Controlling the phenotypic switching of *P. putida* population for the assimilation of plastic-derived compounds.

Maximilian Tieke, Juan Andrés Martinez, Frank Delvigne

1 Introduction

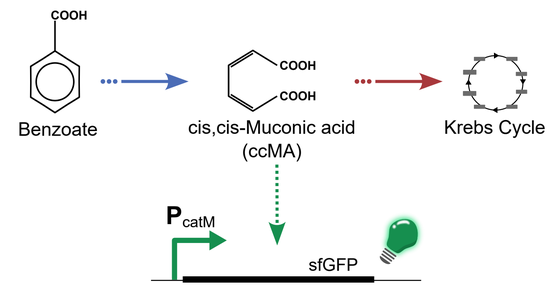
Pseudomonas putida, a versatile soil bacterium, exhibits remarkable **adaptability to** various carbon sources, including **aromatics** and aliphatic compounds. This genetic versatility positions it as a potent candidate for **bioremediation** and sustainable plastics production.

Understanding the **bacterium's phenotypic** changes in response to diverse carbon substrates **is pivotal** for optimizing these processes.

Known diversification mechanisms are linked to [1]:

- Compound degradation
- Compound efflux
- Morphological changes (membrane composition. etc)

Showcasing the potential of analysing phenotypic diversification, I am investigating the case study of *Pseudomonas putida* KT2440' s diversification process in exposure to **increasing benzoate** concentrations.

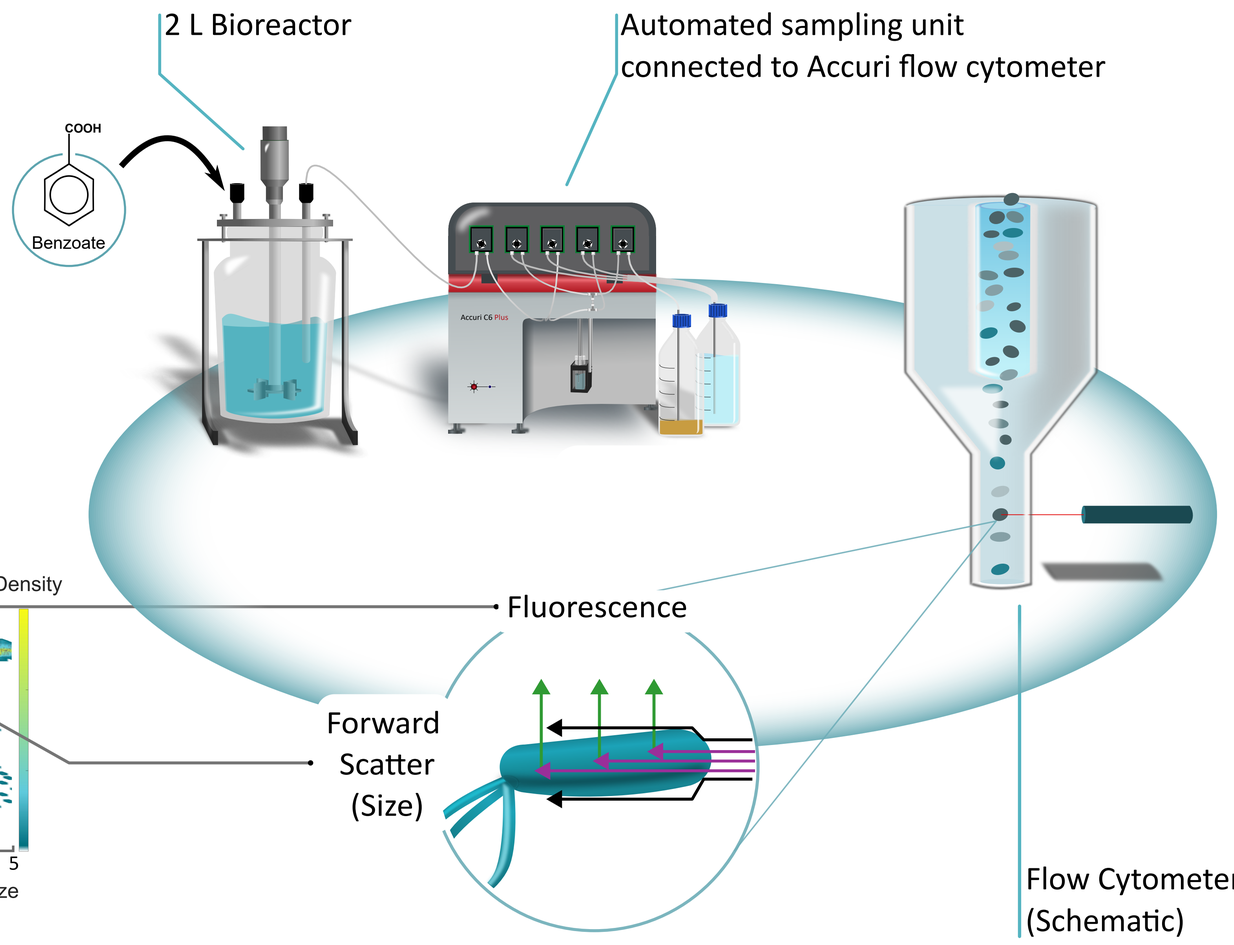


2 Methodology

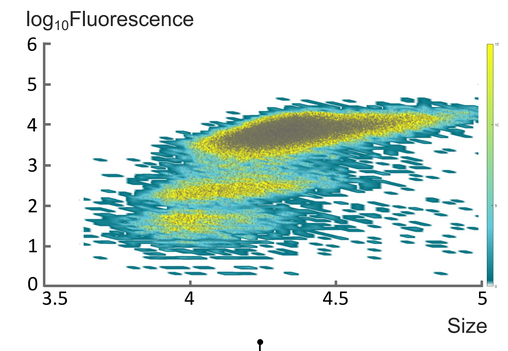
The **plasmid** pCatM_C2 was introduced into *P. putida* KT2440 to **sense** the activity of **benzoate degradation** [2].
The expressed repressor **catM** is inactivated by binding **cis,cis-muconic acid**, an downstream intermediate of the benzoate, resulting in the induction of *sfGFP*.

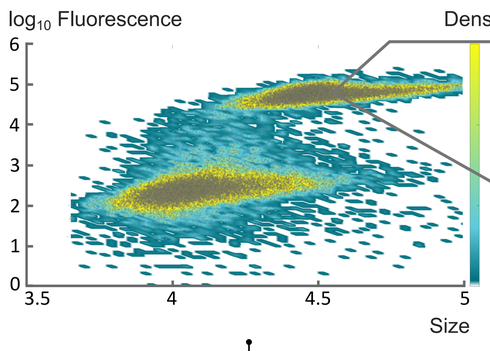
P. putida KT2440 pCatM_C2 was cultures in a continous system with modified M9 media plus 5 g/L glucose. The environmental **benzoate** concentrations **was increased by pulses** every 10 min. Diversifications were analysed with the **online cell-machine interface** [3]. The number of living cells were analysed by impedance flow cytometry (BactoBox) measurements.

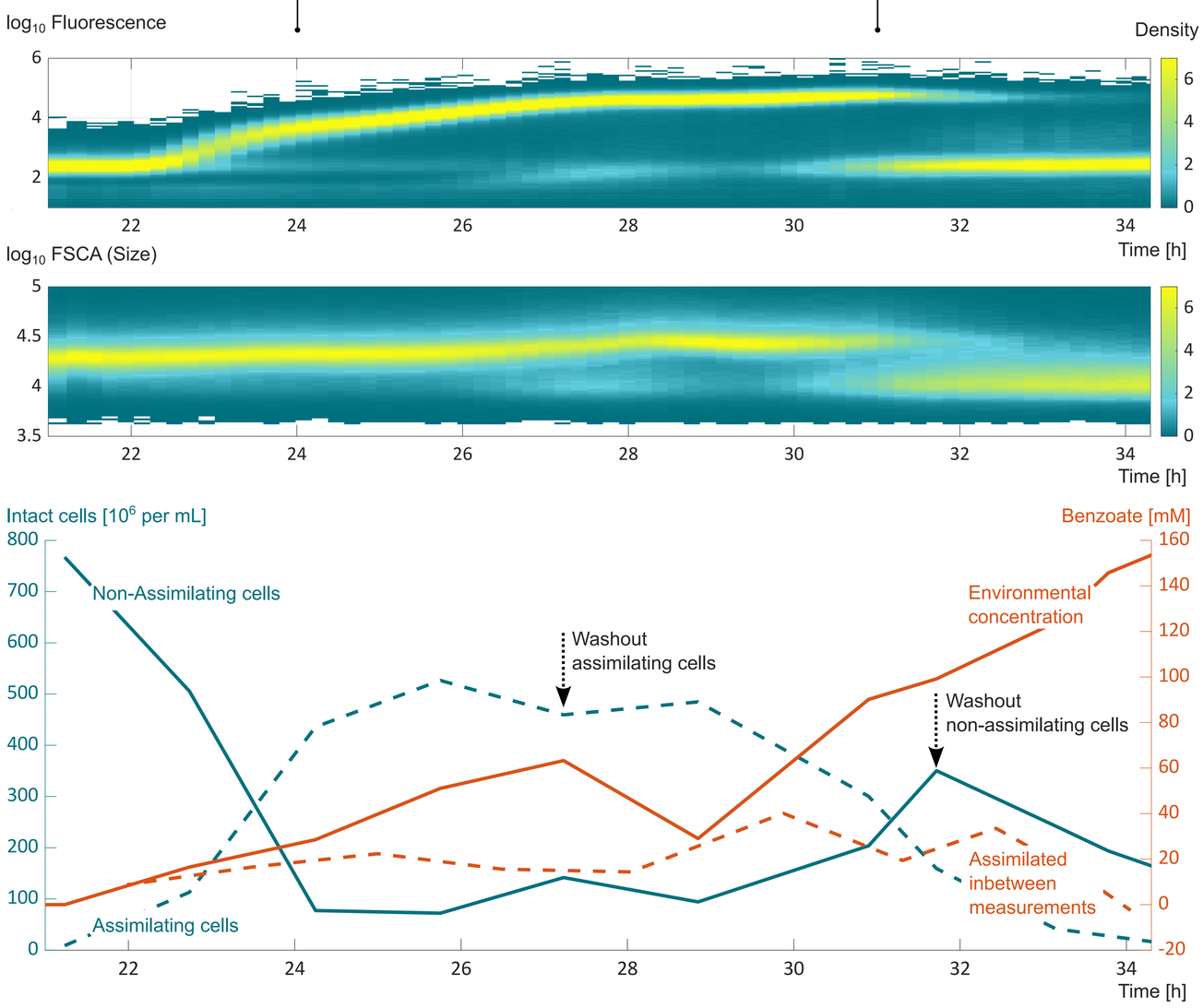
The cell-machine interface Segregostat



3 Results







4 Conclusion

- Upon exposure to a benzoate concentration about 50 mM, benzoate-assimilating cells get outcompeted by those who do not.
- The non-assimilating subpopulation shows a smaller cell-size, resulting in a surface reduction, possibly increasing fittnes at higher concentrations.
- Results suggest population escapes collapse by bet-hedging in a non-assimilating phenotype.
- Preliminary results on the controllability of the system show clear impacts on the assimilation capabilities and phenotypic fate of the population.

References

[1] Reva ON *et al*. (2006) Functional genomics of stress response in *Pseudomonas putida* KT2440. J Bacteriol. Volume 188(11):4079-92. doi: 10.1128/JB.00101-06.

[2] Gayle J. *et al*, (2020) Engineering glucose metabolism for enhanced muconic acid production in *Pseudomonas putida* KT2440, Metabolic Engineering. Volume 59, Pages 64-75, https://doi.org/10.1016/j.ymben.2020.01.001.

[3] Sassi H, Delvigne F. *et al*. (2019) Segregostat: a novel concept to control phenotypic diversification dynamics on the example of Gram-negative bacteria. Microb Biotechnol. 12(5):1064-1075. doi: 10.1111/1751-7915.13442.

Affiliation:
Microbial Processes and Interactions (MiPI), Terra Research and Teaching Centre, Gembloux Agro -Bio Tech, University of Liège, Gembloux, Belgium