Improvement of selected microalgae strains for the production of valuable biocompounds

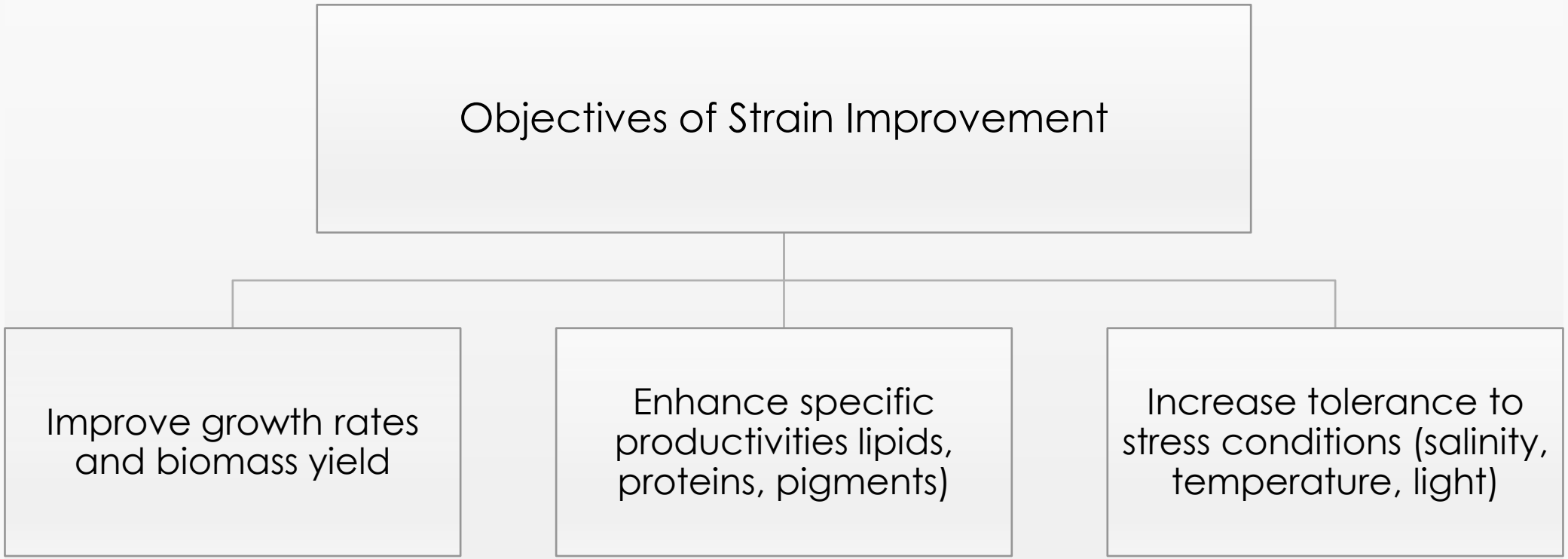
João Manoel

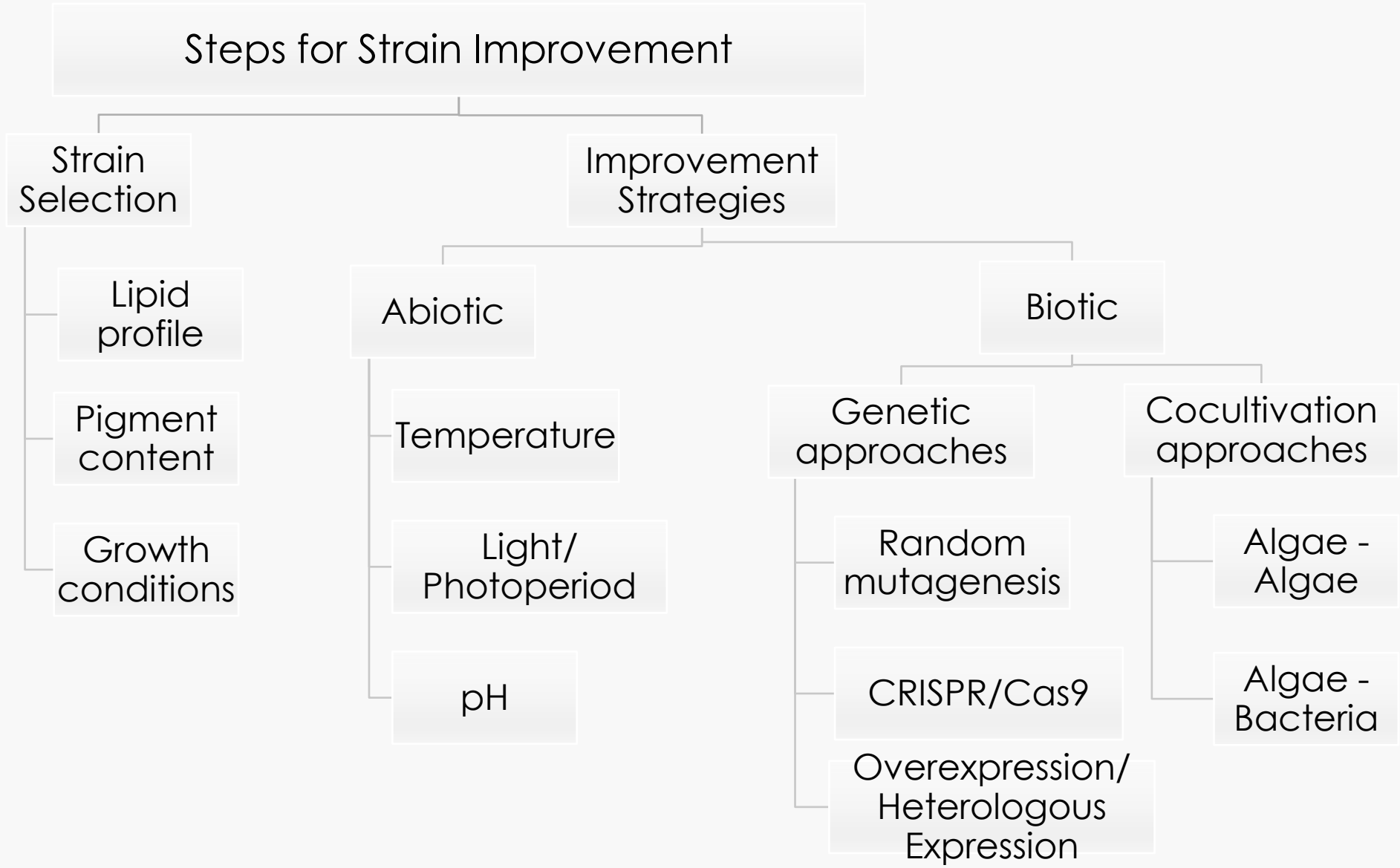
Why microalgae?

→ High yield

→ A lot of interesting bio-products that cannot be obtained in other systems

→ Sustainable economy, sequestering of waste CO_2, bio-fuels of 3rd generation …


Objectives of Strain Improvement
Improve growth rates and biomass yield
Enhance specific productivities lipids, proteins, pigments)
Increase tolerance to stress conditions (salinity, temperature, light)


Steps for Strain Improvement
Strain Selection
Lipid profile
Pigment content
Growth conditions
Improvement Strategies
Abiotic
Temperature
Light/ Photoperiod
pH
Biotic
Genetic approaches
Random mutagenesis
CRISPR/Cas9
Overexpression/ Heterologous Expression
Cocultivation approaches
Algae - Algae
Algae - Bacteria

Abiotic factors

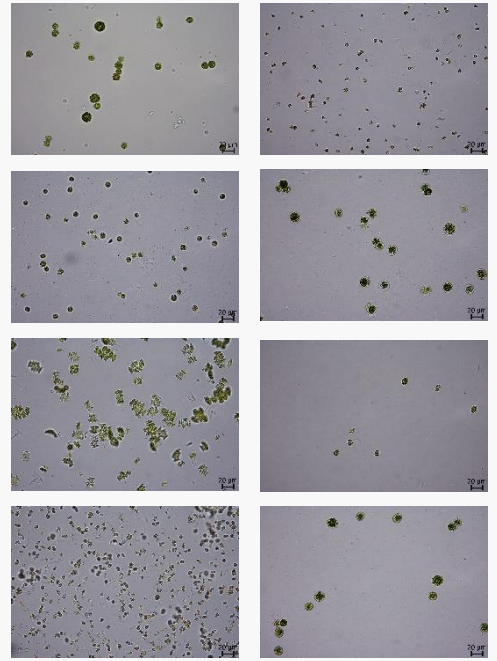
Microalgae as an alternative to Palm Oil

Idea:
To find sustainable alternatives to palm oil

Fatty acid	Amount [%]
Lauric acid (C12:0)	0.2
Myristic acid (C14:0)	1.1
Palmitic acid (C16:0)	44.0
Stearic acid (C18:0)	4.5
Oleic acid (C18:1)	39.2
Linoleic acid (C18:2)	10.1
Linolenic acid (C18:3)	0.4
Arachidic acid (C20:0)	0.1
Total SFAs	49.9
Total MUFAs	39.2
Total PUFAs	10.5

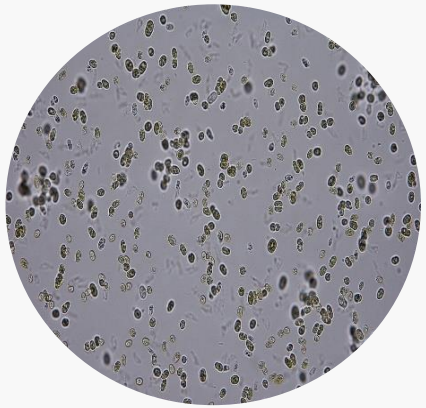
vs.

???

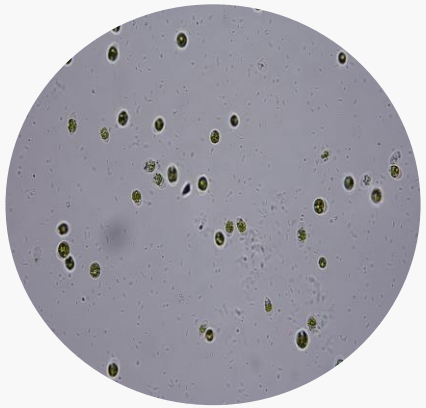


Fatty acid	Amount [%]										
	Palm oil	CCALA 455	CCALA 688	CCALA 456	CCALA 242	CCALA 467	CCALA 243	CCALA 244	CCALA 464	CCALA 463	CCALA 453
Lauric acid (C12:0)	0.2	**0.1**	**3.8**	0.0	0.0	0.0	0.0	**0.1**	**0.1**	**0.1**	0.0
Myristic acid (C14:0)	1.1	**1.1**	**3.9**	0.4	0.4	0.3	0.8	**2.5**	0.7	0.1	0.5
Palmitic acid (C16:0)	44.0	35.3	4.4	29.2	23.9	**37.6**	27.3	**43.2**	35.4	**40.2**	30.3
Stearic acid (C18:0)	4.5	**7.5**	1.9	3.9	**8.0**	**5.2**	**9.1**	**9.5**	**9.0**	**4.4**	**4.8**
Oleic acid (C18:1)	39.2	21.9	13.1	**35.0**	**32.7**	**30.1**	26.8	10.7	24.2	21.6	**34.4**
Linoleic acid (C18:2)	10.1	**12.0**	4.3	**15.6**	5.7	**14.3**	5.2	3.9	**10.4**	**15.6**	**15.6**
Linolenic acid (C18:3)	0.4	**14.4**	**37.3**	5.8	**24.4**	4.6	**26.3**	**24.4**	**13.7**	**10.8**	**8.5**
Arachidic acid (C20:0)	0.1	**0.7**	**3.8**	**1.8**	0.0	**1.1**	**0.1**	**0.9**	**0.7**	**0.9**	**0.9**
Total SFAs	49.9	**44.7**	17.8	**35.3**	32.2	1.6	37.4	**56.1**	**45.9**	**45.7**	36.6
Total MUFAs	39.2	23.8	25.8	**38.2**	21.3	**33.4**	27.9	11.9	26.1	1.6	**35.4**
Total PUFAs	10.5	**26.3**	8.8	**24.1**	**32.5**	**22.4**	**34.7**	**31.9**	**28.0**	**31.1**	**28.0**

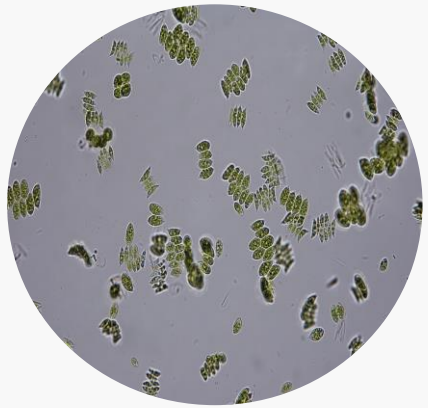
- ***Numbers higlighted in bold are comparable or higher than the values found in palm oil***



Desmodesmus subspicatus
CCALA 467



Chlamydomonas moewusii
CCALA 244



Desmodesmus communis
CCALA 463

1. NORMAL CONDITIONS

- Cultivation at optimim temperature and optimum LI at larger scale in the laboratory (30L AC-PBR)
- Characterization of the growth and verification of FA composition

2. STRESS CONDITIONS

- **Salt stress** – *Chlamydomonas* at 0.1M NaCl
  – *Desmodesmus* 0.03M= 2 g/L

		Growth	FAs
CCALA 244	BG11	$\mu = 0.16\ d^{-1}$ $\tau_{1/2} = 4.43$ Max DW 2.84g/L	TFA ~4-7 % DW C16:0 app. 25% of TFA C18:1n9 8.42% of TFA
	NaCl	**Culture Crashed after 3 days**	
CCALA 463	BG11	$\mu = 0.2\ d^{-1}$ $\tau_{1/2} = 3.4$ Final DW 3.23g/L	TFA 11.7 % DW C16:0 app. 20.52% of TFA C18:1n9 27.33% of TFA
	NaCl	$\mu = 0.4\ d^{-1}$ $\tau_{1/2} = 1.8$ Final DW **8.45 g/L**	TFA between **21.7%** (D11) and **17.7%** (D18) C16:0 app. 25% of TFA C18:1n9 31.2% of TFA (D18)
CCALA 467	BG11	$\mu = 0.2\ d^{-1}$ $\tau_{1/2} = 3.5$ d Max DW 5.23g/L	TFA 17.8 % DW C16:0 app. 25% of TFA C18:1n9 31.7% of TFA
	NaCl	$\mu = 0.2\ d^{-1}$, $\tau_{1/2} = 3$ d Max DW 5.20g/L	4.5% TFA C16:0 app. 25% of TFA C18:1n9 10.9% of TFA

Abiotic factors

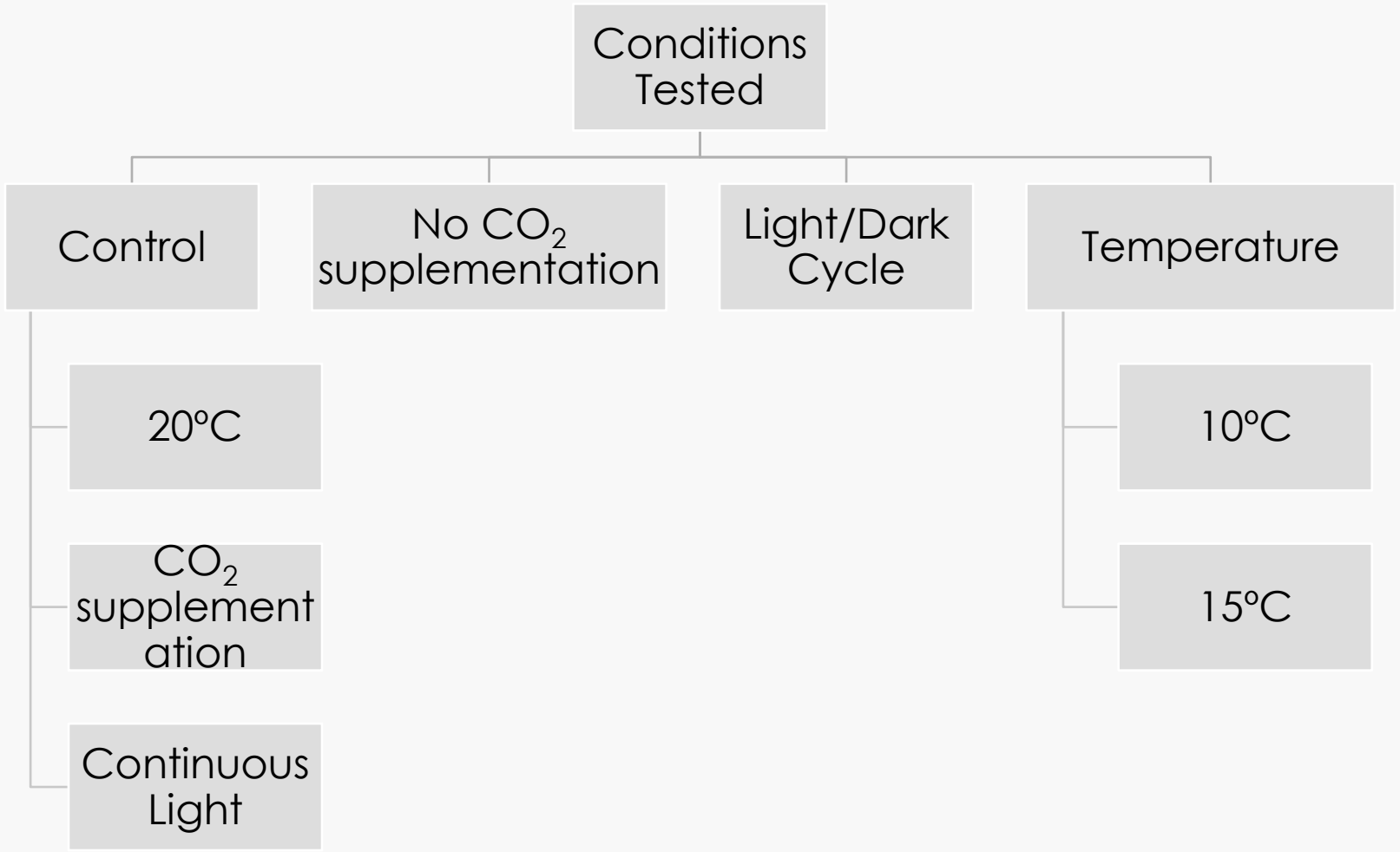
Microalgae as a source for Lutein

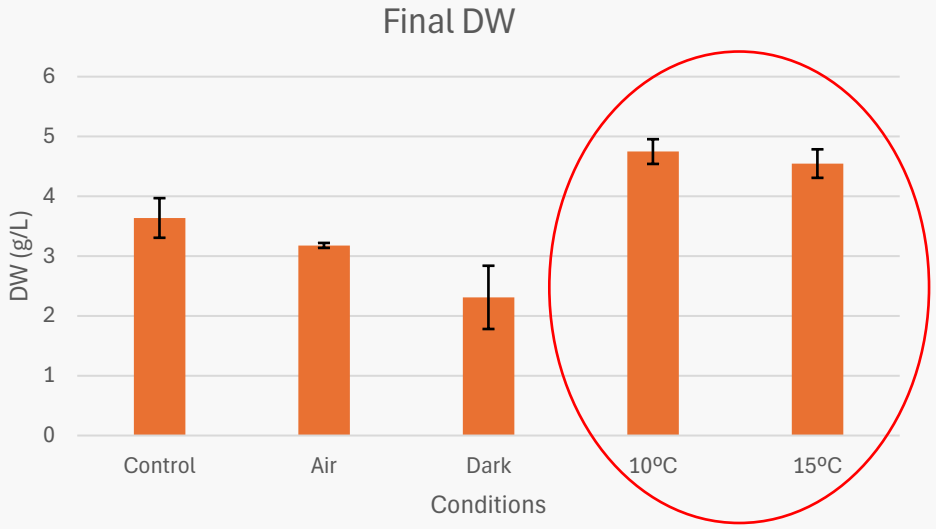
Monoraphidium sp.

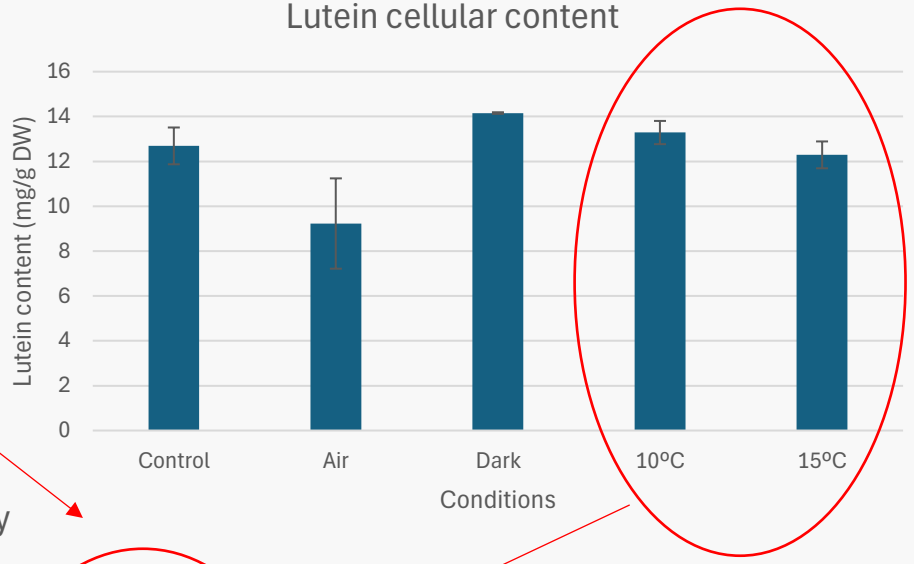
Capable of accumulating Lutein up to 26.39 mg g^{-1} DW in a outdoor TL-RWP*

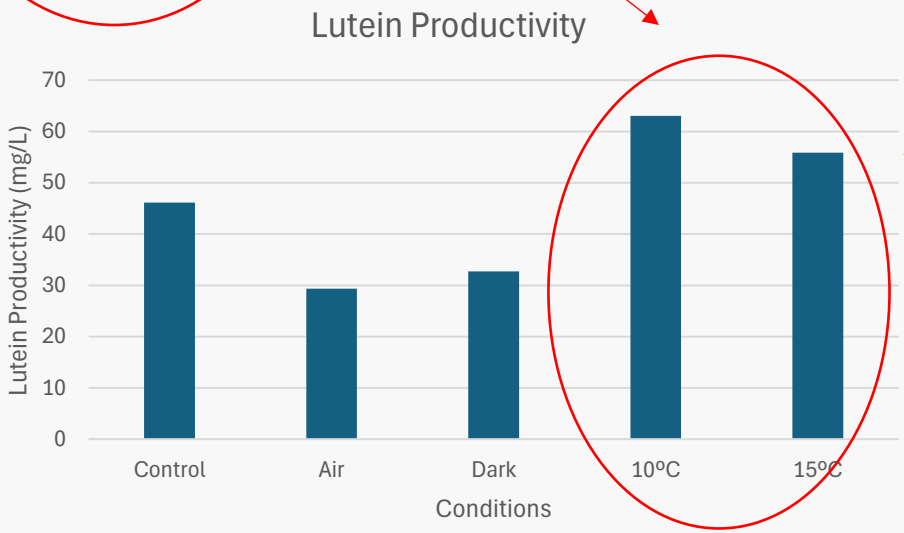
Cold-tolerant species cable of growth at ranges between 20°C-10°C

* DOI: 10.1016/j.algal.2022.102926


Conditions Tested
Control
No CO_2 supplementation
Light/Dark Cycle
Temperature
20ºC
CO_2 supplementation
Continuous Light
10ºC
15ºC





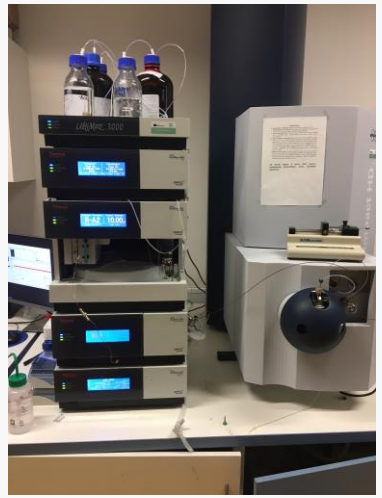


Higher productivities at 10ºC (29%) and 15ºC(21%)

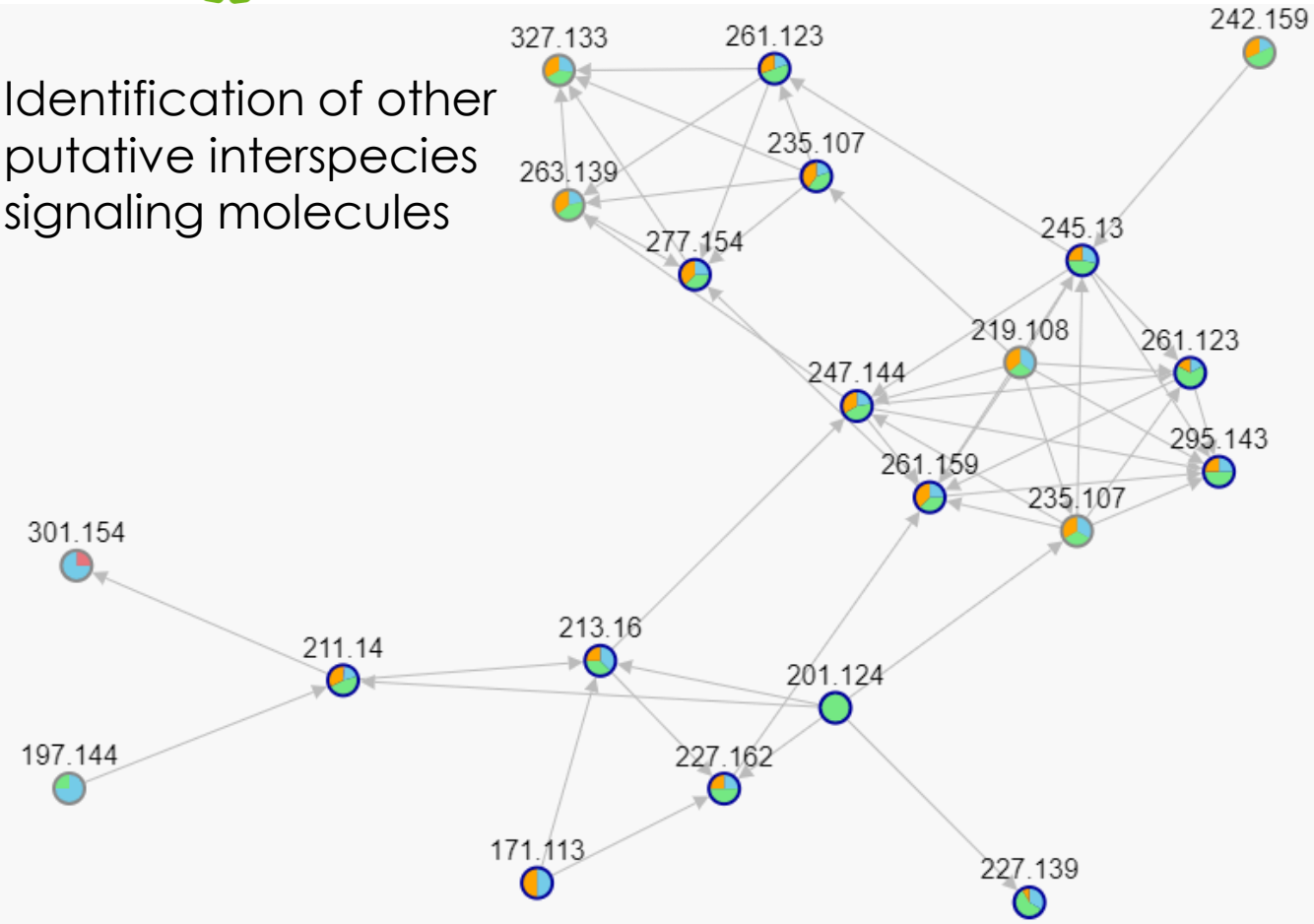
Biotic factors

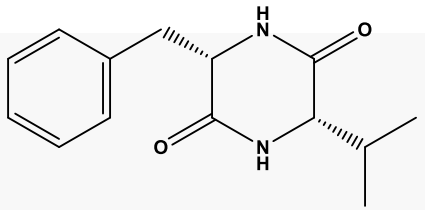
Taping into the microalga's phycoshpere

Growth in LB Media → Plate innoculation → Isolation of bacteria → Supernatant extraction → MS analysis

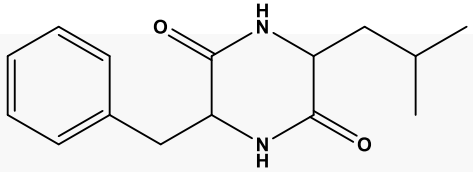


Identification of other putative interspecies signaling molecules

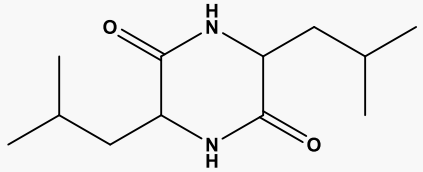




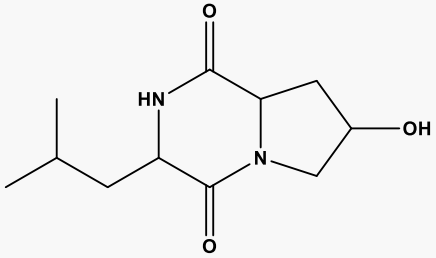
Cyclo(L-Phe-L-Val)



Cyclo(Phe-Leu)



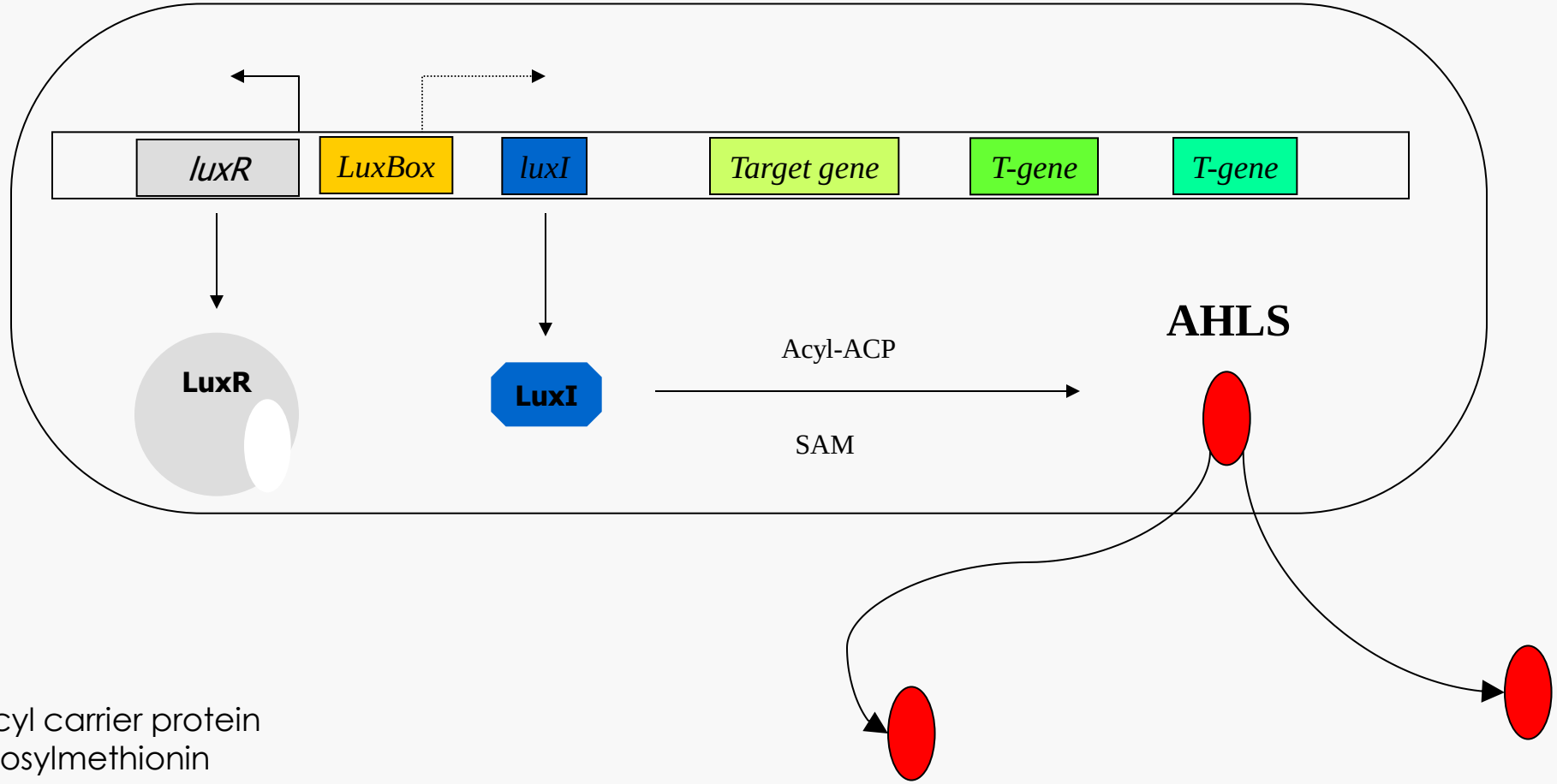
Cyclo(L-leucyl-L-leucyl)



cyclo-[L-(4-hydroxy-Pro)-L-leu]

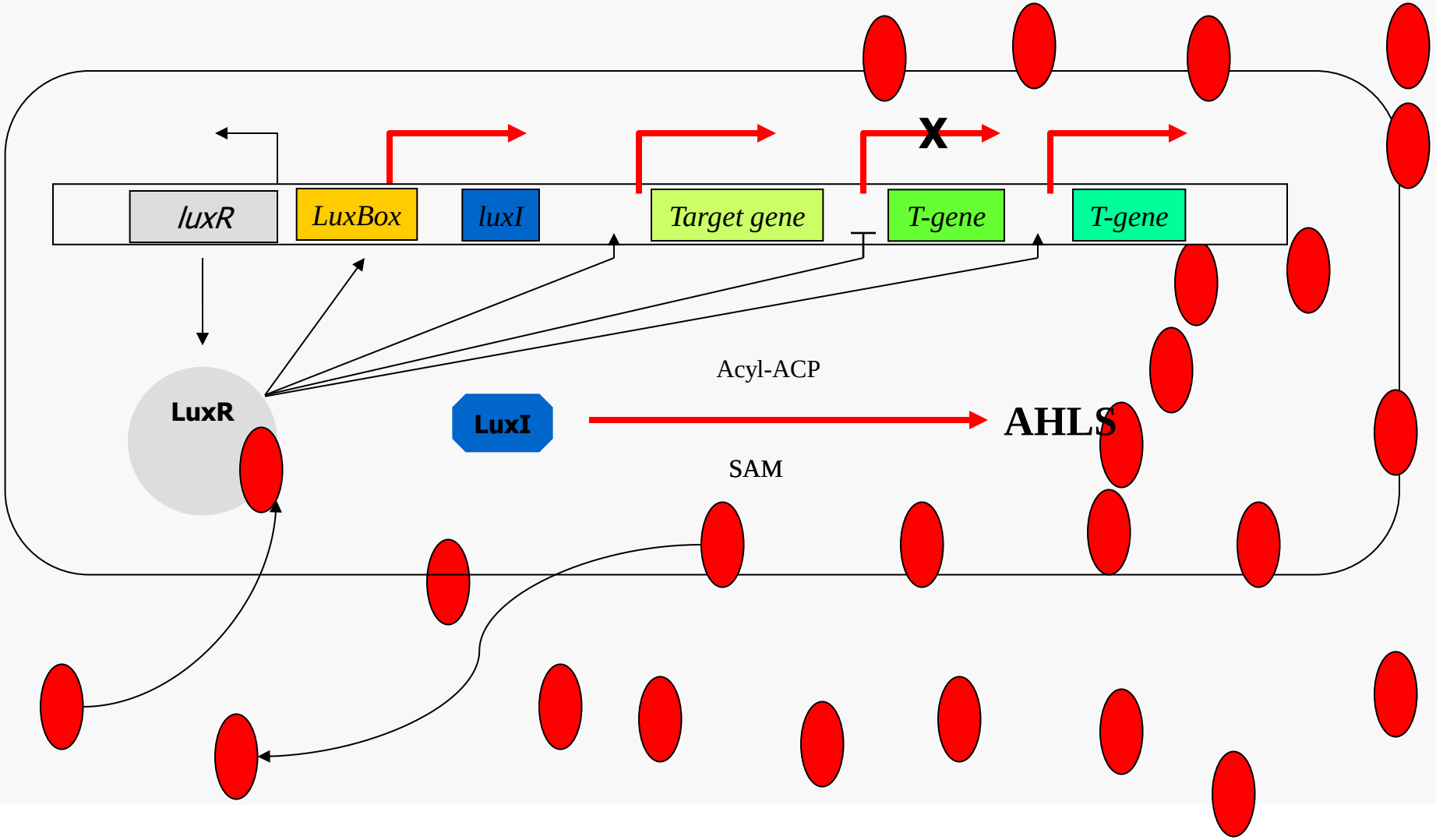
Algal supernatant extract as well as few bacterial isolates showed the presence of cyclic dipeptides known to function as interspecies signaling molecules as well as quorum sensing inhibitors.

Typical QS system : at low [AHL]



Substrates:
Acyl-ACP = Acyl carrier protein
SAM = S-adenosylmethionin

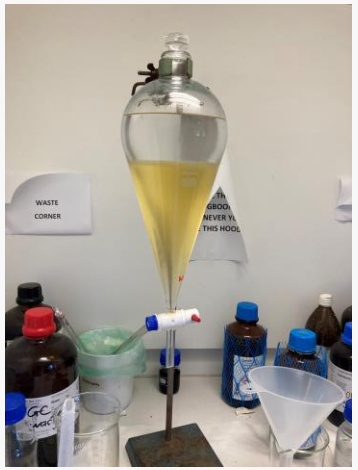
Typical QS system : at high [AHL]



Cultivation of the bacteria

Centrifugation and filtration of the supernatant

Extraction and concentration



Activity assay

Concentration Optimization

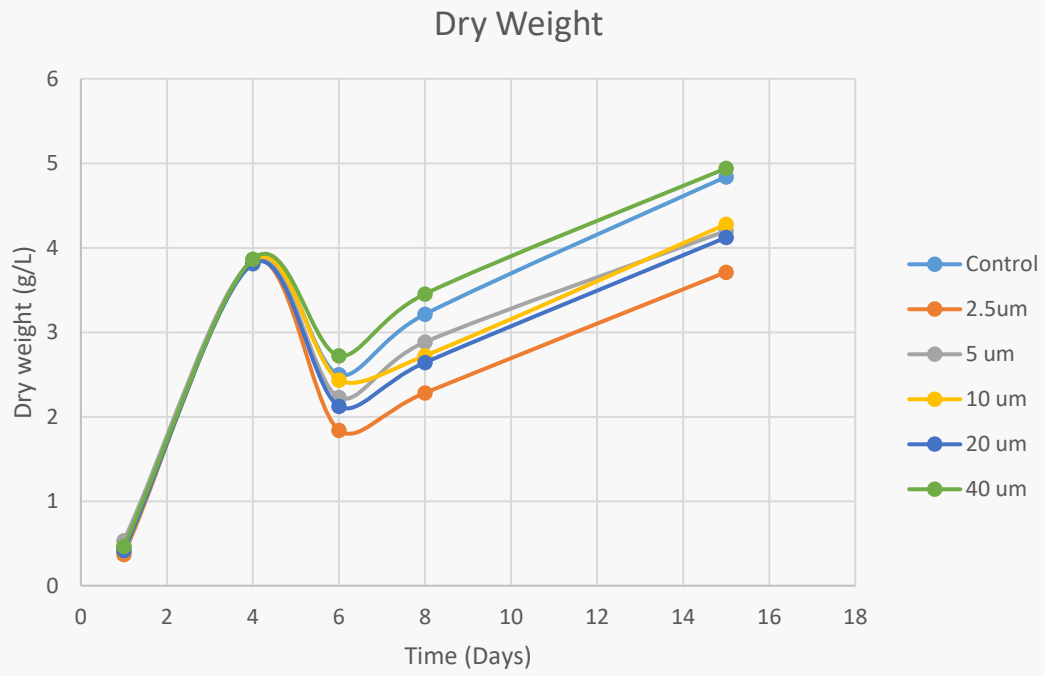


Fig - Dry mass evolution of tested groups during the assay

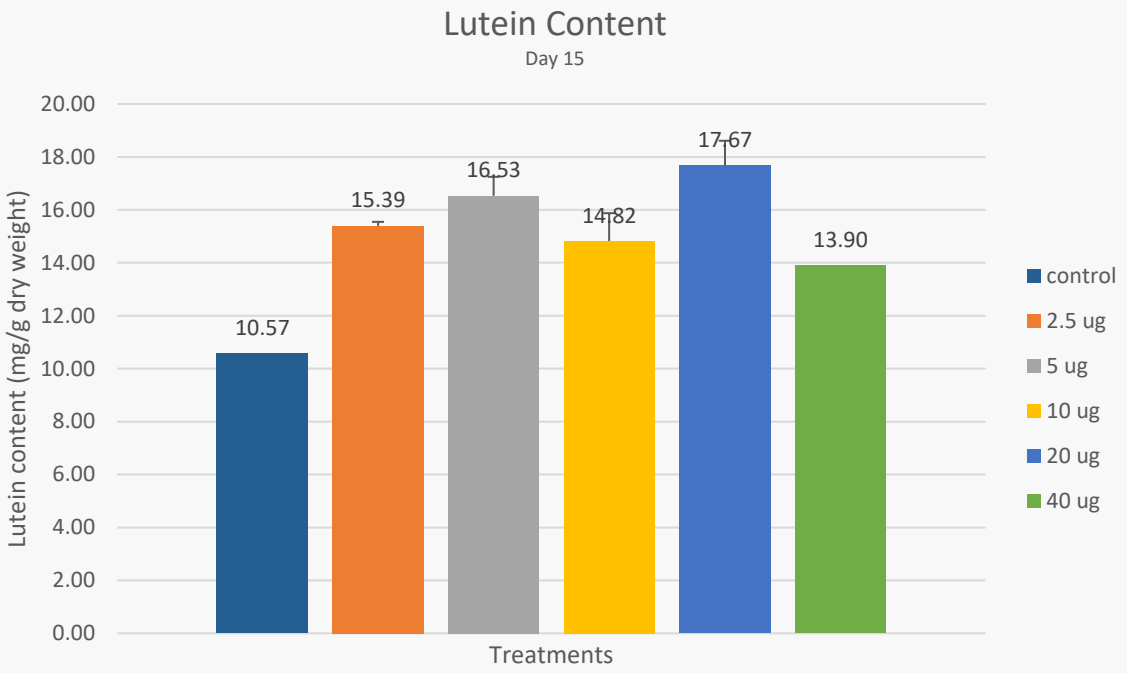


Fig – Lutein content of each test group at the end of the assay

Growth Assay

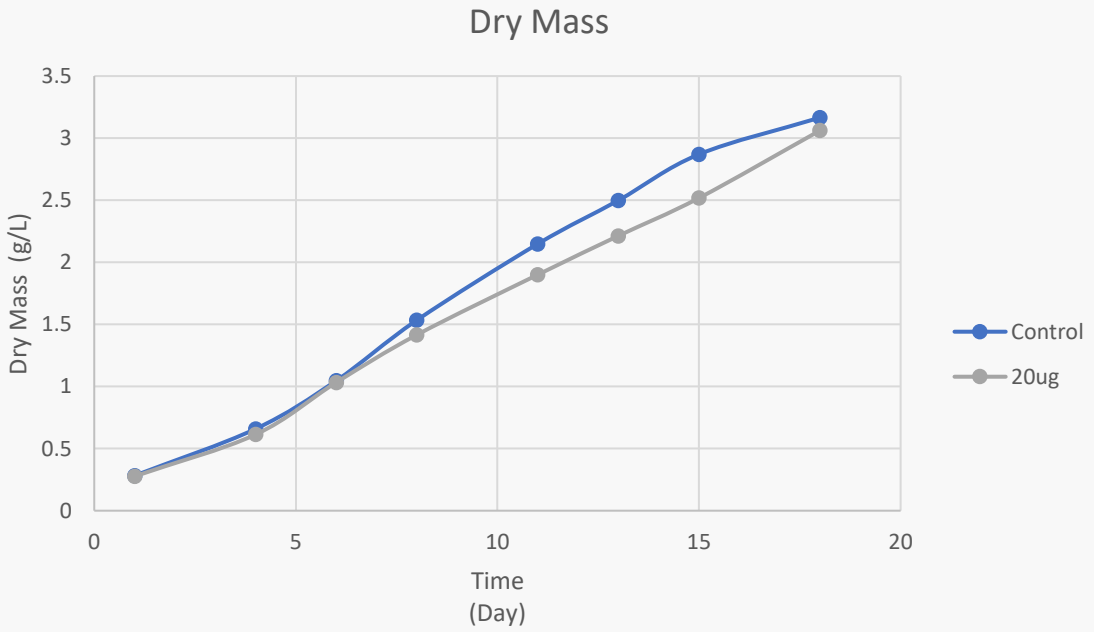


Fig - Dry mass evolution of tested groups during the assay

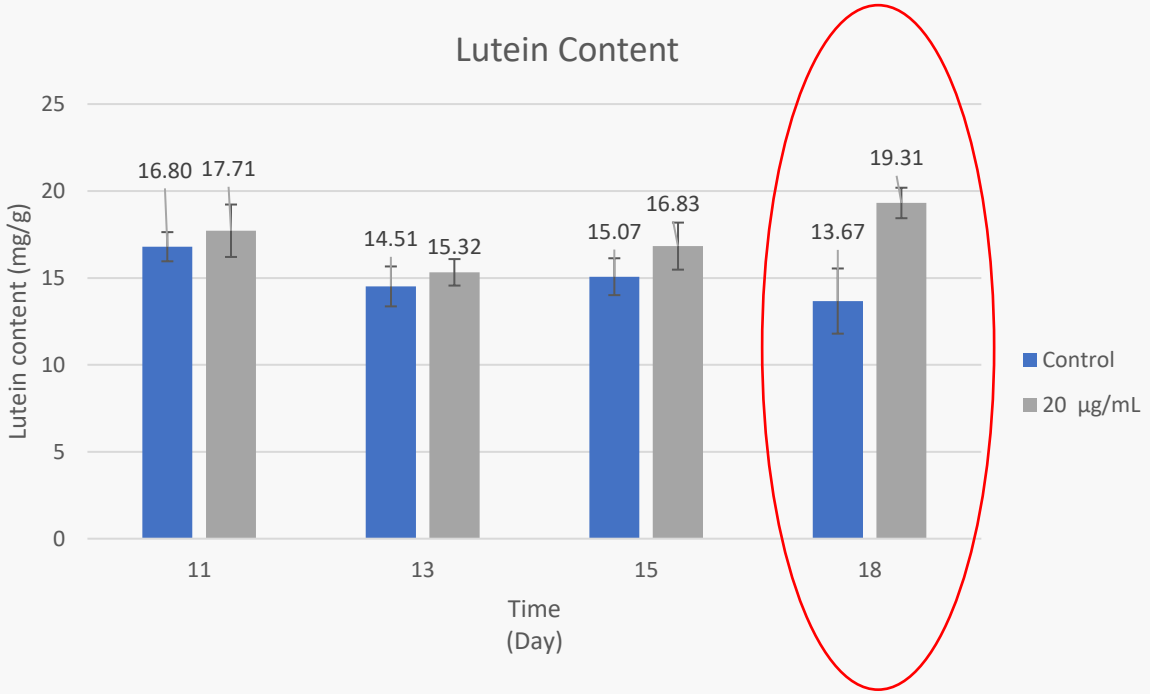


Fig – Lutein content of each test grous in the final part of the assay

41% Improvement

Biotic factors

UV mutagenesis

Chlorella vulgaris

	max growth rate	doubling time	Average % DM		
Strain	**μ (h^{-1})**	**h**	**chlorophyll a**	**carotenoids**	**starch**
***Chlorella vulgaris* heterotrophic**	0.13 ± 0.07	5.33	0.03 ± 0	0.02 ± 0	12.86 ± 5.09
mutant no. 1	0.29 ± 0.38	2.39	0 ± 0	0.02 ± 0.01	8.16 ± 0.87
mutant no. 2	0.28 ± 0.37	2.45	0 ± 0	0.01 ± 0	6.37 ± 0.47
mutant no. 3	0.29 ± 0.38	2.39	0 ± 0	0.05 ± 0.01	26.36 ± 1.37
mutant no. 4	0.33 ± 0.37	2.1	0.02 ± 0.01	0.04 ± 0	25.71 ± 1.17
mutant no. 5	0.29 ± 0	2.39	0.04 ± 0	0.03 ± 0	14.37 ± 0
mutant no. 6	0.29 ± 0.34	2.42	0.04 ± 0.02	0.04 ± 0.02	10.08 ± 5.2
mutant no. 7	0.28 ± 0.37	2.45	0.03 ± 0	0.04 ± 0	31.7 ± 3.33

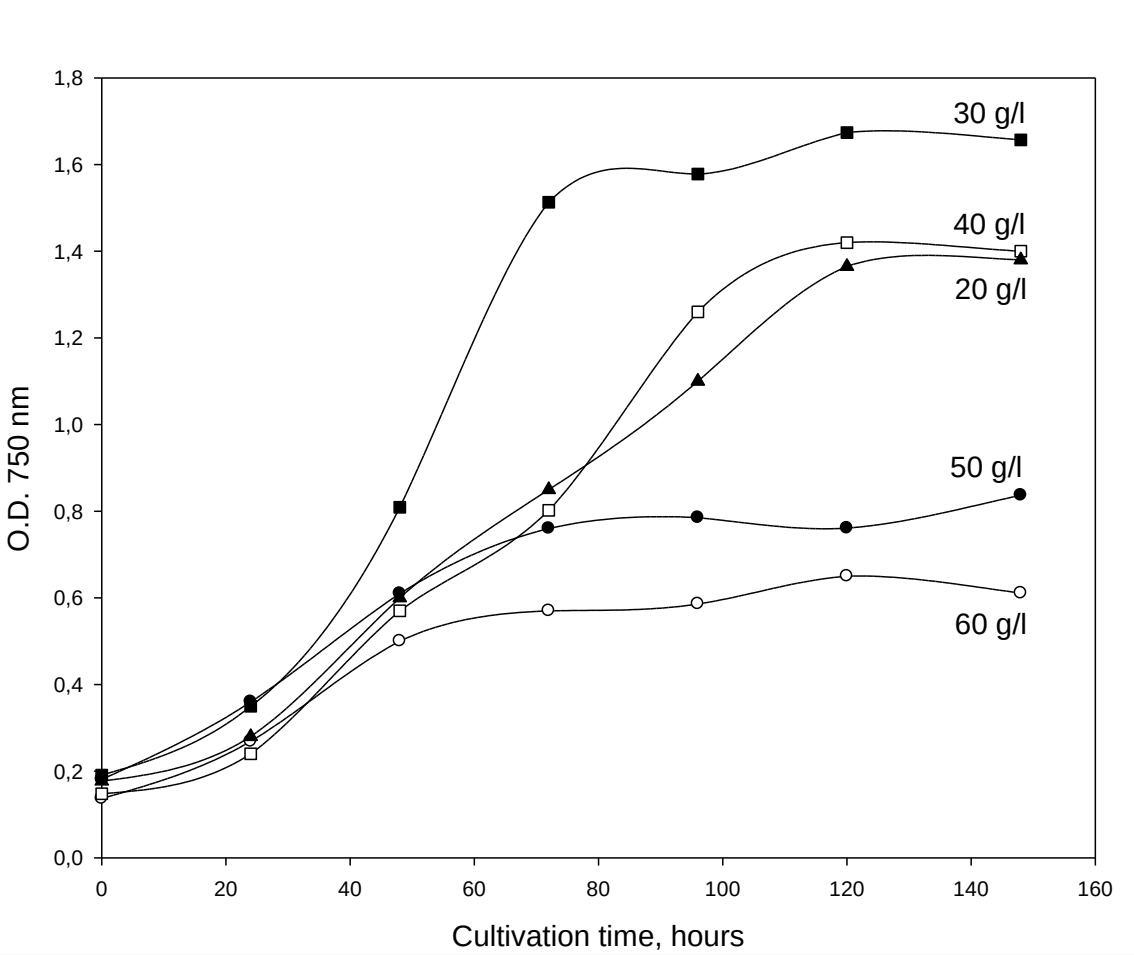
Lutein production in novel and existing mutants

Mutant no.	Lutein [%]	Chlorophyll a [%]
MT1	0.556	0.329
MT2	0.454	0.034
MT3	0.568	0.116
MT4	0.324	0.033
MT5	0.562	0.186
MT6	0.314	0.021
MT7	0.236	0.023
HY-1	0.479	0
EXT1	0.398	0

Strains HY-1 and EXT1 are great lutein producers as they lack chlorophyll.

MT2 mutant has reasonable lutein production, higher growth rates and biomass productivity.

Growth optimization – HY-1 mutant



4-factor Central Composite Design with variables: X_1 glucose content, X_2 nitrogen content, X_3 phosphorus content, X_4 temperature.

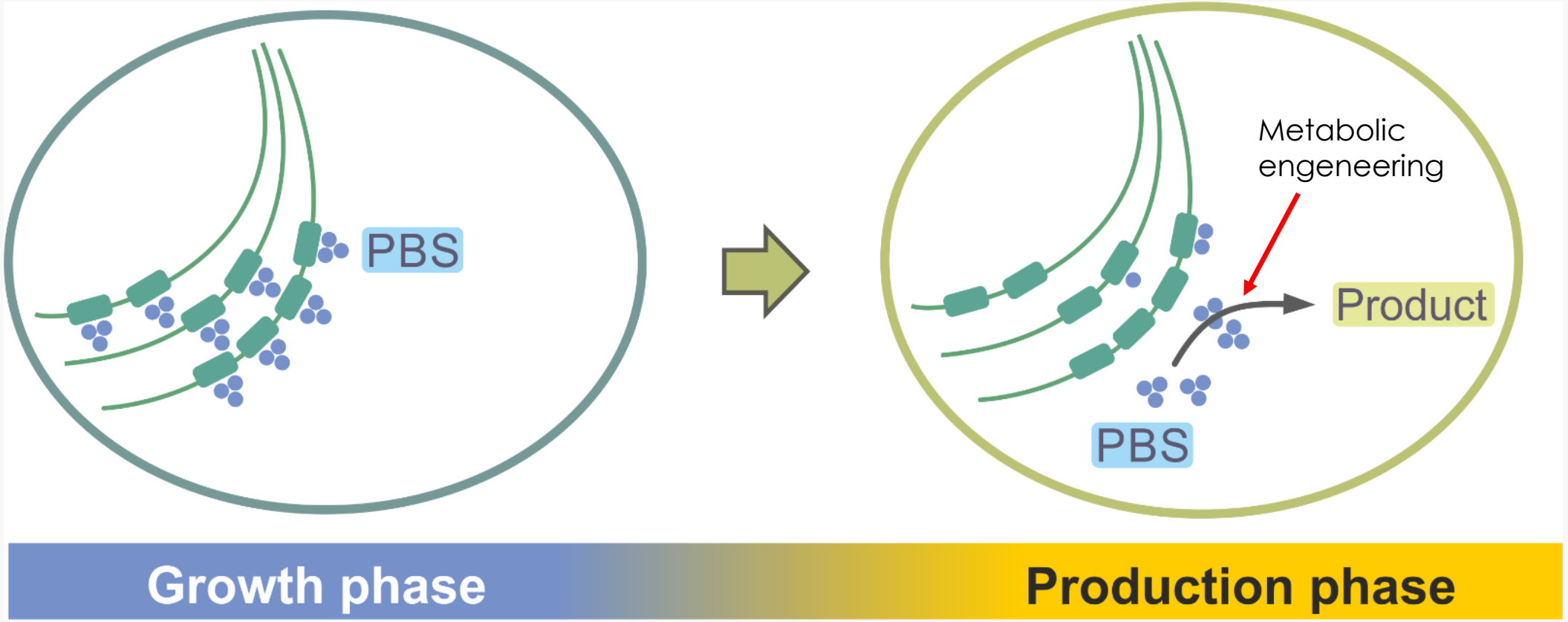
Response variables: growth rate (μ), lutein content. Culture pH=7, mixing speed = 300 rpm, aeration = 1000 mL/min.

Optimal conditions:
Specific growth rate: μ= 0.034 h^{-1}
Optimal glucose concentration: 30 g/L
Optimal KNO_3 concentration: 12.5 g/L
Optimal KH_2PO_4 concentration: 2 g/L
Optimal temperature: 30°C

Biotic Factors

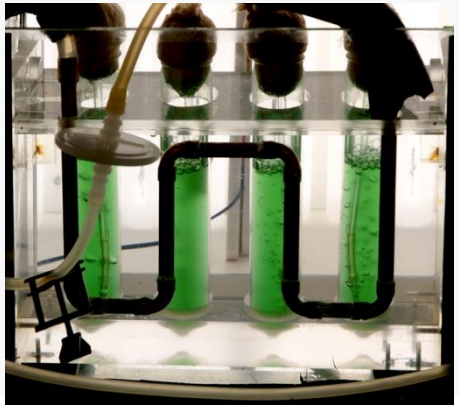
Targeted genetic modifications

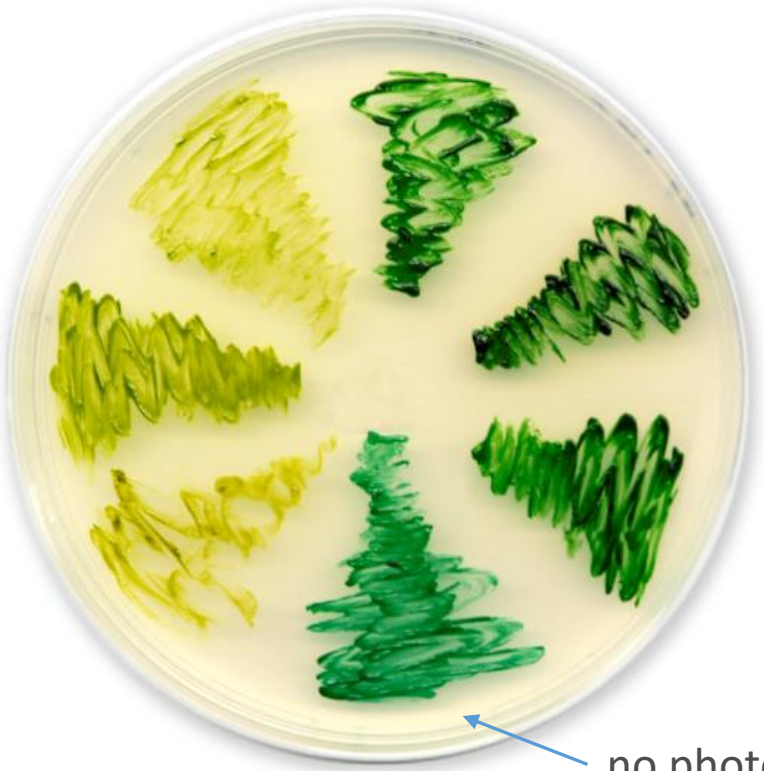
Production of **therapeutic peptides** in cyanobacteria



Co-funded by
the European Union
MINISTRY OF EDUCATION, YOUTH AND SPORTS
PHOTO MACHINES

Bacterial model -> max doubling time around 4 h, easy to construct mutants, available genetics tools (CRISPR/Cas, plasmids, homologue recombination...), multiple mutagenesis ... (~ 3000 mutant strains in Třeboň collection). Capable to grow heterotrophically.





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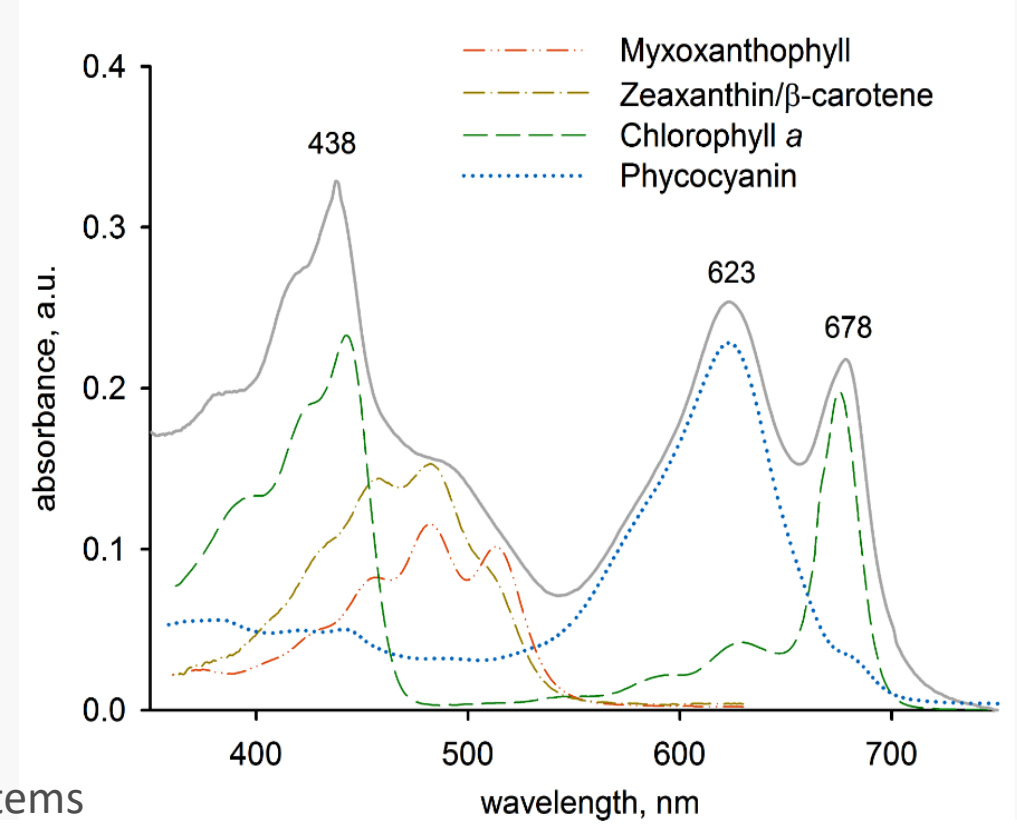
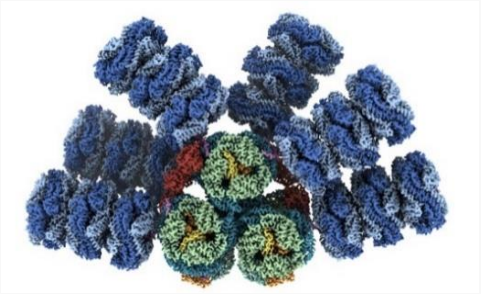
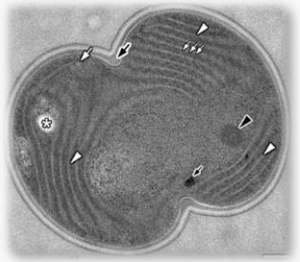






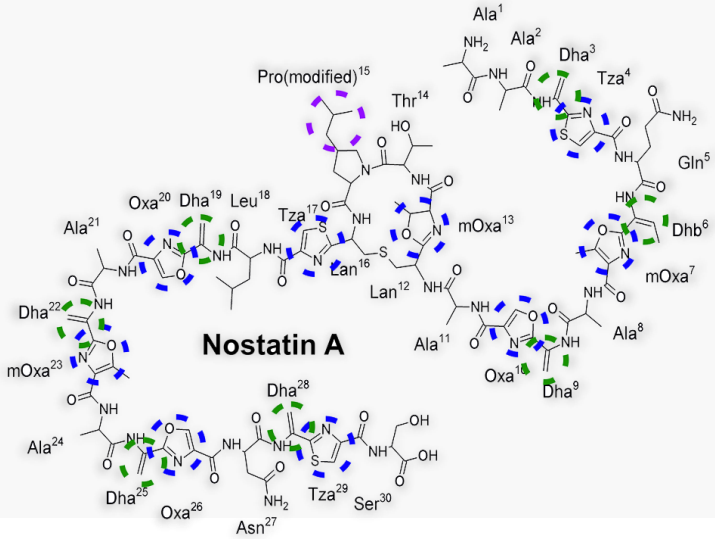
Syn2Cell system

Induction of phycobilisome degradation
Photosystem stability
Balanced cell metabolism
Synthetic light-harvesting antennas

Development of a Raman detector for high-throughput cell screening

New metabolic pathways/enzymes for biotechnology applications

Regulation of the accumulation of energetic reserves in algae

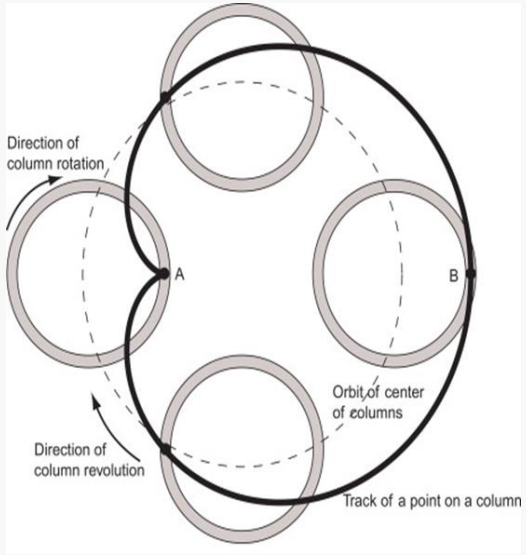


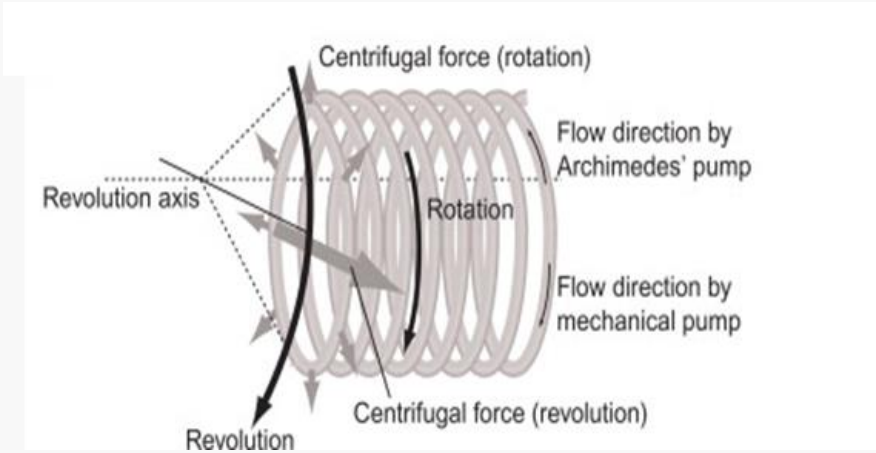
*"Prototype of the **Syn2Cell system** will be developed and then extensively modified to produce **therapeutic peptides**"*

How to get these metabolites

Countercurrent Chromatography (CCC)

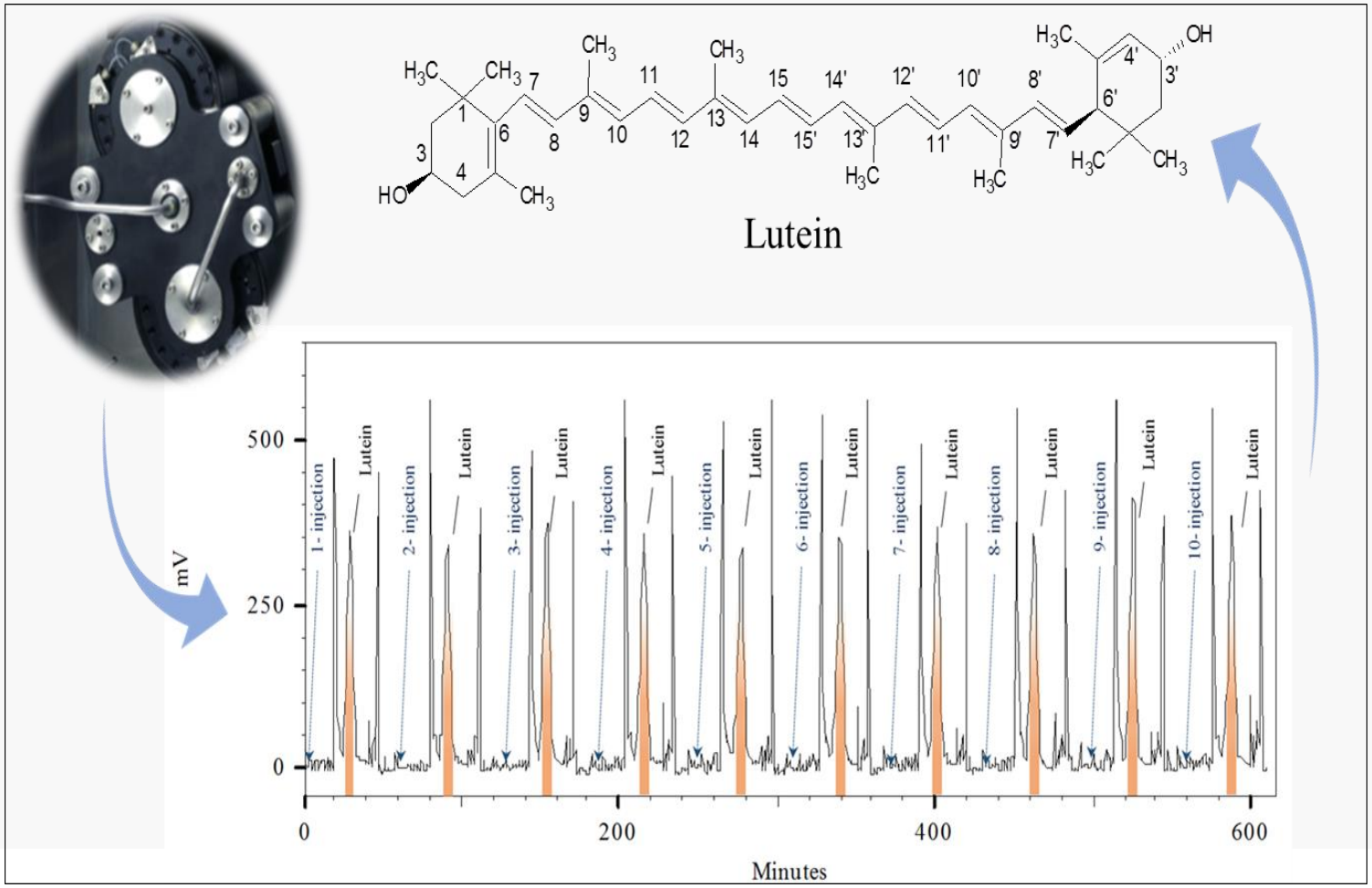
Principles





- Uses two immiscible liquid phases and no solid support.
- Retention of stationary phase by centrifugal force.
- The separation key: partition coefficient (*K*).
- There is a sum of centrifugal forces.
- The maximum centrifugal force is at point B (separation into two phases).
- The minimum centrifugal force is at point A (vigorous mixing).
- Helical column rotation generates pumping force through Archimedes' pumping action.
- Counter current: pumping action applied by an external pump with mobile phase flow in opposite direction to Archimedes' pump.

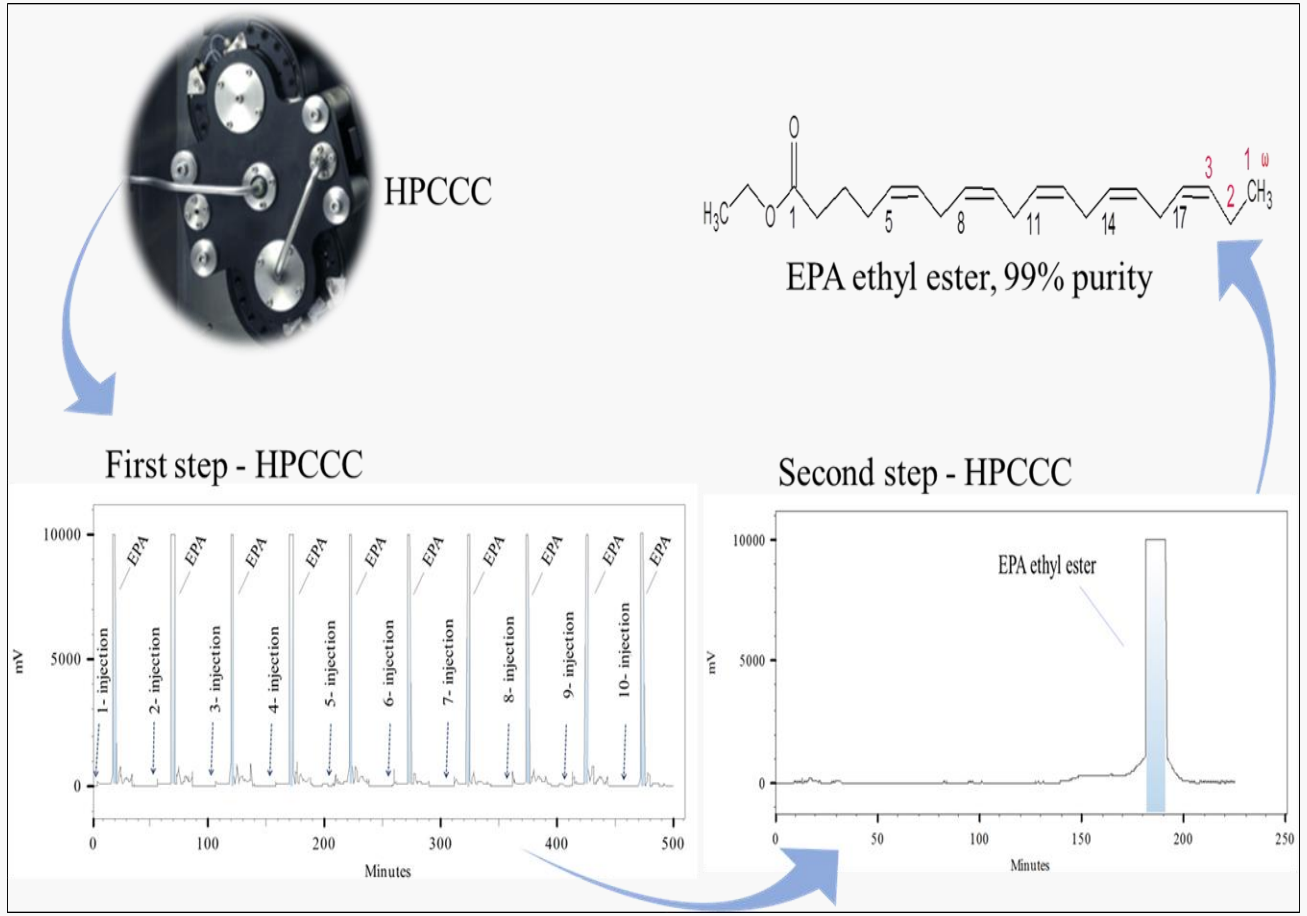
Development of a high performance countercurrent chromatography (HPCCC) method to obtain lutein from *Monoraphidium* sp.



Attributes:

- ✓ Efficient production.
- ✓ Automatic process.
- ✓ Zero solvent waste.
- ✓ Scalable.
- ✓ Flexible.

Separation of eicosapentaenoic acid via two-step high performance countercurrent chromatography.



Attributes:

- ✓ Efficient production.
- ✓ Pharmaceutical use.
- ✓ Automatic process.
- ✓ Zero solvent waste:
  Phase formulation
  Solvent recycling
- ✓ Scalable.
- ✓ Food grade process.
- ✓ Flexible.

Take home messages

The optimization of algal strains allows us to enhance biomass production, which is essential for the expression of valuable metabolites

The vast diversity of algal species offers a wide range of genetic resources that can be harnessed. This diversity is crucial for adapting to different environmental conditions and maximizing productivity

The combination of biotic and abiotic techniques can significantly enhance growth rates and metabolite expression

It is important to consider the end goal of the optimization. Certain strategies, i.e. target mutagenesis cannot be used for human food

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