Assimilation of plastic-derived monomers by *P. putida* KT2440 in continuous cultures

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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.

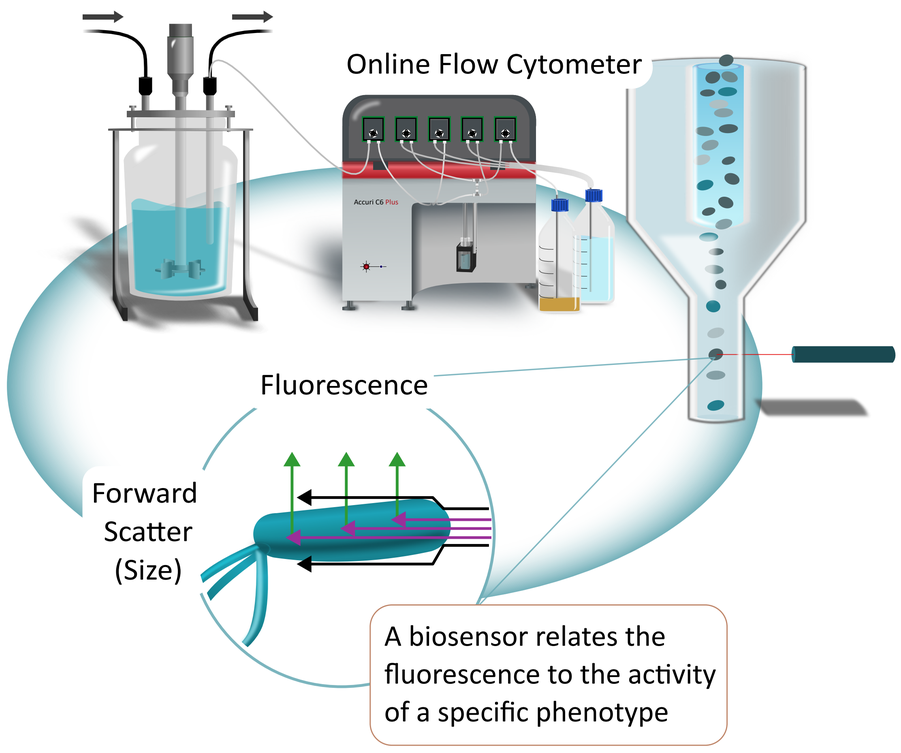
Known diversification mechanisms are linked to [1]:

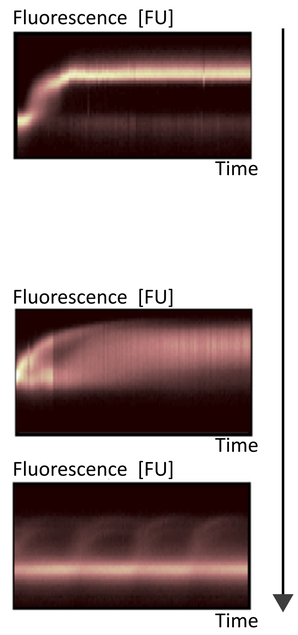
- Compound degradation
- Compound efflux
- Morphological changes (membrane composition. etc)

Showcasing the potential of analysing phenotypic diversification, We are investigating the case study of *Pseudomonas putida* KT2440' s diversification process in exposure to **benzoate and ethylene glycol**.

2 Methodology and State of the Art

The Segregostat cell machine interface [2]





Reducing Growth Rate when transitioning to a new phenotype (with Fitness Cost) [3]

- Populations exhibit different regimes when adopting a new phenotype.
- The regime depends on the disadvantage in growth rate inflicted by the new phenotype.
- Applying induction with pulses leads to a reduction of heterogeneity in regimes of high fitness cost.

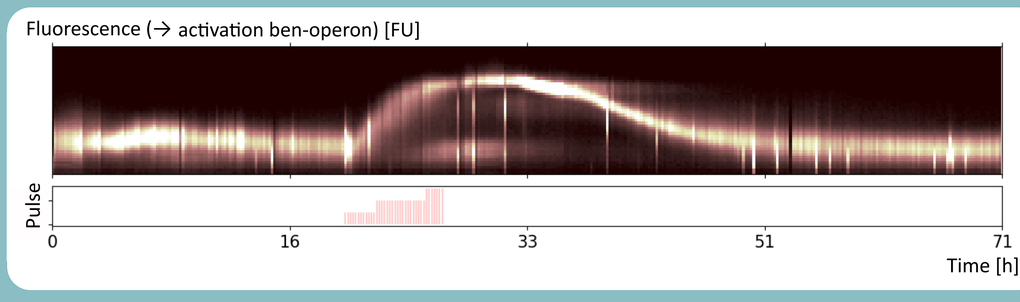
3 Results

P. putida KT2440 pSEVA231[*benA*/*sfgfp*]

Continuous culture with increasing benzoate concentration

- M9 media containing 5 g/L glucose
- Pulse 13 mLbenzoate after FC measurement

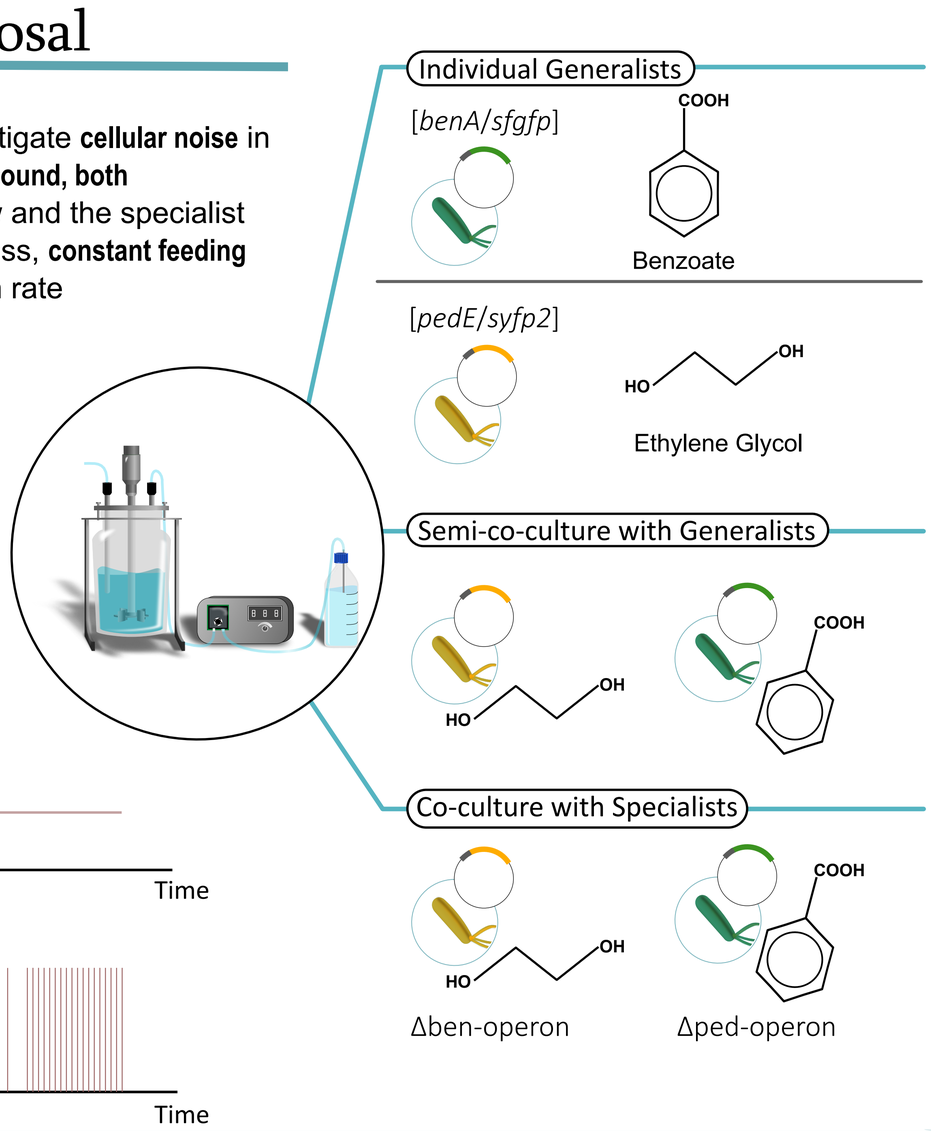
2h	0.25 M
4h	0.50 M
1.5 h	0.75 M

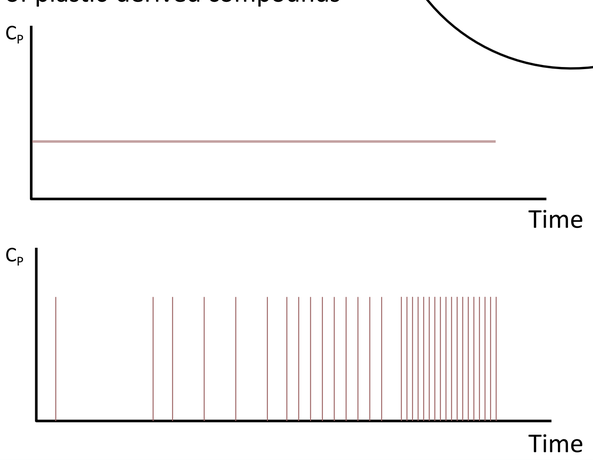


- Constrained diversification regime (low cost for genetic circuit activation)
- Increasing benzoate leads to higher density of GFP-negative cells with smaller cell size.
- Preliminary data indicate effects on GFP stability, impacting fluorescence ability.

4 Process proposal

The objective is to investigate **cellular noise** in processes with **one compound, both compounds simultaneously** and the specialist approach. In each process, **constant feeding** on maximum conversion rate will be compared to a process allowing phases of ***induction and relaxation***.





5 Conclusion

Our research delves into how catabolic repression and damage impact degradation activity in continuous culture.

The diversification regime offers valuable insights into the fitness costs associated with adapting to individual phenotypes.

This study provides a deeper understanding of the innovative specialist approach.

Our investigation may pave the way for achieving stable continuous processes by determining optimal frequencies for induction and relaxation.

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] 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.

[3] Henrion, L., Martinez, J.A., Vandenbroucke, V. et al. Fitness cost associated with cell phenotypic switching drives population diversification dynamics and controllability. Nat Commun 14, 6128 (2023). https://doi.org/10.1038/s41467-023-41917-z

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