How could flow cytometry enable real-time monitoring of phenotypic diversity in microbial co-cultures?

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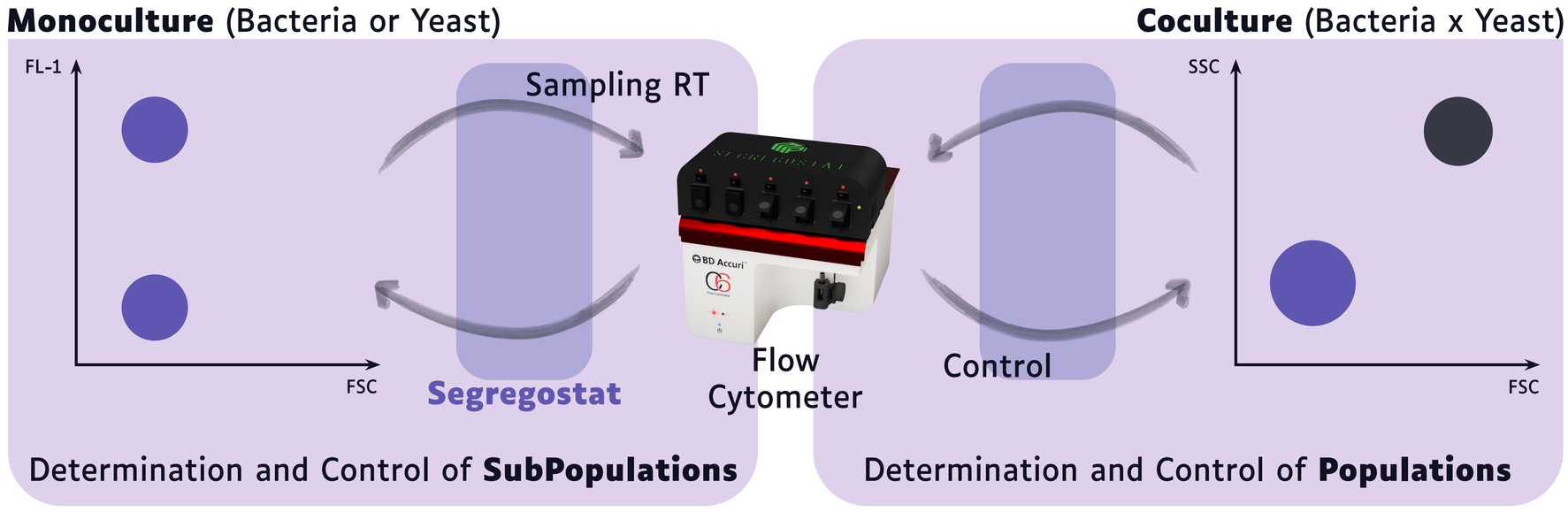
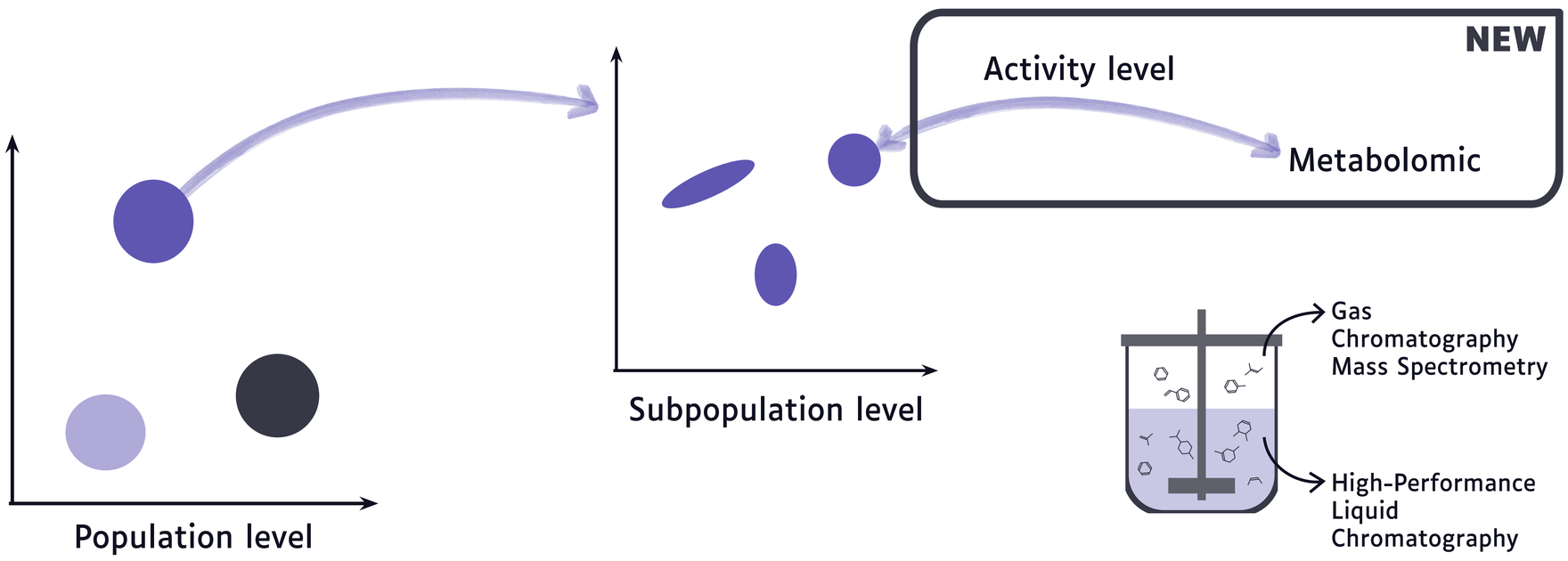
BACKGROUND Previously done in the team

Our team focuses on studying and controlling the dynamics of microbial populations and their heterogeneity[1]. To this end, we developed the **Segregostat**[2], a device enabling automated flow cytometry (FC) and feedback control loop as a strategy that can be applied to mono- and cocultures[3], providing new opportunities to control community composition and to monitor phenotypic dynamics in real time.



OBJECTIVES Combine these two levels in cocultures of 3 microorganisms (Bacteria x Yeast x Bacteria or Yeast)



Beyond combining populations and subpopulations, we aim to explore metabolite dynamics to gain deeper insight into microbial interactions.

I - Understanding of the system

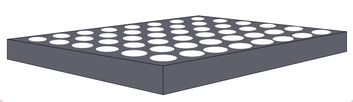
II - Control of the system

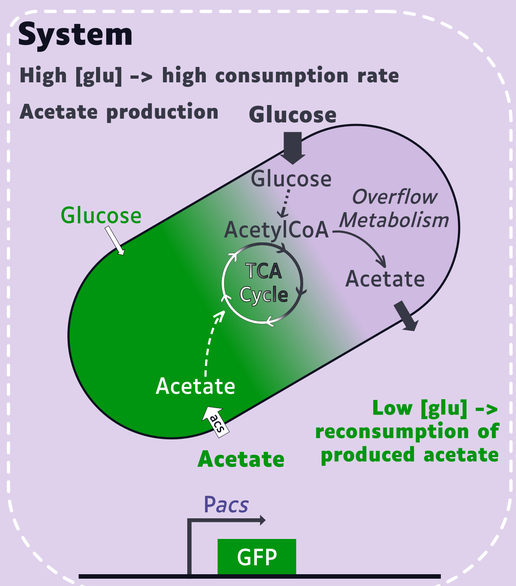
STRATEGY AND PRELIMINARY RESULTS

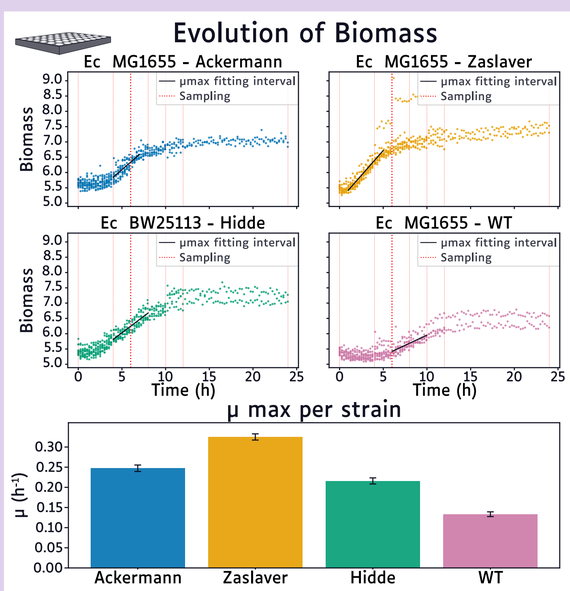
Strategy

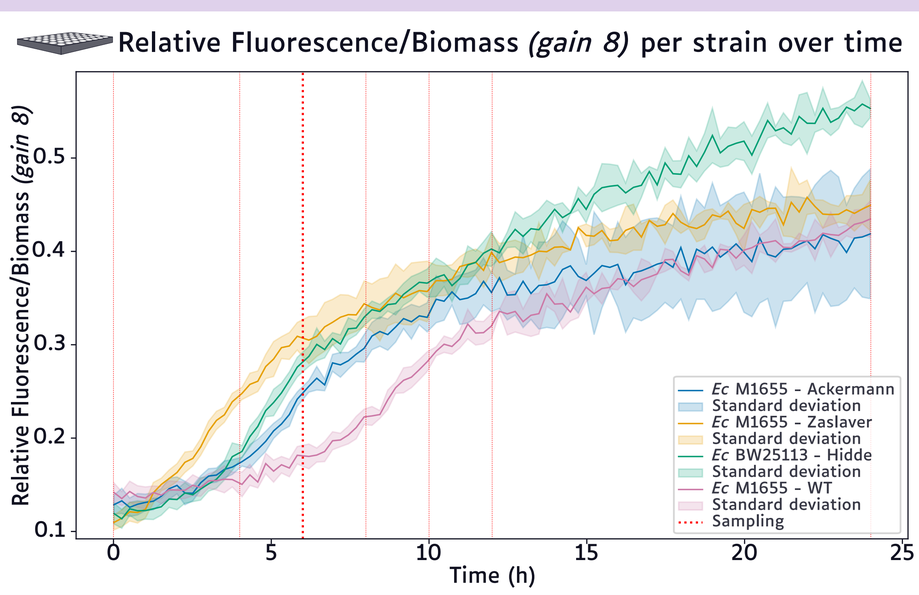
To achieve this goal, we will first focus on resolving 2 subpopulations, namely *E coli* producing or reconsuming acetate.

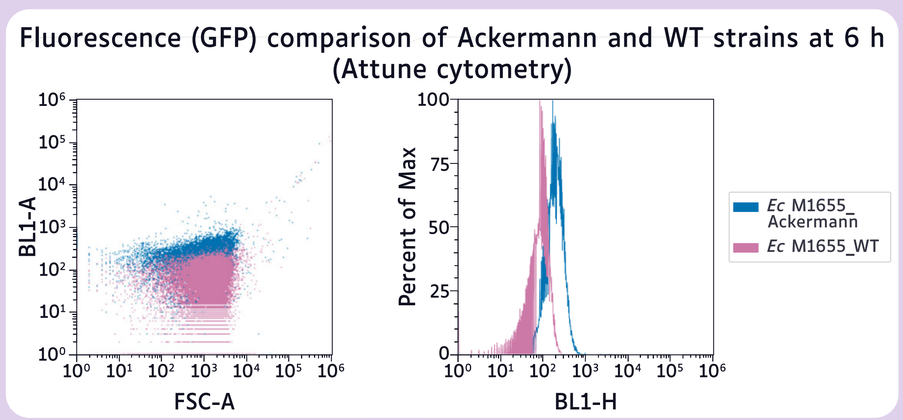
As a first step, we will characterise collection strains in BioLector.











PERSPECTIVES

1. Evaluate additional strains and circuits, and select candidates based on carbon consumption and fluorescence signal intensity
2. Continuous culture in bioreactor with the selected strain
   Online monitoring of fluorescence by FC via the use of the Segregostat
3. Metabolic characterisation of the culture and link to observed subpopulations
4. Introduction of a new organism (bacteria or yeast) to observe inter-species dynamics and their impact on characterised subpopulations

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(1) Delvigne, F., et al, Metabolic Variability in Bioprocessing: Implications of Microbial Phenotypic Heterogeneity. *Trends Biotechnol.* **2014**, 32 (12), 608–616. https://doi.org/10.1016/j.tibtech.2014.10.002.
(2) Sassi, H., et al, Segregostat: A Novel Concept to Control Phenotypic Diversification Dynamics on the Example of Gram-negative Bacteria. *Microb. Biotechnol.* **2019**, 12 (5), 1064–1075. https://doi.org/10.1111/1751-7915.13442.
(3) Martinez, J. A., et al, Automated Adjustment of Metabolic Niches Enables the Control of Natural and Engineered Microbial Co-Cultures. *Trends Biotechnol.* **2025**, S0167779924003652. https://doi.org/10.1016/j.tibtech.2024.12.005.