Controlling microbial population instability in continuous cultivation: impact of switching cost and phenotypic escape

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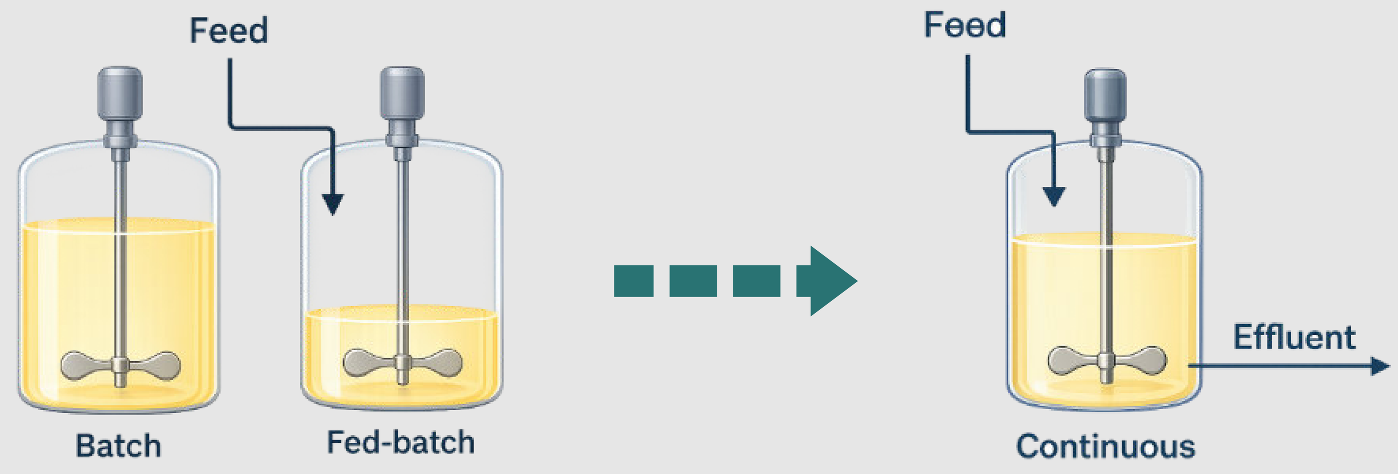
1 CONTEXT

FROM BATCH TO CONTINUOUS BIOMANUFACTURING...



Benefits of continuous bioprocessing :

- Less production dowtime
- Smaller facility footprint
- Higher space-time yield

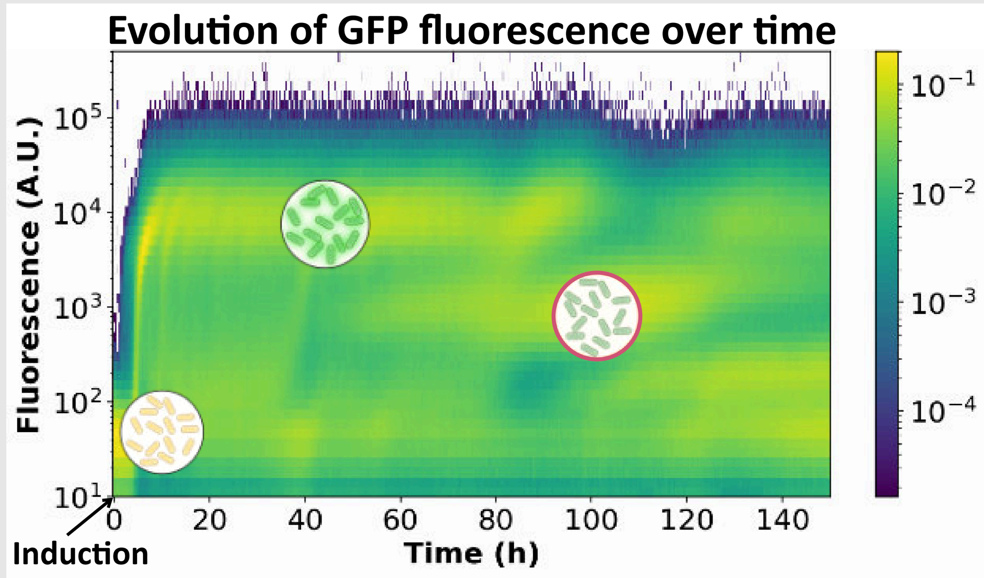
Transition limited by
CELL POPULATION INSTABILITY

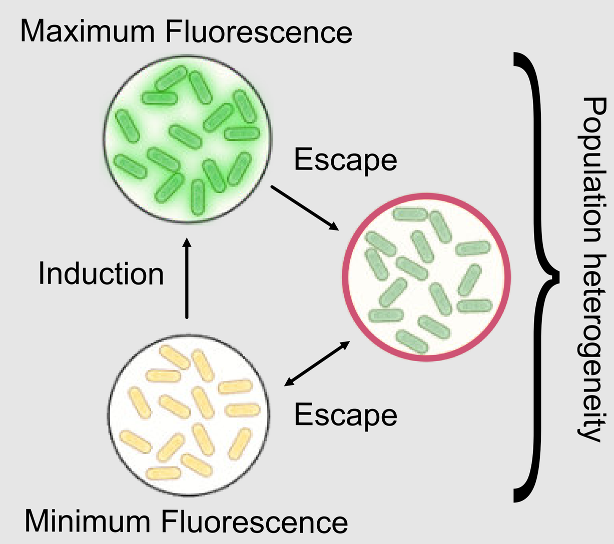
2 CHALLENGE

PHENOTYPIC ESCAPE: A DRIVER OF CELL POPULATION INSTABILITY

During continuous cultivation, fraction of cells may progressively reduce or cease recombinant protein production, leading to **heterogeneous** cell populations.

Using **GFP** as a reporter, flow cytometry reveals distinct **subpopulations** with different expression levels [1].

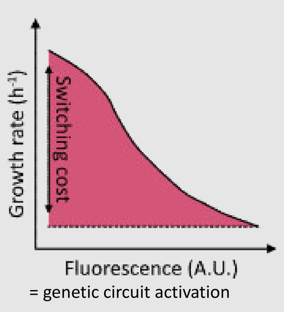




One of the mechanism underlying this behavior is **phenotypic escape**, defined as a **reversible** and **non-genetic** change in gene expression.

3 PRELIMINARY OBSERVATIONS

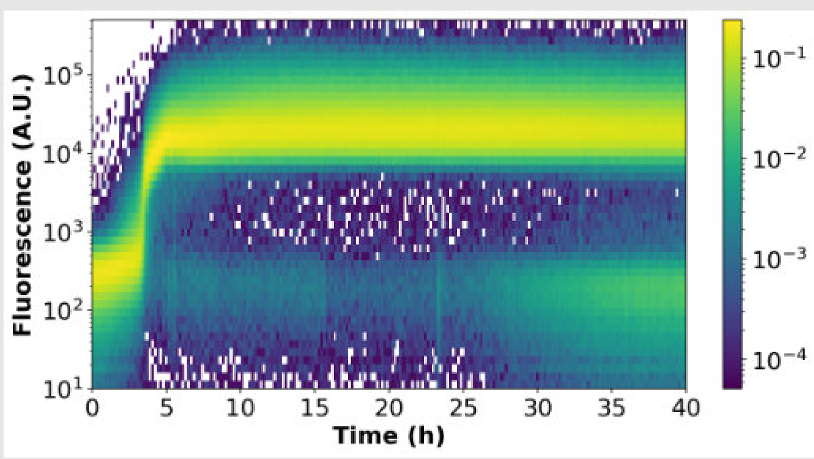
INFLUENCE OF THE SWITCHING COST ON POPULATION DYNAMICS [2]



Switching cost (SC) = Reduction in growth rate associated with activation of the target gene circuit.

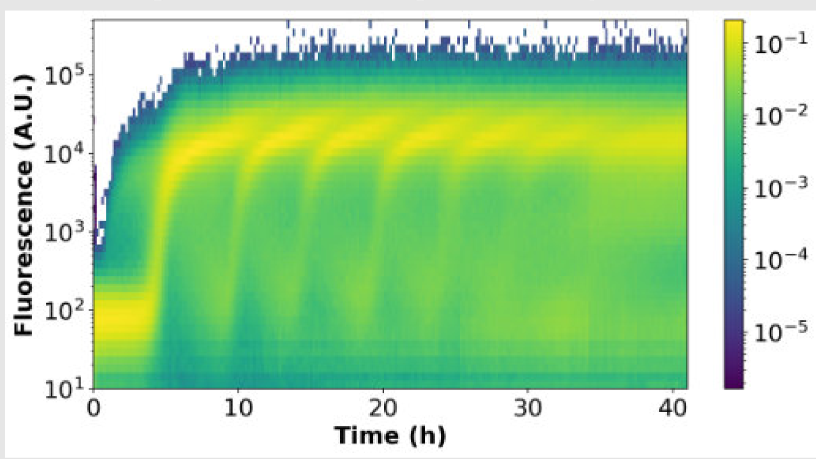
The greater the cellular resource demand, the higher the switching cost.

Low switching cost system



- Homogeneous induction

High switching cost system



- Delayed induction
- Coexistence of subpopulations
- **Escape of induction**

4 CURRENT OBJECTIVE

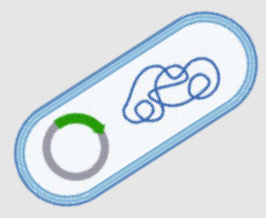
Characterize the **phenotypic escape** mechanism.

↳ **Determine** how **switching cost** affects growth dynamics and contribute to **phenotypic escape**.

5 EXPERIMENTAL APPROACH

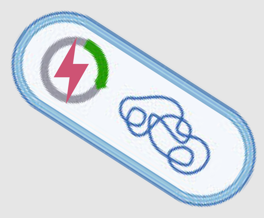
BIOLOGICAL MODELS

E. coli BL21 pUA139



Low SC system

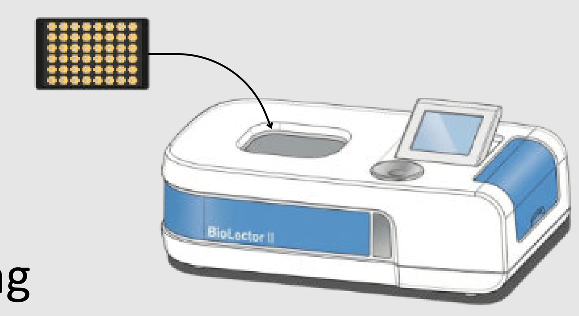
E. coli BL21(DE3) pET28



High SC system

CULTIVATION CONDITIONS

- Different carbon source mixtures (glucose/lactose, arabinose/lactose, xylose/lactose,...)
- Different lactose induction time
- Culture in microplate using BioLector
  ↳ Biomass and fluorescence monitoring

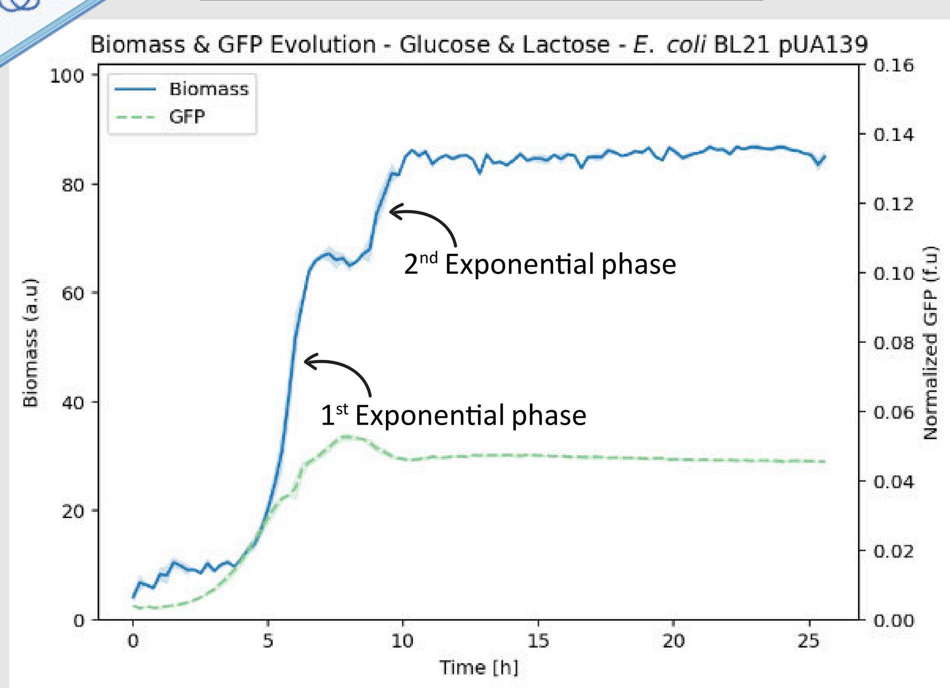


6 RESULTS

COMPARISON OF GROWTH DYNAMICS UPON LOW AND HIGH SWITCHING COST

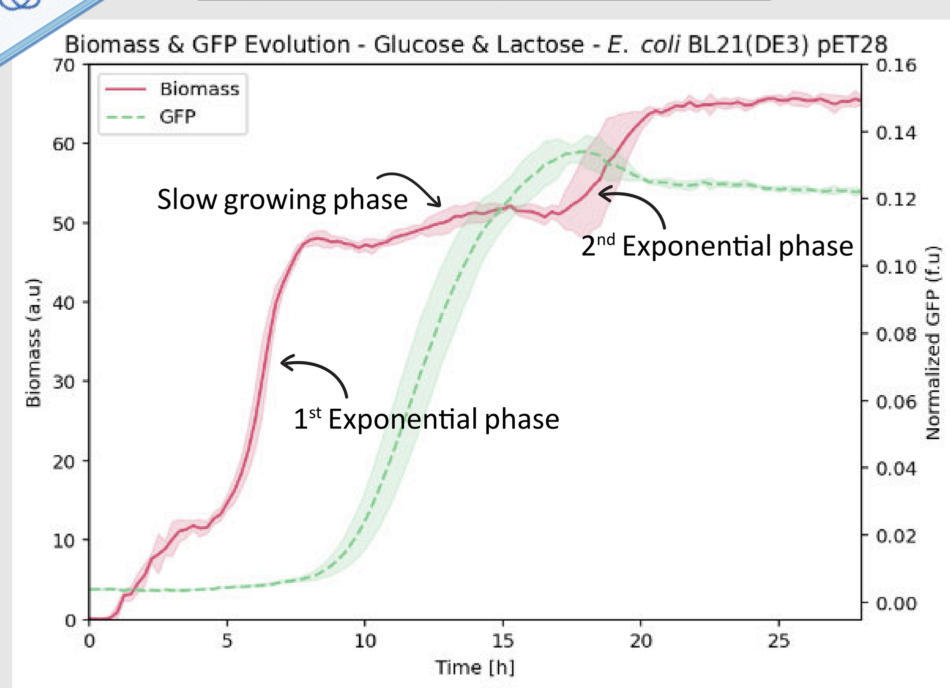
E. coli BL21 and *E. coli* BL21(DE3) on a mix of glucose and lactose

Low switching cost system



- Classical diauxic growth

High switching cost system

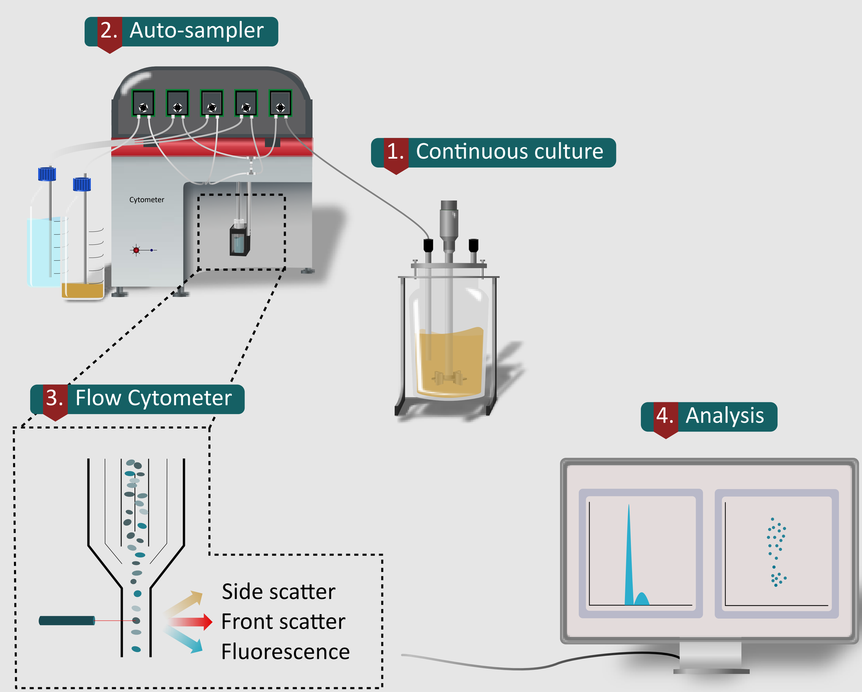


- Three distinct growing phases

The additional growth phase suggests the emergence of **complex population dynamics** under **high switching cost** conditions.

7 NEXT STEPS

- **AUTOMATED FLOW CYTOMETRY**
  Real-time single-cell population dynamics



- **OFFLINE HPLC ANALYSES**
  Glucose and lactose consumption profiling

Investigating the **link** between the additional **growth phase** and **phenotypic escape**.

More information?

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[1] Delvenne, M., Vandenbroucke, V., Henrion, L., Sehrt, M., Martínez, J. A., Sloodts, A., Telek, S., Zicler, A., & Delvigne, F. (2025). A Fitness–Entropy Compensation effect set the trade-off between growth and gene expression in cell populations (p. 2025.07.05.663304). bioRxiv. https://doi.org/10.1101/2025.07.05.663304

[2] Henrion, L., Martinez, J. A., Vandenbroucke, V., Delvenne, M., Telek, S., Zicler, A., Grünberger, A., & Delvigne, F. (2023). Fitness cost associated with cell phenotypic switching drives population diversification dynamics and controllability. Nature Communications, 14(1), 6128. https://doi.org/10.1038/s41467-023-41917-z