Whole-Cell Biocatalysis: CRISPR/Cas9 for the Development of a Sialyllactose-Producing Strain

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Motivation

- An *E. coli* strain will be developed that enables the production of sialyllactose (SL) starting from glucose (Glc).
- After theoretical strain construction with SnapGene®, genome-editing is planned using CRISPR/Cas9 to knock-in/out selected genes for an optimal reaction cascade.
- Human milk oligosaccharides (HMOs) such as SL are important for human health due to their beneficial effects on the growth of naturally occurring bacteria in humans, such as bifidobacteria. In addition, HMOs prevent pathogen adsorption and thus act as acceptor mimics for pathogen binding [1].
- Sialylated HMOs are important components of breast milk and ensure that toxins are neutralized and adhesion of bacteria and viruses to the epithelial surface is prevented when breastfeeding infants. SL is present as 3'-SL and 6'-SL in human milk [2, 3].
- For this reason, and because of limited availability of natural HMOs, its production is receiving increasing attention in biotechnology. Moreover, HMOs are required for studies of diseases (infectious, autoimmune and cancer) and biologically functional materials [1, 3].

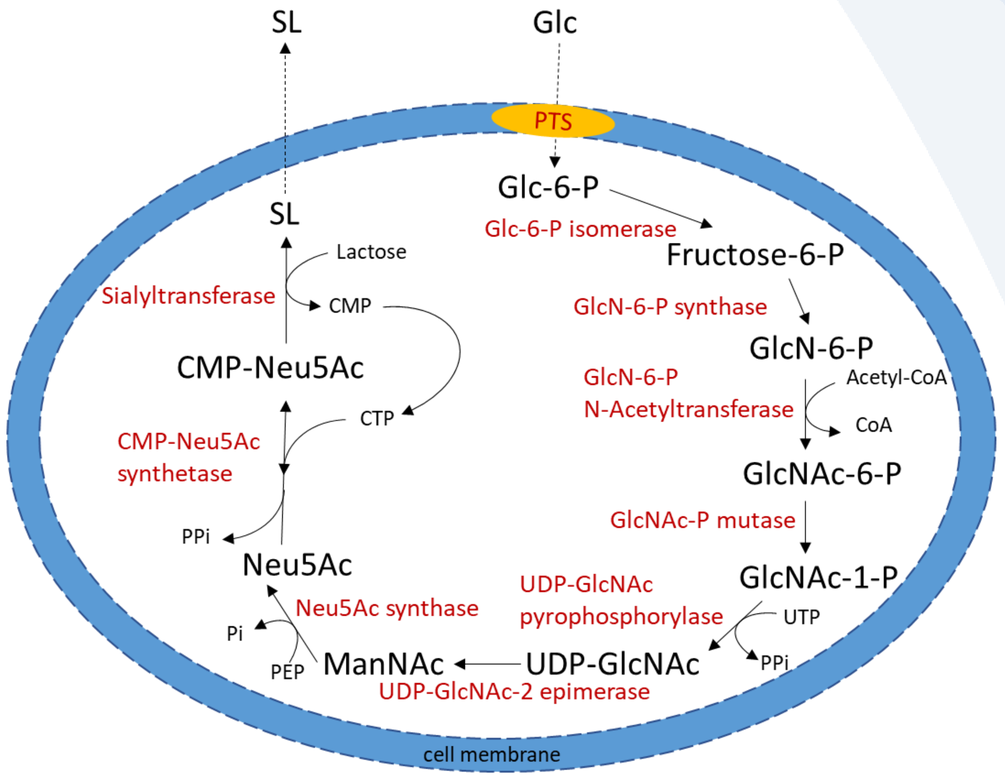

Fig. 1: Reaction scheme of a SL-producing *E. coli* cell for the use in whole-cell biocatalysis starting the synthesis by using glucose as starting material

Principle of CRISPR/Cas9

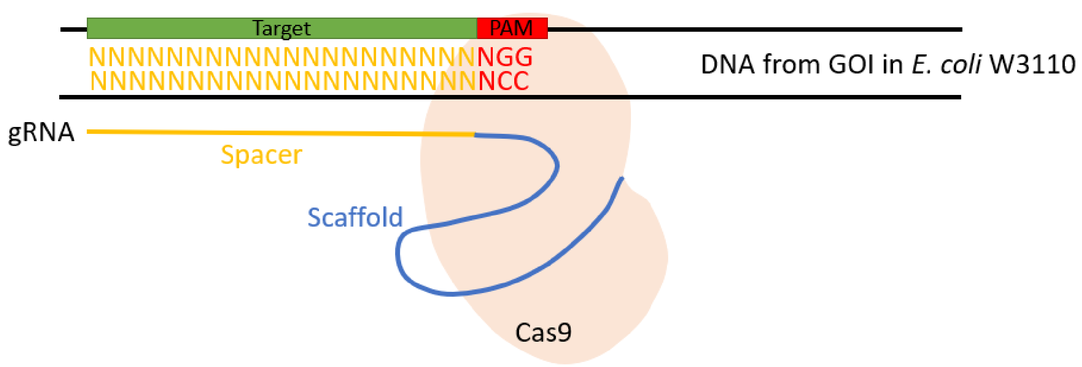

Fig. 2: gRNA-Cas9 complex binding the genomic DNA of *E. coli*

- Guide RNA (gRNA) directs the Cas9 nuclease to a specific genomic location (target sequence), which is upstream of a specific protospacer adjacent motif (PAM)
  - Consisting of a spacer sequence that is specific to the DNA target and a scaffold sequence that interacts with the Cas9 protein
  - Binds to a recombinant form of Cas9 protein that has DNA endonuclease activity
  - Resulting complex will cause target-specific double-stranded DNA cleavage. The cleavage site will be repaired
- Cas9-induced double strand breaks are repaired via the λ Red-mediated homologous recombination.

Approach

- Production of gRNA-plasmids for specific targeting of the genomic *E. coli* DNA via PCR, restriction digest and ligation
- Isolation of pEcCas-plasmid containing Cas9 enzyme
- Production of double-stranded donor DNA (dsDonorDNA) for gene knock-out via extension PCR
  - Consists of homologous overhangs
  - Leads to the deletion of the gene sequence except of the start codon and the last 7 amino acids
- Transformation of *E. coli* with pgRNA-Plasmid, pEcCas-plasmid and dsDonorDNA via electroporation
- Check-PCR of potential edited colonies and sequencing

Challenges & Outlook

Strain development: the main challenges for HMO production were the strain development of a whole cell biocatalyst, the requirement of multiple enzymes, cofactor regeneration, substrate uptake and product release, as well as the prevention of intermediate synthesis or degradation.

Strain construction: genome-editing was used to ensure that the necessary substrates and cofactors were synthesized and regenerated in the cell to prevent unwanted degradation of substrates/products.
The newly constructed strain will be characterized, and the expression optimized.

References:
[1] L. Guo et al., Applied and Environmental Microbiology 2018, Vol. 84 (13).
[2] Z. Li et al., Molecules 2020, Vol. 25 (16).
[3] L. E. Horsfall et al., FEBS J. 2010, Vol. 277 (13).
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