Interview with Mr Jean-Marie François

WHO ARE WE INTERVIEWING? (job, studies...)

Mr. Jean-Marie François is a professor of Industrial Microbiology and BioNanotechnology at Federal University of Toulouse, School of Engineer. His research activity concerns integrated physiology and functional genomics in microbial systems, with a specific focus on genetic and metabolic regulation and refactoring of carbon and energy metabolism.

CONTEXT

Mr François is a specialist in *Saccharomyces cerevisiae*, so we contacted him especially for the culture of the baker's yeast in our specific condition and also for the design of our clonings.

RATIONAL (What questions did we ask him? What answers did we want to have?)

1. Questions about nitrogen source

Since we would like to grow yeasts with the less resources as possible, and especially with the resources available on a space station, we came to ask ourselves the question of using urine as a source of nitrogen for our acetogenic-yeast co-culture.
Saccharomyces cerevisiae can grow on urea as a nitrogen source by releasing CO2. Urine also contains ammonia. **If S. cerevisiae is in the presence of urea and ammonia, can it consume the urea leaving the ammonia for the acetogen?**

2. Questions about the production of provitamin A in *Saccharomyces cerevisiae*

We are studying how to synthesize beta-carotene in Saccharomyces cerevisiae by adding heterologous enzymes from Xanthophyllomyces dendrorhous (namely: crtE, crtYB and crtI). The problem is that all publications to date have been on yeast growing on glucose. However, in the context of our project (co-culture with Clostridium ljungdahlii growing on acetate and/or ethanol), we would like to know **the effect of the growth on C2 carbon source on the mevalonate pathway**. **Would you know if it is up or down-regulated under such conditions? Which enzymes are most affected? Which enzymes restrict the flow to FPP (and GGPP)?**

3. Questions about the design of our clonings and the yeast strain

We showed our cloning design to Mr. François to validate or invalidate it and to help us choose the appropriate strain.

INTERVIEW (summary of the interview)

1. Questions about nitrogen source

S. cerevisiae can indeed use urea but prefers ammonium. Everything will depend on the C/N ratio because if N is low, it will probably use ammonium first and therefore will not leave any to the acetogens. On the other hand, if the DRu1,2 urease gene encoding urease is strongly increased, then it will favorably convert urea into ammonium. However, yeast is a cell that keeps everything and excretes very little so the yeast could not excrete ammonium for the bacteria. One solution would be to express urease on the surface of Saccharomyces cerevisiae.

2. Questions about the production of provitamin A in *Saccharomyces cerevisiae*

The yeast will obviously need its mevalonate pathway even when growing on ethanol or acetate because this pathway leads to essential growth compounds such as ergosterols, dolichol and heme useful for respiratory enzymes.
No study has been carried out on the effect of Carbon source on the expression of genes of this pathway, and certainly not in ethanol versus glucose conditions e.g. it would probably be interesting as information.
In normal conditions on glucose, the limiting enzyme in this pathway is HMG coA reductase which is encoded by 2 genes and the complexity comes from their different localization and regulation, then further down, it is FPP synthase. But in general, we act on the HMGR to promote the flow to the mevalonate ! It is very well known and it has been applied in several cases.

3. Questions about the design of our clonings and the yeast strain

Mr François validates our cloning strategy. He advised us to use TDH1 and ADH1 promoters which are strong and constitutive. He also thought that we should use BY4241 which is auxotrophe for leu, his, met and ura and is Δ Gal4.

4. His general opinion on our subject

Mr. François thinks that our project is quite feasible. He told us that it was unlikely that the acetate produced by the bacteria would be toxic to the yeast when it consumes ethanol. He added that the limonene and geraniol would certainly not be excreted.

“It's certainly original as a co-culture system... These are topics for the future! Good luck.” Jean-Marie François about our iGEM project.