Study of genomic stability of heterologous production at simulated industrial-scale cell concentration with *Yarrowia lipolytica*

In this project, it was shown that an industrial yeast was not able to sustain production at industrially-relevant volumes after continuous growth over time.

The global economy is controlled by petroleum nowadays. For decades, it has supplied all our needs by creating the chemical compounds necessary to manufacture the things we encounter in our everyday lives. However, our society is on the brink of collapse as we know it. Climate crisis is a reality and our actions are not enough to mitigate its consequences. One alternative to unsustainable oil is cell factories. Scientists have always wondered what's inside microorganisms. Why do they live? What is their function in the planet? Are they beneficial/detrimental for us? One thing we know for sure is that they can deliver chemical compounds that have industrial applicability as products of biotechnological interest. We also have the tools to modify them so that they become production machines; the so-called cell factories. Nevertheless, engineered microorganisms do not perform as expected in industrial settings, since they usually drop production in exchange for growing better. These bugs don't like to be stressed to produce compounds, they just want to live happily and grow as fast as possible!

In my project, the yeast *Yarrowia lipolytica* was engineered to produce either β-carotene or fatty alcohols; both products of biotechnological interest, by introducing many copies of genes by two different strategies (to find out the most stable) that enable their production. These yeasts were left growing for several generations in lab-scale in plates the same way they would grow for an industrial setup in bioreactors. After measuring the production of the compounds of interest, both decreased and yeasts showed more production fitness (only for β-carotene). Moreover, both strategies were equally unstable. This means that this problem is hampering the industrial application of microorganisms and therefore to help us stop pumping oil out of the ground. Innovative ways to engineer microorganisms so that they stop disliking producing a lot of a compound of interest are needed in order to generate a bio-based economy, which would create new jobs and rethink the whole system of chemicals manufacturing; towards a more sustainable industry.

My work could be used to show how easy it is to lose production in an industrial strain, but more importantly, future work should focus on analyzing the whole genome to unravel the mechanism by which production is lost in exchange of growth. This would light up new inventions to overcome this problem.