Temperature impact on yeast fluidity

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Standard Project

Experimental Report

Proposal title: **Temperature impact on yeast fluidity**		**Proposal number:** 99140076
Beamline: DISCO	**Date(s) of experiment:** from: 31/05/2014 to: 01/06/2014	**Date of report:** 12/09/2014
Shifts: 6 shifts	**Local contact(s):** Frédéric JAMME Matthieu REFREGIERS	*Date of submission:* *12/09/2014*

Objective & expected results (less than 10 lines):

The main objective of the experiment is to design a procedure for measurement of membrane fluidity, i.e. fluorescence anisotropy, on living yeasts at 4°C and 22°C. For this purpose, we will performed preliminary tests to evaluate the technical environment necessary with the TELEMOS microscope such as convenient objectives, controlled temperature stage using Peltier effect, polarizer rotation, software image acquisition procedure, etc. Various staining conditions with TMA-DPH and Laurdan dyes have also to be tested. During this beamtime, we will expect a set of good quality parallel and perpendicular images convenient for fluorescence anisotropy calculation.

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

Biological material

Saccharomyces cerevisiae and *Debaryomyces arctica* cells were grown 48h at 28°C and 15 days at 4°C in complete medium (YPG) in INRA laboratory before arrival at SOLEIL synchrotron. One millilitre of each culture was incubated with 10 µM final concentration of 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) or 6-Dodecanoyl-2-Dimethylaminonaphthalene (Laurdan). Incubations were performed on ice for cells grown at 4°c or at room temperature for cells grown at 28°C. Cells were washed once with PBS before observation. Cell suspensions (6 µL) were placed between two quartz slides.

Image acquisition

Experiments were done on TELEMOS microscope equipped with polarization filters and controlled temperature stage using Peltier effect. Unfortunately, we encountered microscope and software problems and lost a lot of experimental time. So, during the remaining time, we decided to work on cells stained with TMA-DPH to obtain a coherent set of experimental data. Parallel and perpendicular image acquisitions were performed for *S. cerevissiae* and *D. arctica* grown at 4°C and 28°C.

Results

Fluorescence anisotropy intensity was calculated using ImageJ software as described in Figure 1 and Table 1 on a subset of data.

Figure 1: fluorescence anisotropy preliminary data on D. arctica (Ipar, parallel image; Iper, perpendicular image)

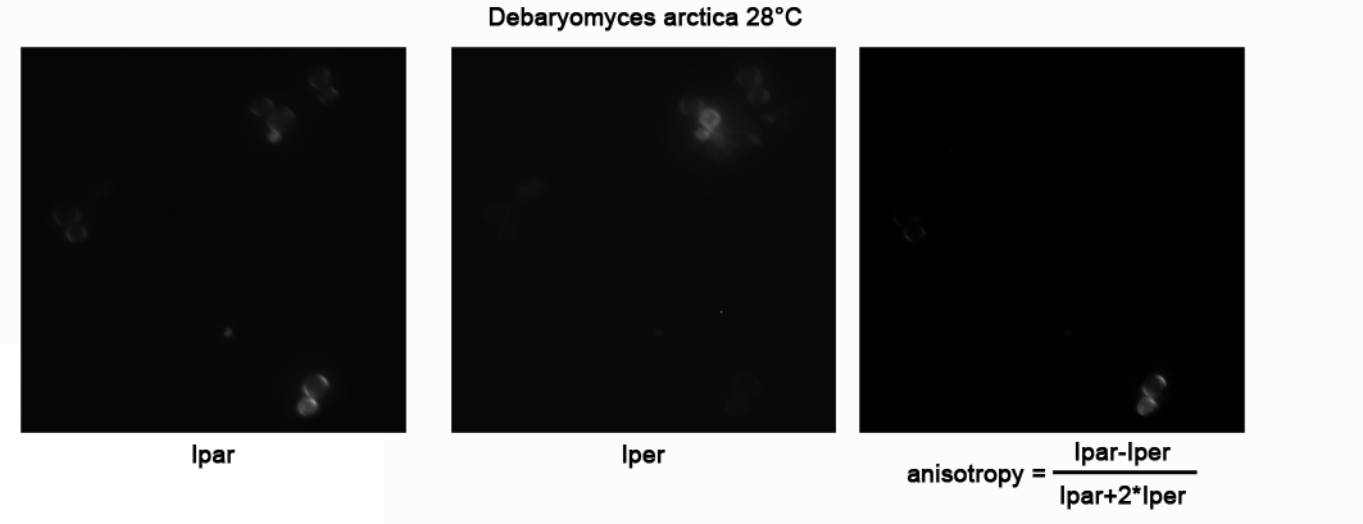


Table 1: anisotropy intensity for individual yeast cells

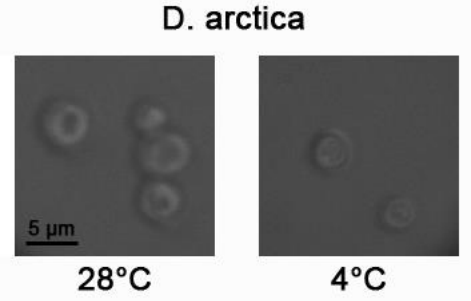
	S. cerevisiae 28°C	S. cerevisiae 4°C	D. arctica 28°C	D. arctica 4°C
Cell 1	0.151	0.257	0.320	0.108
Cell 2	0.091	0.300	0.279	0.140
Cell 3	0.150	0.134	0.114	0.214
Cell 4	0.132	0.130	0.108	0.168
Average	0.131	0.205	0.205	0.158

Conclusions

At this time, it is not reasonable to draw robust conclusions but we are convinced that it will be possible with technical improvements such as those listed below.

- We observed that cell grown 15 days at 4°C are not in a good physiological state for microscopy and decided to optimize our growth conditions for further experiments. In particular, we observed a lot of cell death in the samples.
- It will be necessary to preserve the quality of biological samples by reducing the acquisition time. For this purpose, we concluded that motorization of polarizers and automatized acquisition have to be developed
- Acquisition of images out of focus has a negative impact on the calculation of fluorescence anisotropy. So, acquisition of Z-stacks will resolve this problem
- We observed that the cells present a large heterogeneity in size (figure 2) in a same culture, between species and between culture conditions (28°C and 4°C), so it will be necessary to normalize anisotropy signal according to cell surface to make valuable comparisons

Figure 2: *D. arctica* size heterogeneity



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

The beamtime allowed for this experiment was 6 shifts and was efficiently used to explore some technical requirements and for the development of convenient protocols for a further in-depth study of fluorescence anisotropy on yeast cells with TMA-DPH but also with other lipid dyes such as DPH or Laurdan. At this time, DISCO staff is hardly working to upgrade the beamline and answer our technical requirements. Now, the motorization of the polarization filters is available on the beamline.

Publication(s):

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