Exploring Microbial Robustness for a Sustainable and Efficient Bioproduction

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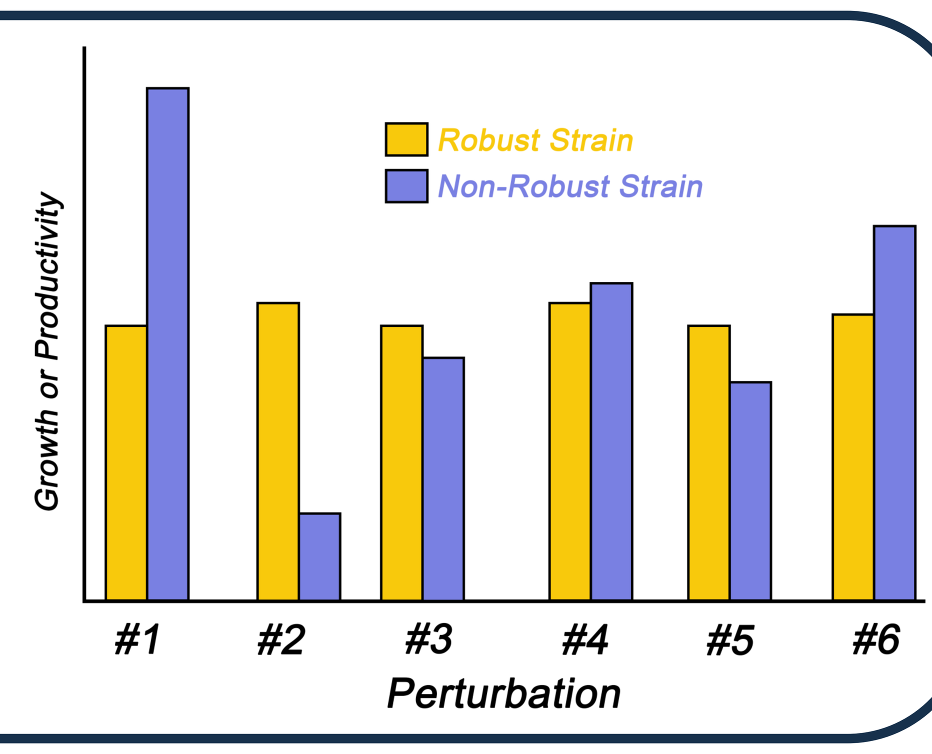
Background

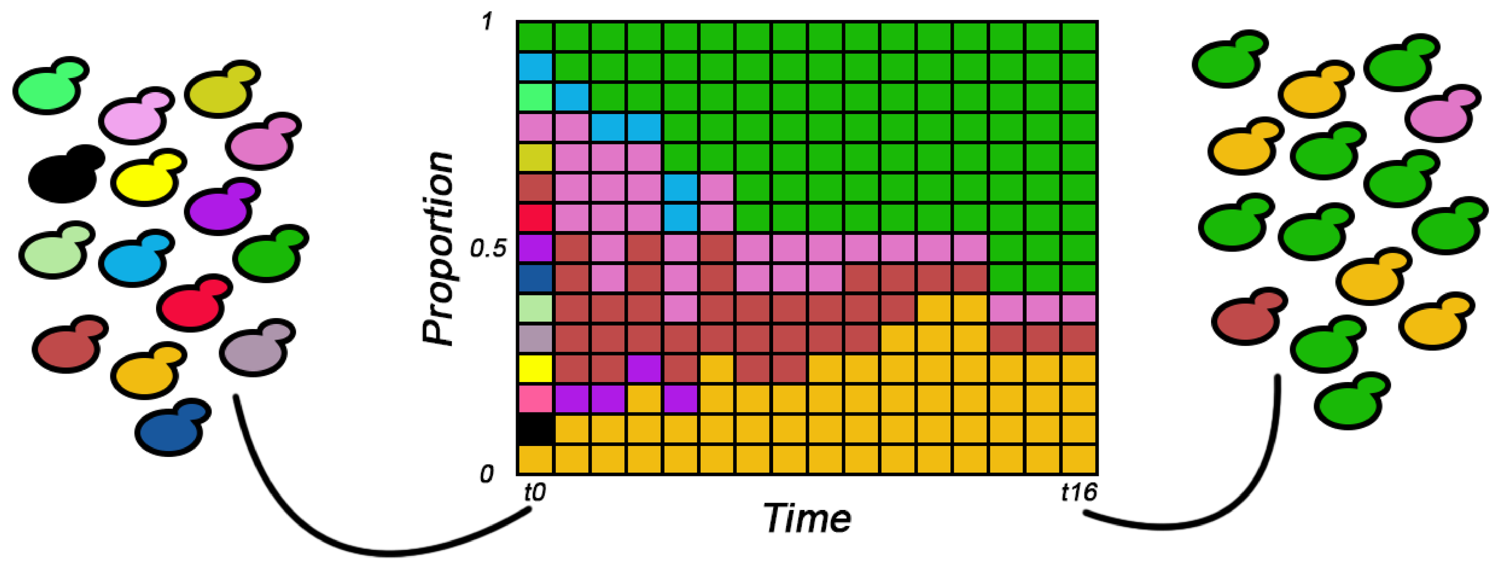
Microbial Robustness refers to the ability of a microorganism to maintain a stable performance in face of different perturbations. Industries constantly need more robust strains to improve their bio-based production. However:

- **Population dynamics** have been poorly studied in industrial processes;
- More in-depth studies at a single-cell level of **cellular status** are now missing from the industrial scenario;
- Industrialists and researchers lack an objective method to **quantify robustness** and compare strains.

Here we propose different ways to approach these problems in yeast *Saccharomyces cerevisiae*.

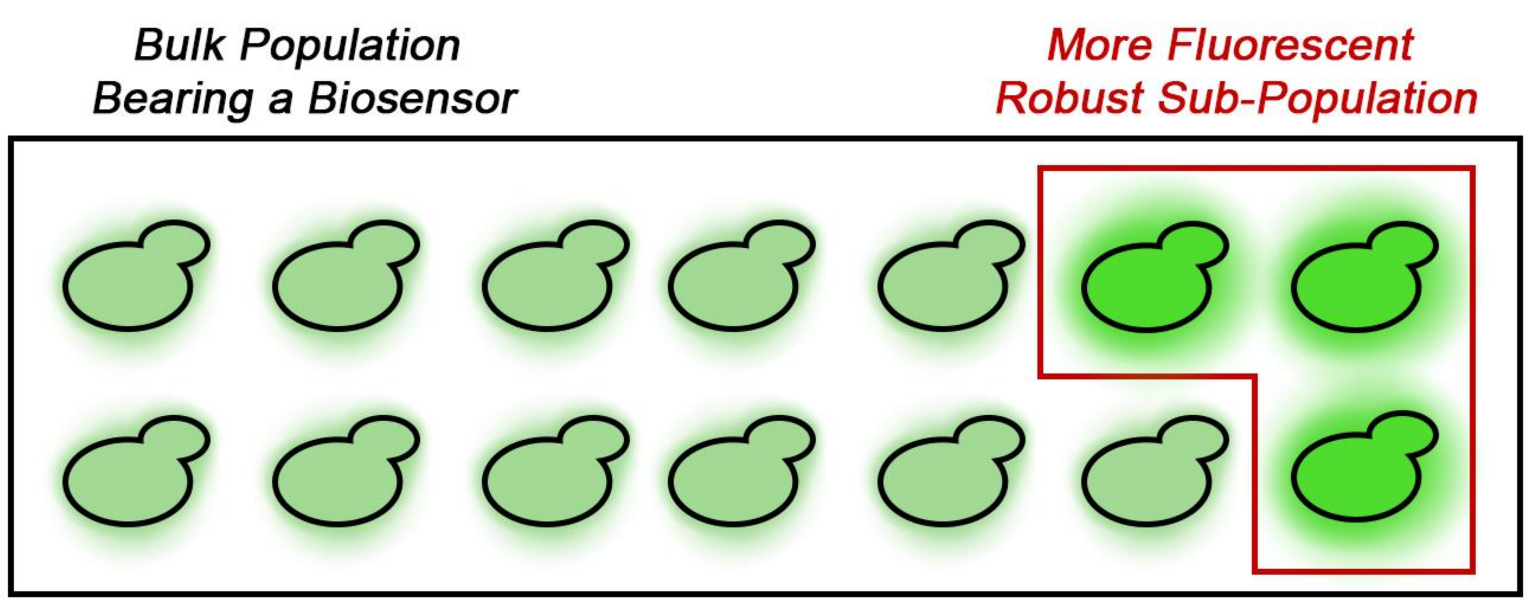


Population Dynamics



In order to track population dynamics and identify antagonistic and synergistic traits of robustness in diverse environments, we developed *S. cerevisiae* strains with different barcodes integrated in the genome. This technology will be used to highlight the different sub-populations that are developed during relevant industrial processes.

Cell Status

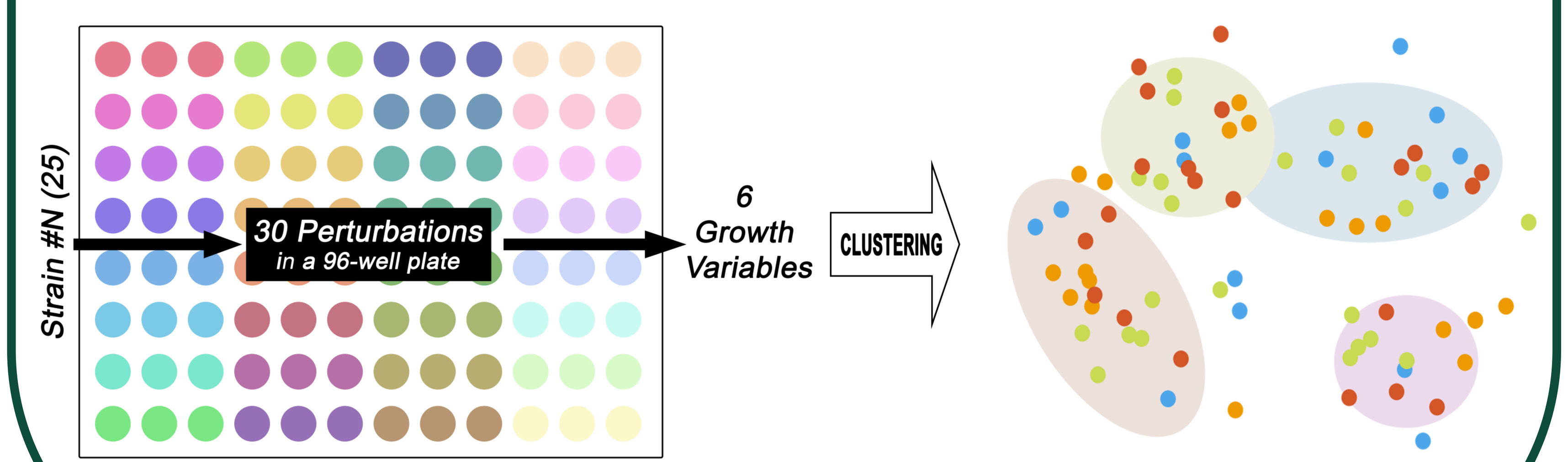


Thanks to the use of fluorescent biosensors, it would be possible to analyse cell populations at the single-cell level and identify robust sub-populations that might give insights on new robustness features for the development of new strains. Biosensors would monitor:

- Intracellular parameters → intracellular pH, ATP levels, ribosome concentration, oxidative stress or glycolytic flux;
- Cellular status → identification of replicating, non-replicating, metabolically active, quiescent, senescent and dead cells.

Robustness Quantification

Natural yeast diversity will be exploited to study robustness from a physiological point of view. 25 yeast strains from different origins (laboratory, industry, wild) will be grown in 30 different relevant industrial perturbations in a 96-well plate. The six growth variables obtained (μmax, doubling time, lag phase, adaptability, ethanol yield and cell dry weight) will be used to develop a method which classifies strains based on their robustness and MVA (multivariate analysis) will be performed to identify common features underlining important robustness factors.



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