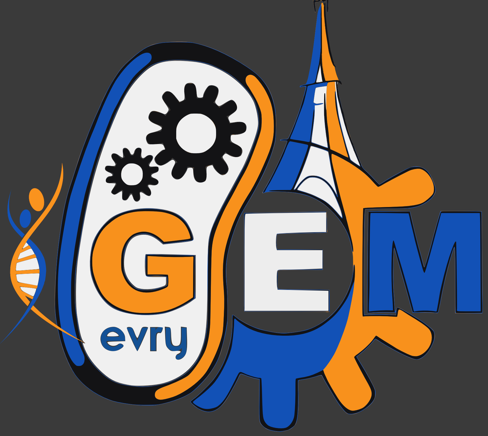

PSICOSE

iGEM EVRY PARIS-SACLAY

A brand new molecule screening method based on biosensors

A method to improve and standardize enzymatic bio-production

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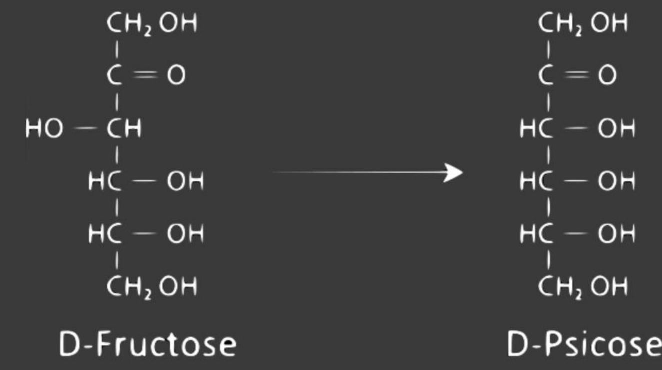
ABSTRACT

8.5% of the world population suffered from diabetes in 2014

5 millions deaths in 2015.
1 person dies **every 6 seconds** in the world, more than AIDS, tuberculosis and malaria.
Symptoms include stroke, dementia, kidney and heart failure.

To assist in the prevention of diabetes, we turned to a rare sugar called Psicose which possesses some incredible properties. It has zero calories, increases the metabolism of fats, and regulates glycemia. **Psicose** is a natural sweetener present in low quantities in plants and can fit within a diabetic diet. With that in mind we wanted to **bioproduce** Psicose and enhance production efficiency by optimizing the culture conditions and constructing a **user-friendly bioscreening system to aid in enzyme selection**. This high throughput technique allows faster enzyme screening at a lower cost. This simplified system could not only revolutionize current enzyme screening for industry but also help us perform the most efficient bioproduction possible to provide diabetics with Psicose.

CHEMISTRY



Epimerisation of the D-Fructose in D-Psicose

Knowing that D-Psicose is a C-3 epimer of D-Fructose we first produced it chemically using an epimerization reaction with Fructose as the substrate. We applied the experimental conditions described in the literature for the epimerization process.

The results were analyzed by NMR and compared to the NMR spectra of different commercial sugars (Mannose, Glucose, Fructose and Psicose). A small quantity of Psicose was detected and it showed us why there is a lack of publications about this chemical process. The yields are too low for global production. Nevertheless some other techniques are described to synthetize Psicose using total synthesis but this method is still too expensive and doesn't fit with our project.

HUMAN PRACTICES

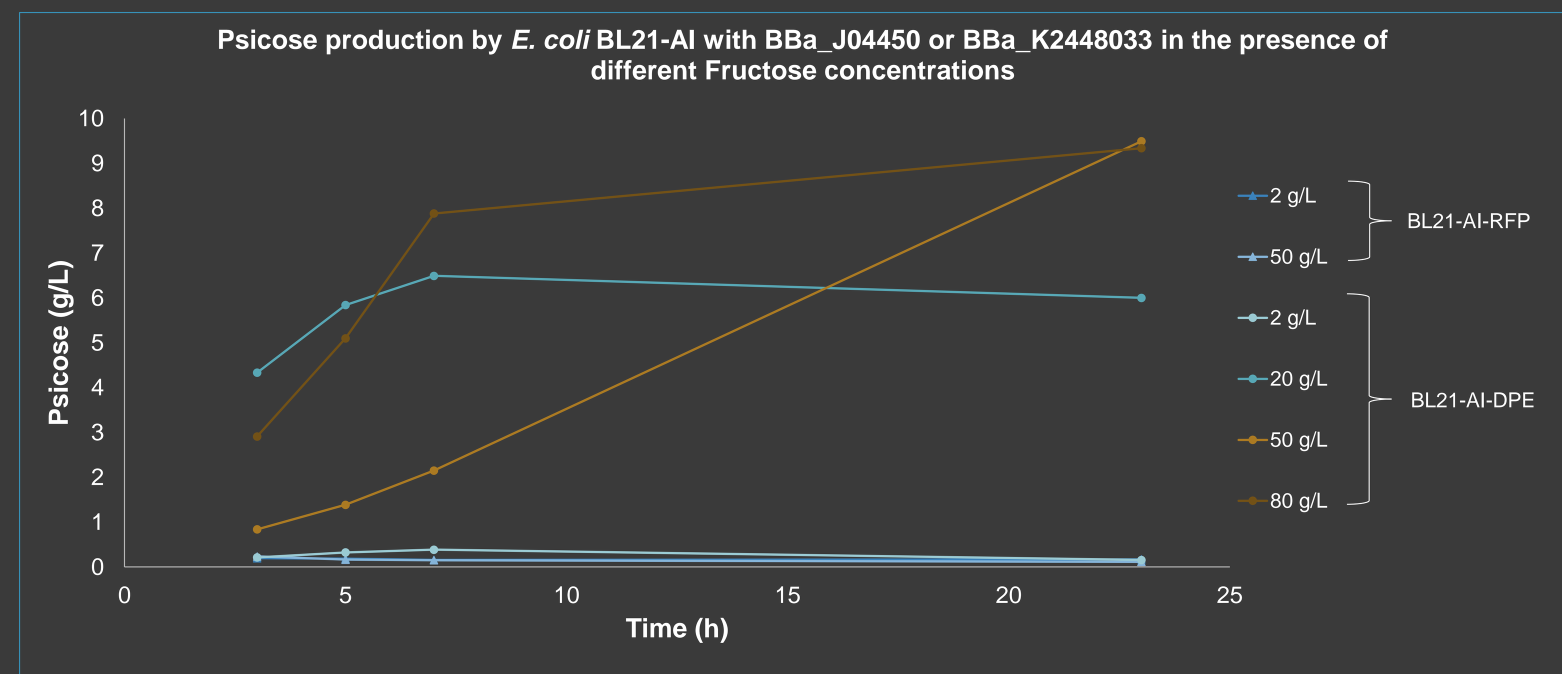
To better understand the problems diabetics face and to propose the best solution, we met with the French Federation of Diabetics. As a result of this meeting we decided to choose Psicose from a range of possible sweeteners as the best to use for diabetes prevention. We then began to investigate the legal issues involved in placing Psicose on the market and wrote a food additive assessment for the European Commission as a first step to legally provide Psicose in France. Since we were performing bioproduction, we wrote a report on sugar consumption where we explained why our project is safe. We also conducted a survey to learn the publics' perception of our project and we got some great feedback on the bioproduction process.

In parallel with the bioproduction, we spoke to different companies about their bioproduction methods and what improvements we could implement. We learnt that enzyme screening processes were slow and expensive. So we decided to work on a bioscreening system based on biosensors that would be able to perform fast and high throughput enzyme screening. Our meeting with Sanofi-Aventis allowed us to go even further by standardizing our bioscreening system using Golden Gate Assembly : we were able to assemble a larger number of parts, faster for our bioscreening systems. This method allowed us to create the Universal Biosensing Chassis : a modular biosystem able to screen every molecule as long as there are specific transcription factors and promotors to sense it.

BIOPRODUCTION

The bioproduction of this rare sugar can be easily achieved using an epimerase since D-psicose is a C3 epimer of D-fructose. After researching the literature we chose D-psicose 3-epimerse (DPE) from *Clostridium cellulolyticum* to test the bioproduction in E. coli. First of all we cloned the epimerase in pSB1C3 with RBS and pTac1 promoter (BBa_K244033) and transformed in E.coli BL21-AI, the best strain to obtain great yields. The production was performed in batch culture, with different concentrations of Fructose.

HPLC measurements allowed us to conclude that *E. coli* BL21-AI harboring BBa_K244033 is able to produce up to 9 g/L of Psicose from 50 g/L and 80 g/L of Fructose in the medium and that there is a proportional relationship between the fructose concentration and the psicose measured. No psicose was produced with our negative control which was expressing RFP.
We also determined that further increases in Fructose concentration in the media has a negative impact on *E. coli* growth and that the IPTG induction level of the pTac1 promoter did not influence bioproduction.

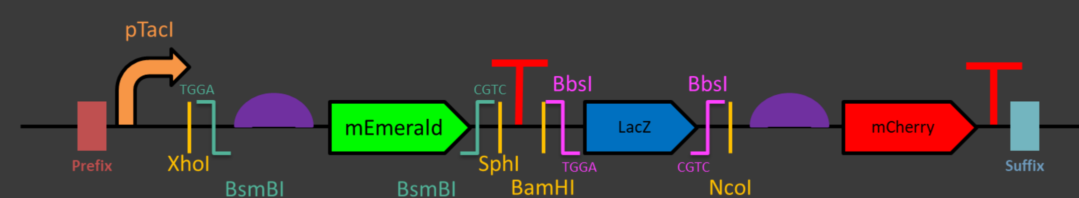


Psicose production of BL21-AI transformed with a DPE from C. cellulolyticum and BL21-AI transformed with an RFP, depending on the fructose concentration: 2 g/L, 20 g/L, 50 g/L or 80 g/L. BL21-AI-RFP is used as a negative control with no DPE.

BIOSENSORS

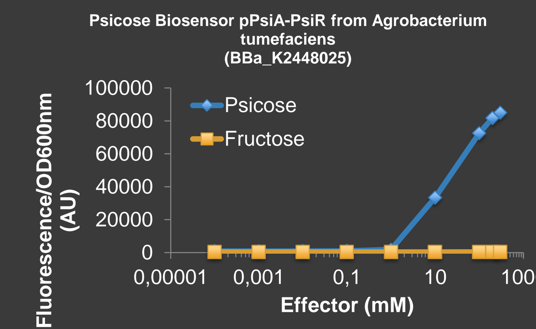
To improve the bioproduction process, we designed transcription factor-based biosensors able to detect Psicose. We used it to screen for the best epimerases for the conversion of fructose into Psicose. We based our biosensor on a simple process: When **pTacI** is induced by IPTG, it drives the transcription of a high psicose affinity transcription factor, **PsiR**. If Psicose is present in the cell, the transcription factor will bind preferentially to it and thus be inactivated. The repression of the related promoter, **pPsi**, will be released, enabling the transcription of a fluorescent protein, **mCherry,** proportional to the concentration of the compound. If Psicose isn't present in the cell, PsiR will bind to pPsi, preventing any transcription of mCherry.

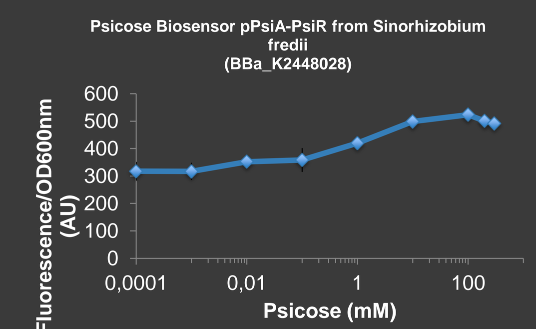
For the standardization of our system and to facilitate the construction of the different biosensors, a **Universal Biosensing Chassis** has been engineered. By its design the **UBC** allows for **fast cloning** by traditional methods or **Golden Gate Assembly**.

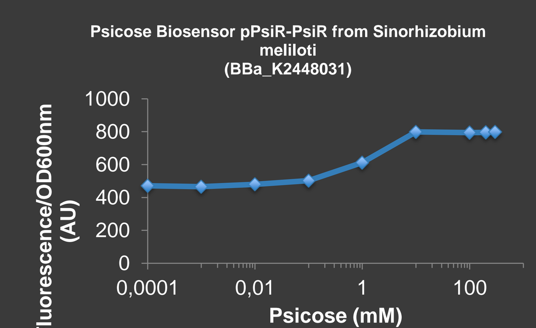


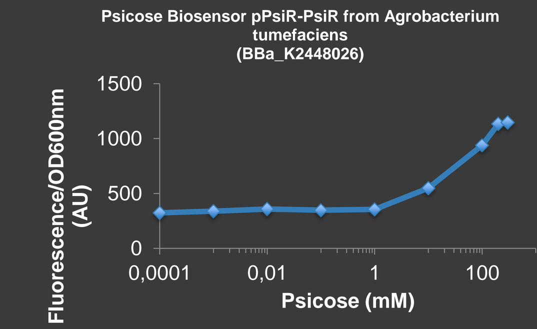
Schematic Design of the Universal Biosensor Chassis

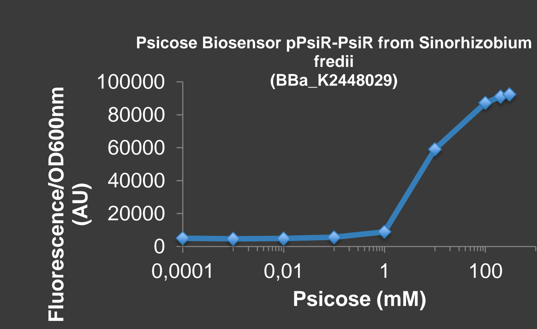
pTacI : promoter inducible to IPTG
mEmerald : insertion marker of the transcription factor
LacZ : insertion marker of the promoter
mCherry : reporter gene

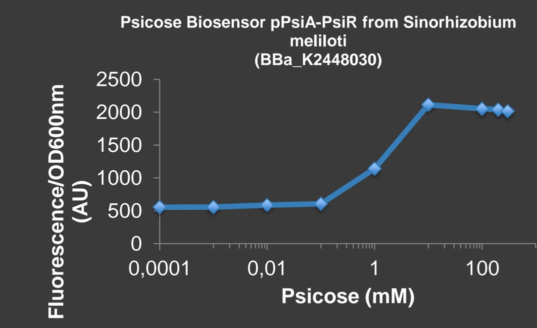


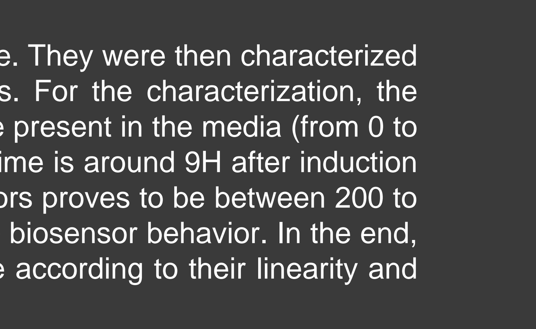


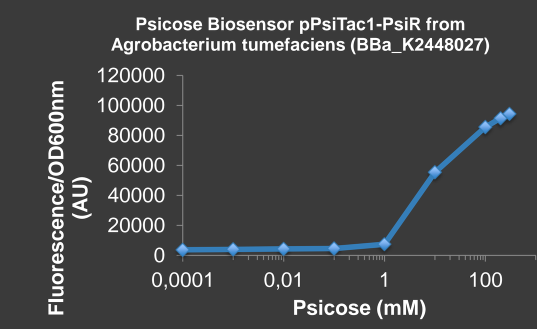








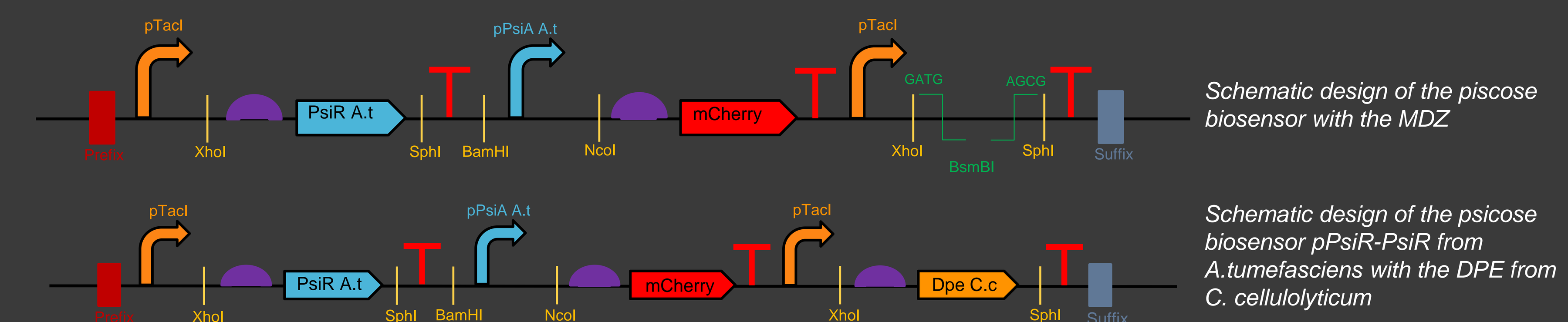




This system allowed us to construct **7 functional biosensors** to detect Psicose. They were then characterized in order to determine the most suitable biosensor for our screening process. For the characterization, the emission of fluorescence has been measured for different quantities of Psicose present in the media (from 0 to 300 mM). With this experiment we determined that the optimal measurement time is around 9H after induction but relevant results can be obtained at 6H. The basal activity of those biosensors proves to be between 200 to 1800 A.U of fluorescence. Last but not the least, fructose doesn't influence the biosensor behavior. In the end, we show that **3 biosensors** are perfectly suitable for the screening procedure according to their linearity and dynamic range.

SCREENING SYSTEM

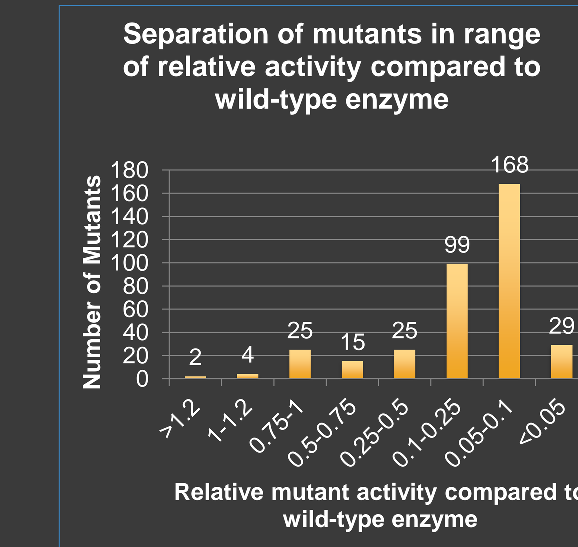
To demonstrate the proficiency of our project, the final step is the selection of the best enzyme in order to improve the production of Psicose. For this purpose we modified our biosensor by adding a **Mutant Drop Zone (MDZ)** allowing quick and easy insertion of multiple mutant enzymes in order to test their efficiency.

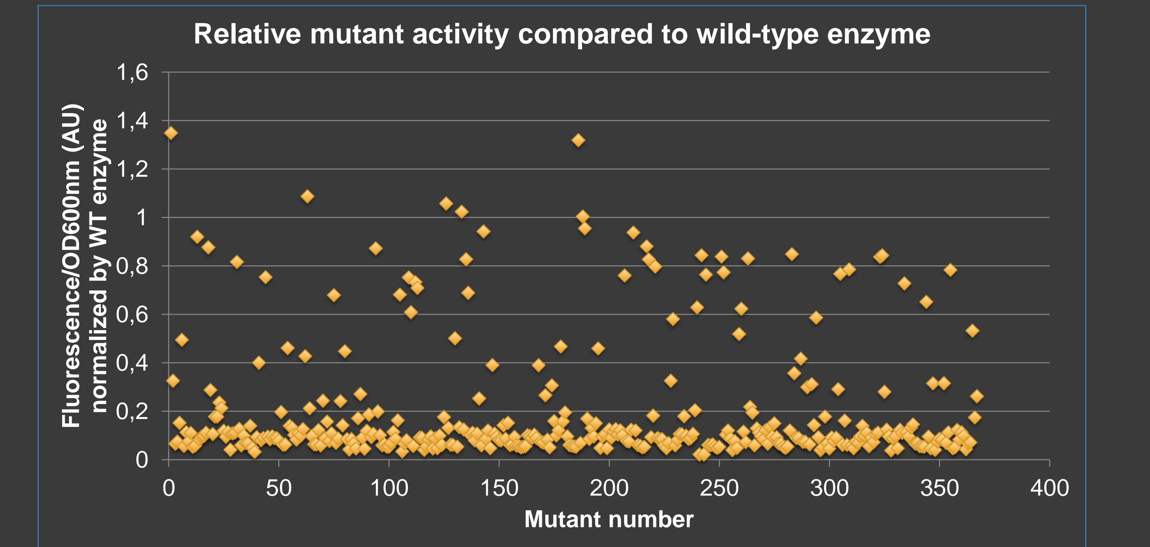


Schematic design of the piscose biosensor with the MDZ

Schematic design of the psicose biosensor pPsiR-PsiR from A.tumefasciens with the DPE from C. cellulolyticum

We then created a bank of mutants using Error Prone PCR, for the epimerase DPE from C. cellulolyticum. This method allows us to obtain variants with a controlled rate of mutation and a-priori free. We then inserted each mutant into the MDZ, and performed our screening process.





Based on the data, among the bank of 400 screened mutants, 2 enzymes showed an almost 40% improvement in activity.
To improve this process, more mutants should be tested and the utilization of a flow-cytometer would facilitate the selection as a function of the emission of fluorescence quicker and more efficiently, transforming the whole method in a high throughput process

We achieved bioproduction of Psicose

We improved our bioscreening system making it universal

We achieved an improvement of bioproduction using bioscreening

ACKNOWLEDGMENTS

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