

# Improvement of xylose utilisation for bioprocesses (XyloImpro)

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### Abstract

The project deals with biotechnological production of fuel bioethanol and other chemicals from renewable lignocellulosic raw materials. This is highly important for ecoefficient and economically feasible bioethanol production, as well as production of also other bulk chemicals from lignocellulosic biomass. The main aim of the project is to further develop and improve *Saccharomyces cerevisiae* for production of bioethanol (and other chemicals) from the xylose fraction of plant biomass. The yeast *S. cerevisiae* is the most desired process organism for industrial scale since it is robust, has high ethanol tolerance and has been extensively used in large scale processes. It also tolerates well the inhibitors found in lignocellulosic hydrolysates. We have hydrolysate tolerant proprietor strains available that have been engineered to use xylose. This research focuses on fundamental understanding of those cellular factors that we consider still the most essential in improvement of xylose utilisation by yeasts to the level required in commercial production of bioethanol and other chemicals from lignocellulosic raw materials. These are:

1. Improvement of xylose transport to the cell
2. Engineering of cellular redox metabolism for improved xylose fermentation
3. Understanding of cell responses and regulatory mechanisms involved in xylose utilisation

### Results

During the first year of the project, we have focused on the second of the aforementioned topics, *i.e.* studying the possibilities to improve the xylose fermentation of recombinant yeast *S. cerevisiae* by engineering the cellular redox balance. This was assessed by two ways.

The so called oxidoreductive pathway of xylose conversion in the recombinant yeast *S. cerevisiae* consists of two enzymes, xylose reductase (XR) and xylitol dehydrogenase (XDH). Even though redox neutral as such; the former enzyme reducing xylose to xylitol and the latter oxidising xylitol to xylulose, which enters the pentose phosphate pathway after phosphorylation by xylulokinase, the pathway results in redox cofactor imbalance in the cell. This is due to the different cofactor specificities of the two enzymes, the reductase preferring NADPH, the dehydrogenase being strictly NAD<sup>+</sup>-dependent. This pathway has been the only route to xylose-metabolising yeast *S. cerevisiae* strains until very recently, thus enhancement of the oxidoreductive conversion continues to be important, not least due to also the complex global IPR situation on the topic.

To overcome the cellular redox cofactor imbalance in the xylose yeast we have constructed xylose yeast strains that express an endogenous NADH-kinase encoding gene *POS5* in a truncated form encoding a cytosolic enzyme, or a MMP1489 gene encoding a bifunctional NADP<sup>+</sup> phosphatase/

NAD<sup>+</sup>-kinase enzyme from Archaeal *Methanococcus maripaludis*. Based on homology to the corresponding protein of *Methanococcus jannaschii*, this protein has a NADP<sup>+</sup>-phosphatase activity in its N-terminus and a NAD<sup>+</sup>-kinase activity in its C-terminus. We are also testing whether the NAD<sup>+</sup>-kinase activity of *M. maripaludis* MMP1489 could be decreased and the NADH-kinase activity increased by two different amino acid changes in the protein. The *POS5* and MMP1489 genes are expressed separately and together in order to create a transhydrogenase-like cycle in the xylose-fermenting *S. cerevisiae*. In an optimal situation, Pos5p would convert NADH to NADPH for the XR reaction, and MMP1489 phosphatase activity would convert NADP<sup>+</sup> to NAD<sup>+</sup> for the XDH reaction. It is, however, not known what the physiological activity of MMP1489 protein will be when expressed in *S. cerevisiae*. The genes are integrated into the *ADH2* and *UTR1* loci. *ADH2* encodes the alcohol dehydrogenase isoenzyme that is mainly involved in oxidation of ethanol to acetaldehyde while *UTR1* encodes a cytosolic NAD<sup>+</sup>-kinase. Consequently, disruption of these genes may further improve the xylose fermentation properties of the strain. Preliminary shake flask batch cultures indicated that there was increased xylitol production by the strains with *POS5* or MMP1489 variants suggesting that the enzymes encoded by these genes had at least some *in vivo* activity. The results indicate that expression of these genes affected the redox balance and most likely increased the intracellular NADPH availability.

An alternative way to overcome the redox cofactor imbalance created by the oxidoreductive pathway would be to convert the xylose directly by isomerisation to xylulose, by a xylose isomerase enzyme. Extensive work on this option has only recently resulted in efficient xylose-metabolising yeast strains converting xylose by isomerisation to xylulose by *Piromyces sp.* xylose isomerase. We have looked for alternative/novel xylose isomerase encoding genes. Such gene candidates from *Tetraodon nigroviridis*, *Strongylocentrotus purpuratus*, in addition to barley xylose isomerase encoding gene, have so far been expressed in *S. cerevisiae* yeast. Preliminary shake flask batch cultivations and enzyme activity measurements did not, however, show any xylose isomerase activity in these strains. Work is ongoing to further characterise the strains and these xylose isomerase homologues.

In summary, work during the first year of the project has focused on means to overcome the cellular redox cofactor imbalance of xylose yeast. Finalisation of this work will continue during the year 2008, but in addition, we will focus on the other two topics of the project; improvement of xylose transport to the cell, and understanding of cell responses and regulatory mechanisms involved in xylose utilisation.