Determination of storage lipid and storage carbohydrate fluxes in single Saccharomyces cerevisiae cells using Synchrotron FTIR microspectroscopy

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Standard Project

Experimental Report template

Proposal title: Determination of storage lipid and storage carbohydrate fluxes in single Saccharomyces cerevisiae cells using Synchrotron FTIR microspectroscopy		**Proposal number: 20100195**
Beamline: SMIS	**Date(s) of experiment:** from: 7 of december to: 11 of december	**Date of report:** 21 of march
Shifts: 12	**Local contact(s): Frédéric JAMME**	*Date of submission: 21 of march*

Objective & expected results (less than 10 lines):

Rarefaction of fossil resources has led to a search of renewable carbon sources. One strategy consists on the development of optimized yeast strains for lipid production used as alternative source of biodiesel. These biotechnological approaches need the identification of key factors involved in the control of quantity and quality of storage lipids. Using genetic modification of Saccharomyces cerevisiae, heterologous expression of structural lipid body plant proteins, we obtained cell population presenting storage lipid overaccumulation. In a same culture, we observed cell to cell heterogeneity in lipid content due to the use of an inducible expression system. Because of this particularity, this strain appears as a powerful tool to evaluate the dynamic of lipid storage and carbon fluxes by measuring the biochemical changes (composition in lipids and carbohydrates) on single cells using the Synchrotron FTIR microspectroscopy.

The objectives of this study are

- Determination of the minimum and maximum of lipid storage in this biological system, and in the longer term, and understanding of lipid dynamic for biotechnological applications and development of yeast strain for biofuel production
- Evaluation of the link between carbon fluxes and in the longer term, modulation of carbon fluxes for the development of optimized yeast strain for lipid production.

Results and the conclusions of the study (main part):

Technical procedure developped for biological material preparation

Yeast cell cultures were conducted in parralel with analysis on the SMIS beamline. We developped at SOLEIL a protocol to allow IR analysis on fresh cells thanks to the infrastructure provided by the biology laboratory. After one (18 hours) or two days (42 hours) of culture, cells were collected only few minutes before analysis by centrifugation and washed one time with water. Two microliter drops were put down and dried on ATR hemisphere (see below) under vacuum. Yeast strains used for this study expressed structural lipid body plant proteins, AtClo1 and AtS3, fused with the GFP protein leading to a possible detection of the chimeric proteins under UV illumination. We also used the corresponding control strains (without expression of plant protein, with or withous GFP)

FTIR microspectroscopy coupled to fluorescence analysis

Synchrotron FTIR microspectroscopic analysis, using the SMIS beamline at the SOLEIL Synchrotron, was carried out on single cell, with a high spatial resolution, thanks to a Zinc Selenide (ZnSe) 4-mm diameter ATR hemispherical Internal Reflection Element. All spectra were recorded on the Continuum XL microscope. Individual spectra were saved in log(1/R) format at 8 cm^{-1} spectral resolution, with 128 co-added scans encompassing the mid-IR region from 4000 to 800 cm^{-1}. We were able to perform 50 single cell IR spectra on each culture (4 strains and two times of cultures on two independent cultures = 800 spectra).

As the SMIS beamline, microscope is equiped for fluorescence imaging, we were able to evaluate expression level of plant protein in single cell by GFP fluorescence observation and acquisition. We could

observe a tight correlation between protein expression level (GFP signal) and storage lipid content. Modification of lipid content in single cell was evaluated by changes in C=O and C-H stretching bands on IR spectra, corresponding to triacylglycerol and fatty acid content. Cells with a high level of fluorescence also presented a increase in C=O and C-H stretching bands and a modification in the CH2/CH3 ratio (figure 1).
We also confirmed the high heterogeneity of the inducible expression system leading to a false estimation of the lipid accumulation capacity of these modified yeast cells by biochemical analysis on large samples.
To our knowledge, the work presented here using the SMIS beamline for FTIR microspectroscopy coupled with fluorescence analysis had never been previously described in the literature.

Concerning storage carbohydrates (trehalose and glycogen) it was impossible to detect clear modifications on IR spectra and multivariate statistical data analysis will be necessary to make a robust conclusion concerning a link between storage lipid and storage carbohydrate fluxes. This work is now in progress on all the set of data obtained in december.
In order to validate the results obtained with the Synchrotron FTIR microspectroscopy, we planed to carry out biochemical experiments. We are now performing biochemical quantification of storage lipids and storage carbohydrates by chromatography analysis. We are using biological material obtained in the same culture conditions as those used at SOLEIL in december. Lipid analysis were routinely carry out in the lab and the experiments will be done in april and may 2011. For storage carbohydrates, we are now elaborating a protocol to quantify glycogen and trehalose after specific enzymatic hydrolysis. Preliminary results are available (figure 2).

Figure 1 : FTIR microspectroscopy coupled to fluorescence analysis
Image of *S. cerevisiae* cells on the microscope slide after deposition on the ATR hemisphere under visible light (**1**) or by fluorescence with GFP filter set after UV light exposure (**2**). We could detect an heterogeneity of GFP fluorescent signal in each cell, **A**, cell with a high signal, **B**, cell with no GFP signal . Single cell IR spectra were recorded (**3**) and we could observe a tight link between a high GFP signal and C=O, C-H stretching band increase and CH2/CH3 ratio modifications (signature of triacyl glycerol content increase in cell)

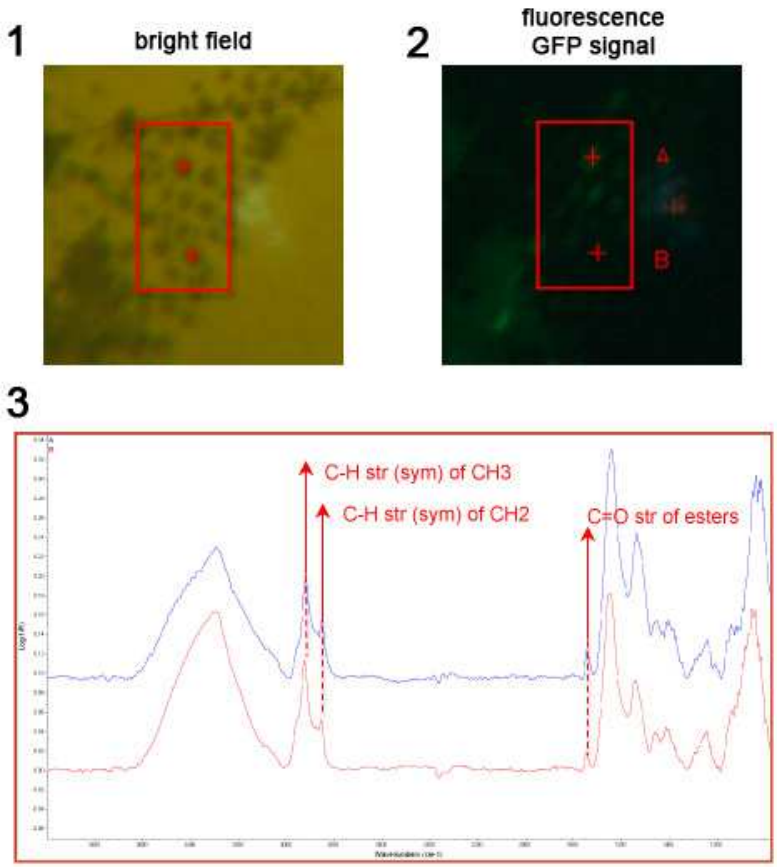
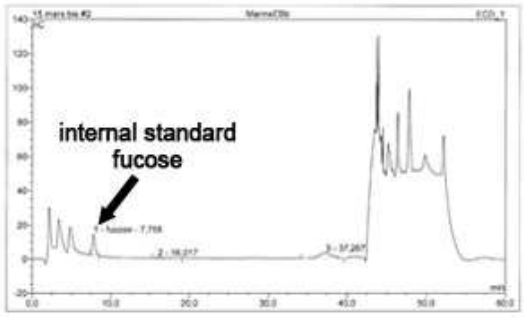


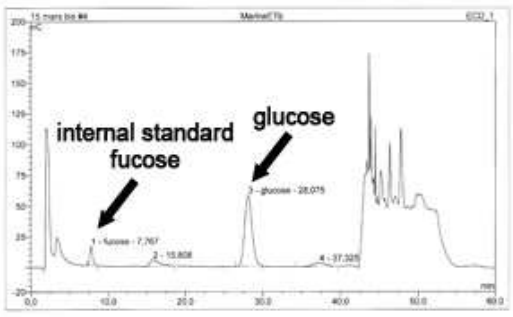
Figure 2 : Preliminary results of storage carbohydrate quantification by ion exchange chromatography on cells expressing AtS3 protein

Yeast extracts were incubated in presence of trehalase, amyloglucosidase or without enzyme and analyzed by ion exchange chromatography (**1 left panel, without enzyme; 1 right panel, after trehalase treatment**). The level of free glucose in the non treated extract or obtained after hydrolysis of trehalose or glycogen was quantify using fucose as internal standard (**2.**)

1

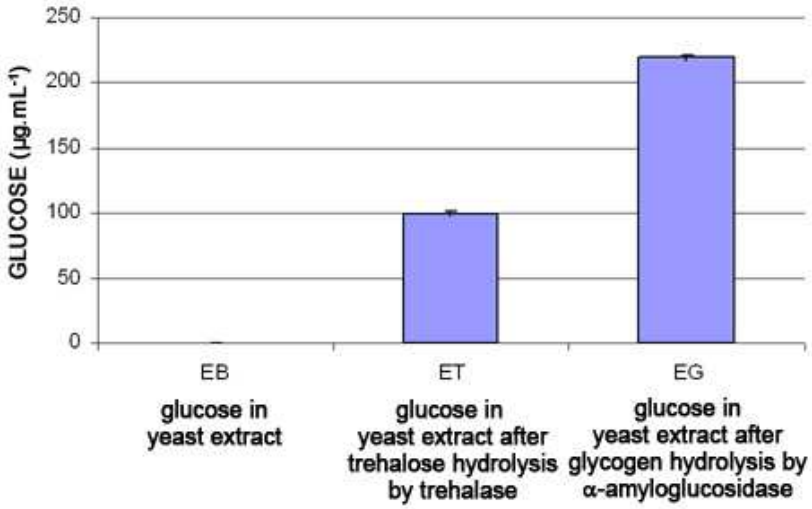


Chromatography result for yeast extract (EB)
no free glucose



Chromatography result for yeast extract
after trehalase treatment (ET)

2



Justification and comments about the use of beam time (5 lines max.):

The beam time of 12 shifts, allocated for the present proposal, corresponded to the realization of the depicted sFTIR experiments on all the biological samples, modified yeast strains with plant protein (with AtS3 or AtClo1) and the two control strains (with or without GFP expression), after one or two days of culture. These cultures were performed twice to evaluate the reproducibility of the experiments. We effectively needed an average duration of 6 hours for analysis of each sample, corresponding to the time required for preparing and transfering cells from the biological laboratory to the SMIS beamline, preparing cell-coated hemispheres (two or three were needed to obtain 50 spectra per biological sample), positioning, zone selection and microscope alignment.

Publication(s):
Work described above to be submitted.