DISCOmega: Linking omega 3 content and membrane fluidity in yeasts using DISCO beamline

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Standard Project

Experimental Report

Proposal title: DISCOmega: Linking omega 3 content and membrane fluidity in yeasts using DISCO beamline		**Proposal number:** 20141082
Beamline: DISCO	**Date(s) of experiment:** from: 02/04/2015 to: 04/04/2015 from: 17/07/2015 to: 20/07/2015	**Date of report:** 03/09/2015
Shifts: 15	**Local contact(s):** Frédéric JAMME Matthieu REFREGIERS	*Date of submission:* *03/09/2015*

Objective & expected results (less than 10 lines):
With this project, which requires the synchrotron light, we intend to study precisely the phenotypic variability (membrane fluidity) of a subset of species from Saccharomycotina, some *Debaryomyces*, which are of high interest for food biotechnology and produce omega 3 fatty acids. We hypothesize that contrasted omega 3 fatty acid content lead to specific physiological properties like contrasted membrane fluidity. To confirm our model we need DUV DISCO beamline to perform measurement of fluorescence anisotropy.

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

Background

Previous work (proposal 99140076, Temperature impact on yeast fluidity, 31/05/2014 to 01/06/2014) performed on DISCO beamline allowed us to develop convenient protocols for a further in-depth study of fluorescence anisotropy on yeast cells with TMA-DPH. DISCO staff worked hardly to upgrade the beamline and answer our technical requirements such as motorization of the polarization filters on the beamline (https://www.facebook.com/588843867817719/videos/vb.588843867817719/732068060161965/?type=2&theater).

Biological Material

Species	Ecological niche	PUFA
Debaryomyces hansenii	beer	PUFA omega 3 (< 10%)
Debaryomyces arctica	Arctic zone	PUFA omega 3 (> 30%)
Yarrowia lipolytica	laboratory	PUFA but no omega 3

Cells were grown 24h at 28°C or one week at 4°C in minimal medium (YNBG) or in complete medium (YPG) in the Biology 2 laboratory at SOLEIL synchrotron.

Membrane staining with TMA-DPH

500 µL of cell culture was incubated with 10 µM final concentration of 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH. Incubations were performed 15 minutes at room temperature or on ice. Cells were washed once with PBS before observation. Cell suspensions (3 µL) were placed between two quartz slides.

Microscope configuration

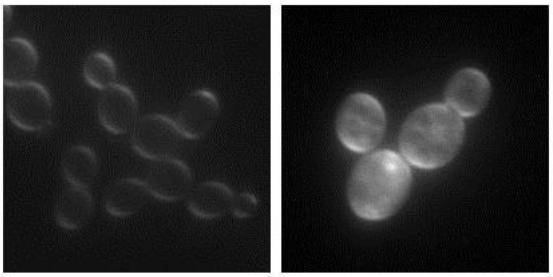
TMA-DPH = excitation: 340 nm; Emission: DM410_420-480

Results of first session, 2 to 4 of April

Previously we observed that acquisition of images out of focus has a negative impact on the calculation of fluorescence anisotropy. So, we performed acquisition of Z-stacks to solve this problem. Unfortunately, we were exposed to cell drying and bleaching effect during acquisition. A lot of images were not useful for analysis and anisotropy measurement.

We also observed high level of non-specific fluorescence (cytoplasm autofluorescence, see Figure 1) when cells were grown in minimal medium, at 4°C, and for old sample (cell stress). This signal will impair anisotropy calculation.
We decided to optimize our growth conditions for the next session with cells grown at 28° c in YPG medium.

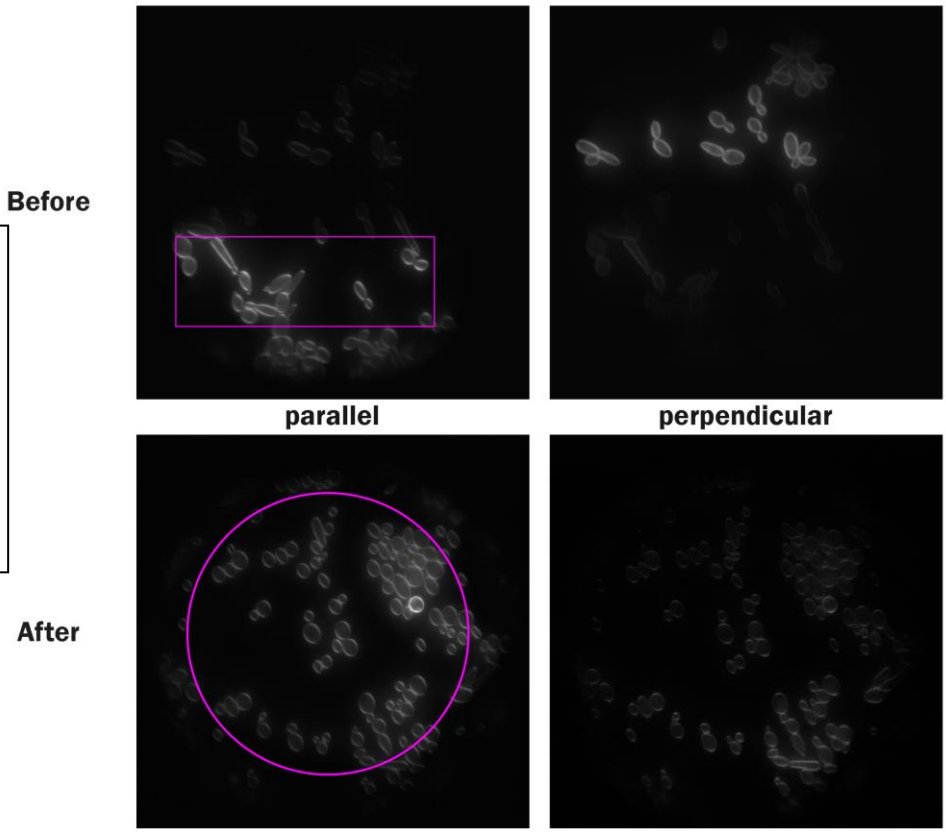
Figure 1 : *Debaryomyces* cells stain with TMA-DPH observed using Telemos microscope with polarized light. Left panel, correct staining of membrane. Right panel, cell autofluorescence



Results of second session, 17 to 20 of July

Before our arrival, the beamline staff performed technical improvement with light homogenization (Figure 2). This modification of the microscope increased the number of cells available for anisotropy calculation on images. Unfortunately, the camera associated with Telemos microscope broke down before our session and we obtained low quality images with the material available (high background level)

Figure 2: *Yarrowia lipolytica* cells stain with TMA-DPH observed using Telemos microscope with polarized light before (up panels) and after (bottom panels) light homogenization. Left panel, parallel polarizers. Right panel, perpendicular polirizers.



Conclusions

At this time we obtained data for the thre species considered (*D. hansenii*, *D. arctica* and *Y. lipolytica*) in one culture condition (YPG). We are now workin on the best way to perform anisotropy measurement on thes images as we have to deal with

- Autofluorescences and high background.
- Non homogenous staining on membranes with rotation of signal (due to cell round shape?, Figure 3 green arrows)

To solve this problem, we will analyze sub-region of membrane corresponding to regions with the highest signal when polarizers are in parallel configuration (Figure 3, pink ellipse).

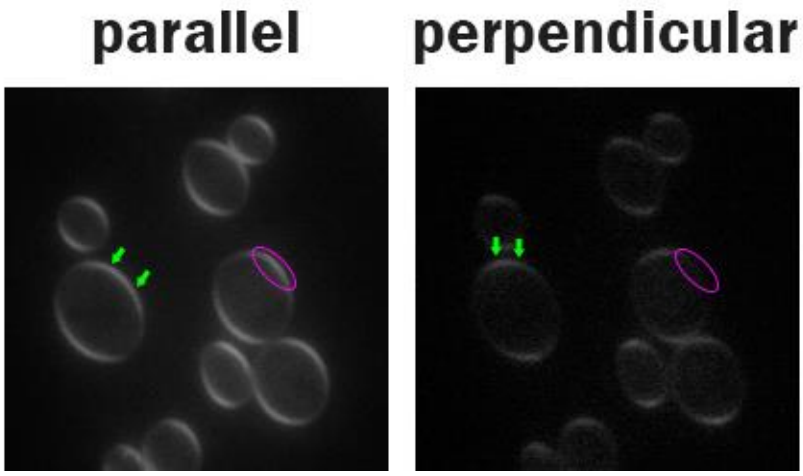


Figure 3: TMA-DPH signal is not homogenous on membranes and varies among polarizer position. Green arrows indicate the position of higher signal. Pink ellipses indicate the region selected for anisotropy calculation.

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

The beamtime allowed for this experiment was 15 shifts and was efficiently used to study fluorescence anisotropy on 3 species of yeast cells with TMA-DPH. We encountered technical bottlenecks (cell stress, sample drying, failure of camera, etc) that preclude testing the impact of medium, culture temperature, temperature shift, on membrane fluidity.

Publication(s):

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