Galdieria sulphuraria heterotrophic metabolism in the presence of different carbon sources

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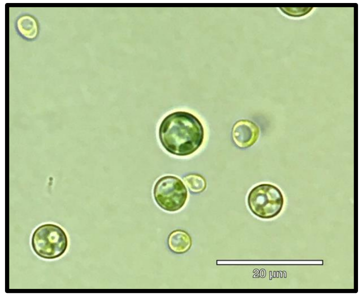
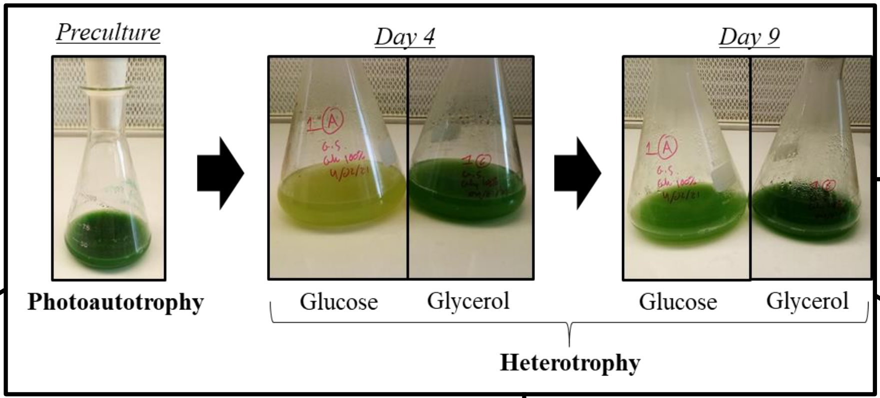
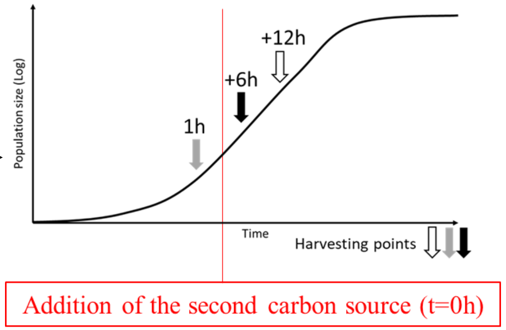


Galdieria sulphuraria 074W

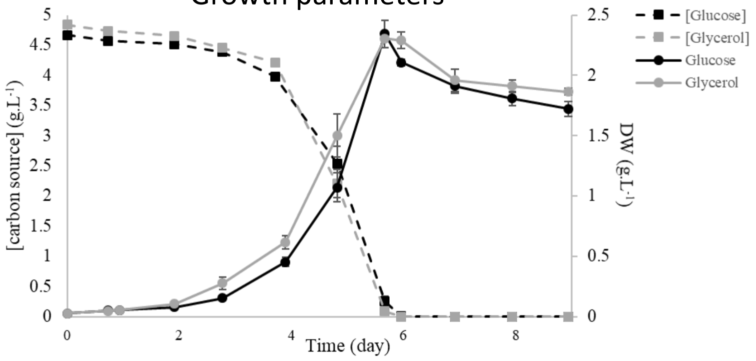
Colour difference depending on the growing conditions



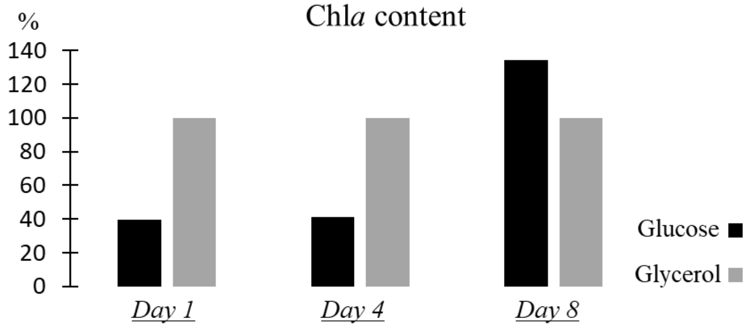
Time-course experiment



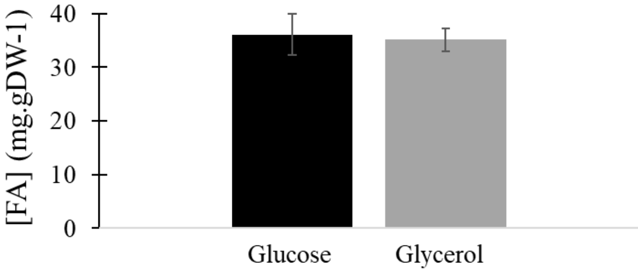
Growth parameters



Chl*a* content



Total FA DW content in stationary phase



Introduction

Why studying extremophilic organisms ?

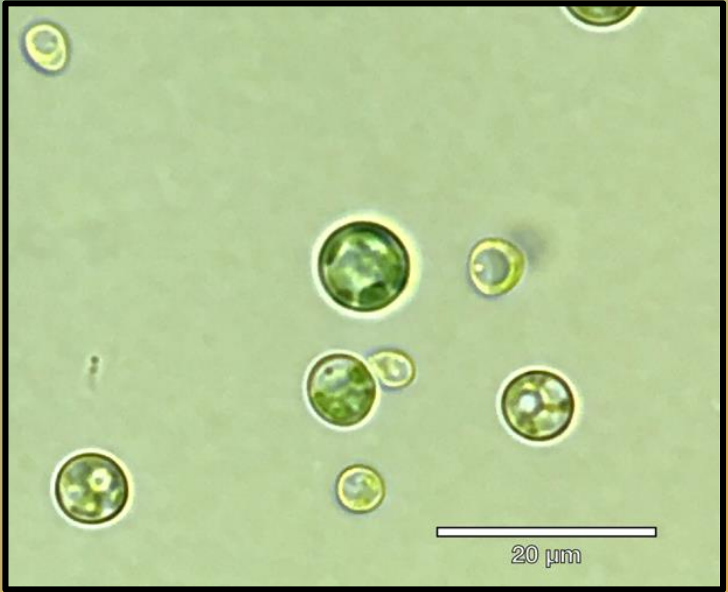
- Growth in too extreme conditions for the majority of the life forms
- Decreased risk of contamination
- Can be grown in harsh environment (e.g. wastewater)

Galdieria sulphuraria

- Thermoacidophilic red microalga
- Able to grow at high T° up to 56°C and low pH (0-4) [1]
- Resistant to high heavy metals and high salt concentrations [2]
- Phototroph or heterotroph (with more than 50 carbon sources) [3]
- Studied for high-added value biocompounds (e.g. phycocyanin, α-tocopherol) [4] or wastewater bio-remediation [5]

In this study

- Heterotrophic growth of *Galdieria sulphuraria*
- Carbon sources : glucose or glycerol



Galdieria sulphuraria 074W

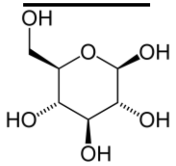
Sulphurous acidic hot spring in Yellowstone National Park

Objectives

- Determination of the differences of *Galdieria sulphuraria* heterotrophic growth under two different carbon sources, either glucose or glycerol
  - Growth parameters
  - Pigment content
  - Fatty acid content
- Set up of an experimental design to compare transcriptomes while growing under glucose or glycerol

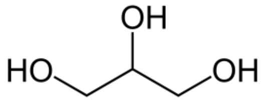
Growth conditions

Glucose



MM = 180,156 $g.mol^{-1}$

Glycerol



MM = 92,094 $g.mol^{-1}$

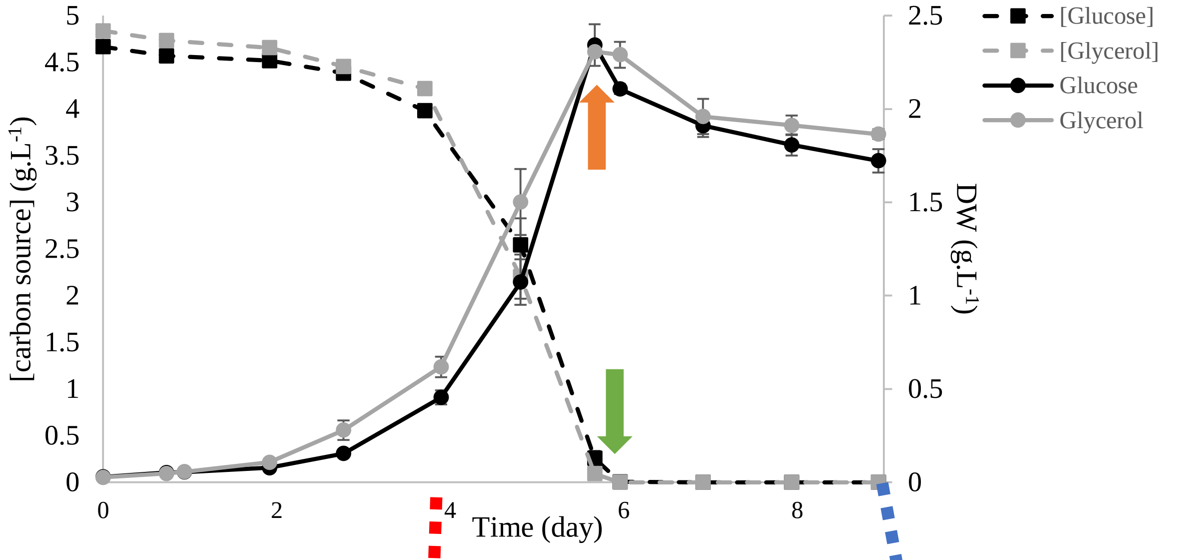
2 Conditions	Glucose	Glycerol
(mM)	25	50
($g.L^{-1}$)	4,50	4,60
Carbon atoms (mM)	150	150

- *G. sulphuraria* strain 074W
- Heterotrophically (in the dark) with glucose or glycerol
- 2xGS Modified Allen culture medium
- T° = 42°C
- pH = 2
- Constant shaking

Colour changes in heterotrophic cultures of *G. sulphuraria*

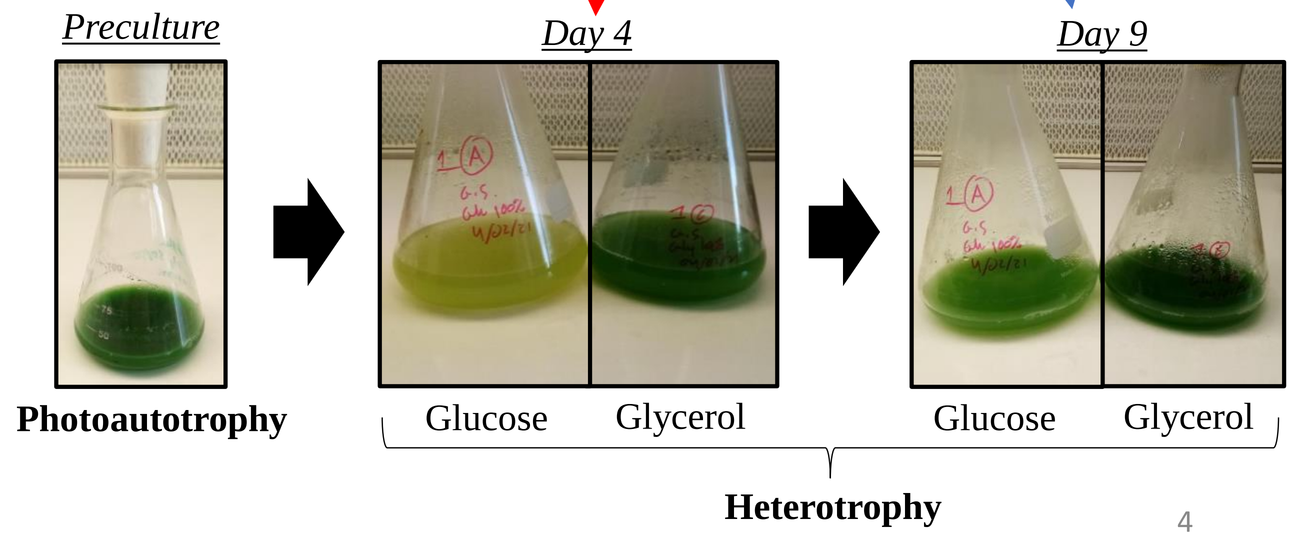
Graph in the **top right** :

- Solid lines : DW ($g.L^{-1}$)
- Dashed lines : carbon source consumption ($g.L^{-1}$)
- Maximal biomass concentration : ~2,4$g.L^{-1}$ at day 6 for both conditions (orange arrow)
- Stationary phase reached when carbon source is depleted (green arrow)



Pictures in the **bottom right** :

- Day 4 : colour of the culture turns yellow when glucose is added (red dashed arrow)
- Day 9 : green colour recovered in both conditions after glucose depletion (blue dashed arrow)



Fatty acids and chlorophyll *a* cellular content

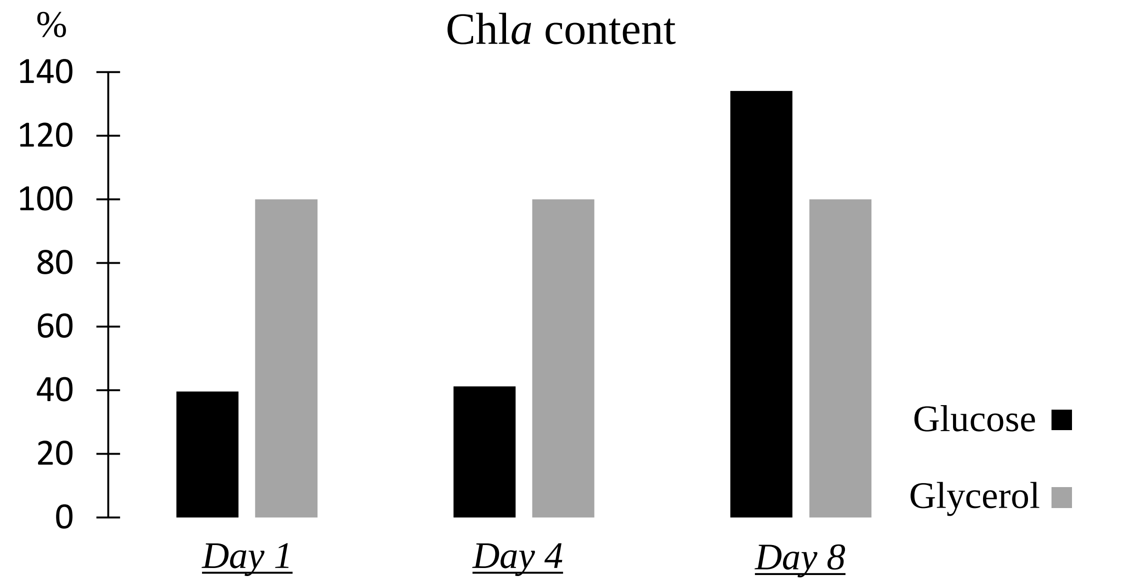
Graph in **bottom left** :

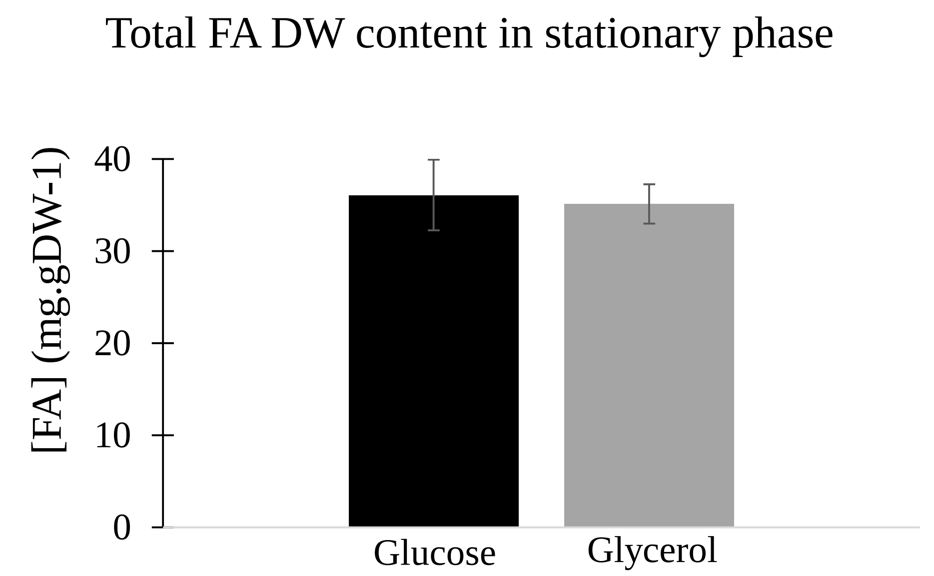
- Day 1 & 4 show exponential phase (carbon source is present)
- Day 8 show stationary phase (carbon source depletion)
- Chlorophyll *a* content is about twice less abundant if glucose is present in the culture (days 1 & 4)
- Glucose extinction correlates with pigment recovery (day 8)

Graph in **bottom right** :

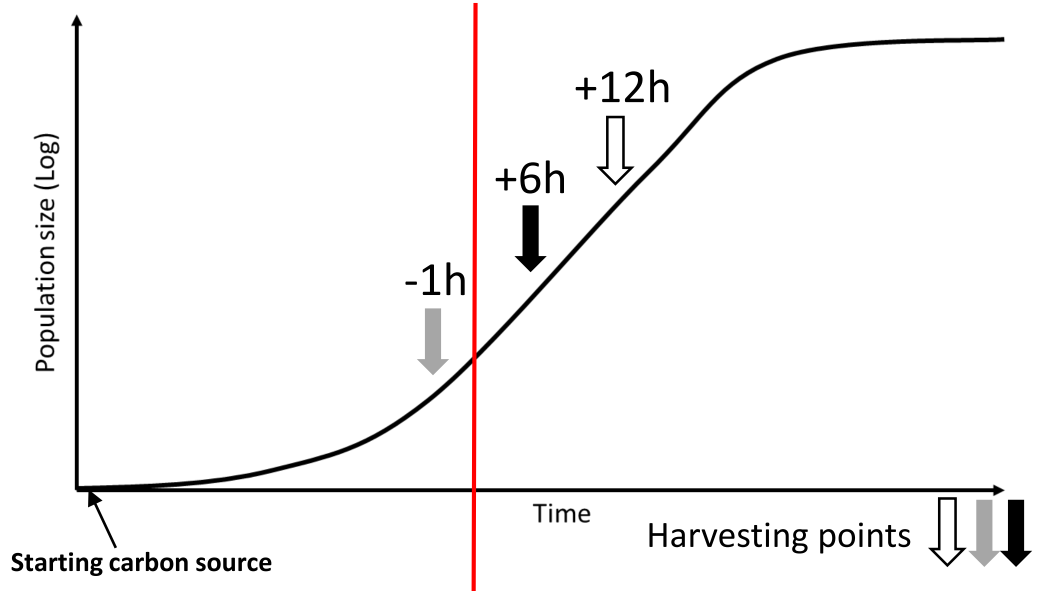
- Total fatty acid content not significantly different between glucose and glycerol condition.
- Fatty acid profile is nearly similar in both conditions (data not shown).

As a perspective, it might be interesting to analyse storage polysacharride profil under the two different conditions.





Transcriptomic analysis – experimental design



Addition of the second carbon source (t=0h)

Condition	Starting carbon source	Second carbon source
A	Glucose (12,5mM)	Glucose (12,5mM)
B	Glucose (12,5mM)	Glycerol (25mM)
C	Glycerol (25mM)	Glucose (12,5mM)
D	Glycerol (25mM)	Glycerol (25mM)

How to study transcriptome variations between the presence of glucose or glycerol ?

- Time-course experiment
- Two carbon source additions in one growth curve (starting and second carbon source)
- 4 different conditions, A and D are controls
- Harvesting at three timepoints (-1h, +6h, +12h) before and after second carbon source addition (t=0h)
- Study of the carbon metabolism and key enzymes in chlorophyll *a* biosynthesis when glucose is present

References :

[1] Hirooka S, Miyagishima S ya (2016) Cultivation of acidophilic algae galdieria sulphuraria and pseudochlorella sp. YKT1 in media derived from acidic hot springs. Front Microbiol 7:1–11.

[2] Ju X, Igarashi K, Miyashita S ichi, et al (2016) Effective and selective recovery of gold and palladium ions from metal wastewater using a sulfothermophilic red alga, Galdieria sulphuraria. Bioresour Technol 211:759–764.

[3] Gross W, Seipold P, Schnarrenberger C (1997) Characterization and purification of an aldose reductase from the acidophilic and thermophilic red alga Galdieria sulphuraria. Plant Physiol 114:231–236.

[4] Cheng F, Mallick K, Henkanatte Gedara SM, et al (2019) Hydrothermal liquefaction of Galdieria sulphuraria grown on municipal wastewater. Bioresour Technol 292:121884.

[5] Bottone C, Camerlingo R, Miceli R, et al (2019) Antioxidant and anti-proliferative properties of extracts from heterotrophic cultures of Galdieria sulphuraria. Nat Prod Res 33:1659–1663.

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