KU LEUVEN

Polygenic analysis of natural and artificial genetic variation in yeast acetic acid tolerance

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Goal

The knowledge gained will tell us which yeast genes can be modified in order to enhance acetic acid tolerance to a very high level. These genes will be used to make the strains with artificially induced tolerance and therefore improved performance of conversion of biomass into bioethanol, where the final goal is more efficient second generation bioethanol production.

Introduction

Yeast fermentation is used to produce bioethanol for transport fuel with first-generation substrates like sugar cane, corn and wheat. However, the crops used for foodstuffs are currently also used for bioethanol production which is the reason for a big ongoing debate. Attractive alternative substrates are lignocellulosic wastestreams and bioenergy crops (rich in pentoses and inhibitors present in the hydrolysates). Therefore, the research is based on developing yeast strains able to overcome disadvantages of mentioned substrates.

The focus is on the complex trait of acetic acid tolerance and the present project should therefore give the insight in the genetic basis of high acetic acid tolerance in yeast.

Methods

The majority is confidential and therefore not shown here.

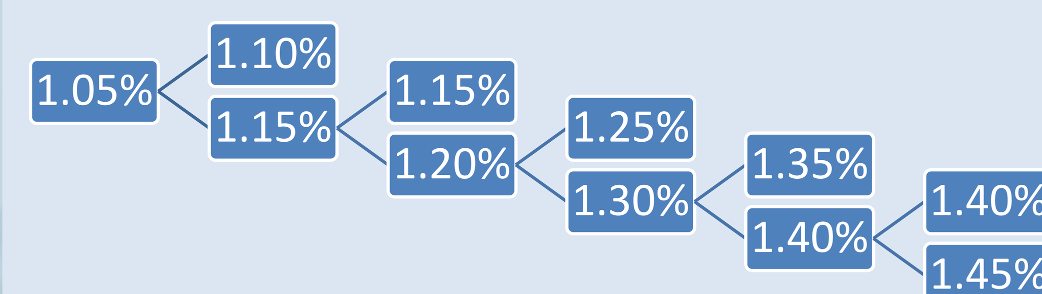
Strains with artificially induced high acetic acid tolerance will be generated by either means of evolutionary engineering (gradual adaptation) or by combining all mutant genes identified into a single industrial bioethanol strain.

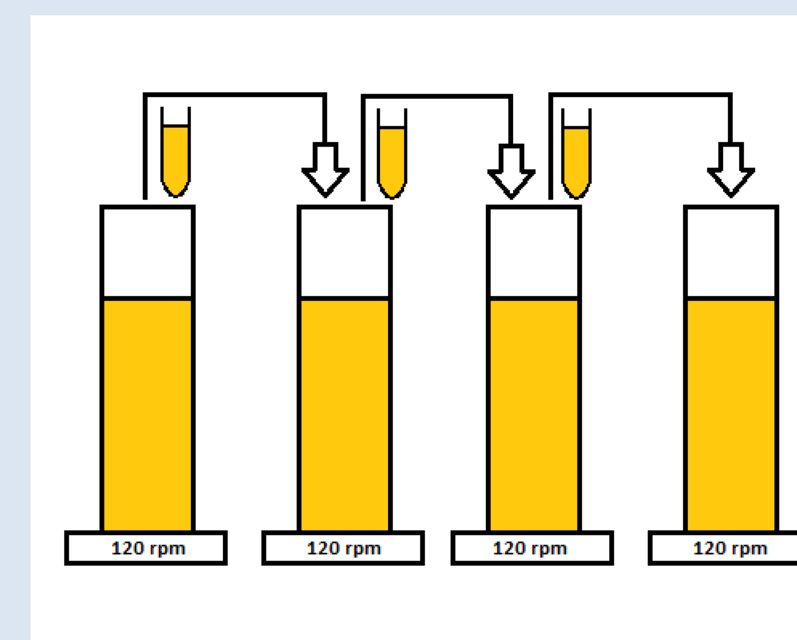
Sequencing technologies will be combined with the advantages of yeast as a genetic model system. Pooled-segregant whole-genome sequence analysis combined with reciprocal hemizygosity analysis has become a very valuable technology for polygenic analysis of complex traits in yeast.

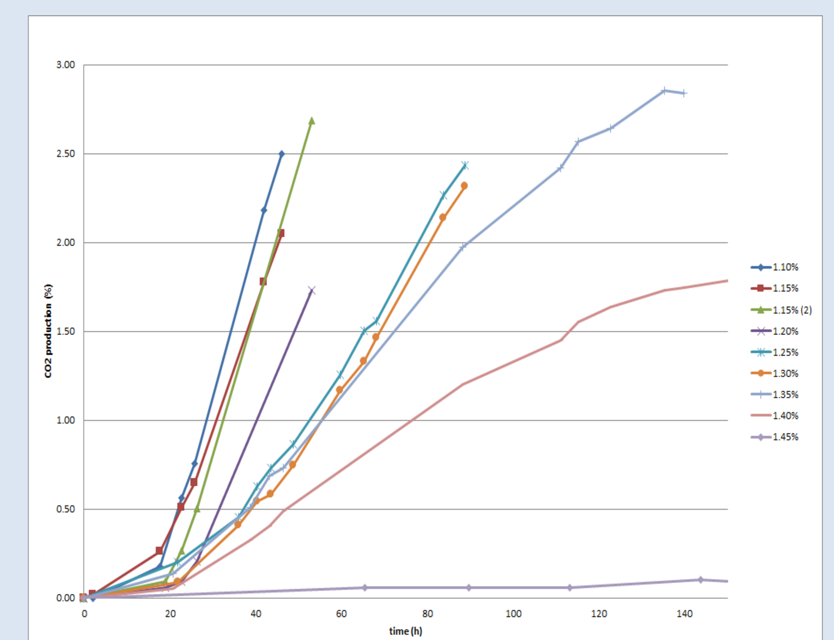
Results

The majority is confidential and therefore not shown here.

Evolutionary engineering







Conditions:

Parameter	Value
Temperature	30°C
Starting OD	0.2
Rotations	120rpm
Transfer time	exponential phase
Medium	2% YPD
pH	~4
Volume	100ml

Parallel with stress increase and re-inoculation strains were stored at -80°C.

After a few days first stability check was performed where improved strains clearly fermented better (shorter lag phase and higher fermentation rate) compared to the starting control strain. However, after 3 months another stability check was done and strains did not show any improvement.

Conclusion

The strain can be improved by means of evolutionary engineering.

Long term storage on -80°C influence improved evolutionary engineering properties or there were no mutations during evolutionary engineering but adaptation occurring.