

FAT AND FABULOUS



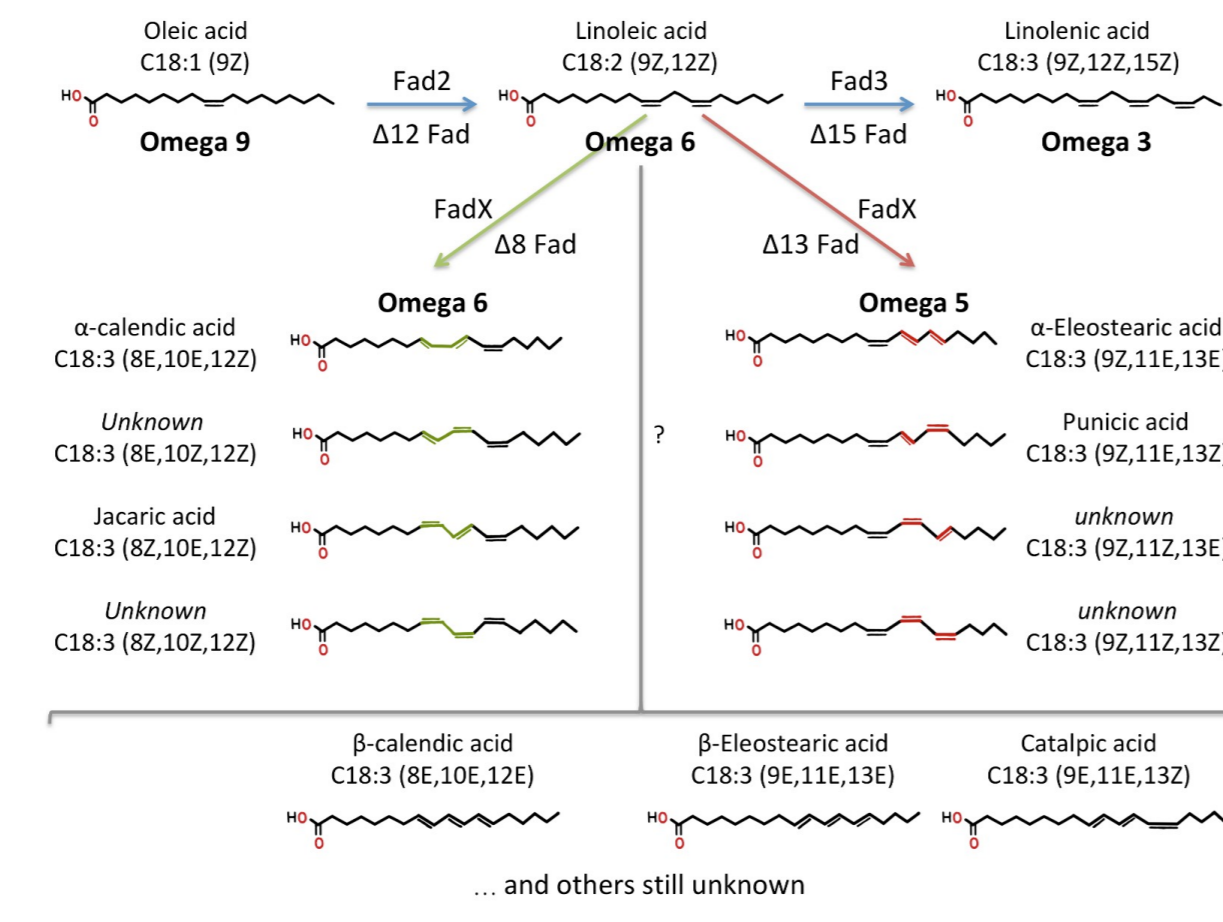
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Supervisors : Ioana Popescu, Manish Kushwaha, Anna Niaraki and Jean-Marc Nicaud ; Advisors : Nazim Sarica and Young-Kyoung Park

Fat is not so bad. In fact, it can be fab!

Fatty acids were primordial constituents of the earliest cell and continue to play key roles in all cellular life today. It is not surprising then that fatty acids, particularly unsaturated ones (think 'omega'), are widely used in the pharmaceutical, agrifood and cosmetic industries. However, their large-scale production is giving rise to many environmental challenges, especially as our planet faces the threat of climate change. Our team is interested in the production of medically-relevant Conjugated Linolenic Acids (CLnAs), which is a class of rare fatty acids with three conjugated bonds. In this project, we develop a launchpad for their bioproduction using the oleaginous yeast *Yarrowia lipolytica*, a powerful chassis organism, whose metabolism is naturally poised for lipid production.

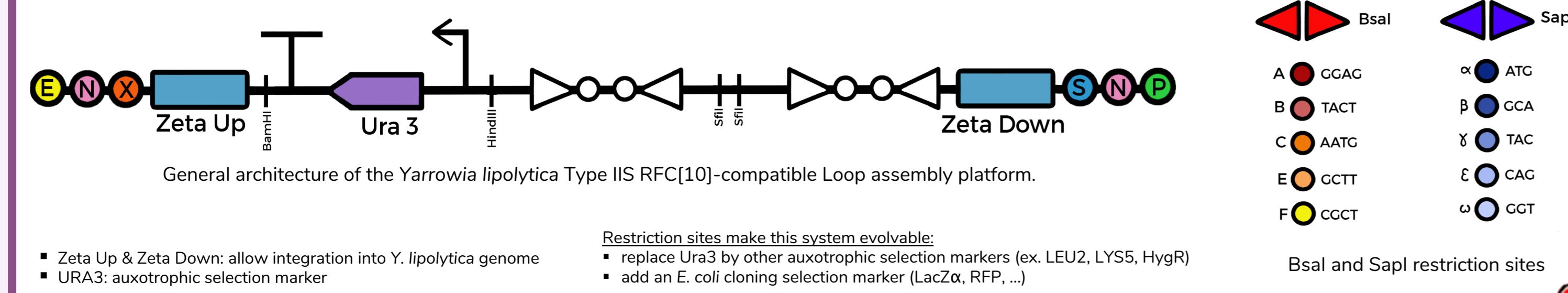
CLnA

- CLnAs are fatty acids having 18 carbons and 3 conjugated double bonds [1]
- Double bond geometric isomerism: cis (Z) or trans (E)
- Only 7 CLnAs were discovered in nature as of today
- CLnAs are synthesized by bifunctional fatty acid conjugase / desaturase (FadX) enzymes from linoleic acid
- The FadX of only 3 CLnAs were uncovered:
 - α -caldenic acid [2,3]
 - α -eleostearic acid [4]
 - punicic acids acid [5,6]



A Type IIS RFC101 Loop assembly system for Yarrowia lipolytica

We have designed a Loop assembly system [7] for the oleaginous yeast *Yarrowia lipolytica* that makes fast and efficient cloning possible by Golden Gate. It offers modularity for assembling complex genetic circuits and their subsequent transfer and integration into the *Yarrowia lipolytica* genome. This platform facilitates future cloning of genetic constructs for *Yarrowia lipolytica* and makes it more accessible to the scientific community in general, and the iGEM community in particular.



Yarrowia lipolytica: Yes, but which promoter(s) ?

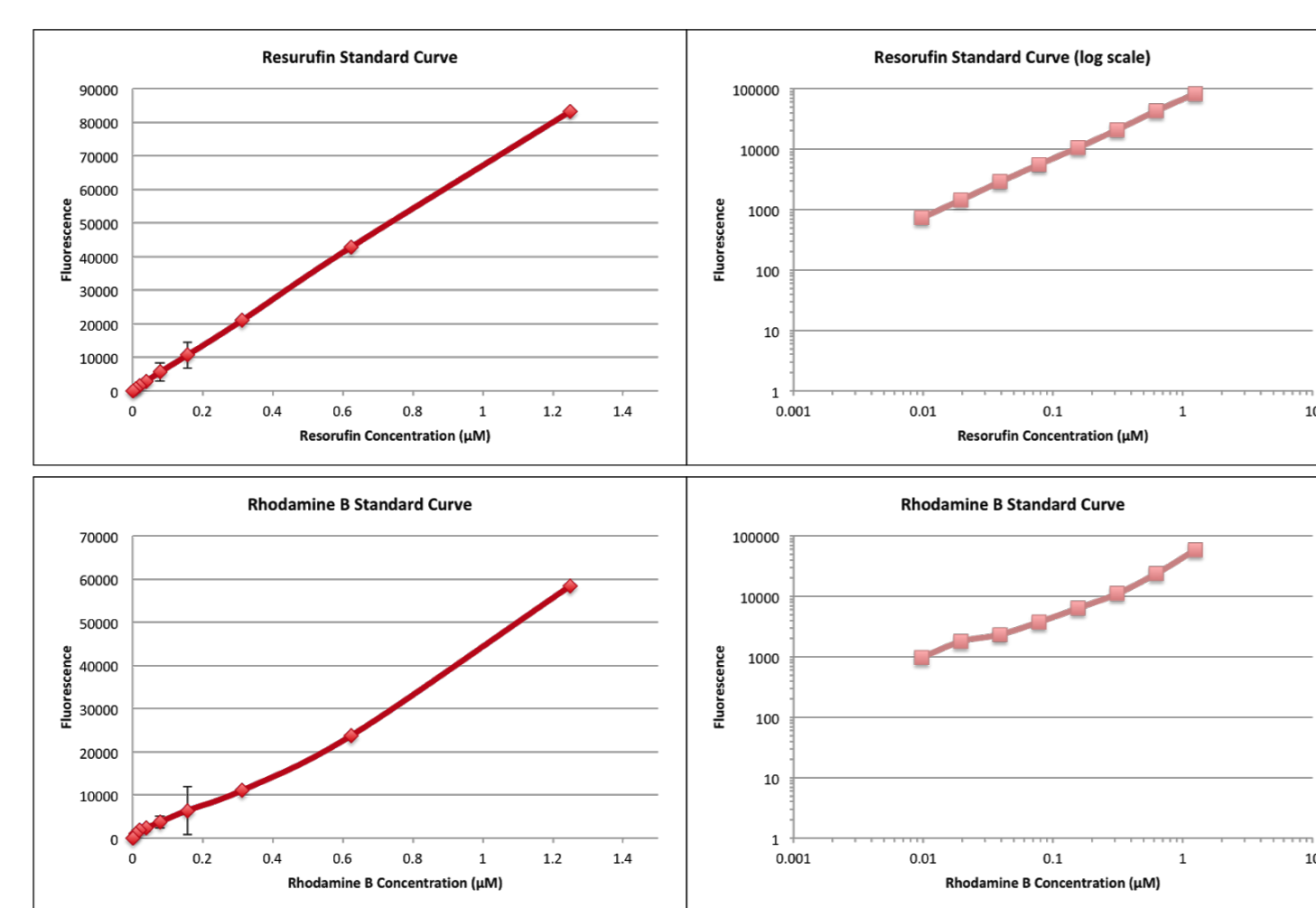
pTef1, a versatile and robust promoter



- We have successfully built 4 versions of the pTef1 promoter:
 - pTef1c (BBa_K2983050)
 - pTef1d (BBa_K2983051)
 - pTef1e (BBa_K2983052)
 - pTef1f (BBa_K2983053)
- which are able to drive the expression of a reporter gene in the oleaginous yeast *Yarrowia lipolytica* at an equivalent strength as the part already present in the iGEM Registry, pTef1a (BBa_K2117000).
- we have made an improvement of the BBa_K2117000, a version of pTef1 promoter not compatible with the Type IIS RFC(1000).

New Calibration

RedStar2 (BBa_K2983060) emits bright red light upon excitation ($\lambda_{excitation}$ 558 nm and $\lambda_{emission}$ 586 nm) and was introduced as a the "brightest and most yeast-optimized version of the red fluorescent protein" [8]. It's for this reason that we chose it for characterization in the yeast *Yarrowia lipolytica*.



Using two new compounds [11]:

- resorufin ($\lambda_{excitation}$ 571 nm and $\lambda_{emission}$ 585 nm)
 - rhodamine B ($\lambda_{excitation}$ 555 nm and $\lambda_{emission}$ 580 nm)
- we bring to iGEM new standardization methods that are particularly useful in the context of diversification of synthetic biology chassis which need fluorescent proteins other than GFP.

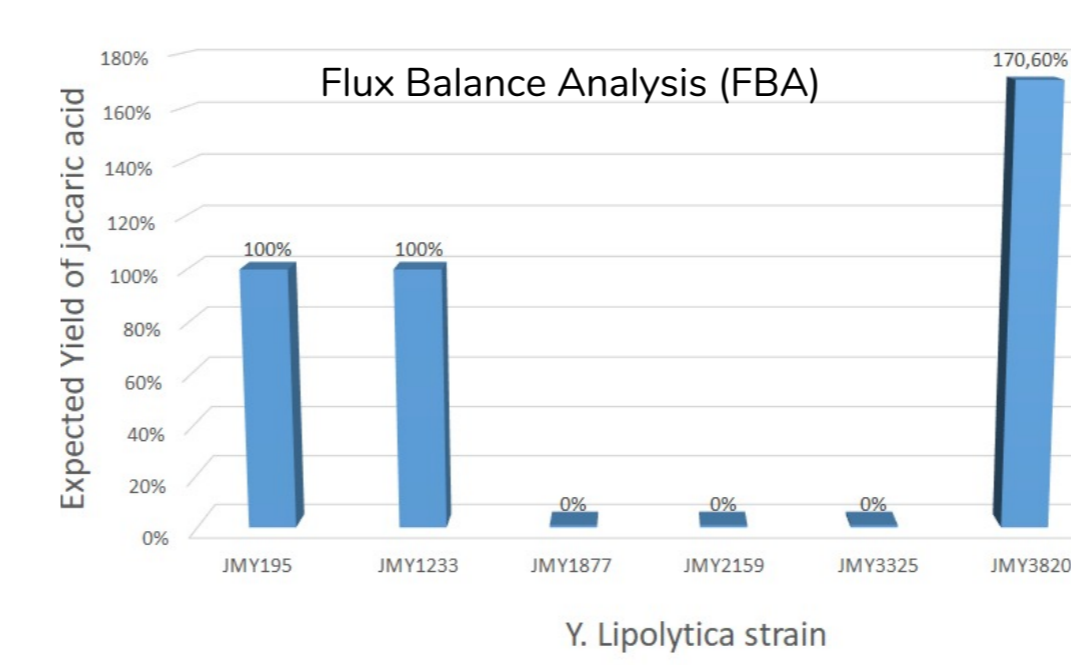
References

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Yarrowia lipolytica: Yes, but which strain(s)?

Modeling \Rightarrow JMY3820 is the best strain to produce CLnAs by FadX from linoleic acid

- the mechanisms of fatty acids' degradation through the β -oxidation pathway are disrupted (pox1-6A)
- the triacylglycerol mobilisation is inhibited by the disruption of the gene encoding the triglyceride lipase (Tgl4)
- two enzymes of the triacylglycerol biosynthetic pathway, the acyl-CoA: diacylglycerolacyltransferase (DGA2) and glycerol-3-phosphate dehydrogenase (GPD1) are overexpressed to push and pull triacylglycerol biosynthesis

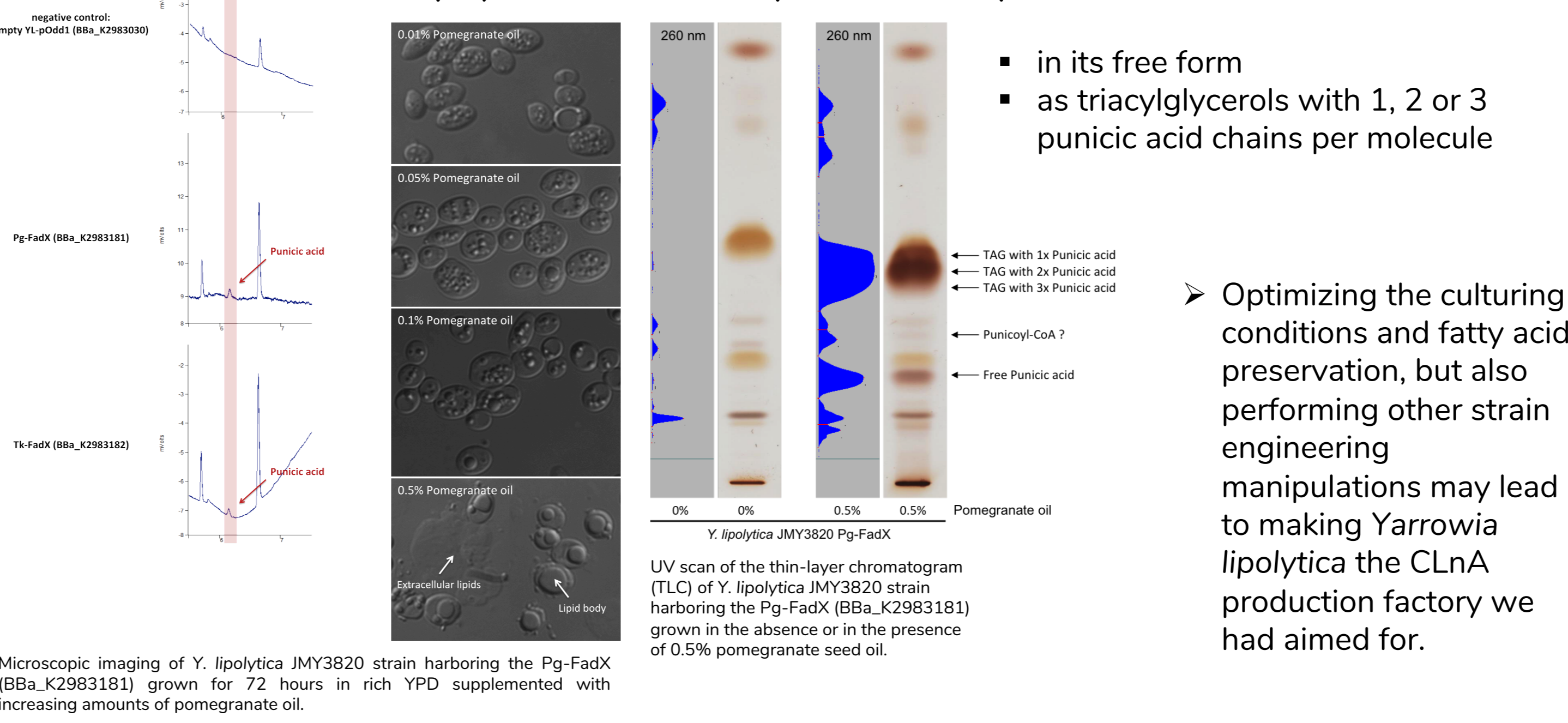


Punicic acid bioproduction in Yarrowia lipolytica

Y. lipolytica is able to produce punicic acid:

- Peak at the same retention time that positive control (pomegranate seed oil)
- Peak only present if a FadX [5,6] is present:
 - Punica granatum (Pg-FadX)
 - Trichosanthes kirilowii (Tk-FadX)
- Both Pg-FadX and Tk-FadX are active
- The best strain is JMY3820 = the one we selected according to FBA modeling
- Punicic acid yield is low

Y. lipolytica is able to stock punicic acid in lipid bodies



Jacaric acid: sequencing of Jacaranda mimosifolia exome & identification of a putative FadX

Wet Lab experiments:

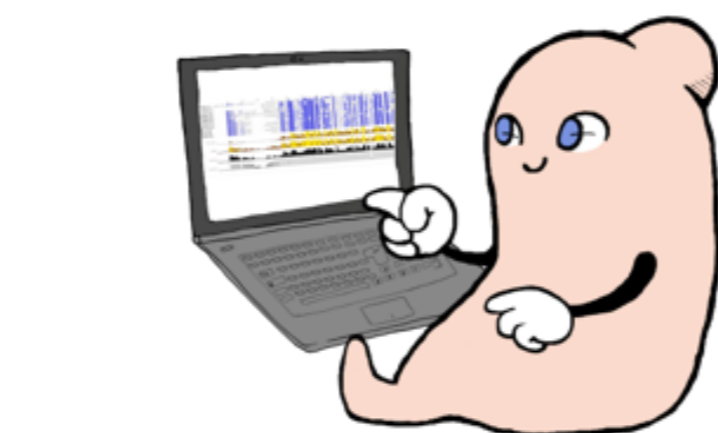
RNA extraction from fresh and germinated seeds of *Jacaranda mimosifolia* \Rightarrow Illumina RNAseq \Rightarrow

Dry Lab analysis:

- de novo transcriptome assembly
- alignment to a reference genome: *Handroanthus impetiginosus*

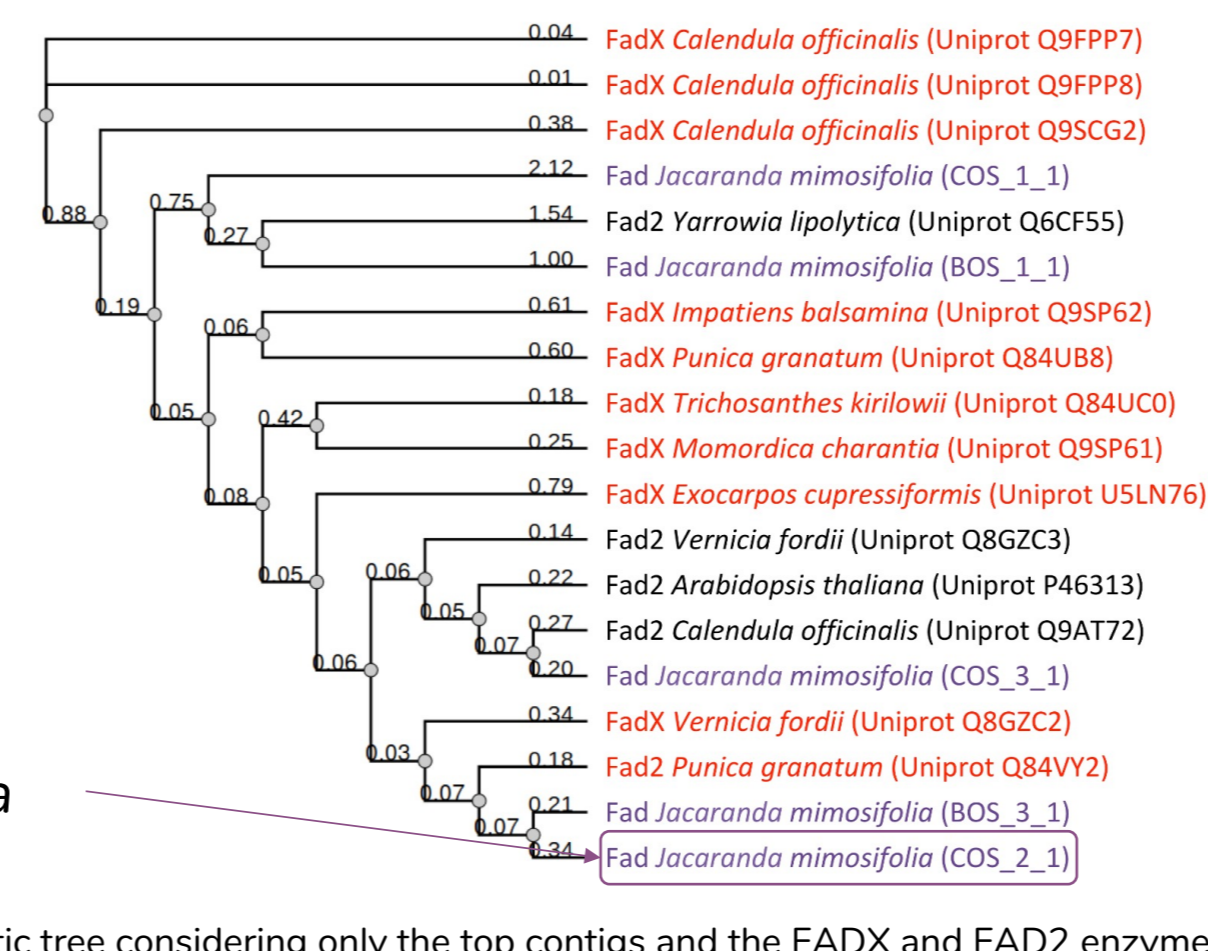
Integrated Human Practices:

Florian Jabour is a botanist at the National Museum of Natural History in Paris. We met him in his laboratory and discussed our plan to identify a specific ORF in the *J. mimosifolia* genome and clone and express it in *Y. lipolytica*. He pointed us to *Handroanthus impetiginosus*, a tree from the same family as *J. mimosifolia*, the Bignoniaceae, who is the only one from this family having the genome sequenced [17].



FadX and Fad2 sequences are very similar

- COS_2_1 is a full length ORF.
- It is therefore a putative FadX form *J. mimosifolia*
- BBa_K2983063



more Human Practices

SWOT analysis of economical aspects

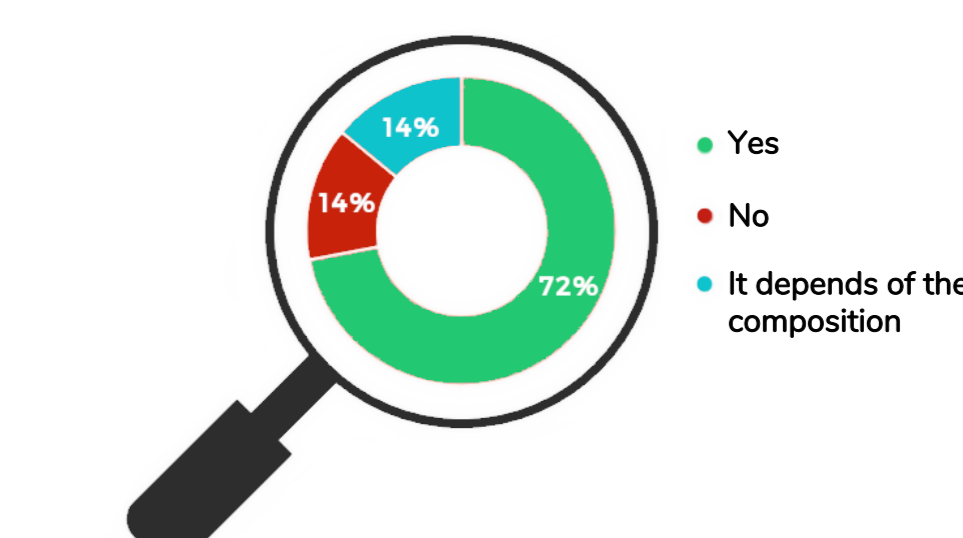
Strengths	Weakness
<ul style="list-style-type: none"> No dependence on agriculture Production of pure fatty acid Low production cost Preserve environment 	<ul style="list-style-type: none"> Find production pathways for all the fatty acids
Opportunities	Threats
<ul style="list-style-type: none"> Creation of new drugs to treat cancer and inflammatory disease Low competition market Reduce the price of punicic acid and thus recover sales volumes 	<ul style="list-style-type: none"> Limited clientele Pomegranate fruit based products are not the best selling products

We conducted a SWOT study to determine the strengths and weaknesses of our fatty acids production technique compared to what is already on the market. This work helped us understand the financial risks taken, the possible lack of innovativeness and the time it would take to commercialize such products.

VOX POP



If we would bring these rare fatty acids on the market (as treatments, or in cosmetics), would you use them knowing they were made by GMOs ?



Collaborations

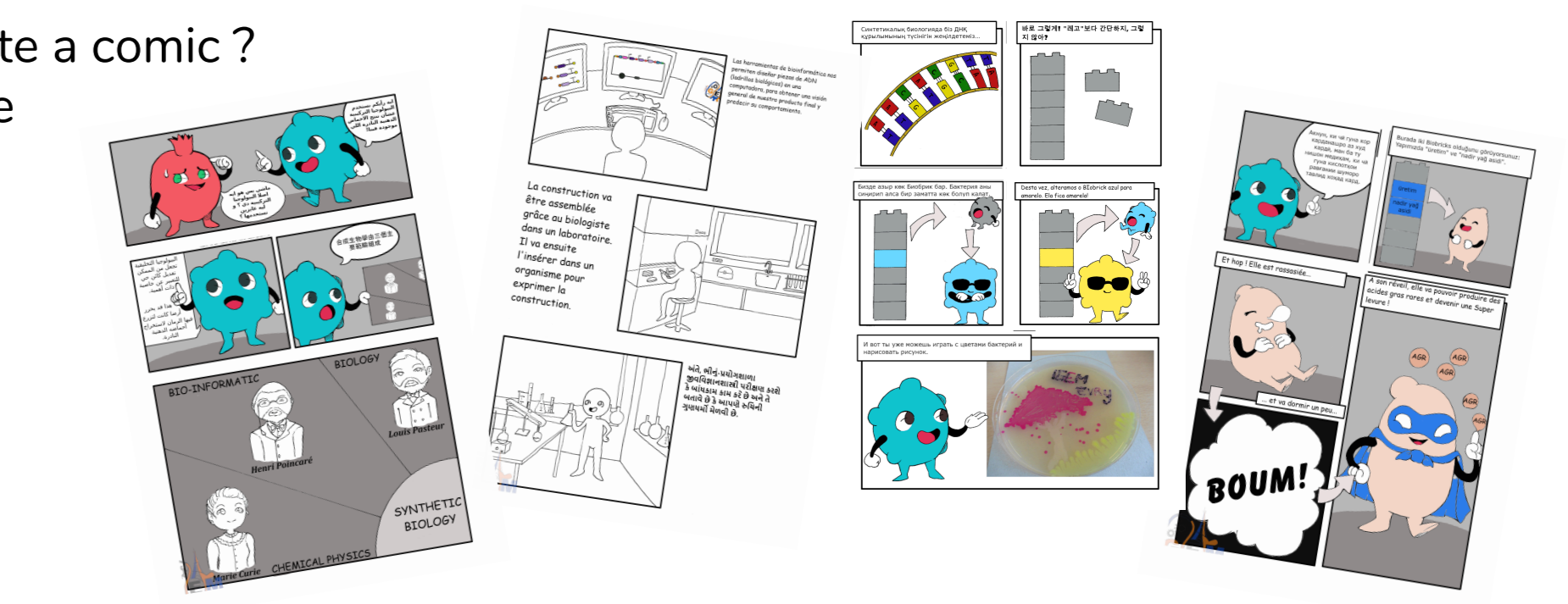
Our team organized 2 collaborations:

- InParis Meet-Up, where our team was responsible for managing the funds and for the administrative aspects related of the 17 teams that came to the Meet-Up.



- In how many languages can you translate a comic ?

- We made a comic strip explaining the synthetic biology and its usefulness in our project.
- Comic translated in 12 languages: French, Arabic, Spanish, Portuguese, Gujarati, Slang, Chinese, Kazakh, Korean, Tajik, Kyrgyz and Turkish.
- By:
 - By AFCM-Egypt
 - CCA_San_Diego
 - Bioriild_Somaiya
 - CU
 - Hong_Kong_HKU



Take a break with our comic!



Education & Public Engagement

We focused our mediation and awareness-raising activities on science, and especially on synthetic biology, our project, but also on current hot topics such as vaccination or GMOs. Our audiences ranged from 5 to 70 years old.

We participated at Science Festival where we presented 4 activity around science:

- banana DNA extraction
- red cabbage pigments will give you colors
- draw me a yeast
- exhibition around bacteria



We proposed an afternoon science mediation around microorganisms in elementary school.