Assessing *Botryococcus braunii* hydrocarbon production potential using original functional and physiological screening approaches.

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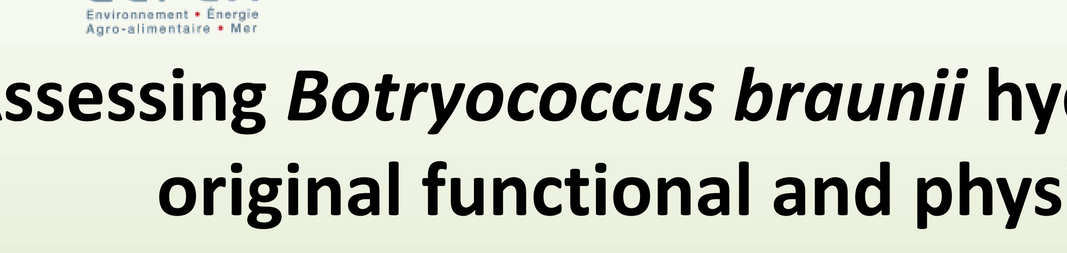
BOTRYOCCCUS BRAUNII: A HIGH BIODIVERSITY AND A VERY SPECIFIC PHYSIOLOGY

The microalga *Botryococcus braunii* is known for its potential as a source of renewable energy by producing hydrocarbon fuels[1].

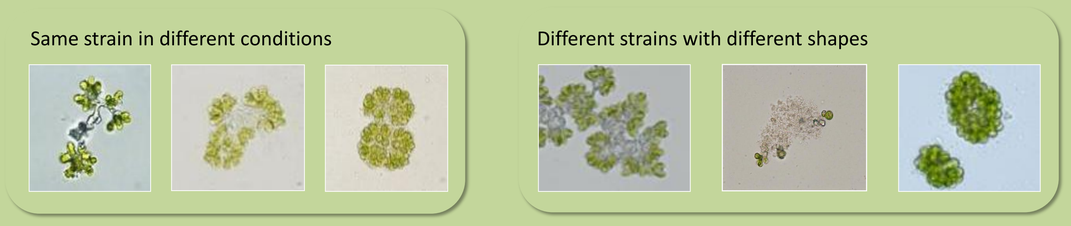
- Hydrocarbon accumulation in some *B. braunii* strains can achieve 60% of dry biomass[2]
- This species has been divided in three chemical races A, B and L related to the structure of their principal hydrocarbons[3].
- Other strong features of *B. braunii* are its low growth rate, with a minimum generation time of 2 days, and its original colonial morphology where the cells are embedded in a mucilaginous sheath.



A typical *B. braunii* culture : with big colonies naked-eye visible in flasks. The colonies are grape shaped and the cells are pear shaped and maintained with a mucilaginous sheath (strain P2 in these photos).

x400

- 12 strains were studied, originating from european banks of strains : Algobank (Caen), CCAP (Oban), CCALA (Trebon), SAG (Gottingen) and from a private source.
- Strains are part of the A and B race, some strains have no race identified and from one strain (P2), no specific B. braunii hydrocarbons have been identified.



Examples of B. braunii variability: the colony shape, showing the variable part of the extracellular sheath in the colony.

STRAIN AND CULTURE CONDITIONS SCREENING METHODOLOGY

Given the originality and the diversity of the morphology, the growth rate and the lipid contents, a screening of strains and culture conditions is required.

Objective

Determinate the best culture conditions for the highest hydrocarbon productivity with the strains studied.

12 strains

Growth rate

Biological variability

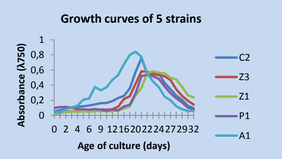
Hydrocarbon rate

Chemical variability

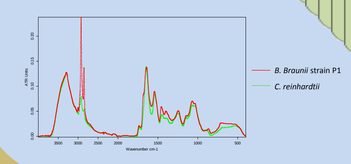
STRAINS SCREENING → SELECTED STRAINS

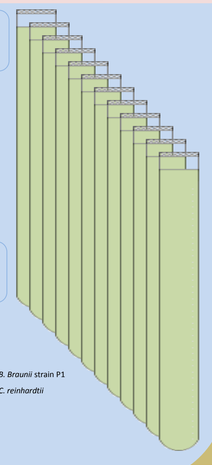
Cultivation system : low volume systems run in paralell, especially adapted to *B. braunii* original colonies-growing form.

- Ability to grow in PBR (λ750 measurement)



- Hydrocarbons nature and rate (FTIR profile)



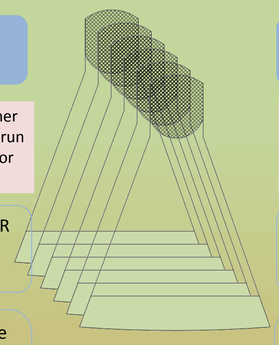


PHOTOBIOREACTOR CULTIVATION AND OPTIMISATION OF CULTURE CONDITIONS

CULTURE

Cultivation system : higher volume systems in flasks run in paralell, shaked and/or CO_2 supplemented.

- Ability to grow in PBR (λ750 measurement and dry weight)
- Hydrocarbons nature and rate (FTIR profile and GC-FID and GC-MS analysis of extracted lipids)

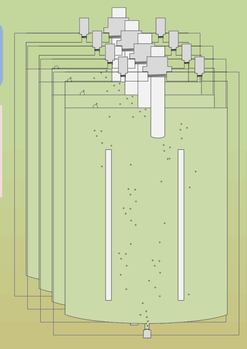


- Monitoring of colony and cells morphology (optic microscopy)

PRODUCTION

Cultivation system : specific shaped PBR with pH and temperature controlled, run in paralell.

- Ability to grow in PBR (λ750 measurement and dry weight)
- Hydrocarbons nature and rate (FTIR profile and GC-FID and GC-MS analysis of extracted lipids)

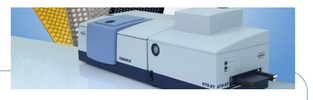


- Monitoring of colony and cells morphology (optic microscopy)

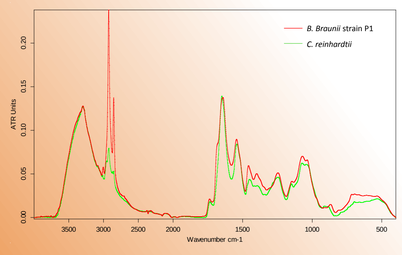
FUNCTIONAL SCREENING METHODOLOGY

FRESH BIOMASS

FTIR-HTSXT



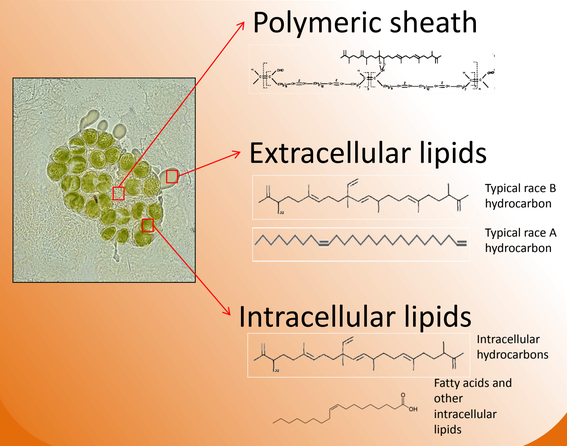
Quick functional screening of hydrocarbon and other lipids



Targetted vibrators to know for spectra interpretation :
- 2900cm^{-1}, aliphatic chains
- 1740cm^{-1}, lipids

EXTRACTING PROCEDURE

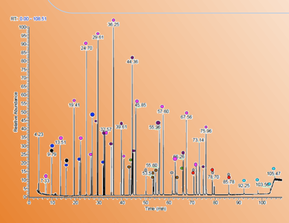
Extraction of lipids in fractions corresponding to different localization in the colony and different polarity of the lipids[3]



EXTRACTED BIOMASS

GC-MS
Py-GC-MS
GC-FID

- Analysis of the lipid fractions with and without derivatization (esterification of fatty acids).
- Pyrolysis is used to study long carbon chain lipids (up to C40).

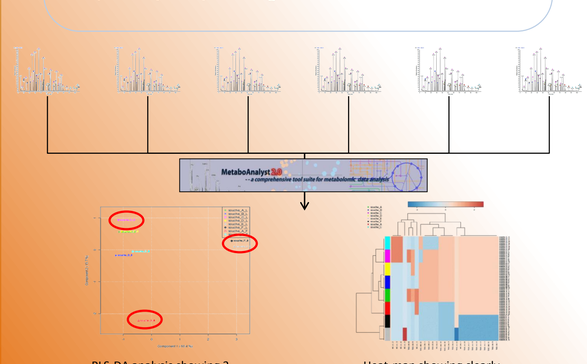


GCMS profile of the apolar and intracellular fraction of a strain P1 culture.

The first results shows mainly profiles with long chain fatty acids and long chain hydrocarbons, linear or squalen-like. Precise identifications are in progress.

MULTIVARIATE ANALYSIS

This lipidomic approach allow to use a high quantity of information to highlight tendencies without apriori. As a result, groups of strains associated to specific culture conditions can be discriminated depending on the nature and quantity of lipids targetted.



PLS-DA analysis showing 3 distinct groups of strains. One probably corresponding to A race strains, one to B race strains, one to non-hydrocarbon synthesizing strain.

Heat-map showing clearly groups of strains synthesizing the same molecules (molecules on abscissa, strains with 10 replicates on orinate)

PERSPECTIVES

Conception and setting up of screening and characterization tools (PBR, analysis platform)

→ Selection of strains having high hydrocarbon productivity with corresponding growth conditions.

→ Suggestion of a strain and the corresponding culture conditions leading to hydrocarbons as a new biokerosene source.

1 Metzger, P., Largeau, C., 2005. Botryococcus braunii : a rich source for hydrocarbons and related ether lipids. Applied Microbiology and Biotechnology 66, 486-496.
2 Banerjee, A., Sharma, R., Chisti, Y., Banerjee, U. C., 2002. Botryococcus braunii: A Renewable Source of Hydrocarbons and Other Chemicals. Critical Reviews in Biotechnology 22, 245-279.
3 Metzger, P., Largeau, C., 1999. Chemicals of Botryococcus braunii. In: Z, C. (Ed.), Chemicals from microalgae, pp. pp 205-260.

http://www.gepea.fr/