# Microbial robustness in bioprocesses

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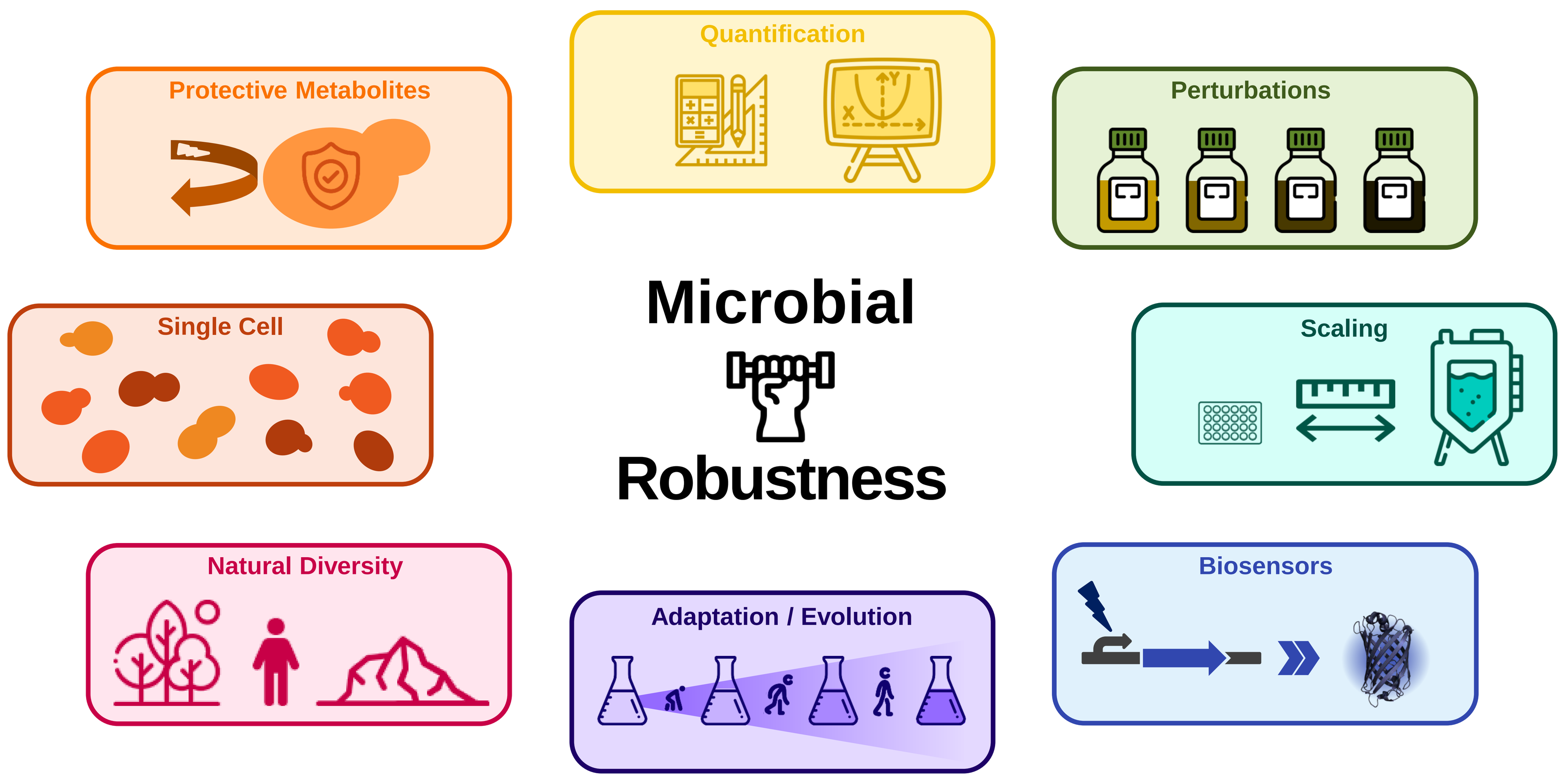
## Background

- A broad range of predictable and stochastic **perturbations affects the performance** and productivity of microorganisms in bioprocesses.
- **Microbial robustness** describes the stability of a phenotype (cellular function) when a system is challenged by different perturbations.



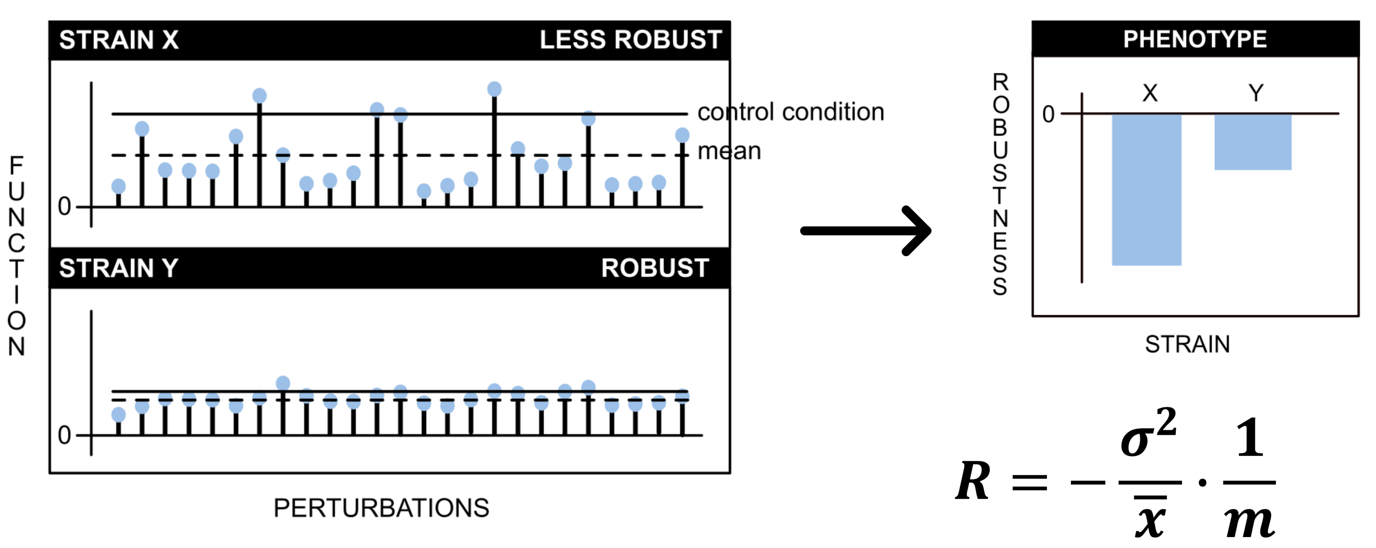

- It **differs** from **tolerance**, which relates to the survival of the cells or growth in the face of perturbations.
- **Lack of tools** to quantify microbial robustness and investigate the physiological traits associated with it under bioprocess-relevant perturbations.

Check the review!

## TOOLS

### Quantification

Trivellin *et al.*, 2022. doi: 10.1021/acssynbio.1c00615



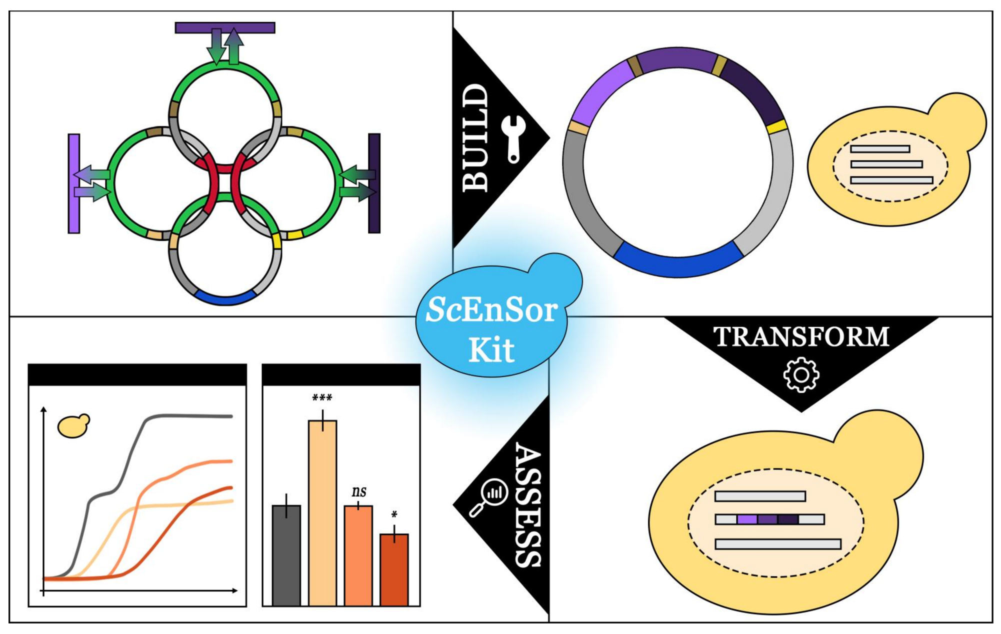

$$R = -\frac{\sigma^2}{\bar{x}} \cdot \frac{1}{m}$$

- This robustness quantification method is dimensionless, free from arbitrary control conditions and frequency independent.
- Robustness is **function-specific** and characterized by **positive and negative** function-specific **trade-offs**.

### Toolkit

Torello Pianale *et al.*, 2022. doi: 10.3389/fmicb.2021.802169

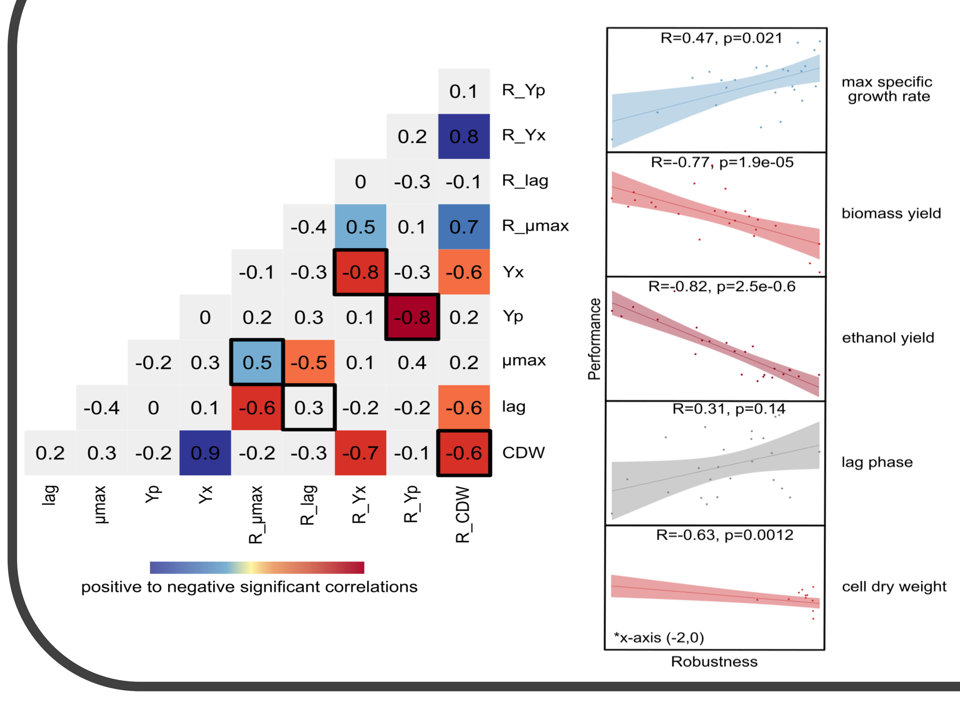
- *Saccharomyces cerevisiae* Engineering + Biosensor Kit → *ScEnSor* **Kit**
- Addgene ID#1000000215
- User-friendly, flexible and customizable kit made of 2 modules.




- **Genome Integration Module**: a set of backbone plasmids to build cassettes to be easily integrated into the genome. Possible to use to introduce new biosensors in the kit.
- **Biosensor Module**: a set of eight biosensors for monitoring the intracellular environment in *S. cerevisiae* (ready to be integrated into the genome)

## APPLICATIONS

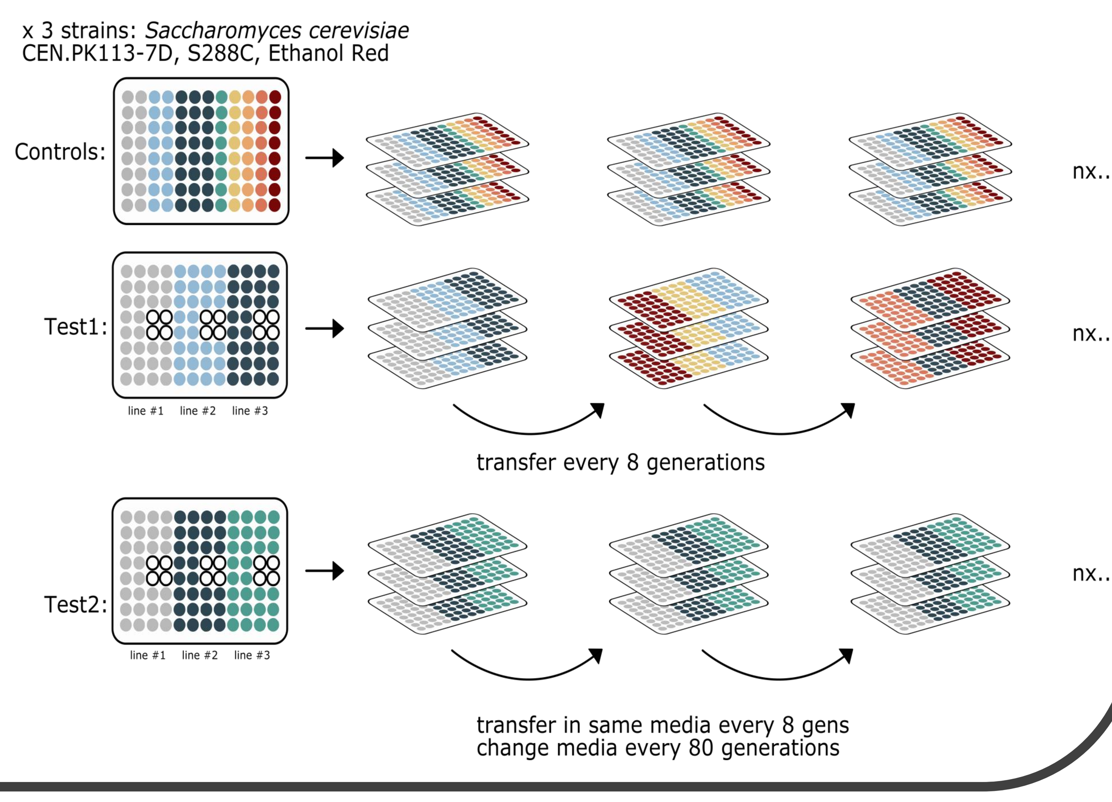
### Performance-Robustness Trade-offs



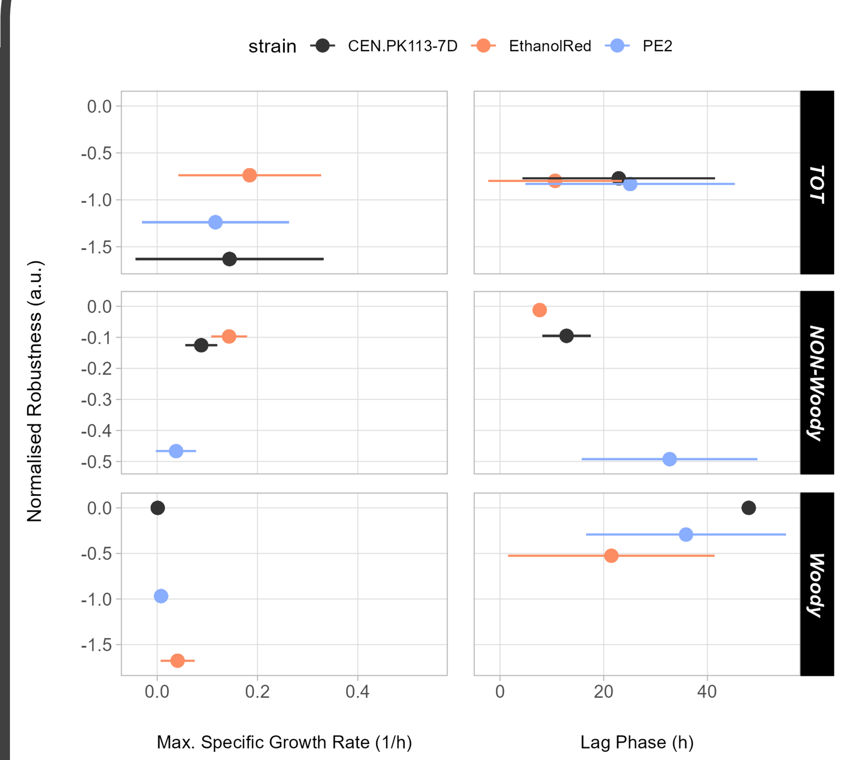

- Phenotypic characterization (μmax, lag, CDW, $Y_P$, $Y_X$) of 24 *Saccharomyces cerevisiae* strains (laboratory, industrial, isolated from nature) was conducted in 29 perturbations.
- Spearman correlations revealed **trade-offs** between performance and robustness for biomass, ethanol yield and cell dry weight.
- Maximum specific growth rate was the only function with a positive correlation.

### Evolution of Robustness in Fluctuating Environments

- Three *S. cerevisiae* strains were evolved for 400 generations in three parallel evolution lines (constant media, transfer in random new medium every 8 generations and every 80 generations).
- Discover potential genetic variants associated with higher robustness using whole genome sequencing and fitness-robustness measurements.

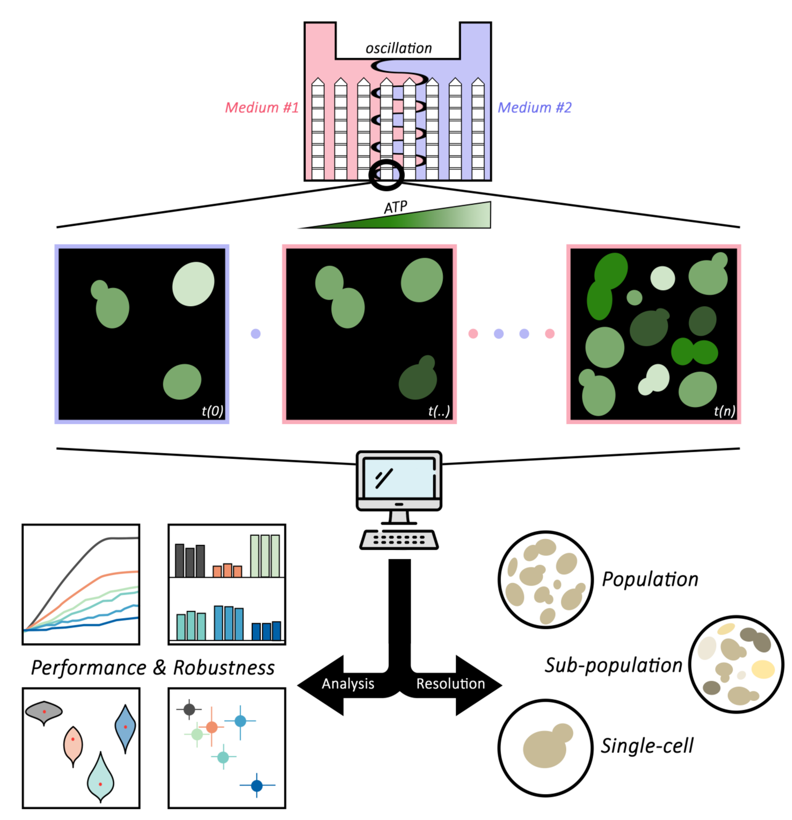



### Strain comparison in lignocellulosic hydrolysates




- Three *S. cerevisiae* strains were grown in 8 different hydrolysates (wheat straw, sugarcane bagasse, out hulls, corn stover, softwood logging residues, spruce and birch).
- Performance and robustness of lag phase and specific growth rate were assessed (figure above).
- Using the biosensors in the *ScEnSor* Kit and the robustness quantification method, the stability over time of different intracellular parameters was also computed.

### Pipeline combining dMSCC and Robustness




- Dynamic microfluidic single-cell cultivation (dMSCC) is a versatile tool to investigate the effects of quickly-fluctuating environments on microorganisms.
- Ideal for downscaling to lab-scale the heterogeneous and dynamic environments found in bioreactors due to poor mixing.
- Robustness quantification was used to assess the stability of functions over time and their heterogeneity within the cell populations.

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