Genetic optimization of metabolic pathways in *Phocaeicola vulgatus* for enhanced succinate production

Mélanie Gindt[1], Rebecca Lück[2] and Uwe Deppenmeier[1]

[1]Rheinische Friedrich-Wilhelms-Universität Bonn, Institut für Mikrobiologie und Biotechnologie, Meckenheimer Allee 168, 53115 Bonn

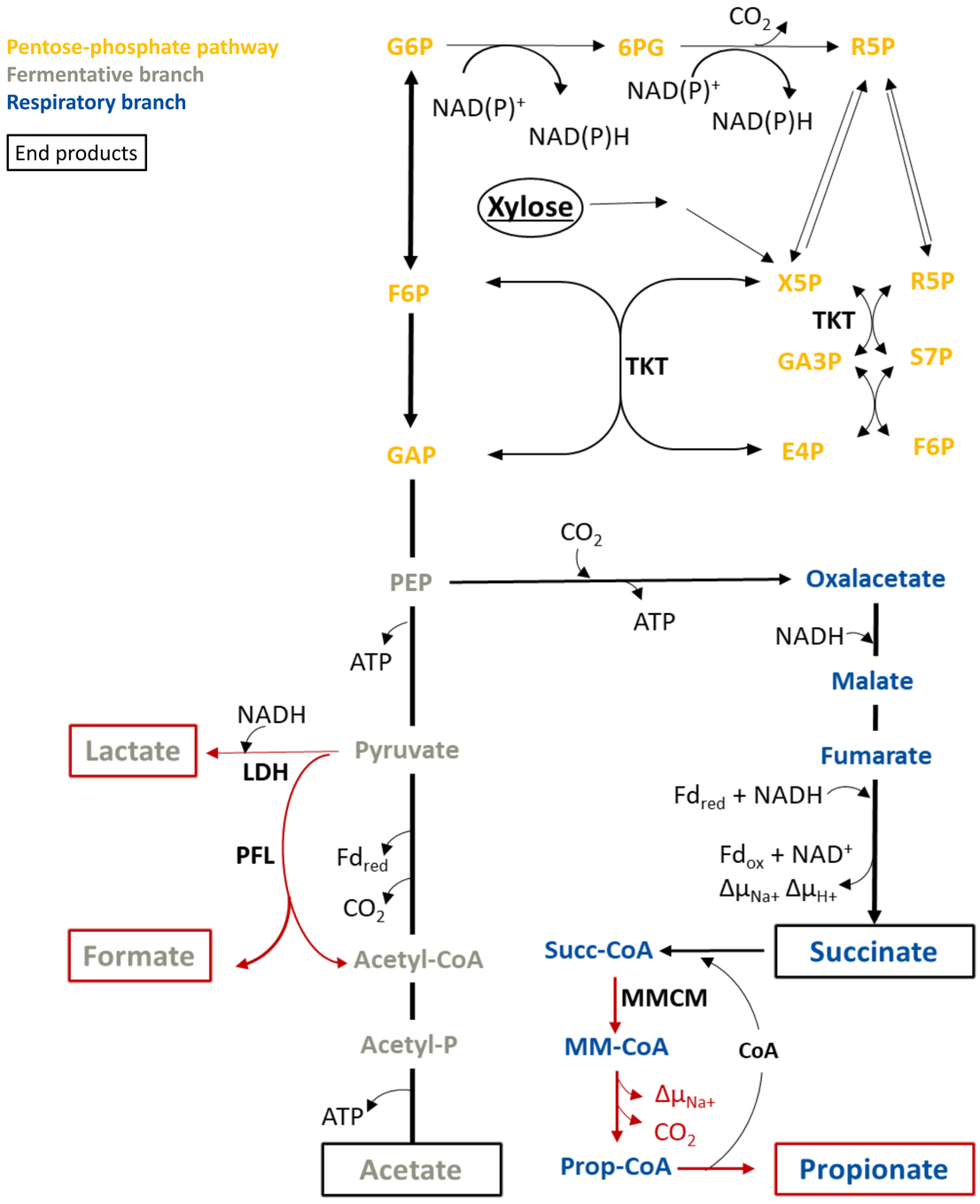
[2]Deutsches Zentrum für Luft und Raumfahrt e.V., Institut für den Schutz terrestrischer Infrastrukturen, 53757 Sankt Augustin

Introduction

The demand for sustainably produced bulk chemicals is rising constantly. Succinate serves as a fundamental component in various food, chemical, and pharmaceutical products. Succinate can be produced from sustainable raw materials using microbial fermentation and enzyme based technologies. *Bacteroides* and *Phocaeicola* species, widely distributed and prevalent gut commensals, possess enzyme sets for the metabolization of complex plant polysaccharides [1]. This study employed novel molecular techniques to enhance succinate yields in the natural succinate producer *P. vulgatus* by channeling the metabolic carbon flow in favor of succinate formation.

Results

Bioinformatic analysis enabled the reconstruction of the central carbon flow in *P. vulgatus* (Fig. 1). With xylose as a carbon source, metabolization starts with the pentose-phosphate pathway (PPP). At phosphoenol-pyruvate (PEP), the metabolic pathway splits into the fermentative and the respiratory branch. In the fermentative branch, PEP is metabolized through pyruvate and acetyl-CoA to acetate. Additionally, lactate and formate are produced. In the respiratory branch, PEP is reduced to succinate which is partially metabolized to propionate.



Figue 1: Schematic overwiev of the metabolic pathway in *P. vulgatus* with xylose as carbon source. After the PPP (yellow), the metabolization of PEP splites into the fermentative (grey) and the respiratory (blue) pathway. End-products are outlined and deleted pathways are shown in red.

Employing a recently published method for markerless gene deletion in *P. vulgatus* [2] the carbon flow of the gut bacterium was channeled towards succinate production. Deletion of the methyl-malonyl-CoA-mutase (MMCM) was performed to prevent the metabolization of succinate to propionate. Moreover, the genes encoding the lactate dehydrogenase (LDH) and the pyruvate-formate-lyase (PFL) were deleted to eliminate the unwanted byproducts lactate and formate. Thus, using molecular techniques the strain *P. vulgatus* Δ*mmcm* Δ*ldh* Δ*pfl* was created.

For succinate production, reducing agents (NADH) are needed, which can be recruited from the PPP. Earlier studies demonstrated increased succinate yields by overexpression of the transketolase (TKT) in *E. coli* [4]. Analysis of the transcript levels of enzyme involved in the PPP of *P. vulgatus* revealed that expression of TKT could be a limiting factor. Therefore, the shuttle vector pG106 harbouring the *tkt* gene, was transformed into the previously generated triple knockout mutant yielding *P. vulgatus* Δ*mmcm* Δ*ldh* Δ*pfl* pG106_TKT.

Triple deletion mutant *P. vulgatus* Δ*mmcm* Δ*ldh* Δ*pfl* (3xKO)

The deletion of the MMCM (*bvu_0309-0310*) resulted in a 100% increase in succinate production in *P. vulgatus*, as metabolization to propionate was effectively blocked. Furthermore, deletion of genes encoding the LDH (*bvu_2499*) and the PFL (*bvu_2880*) eliminated the formation of the fermentative end products lactate and formate.

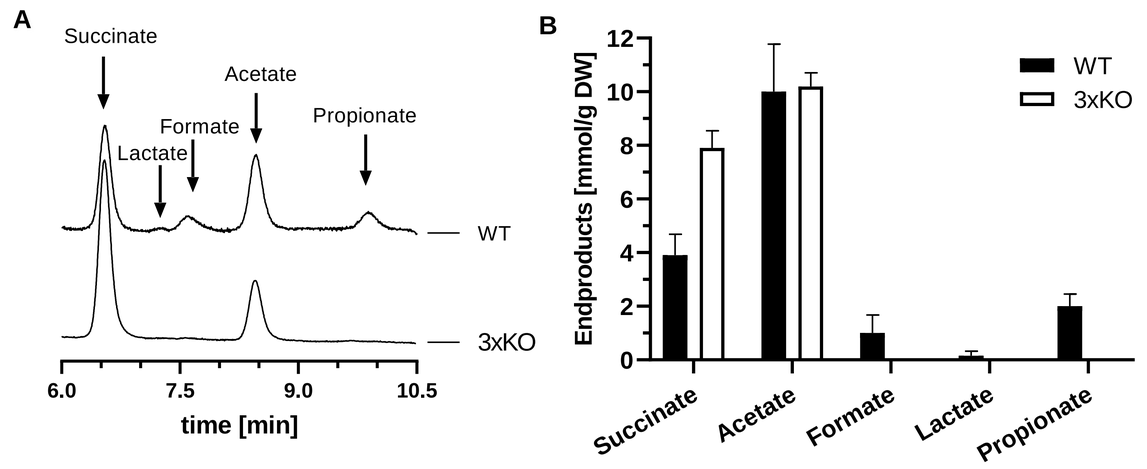


Figure 2: Metabolic endproduct formation by *P. vulgatus* wild type strain (WT) and the triple knock-out mutant strain (3xKO). [A] HPLC chromatogramm of the triple deletion mutant and the wild type strain. [B] endproducts formed by the different *P. vulgatus* strains. DW = dry weight

Overexpression of the *tkt* gene in *P.vulgatus* 3xKO

The overexpression of the TKT (*bvu_2318*) contributed to a 50 fold higher transcript level of the *tkt* gene relative to the WT. Transcript levels of other genes from the PPP were not effected (Fig. 3A). Deletion of the *mmcm*, *ldh* and *pfl* as well as the overexpression of the TKT did not impair growth behavior of the new mutant strain (Fig. 3B).

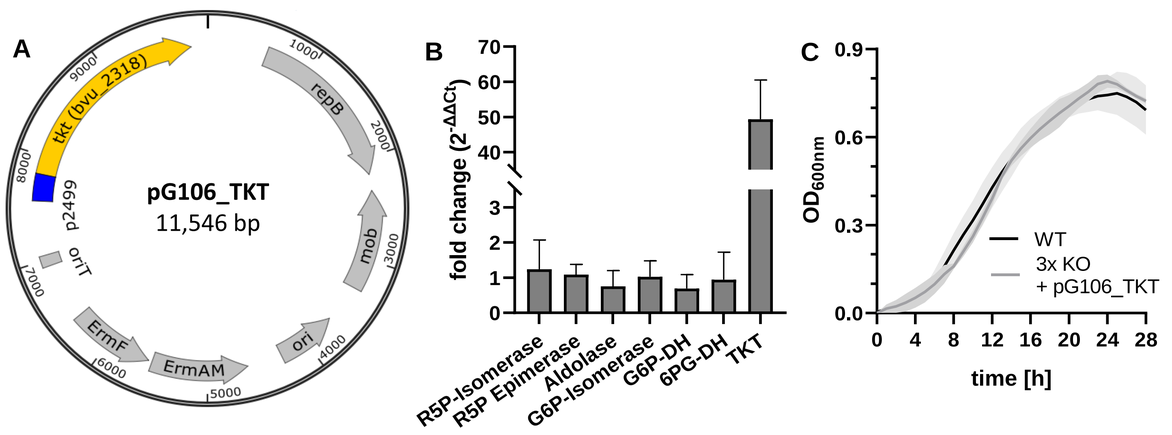


Figure 3: Characteristics of *P. vulgatus* Δ*mmcm* Δ*dlh* Δ*pfl* pG106_TKT. [A] Plasmid map of pG106_TKT. [B] Transcript abundance of *P. vulgatus* 3xKO + pG106_TKT relative to the 3xKO mutant. [C] Growth curves of *P. vulgatus* WT compared to *P. vulgatus* Δ*mmcm* Δ*dlh* Δ*pfl* (3xKO) + pG106_TKT in defined minimal media supplemented with Xylose.

Final succinate yield

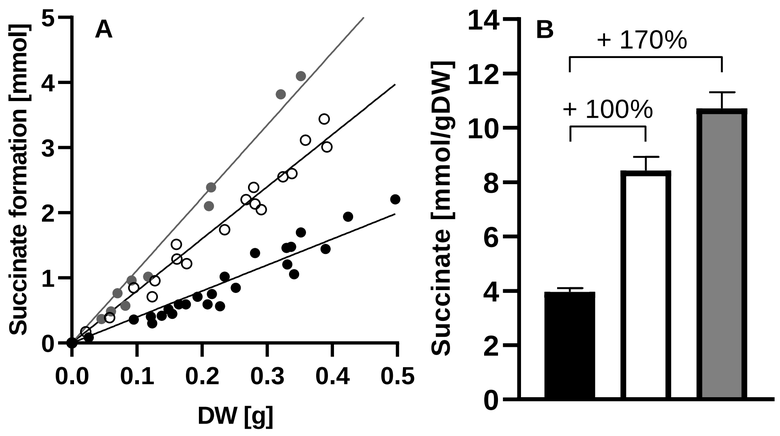


Figure 4: Succinate yield produced by the triple knock-out mutant (3xKO) and the triple knock-out mutant overexpressing the TKT.

By overexpressing the TKT in the 3xKO, succinate production was additionally increased from 9.0 to 10.8 mmol/g DW (Fig. 4A). A total increase of 170 % in succinate yield in the new mutant strain *P. vulgatus* Δ*mmcm* Δ*ldh* Δ*pfl* pG106_TKT relative to the WT strain (Fig 4B).

Contact

Mélanie Elisabeth Gindt
IfMB, University of Bonn
mgindt@uni-bonn.de

References

[1] Chassard et al., 2007. FEMS Microbiology Ecology 61: 121-131
[2] Neff et al., 2022. Anaerobe 81: 102742
[3] Lück and Deppenmeier, 2022. Applied Microbiology and Biotechnology 106: 1211-1225.
[4] Zhu et al., 2014. Metabolic Engineering, 24: 87-96.