Interview with Mr Gilles Truan

WHO ARE WE INTERVIEWING? (job, studies…)

Dr Gilles Truan, CNRS Research Director

A geneticist by training, Gilles Truan joined the CNRS in 1997. His research topic was to understand the functioning of enzymes by means of directed evolution techniques. He then oriented his research towards understanding the role of protein dynamics in their catalytic function. In 2011, the team joined the Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP) at the Institut National des Sciences Appliquées (INSA) in Toulouse and took a new turn towards synthetic biology. Gilles Truan is the head of the Molecular and Metabolic Engineering (I2M) team and is responsible for the Metabolic Engineering axis which includes three LISBP teams. The I2M team is widely recognized in France in the field of synthetic biology. Its research project focuses on the construction of microorganisms with the ability to produce natural or synthetic biological molecules. The team is also known to be a pioneer in the field of protein engineering techniques. In the early 2000s, the team built yeast strains that autonomously produce hydrocortisone, a steroid hormone. Gilles Truan is also the director, with Jean-Loup Faulon, of the Synthetic and Systems Biology Research Group (BioSynSys).

CONTEXT (Why did we do this interview?)

We had a videoconference with Mr Gilles Truan on Friday, April 17 between 3 and 6 pm. The objective was to collect his opinion on our project and to discuss the various points on which we are still uncertain.

INTERVIEW (summary of the interview)

Interview of the 04/17/2020

Cultivation

Mr Truan advises us to calculate how much one mole of substrate H_2/CO_2 produces as biomass of *Clostridium ljungdahlii*. And also how much one mole of substrate H2:CO2 goes into EtOH or acetate, at different stages of the growth. It might be different when cells are already grown and under a form of biofilm.

He also points out that a certain percentage of ethanol and O_2 would have to be reached to start the growth of *Saccharomyces* and that these percentages to be reached are likely to be lethal for *Clostridium ljungdahlii.* We also have to think about how to start the culture.

We note that the method of cultivation still seems unclear. We have not discussed whether the culture was in continuous or discontinuous mode despite some hints that there would be a withdrawal. Cultivation mode should be better defined. Especially for the starter culture, Clostridium. It might be easier to start this one, let EtOH and acetate grow and then couple the two organisms together.

Synthetic biology

First, we questioned the definition of synthetic biology and asked Mr Truan for his opinion. According to him, synthetic biology is "the construction of a biological system not found in nature". In other words, our system of coculture between two species that do not cohabit in nature and their growth based on non-natural symbiosis is synthetic biology.

Next, we want to design a logic gate system for building flavors or for the production of various vitamins. Mr. Truan explains to us that a simple induction system is different from a logic gate system. A logic gate is present when we make an algebraic addition, so with the use of "and" or "or" conditions. Mr. Truan gives us references on the different systems that can be used such as EL222 and Phy-Pif. → contact Pascal Hersen, he and Gilles Truan have collaborated together and they have both systems already on plasmid. Gill will talk to him next

Thursday. I think there is no problem to reuse the plasmids, but maybe not the strains with the opto production of carotene. He will ask, and we'll see...

We need inputs for the logic gate system that do not need to add any compound to the culture. We thought about light or temperature but Benjamin Erable dissuaded us to use light because the medium will be certainly too opaque. Nevertheless, Mr Truan told us that yeasts adapt well to light and that the bioreactor will be small enough to allow photoinduction especially if we use leds inside the bioreactor. And in any case, light can penetrate as long as the culture is not too dense. The temperature seems not to be an option because the change of temperature could be lethal for an organism or the other since they don't have the same optimal temperature. We need to check if LEDs emit in a tight range of wavelength in order to use several colors and to mix them. He added that cyanobacteria have light regulation in order to know if they need to float at the water surface or not, we also can check what kind of regulation it is. There are a lot of light regulations: plants, cyanobacteria etc.

We also need to check the optogenetic systems used in yeast in previous iGEM edition. Nus apparently used some of it, we already collaborate with them, so we may easily get some plasmids.

Note that the choice of system will induce the choice of promoters which we will need to order. We can design the logic gate system without knowing which compounds we are gonna produce. We should start to design it with fluorescent proteins.

Molecules to produce

About vitamins

Mr Truan advised us to make a list of all the vitamins that the astronauts could need for long time space travels and then chose the ones that are potentially easy to produce (1 or 2) and needed in small quantities. We also should pay attention to daily needs (calculate the quantities needed for 5 years for one astronaut), in which food they are found and stability of vitamins to justify our choice. These vitamins could be constitutively produced by the yeast and different tastes could be produced upon different inputs (different wavelengths). The production of two vitamins should be enough to prove our concept. Even one as we are quite unsure of the time slot we will have. Make plans for several, rate the time necessary to obtain them (number of genes to be modified, availability of strains already containing part of the metabolic engineering etc.)

Note that vitamin A is one of the most needed vitamin (to be verified) and one of the least stable (very sensitive to light and O_2). In addition, precursors of vitamin A have been produced in iGEM (team Paris-Bettencourt 2015) and Gilles Truan worked on it and have the modified strain of *Saccharomyces cerevisiae* which produced carotene, the precursor of vitamin A.
Vitamin C is also one of the most needed vitamin and is easy to make (but it is not very exciting).
Vitamin E seems too complicated to produce according to Mr Truan.
About vitamin D3, an enzyme which processes the transformation of 7DHC into D3 may exist according to Mr Truan. But it may take some time to design the pathway...

We tackled the fact that yeast already are nutritive but Mr Truan underlined that autolytic yeast extract and dried yeast are not the same. The process used to lyse the cells induces metabolic changes which produce other compounds. First, an induced autolysis is hard to make and secondly it might not produce the same compounds as the conventional lysis process. Maybe we should check what are the nutritive compounds in dried yeast. Yes, and the same for the yeast extract for which the composition is known. Again, maybe check if recipes for yeast extracts exist on the web.

About flavors

Mr Truan told us that adding salty, sweet, umami or bitter tastes is quite simple and he had expected something like chocolate or fruit flavors. He had thought that we planned to use a logic gate system to mix produced compounds in order to create flavors.

What is sure, is that adding bitter taste is useless because yeast already is bitter. Then we certainly won't have time to insert flavors synthetic pathways but we can think about it while we construct the logic gate system with fluorescent protein.