# Combining metabolic and evolutionary engineering with natural yeast hybridization to generate D-xylose fermenting and inhibitor tolerant *Saccharomyces cerevisiae* strains

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New *Saccaromyces cerevisiae* strains that combine inhibitor tolerance with high D-xylose fermentation rate have been generated and are currently being further developed and scaled-up. A combination of the available tools in rational and evolutionary engineering with the natural recombination systems occurring in yeast populations have been used together to overcome the different challenges and limitations during the improvement of these strains, in a way that the separate techniques could not have achieved.

## Phase 1 – Rational Engineering

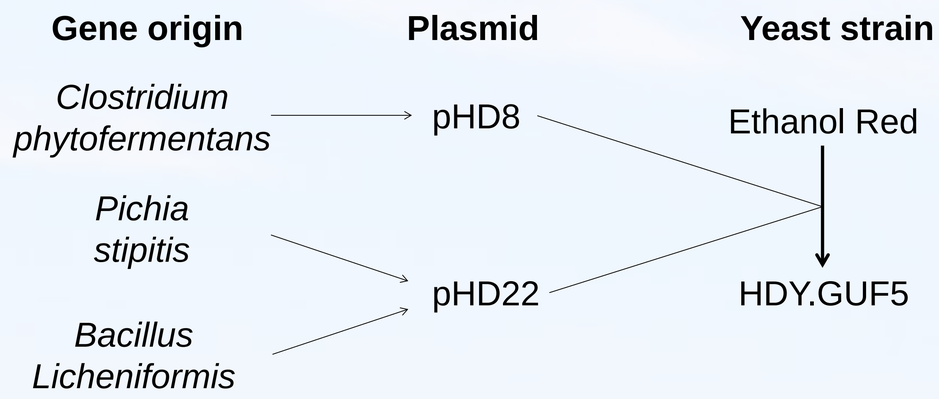


**Figure 1. Rational engineering strategy.** Heterologous genes coming from 3 different microorganisms were transformed, obtaining HDY.GUF5. The new strain did not show a noticeable improvement in either of the sugars fermentation, so it was decided to undergo evolutionary engineering directed to D-xylose utilization improvement.

## Phase 2 – Evolutionary Engineering

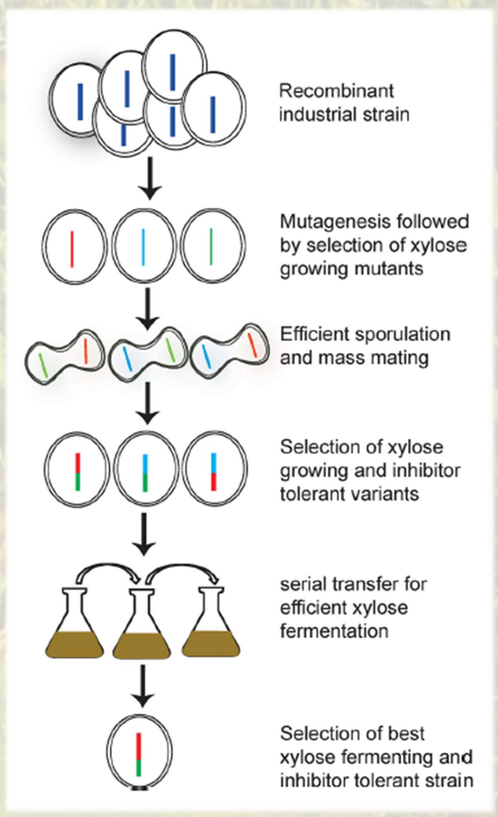


**Figure 2. Evolutionary engineering strategy. [1]** EMS mutagenesis, genome shuffling and evolutionary adaptation were used to obtain strain GS1.11-26. It showed a 17-fold enzymatic activity increase, resulting in a remarkable improvement in D-Xylose fermentation rate, but limitations such as a reduced aerobic growth and inhibitor tolerance as well. These new limitations were addressed by hybridization with other yeast strains.

## Phase 3 – Hybridization

Meiotic recombination to generate hybrids with mixed traits was the last tool used for strain improvement. GS1.11-26 was mixed with fast growing and high inhibitor tolerant Ethanol Red and JT21653, generating GSE16, GSF767 and GSF335.

## Results

Resulting traits in GSE16, GSF767 and GSF335 strains were a D-xylose fermentation decreased to 30-60% compared to GS1.11-26 and a recovered inhibitor tolerance and aerobic growth.

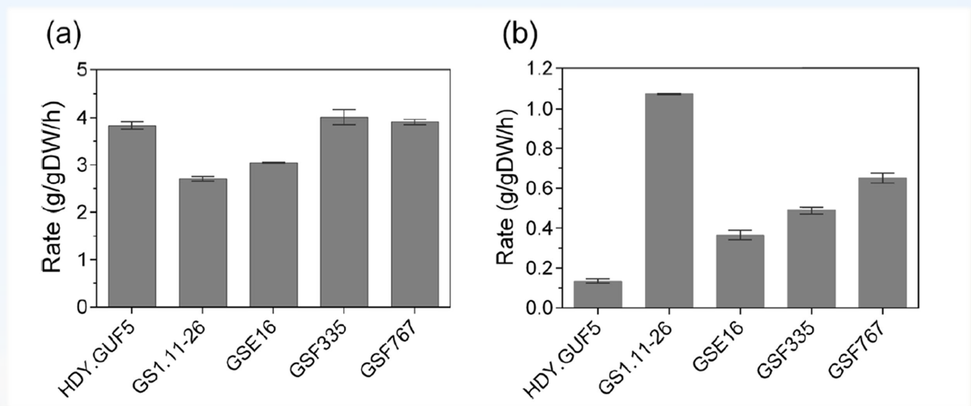


**Figure 3. Maximum sugar consumption rates attained by the three new hybrid strains and GS1.11-26. (a)** Maximum glucose consumption rate **(b)** Maximum D-xylose consumption rate. **[1,2]**

## State of the art

Up to 4 *S. cerevisiae* strains that efficiently ferment D-xylose have been developed and can be scaled up to an industrial level. Further improvement could be made, but first it has to be addressed whether or not a high D-xylose fermentation rate and inhibitor tolerance are incompatible traits.

This work represents the successful combination of all the known and available tools for strain improvement. It demonstrates how useful can be applying rational and evolutionary engineering together with natural yeast hybridization to face different situations, generating a better result than cannot be otherwise obtained by using these technologies separately.

## Acknowledgements

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1. Demeke M. M., Dietz H. *et al*. **Development of a D-xylose fermenting and inhibitor tolerant industrial *Saccharomyces cerevisiae* strain with high performance in lignocellulose hydrolysates using metabolic and evolutionary engineering**. *Biotechnology for biofuels*. 2013. 6:89.

2. Demeke M. M., Dumortier F. *et al*. **Combining inhibitor tolerance and D-xylose fermentation in industrial *Saccharomyces cerevisiae* for efficient lignocelluloses-based bioethanol production**. *Biotechnology for biofuels*. 2013. 6:120.