Model based exploration of pyruvate producing pathways in *Pseudomonas putida*

Supervisors: Sara Moreno-Paz

Contact: sara.morenopaz@wur.nl robert1.smith@wur.nl

Type of thesis: Computational work

Required competences: basic knowledge of python, basic knowledge of microbial metabolism and models of metabolism (for instance BPE-34306 or SSB-50806)

Acquired competences:

- Working with genome scale models and flux balance analysis
- Design of metabolic pathways through gap-filling
- Microbial metabolism

Starting Date: 2021 (to be discussed)

Description:

One of the goals of biotechnology is the design of cell factories to produce metabolites of industrial interest. Metabolic engineering introduces heterologous pathways and rewires cell metabolism aiming to increase product yield, titer and productivity and GEnome Scale, constraint-based metabolic models are valuable tools to guide it (Gu et al, 2019).

A common strategy to increase production of a target metabolite is to couple product formation and growth, which happens when growth precursors are produced as (by)-products of the production pathway (Banerjee et al 2020). The shikimate pathway is part of the aromatic amino acid biosynthesis pathway from which many interesting products including flavonoids can be produced. In this pathway pyruvate, an essential growth precursor, is produced. By constructing a strain only able to produce pyruvate using the shikimate pathway, the flux through this pathway would increase which would lead to increased production of flavonoids.

Pseudomonas putida is the microbial cell factory of choice for many biotechnological applications. It has a considerable metabolic versatility, remarkable tolerance to various stress conditions and shows rapid growth with simple nutritional requirements.

This thesis project starts analyzing all the alternative pathways able to produce pyruvate in a *P. putida* GEM. Then a strain only able to produce pyruvate using the shikimate pathway will be simulated. The predicted metabolism of this strain will be studied, identifying unrealistic active pathways and possible limiting factors (e.g. cofactor limitation). Finally, gap-filling algorithms will be used to find alternative pyruvate production pathways that could be linked to the production of other interesting products.

Bilbiography:

Gu, C., Bae Kim, G., Jun Kim, W., Uk Kim, H., & Yup Lee, S. (2019). Current status and applications of genome-scale metabolic models. *Genome Biology*. https://doi.org/10.1186/s13059-019-1730-3

Banerjee, D., Eng, T., Lau, A.K. *et al.* Genome-scale metabolic rewiring improves titers rates and yields of the non-native product indigoidine at scale. *Nat Commun* **11,** 5385 (2020). https://doi.org/10.1038/s41467-020-19171-4