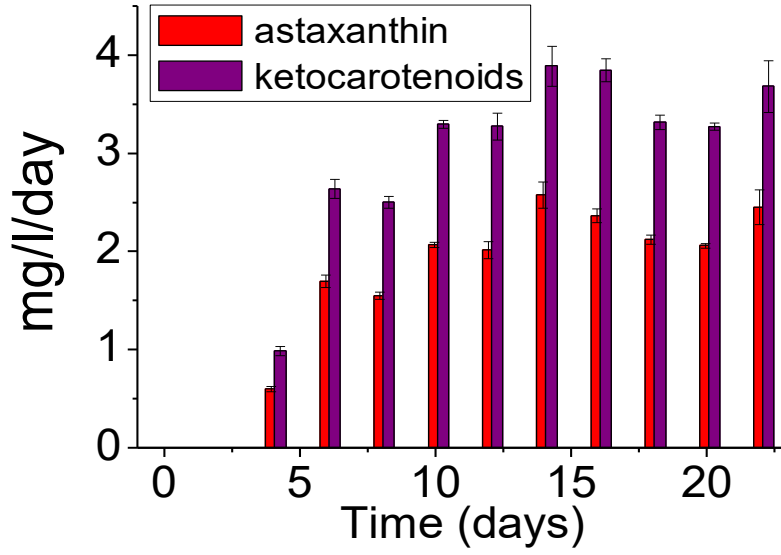
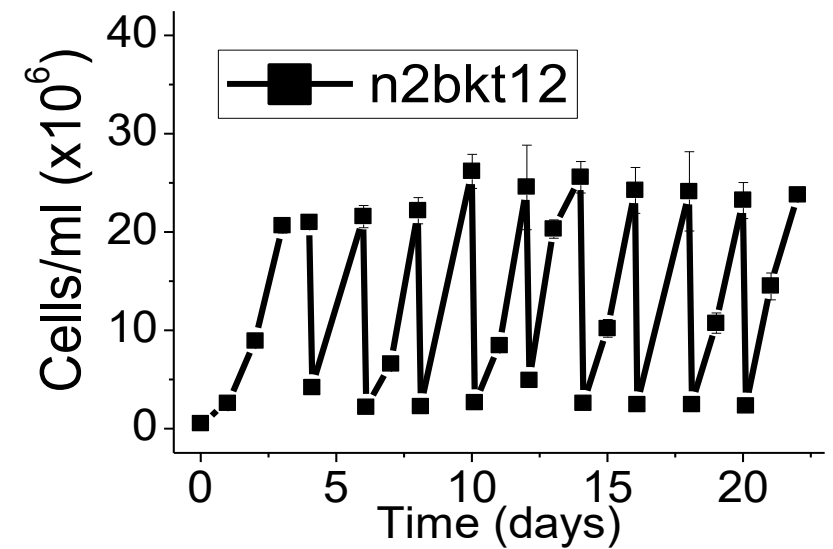
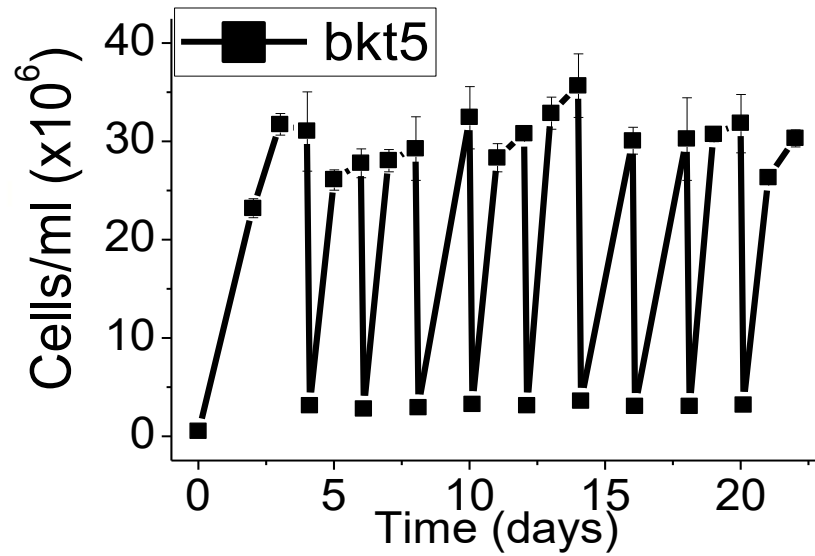
Light: 1000 uE

Media: TAP

Stirring

SEMICONINUOUS CULTIVATION

PRODUCTION OF UP TO 25 mg/l/day ASTAXANTHIN

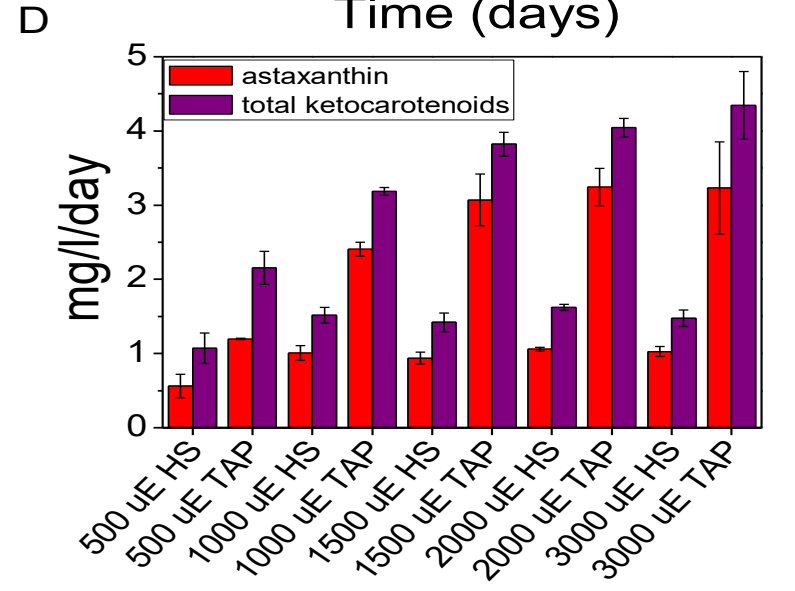
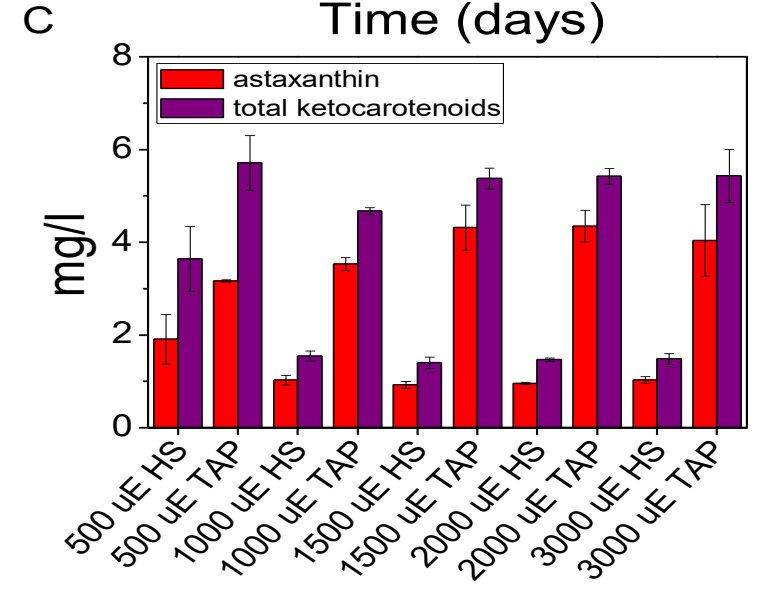
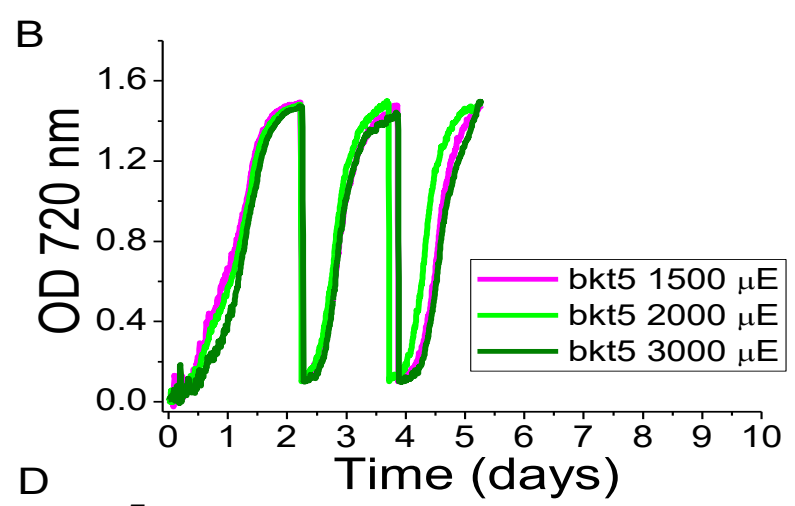
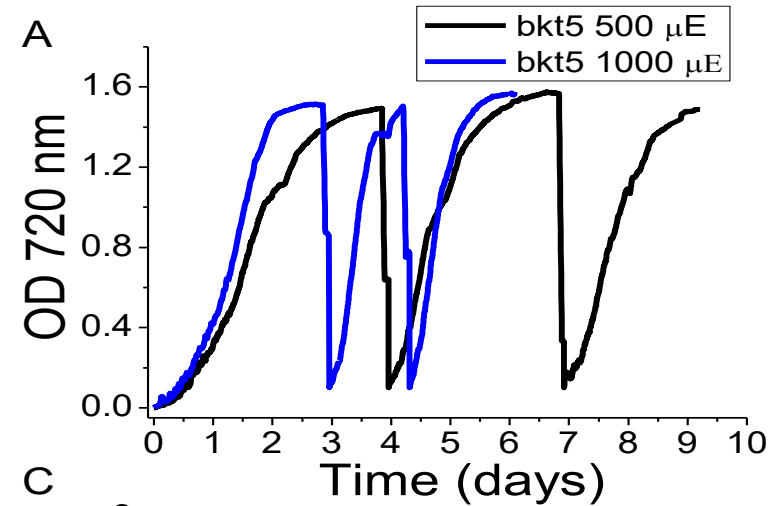


CULTIVATION WITH VERY HIGH LIGHT

- Light: from 1000 to 3000  $\mu\text{E}$
- Media: HS or TAP
- $\text{CO}_2$ : 3%  $\text{CO}_2$

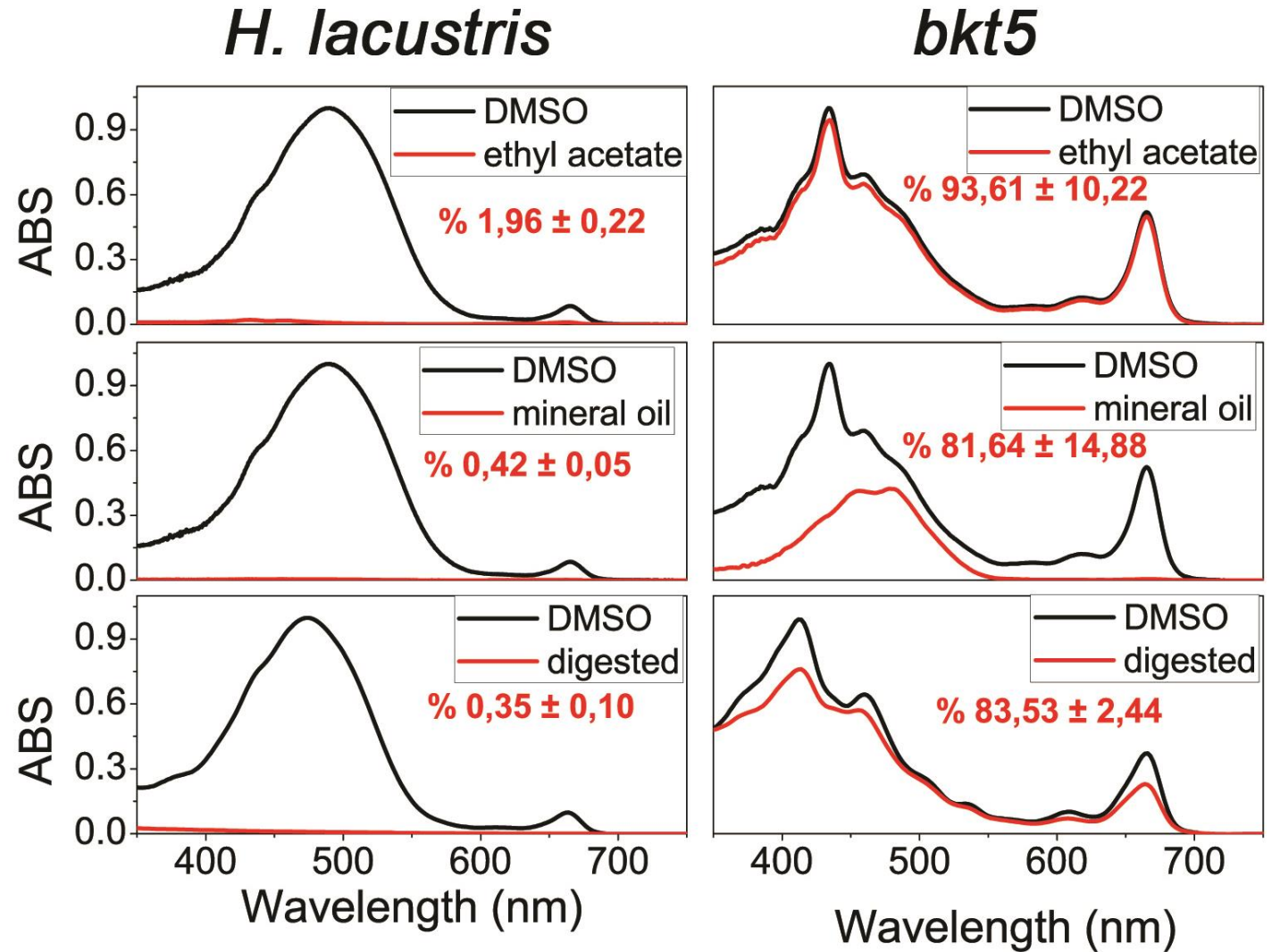
**AUTOTROPHY:**  
 1 mg/l/day ASTAXANTHIN  
 1,5 mg/l/day KETOCAROTENOIDS

**MXOTROPHY:**  
 3,1 mg/l/day ASTAXANTHIN  
 4,3 mg/l/day KETOCAROTENOIDS



## Extractability and bioavailability of astaxanthin from *H. lacustris* vs. *C. reinhardtii* cells

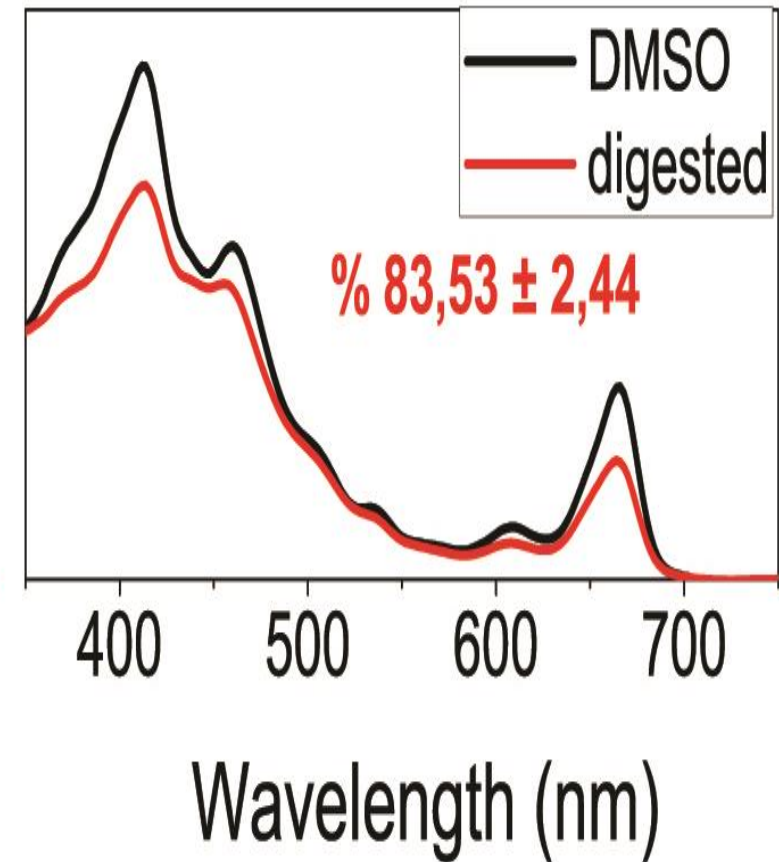
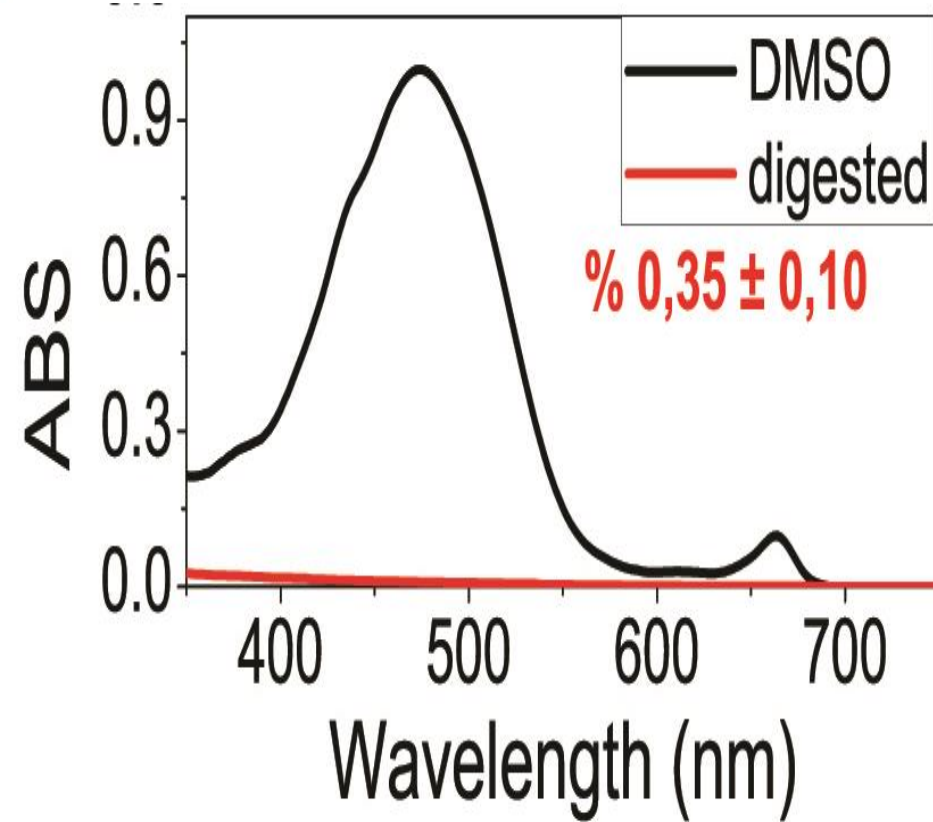
>85% of astaxanthin in *C. reinhardtii* can be extracted by in vitro digestion simulation vs. 0.35% in the case of *H. lacustris*





## Bioaccessibility of astaxanthin from *H. lacustris* vs. *C. reinhardtii* cells.

>85% of astaxanthin in *C. reinhardtii* can be extracted by in vitro digestion simulation vs. 0.35% in the case of *H. pluvialis*



ASTAXANTHIN PRODUCTION IN *C. REINHARDTII* IS POSSIBLE USING ENDOGENOUS BKT

THE LONG C-TERMINAL AND THE LOW STABILITY OF BKT PROTEIN COULD BE A PART OF THE CAUSE OF ABSENCE OF KETOCAROTENOID IN *C.*

*REINHARDTII*

0,12-15 mg/L/day

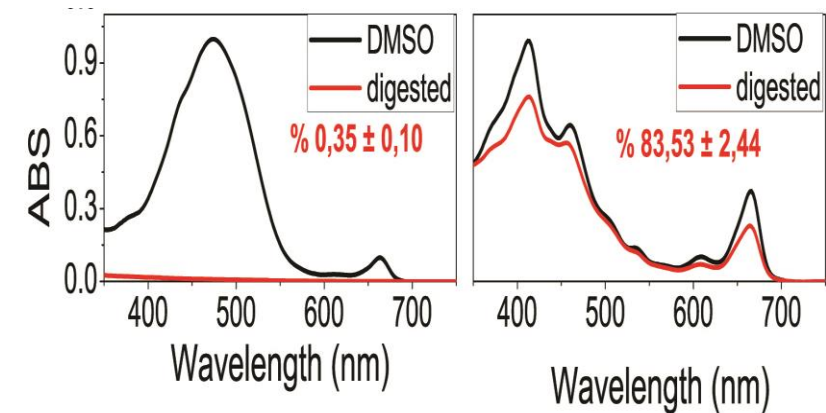
3,1 mg/L/day

*H. pluvialis*

*C. reinhardtii*

COMPARABLE YIELDS CAN BE OBTAINED WITH EXTREMELY SIMPLE SYSTEMS

INCREASED BIOAVAILABILITY





UNIVERSITÀ  
di **VERONA**



**Federico Perozeni**  
Cazzaniga Stefano



**Prof. Zoccatelli Gianni**



**Prof. Kruse Olaf**  
Wobbe Lutz  
Baier Thomas  
Lauersen Kyle J.



European  
Research  
Council

Thanks  
for your attention