A point mutation in the xylose reductase gene increases citric acid production in Aspergillus carbonarius

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A point mutation in the xylose reductase (XR) gene increases citric acid production in *A. carbonarius*

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Introduction:

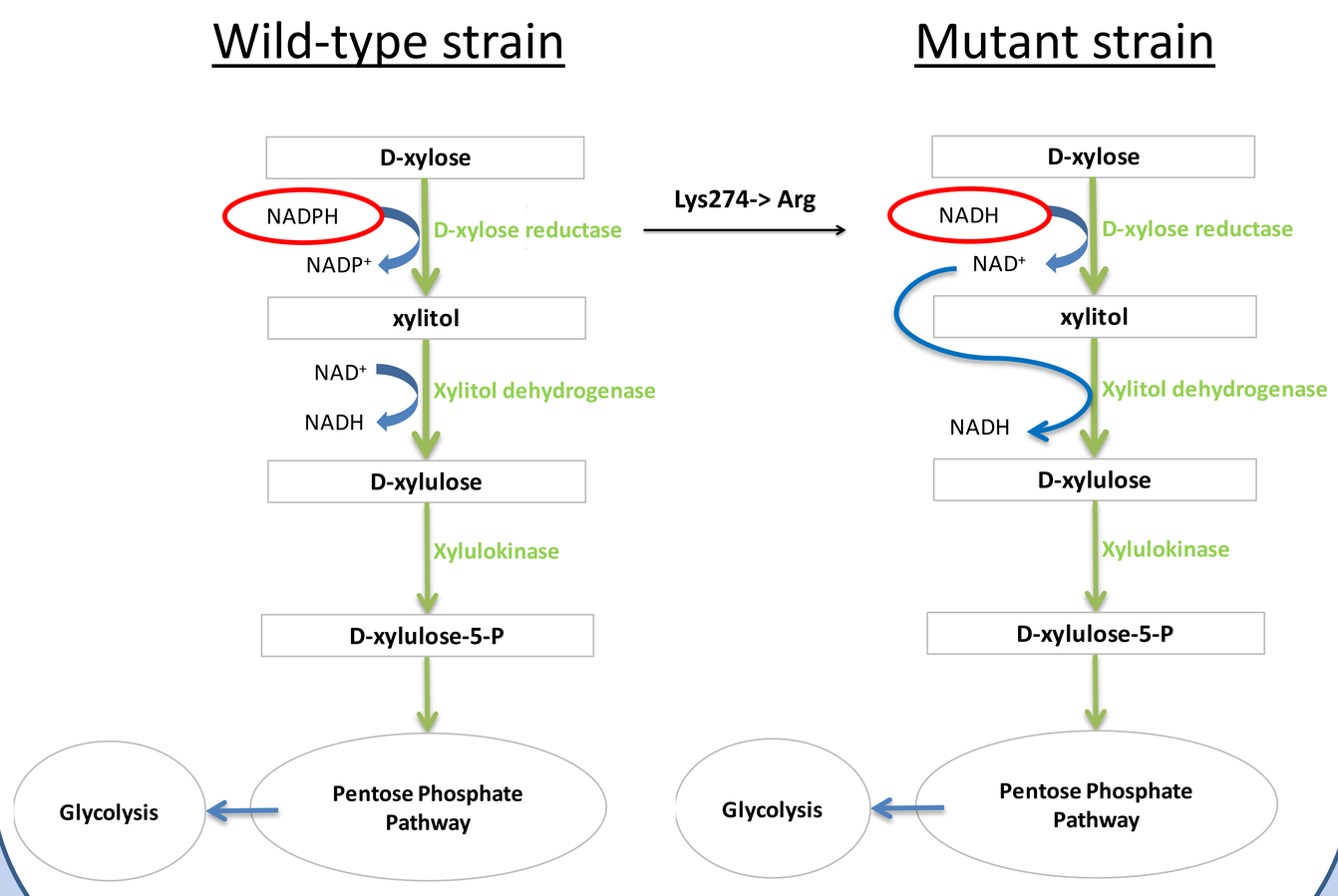
Pentose sugars are among the major components of plant based lignocellulosic biomass. Future biorefineries, which employ plant materials as feedstock, require capable microbial strains for the cost effective conversion of all the sugars of the biomass, including the pentose fraction. Microorganisms metabolize xylose through the pentose catabolic pathway (PCP). In *Aspergillus carbonarius*, the conversion of xylose to xylitol is carried out by a NADPH dependent xylose reductase (XR). Xylitol is further converted by the NAD^+ specific xylitol dehydrogenase. The cofactor impairment between the two enzymes leads to the accumulation of xylitol in xylose fermentation with *A. carbonarius*.

Materials and methods:

- Cloning and fusion of DNA fragments by USER cloning techniques
- Strain: *Aspergillus carbonarius* ITEM 5010
- Mutation of XR by overlap extension method
- Protoplast transformation using PCR-based bipartite substrates for gene targeting
- Batch flask fermentation on defined D-xylose medium in rotary shaker for 7 days.

Purpose of the project:

The aim is to assure the flux of D-xylose through the PCP by preventing the accumulation of pathway intermediates.
In order to overcome xylitol accumulation, the *XR* gene of *A. carbonarius* was identified and the cofactor preference was altered from NADPH to NADH, by introducing a point mutation (Lys274 to Arg) into the enzyme by site-directed mutagenesis.



Results and Conclusion:

The results of the fermentation conducted on defined D-xylose medium with the mutant (SXR1) and wild type strain showed:

- The point mutation in the XR did not affect the rate of D-xylose consumption (Figure 1.)
- Citric acid yield increased 5 fold, while xylitol accumulation was reduced 2.5 fold in SXR1 (Figure 2.)

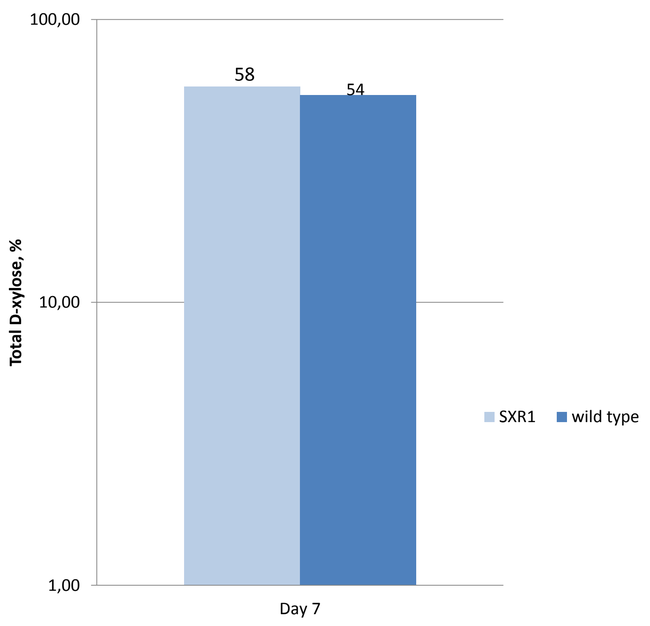


Figure 1. D-xylose consumption of mutant (SXR1) and wild type strain at day 7 of the fermentation, expressed in percentage of initial D-xylose

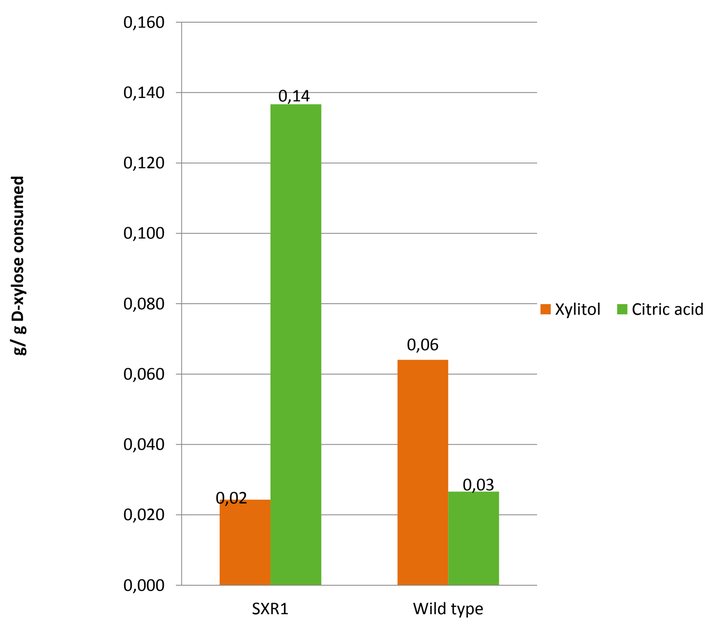


Figure 2. Yield of citric acid and xylitol by mutant (SXR1) and wild type strain at day 7 of the fermentation

References:

[1]Witteveen CFB, Busink R, Vandevondervoort P, Dijkema C, Swart K, Visser J (1989) L-Arabinose and D-Xylose Catabolism in *Aspergillus niger*. J Gen Microbiol 135:2163-2171

[2]Leitgeb S, Petschacher B, Wilson DK, Nidetzky B (2005) Fine tuning of coenzyme specificity in family 2 aldo-keto reductases revealed by crystal structures of the Lys-274-->Arg mutant of *Candida tenuis* xylose reductase (AKR2B5) bound to NAD+ and NADP+. FEBS letters 579:763-767.