HOG1-GFP strain of *Saccharomyces cerevisiae* as an hyperosmotic pressure sensor

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Saccharomyces cerevisiae is a common species of yeast also known as Budding yeast or baker's yeast. *S. cerevisiae* is able to do fermentation, this property induced close relation between yeast and humans, indeed yeast is used to make bread, beer and wine since Ancient history. Fermentation is a metabolic process that transforms sugar into alcohol[1]. In addition to the fermentation, sugar (or more specifically glucose) is involved in cellular respiration[2], so yeast needs sugar to live. However, high sugar concentration induces hyperosmotic pressure to yeast [3] and can go to apoptosis[4]. Yeast reacts to hyperosmotic pressure by producing glycerol as a final product of the HOG pathway[5] [6]. This pathway involves the HOG1 protein[7] which will move from the cytoplasm to the nucleus and will act as an enhancer for different genes[8]. It is very important to understand well the reaction of the yeast metabolism to sugar for the wine agro-industry to know which concentration of glucose yeast can support and also when and how much glycerol, one major component of wine, can be produced.
Here we show the mobility of the HOG1 protein for different concentrations of glucose and the impact of the age of yeast culture in the mobility. In order to observe the HOG1 nuclear translocation, we used a HOG1-GFP strain of *S. cerevisiae*.

Method to induce hyperosmotic pressure to yeast

In order to create different osmotic pressure in yeast, we made YPD media with different concentrations of glucose. We had 5 solutions of YPD media with 0%, 4%, 20%, 30% and 40% of glucose. To induce an osmotic shock, we mixed in an eppendorf 10 µL of glucose solution

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[3] Pratt P, Bryce J, Stewart G. “The Effects of Osmotic Pressure and Ethanol on Yeast Viability and Morphology” *Journal of the Institute of Brewing* vol: 109 (3) (2003): 218-228
[4] Silva, RD et al. “Hyperosmotic stress induces metacaspase- and mitochondria-dependent apoptosis in *Saccharomyces cerevisiae*.” *Molecular Microbiology* 58.3 (2005) : 824-834.
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and 10 µL of the yeast culture. At this point, it gives yeast solution at 0%, 2%, 10%, 15%, 20% of glucose. We made an other yeast solution at 30% of glucose by adding 75µL of the 40% glucose YPD media and 25 µL of the yeast culture. We had to do this solution in a different way because at more than 40% of glucose, it was too hard to dissolve the glucose in the YPD media.
After that, we made a microscope slide with 3 µL of the yeast solution with glucose. 5 to 10 minutes later, we took pictures with a fluorescent microscope, using the GFP filter. We took 10 to 20 pictures per slides depending on the concentration in cells.
We have done one concentration by one to have always the same time duration between the osmotic shock and the observation.
All this protocol has been done 3 times with 3 HOG1-GFP strain of *S. cerevisiae* culture of different ages. The first one has been taken from a cryotube at -80°C to do an overnight culture. The second is an overnight culture of the first and the third is an overnight of the second one. All the overnight cultures have been grown in YPD liquid media with 2% of glucose and incubated at 30°C in a moving incubator. We took the measures after 3.5 days of incubation for the first culture, 2 days for the second and 1.5 days for the third one.

Microscopic observation of the HOG1 fluorescence

First of all, we observed the images of the cells from the fluorescent microscope. We used HOG1-GFP strain of *S. cerevisiae* so with the GFP filter of the microscope we are able to observe the localization of the HOG1 protein (figure 1). We chose to quantify the reaction of the cell to hyperosmotic pressure as the migration of the HOG1 protein from the cytoplasm to the nucleus. To analyze the pictures, we counted the number of cells with homogeneous repartition of the fluorescence in the cell (fluorescence in the cytoplasm) and the number of cells with one intense peak of fluorescence inside the cell (fluorescence in the nucleus). We counted only the budding yeast because we know that mating and budding stage can interfere with normal osmoregulation[9]. Each picture has been counted 3 times by 3 different people.

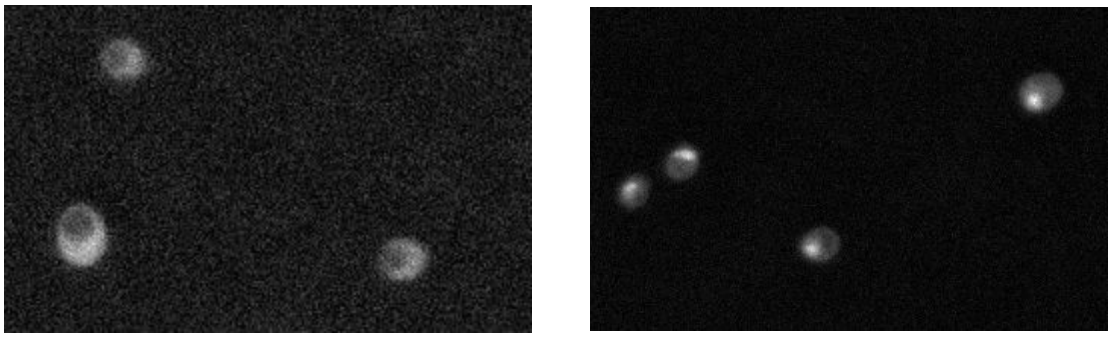

Figure 1: Pictures of *S. cerevisiae* with fluorescent microscope and GFP filter.

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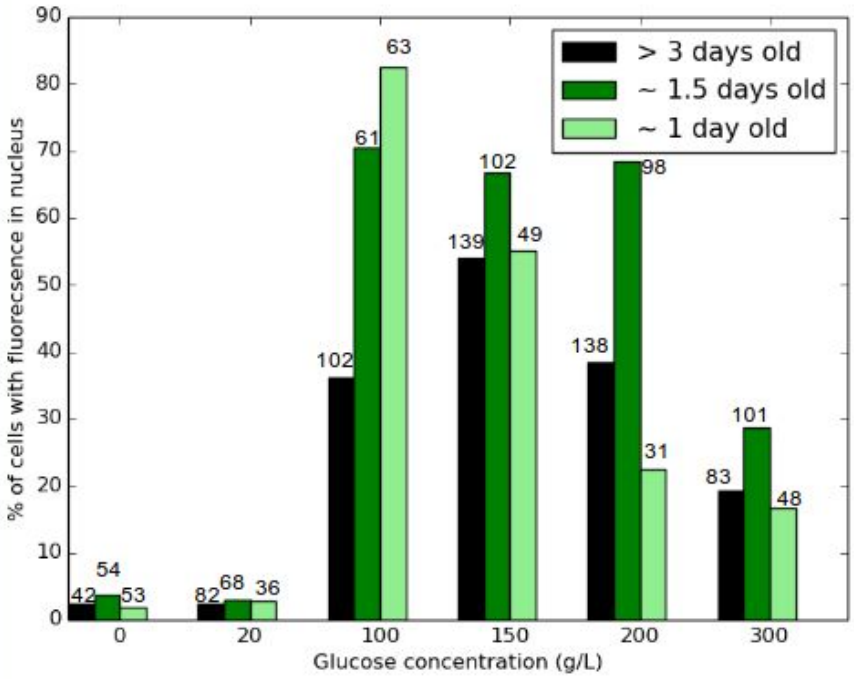

Figure 2: Histogram of the percentage of yeast cells with fluorescence in the nucleus as a function of the concentration of glucose in g/L to induce an hyperosmotic pressure. The black is the yeast culture which is more than 3 days old, the dark green is around 1.5 days old and the light green is around 1 day old. The numbers at the top are the sample size.

We can observe that at 0% and at 2% of glucose, the percentage of cells with fluorescence in the nucleus is around 0 for every culture (> 3, 1.5 and 1 days old) and from 10% there are a part of the cells with more fluorescence in the nucleus than in the cytoplasm for every culture (figure 2).
The maximum of relocation for the 1 day old culture is 10% of glucose whereas it's at 15% for 3 days old culture. The relocation is constant for the 1.5 days old culture between 10% and 20% of glucose.
S. cerevisiae seems to react less when cells are more than 3 days old than when they are younger.

Discussion and perspectives

One of the main concerns of the experiment in the subjectivity of the determination whether fluorescence in the nucleus is more intense than fluorescence in the cytoplasm. To redo this experiment more precisely, it would be better to have more people to count the cells in the picture to limit the confirmation bias and to enlarge the sample size of yeast to reduce the noise. Another alternative could be to automate the process by marking the nucleus and set a threshold to determine if the fluorescence intensity is different in the nucleus or not.

To reduce the individual noise of the cells and to better understand the impact of the age of the culture in the results it could be interesting to take pictures with microscope without fluorescence to determine the ratio of cells without fluorescence and the ratio of dead cells in the sample.
To go further in the study of the impact of hyperosmotic shock in yeast it could be interesting to compare the reaction of mitochondria when osmotic pressure is induced by sugar and by salt[10].

Conclusion

This project sets a basis for exploring the ability of HOG1-GFP strains of *Saccharomyces cerevisiae* to sense hyperosmotic pressure. It's only a basis because we lacked the control on our objectivity, but also the noise of the instruments.
Thanks to our project, we can say that yeast cells react to hyperosmotic pressure with a relocation of HOG1 protein into the nucleus between 10% and 30% of glucose. However, our sample doesn't allow us to determine clearly the accuracy, precision, resolution of our sensor. We would like to test other proteins, with a better control of our variable, to go toward this objective of determining the characteristics of such yeast strains as hyperosmotic pressure sensors.
The perspectives in the industry of wine and beer could be somehow interesting, to have a good control on the osmotic pressure on yeast.

[10] Pastor, M. M., Markus Proft, and Amparo Pascual-Ahuir. "Mitochondrial function is an inducible determinant of osmotic stress adaptation in yeast." *Journal of Biological Chemistry* 284.44 (2009) : 30307-30317.