# Brilliantly lazy: how Cupriavidus necator shines at avoiding work



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## Background

Methods

A general challenge of upscaling in industrial biotechnology is the loss of performance of strains. Increased cultivation times and stressful conditions within the bioreactor stimulate population diversification. This is often paired with loss of production negatively impacting performance, the economic viability of the bioprocess. Here we study the mechanisms in which C. *necator* engineered to produce isopropanol (IPA) escapes plasmid production of the solvent in controlled bioreactor settings.

- o eGFP was used as a biosensor to track plasmid expression of isopropanol  $^{1,2}$ .
- Flask cultures and three continuous cultures ( $\mu = 0.04$  $h^{-1}$ ) were run:
  - No IPA production





- IPA production
- IPA production with selective pressure
- Analysis with FC, FACS, ddPCR, plating methods and sequencing.

## Results



- IPA production causes drastic Ο drop in eGFP<sup>+</sup> population.
- Antibiotic selective pressure does hinder initial population not diversification.
- selective • Antibiotic pressure results in an increase of eGFP<sup>+</sup> population initial after population drop.

## **Group immunity**

The eGFP<sup>+</sup> population in the reactor 100

### Testing residual active kanamycin in 0,14 т filtered media

## Inhibited plasmid expression

• Bioreactor samples recovered



- Microbial tests with wild type C. necator suggest antibiotic degradation in the media.
- The loss of eGFP<sup>+</sup> thus loss of antibiotic degrading capacity could explain the regain of importance of eGFP<sup>+</sup> population after 6 generations.

non-selective media are on inclined to grow on more selective media than nonrecovered samples.

• Most single cell colonies (SCCs) from the bioreactor culture on non-selective recovered plates, regain fluorescence only when cultured on selective media.



• This implies that the plasmid is still present in individuals in the eGFP<sup>-</sup> subpopulation without being expressed.

## **Reduced plasmid copy numbers**

- of the experiment and the end.
- comparable plasmid show сору



## **Plasmid mutations**

 Bulk sequencing of samples from IPA producing conditions shows increased **SNPs** in the consensus as the cultivation continues.



 Plasmid sequencing of four SCCs picked from selective media suggest recombination as possible mechanism for varying levels of fluorescence intensity.



#### References

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