Engineering Yeast Transcription Machinery for Improved Ethanol Tolerance and Production

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Research paper

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- Global Transcription Machinery Engineering(gTME) is used to mutate the genes in Yeast *Saccharomyces cerevisiae*
- improved glucose/ethanol tolerance, a key trait in many biofuels programs

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- Mutagenesis of the transcription factor Spt15p and selection led to dominant mutations that conferred increased tolerance and more efficient glucose conversion to ethanol
- The desired phenotype results from the combined effect of three separate mutations in the SPT15 gene
- Thus, gTME can provide a route to complex phenotypes that are not readily accessible by traditional methods.

Concept

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- Application of Global Transcription Machinery Engineering (gTME)
  - It alters the key proteins regulating the global transcriptome and generates a new type of diversity from them, at the transcriptional level
  - Alteration is done via error-prone polymerase chain reaction (PCR) mutations
- Tolerance to ethanol and glucose mixtures is not a monogenic trait

Method

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- Genetic libraries are created by screening and selection in yeast containing both wild-type and mutated version of proteins
- These libraries were transformed into yeast and were selected in the presence of elevated levels of ethanol and glucose

Method (Continued...)

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- Each of these mutatated genes contained three mutations , and specific triple mutations are referred as taf25-300 and spt15-300 mutations

Results

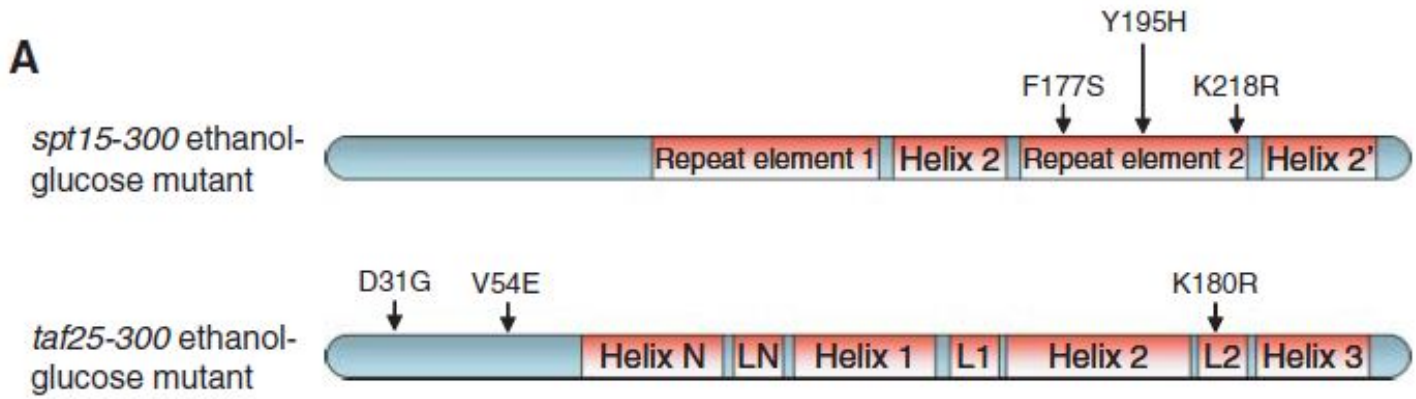

Figure: Triple mutations

- Mutations for the best clone isolated from either the spt15 or taf25 mutant library are shown mapped onto a schematic of critical functional components of the respective factor

Results

- Growth yields of the clones, were assayed in synthetic minimal medium containing elevated levels (6volume) of ethanol and glucose after 20 hours. Under these conditions, the spt15-300 mutant far exceeded the performance of the taf25-300 mutant.

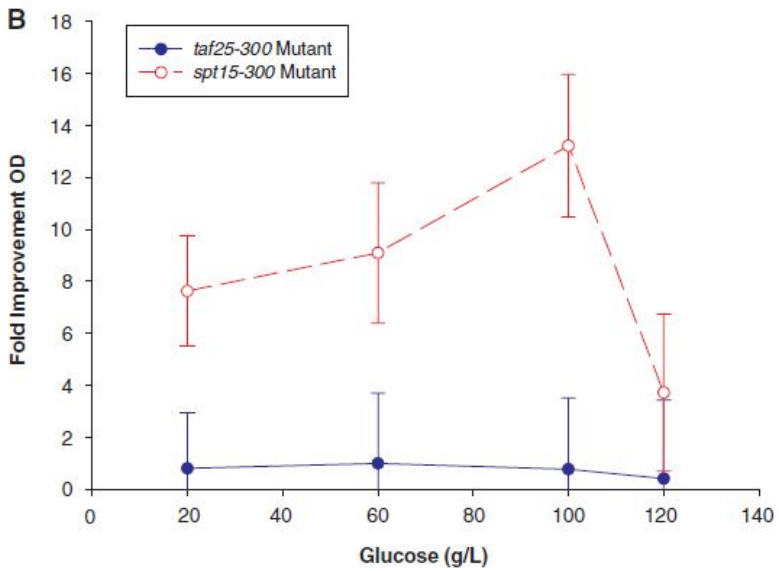

Figure: Yeast gTME mutants with increased tolerance to elevated ethanol and glucose concentrations

Results

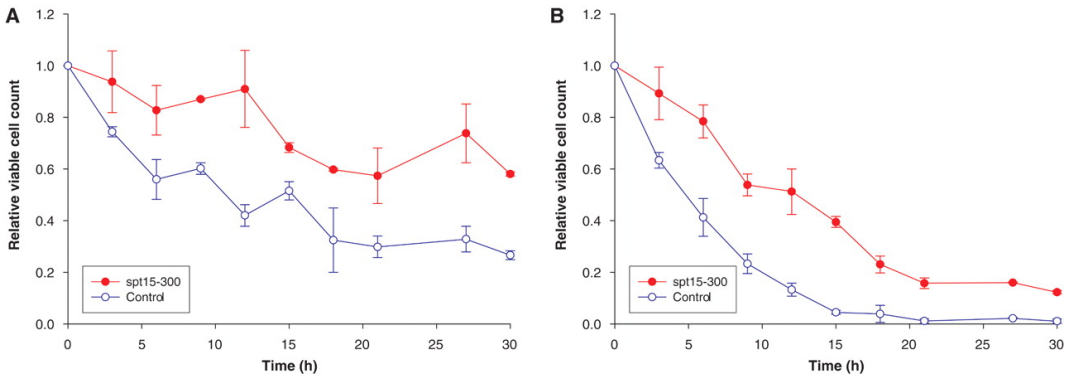

Figure: Cellular viability curves to evaluate the tolerance of the mutant under ethanol stress

- Viability is measured as a function of time (hours) and expressed as the relative number of colony-forming units compared with colony count at 0 hours for stationary phase cells treated and incubated in standard medium in the presence of 12.5% and 15% ethanol by volume.

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- They show that deletion of the great majority of the overexpressed gene targets resulted in a loss of the capacity of the mutant spt15-300 factor to impart an increased ethanol and glucose tolerance.

Result

- All tested knockout strains not harboring the mutant spt15-300 showed normal tolerance to ethanol and glucose stress, which indicated that, individually, these genes are insufficient to constitute the normal tolerance to ethanol.

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- Out of the 14 gene targets assayed, only loss of PHM6 function did not reduce the novel phenotype. Thus, we hypothesize that each gene encodes a necessary component of an interconnected network,although there may be some redundancy of function

Results

- The tolerance (to 5%ethanol, 60 g/liter glucose) of 14 strains deleted in one of the 14 genes, respectively, was tested by comparing the knockout strain containing the spt15-300 and wild-type SPT15. All gene knockouts, except PHM6, resulted in slight to full loss of phenotype

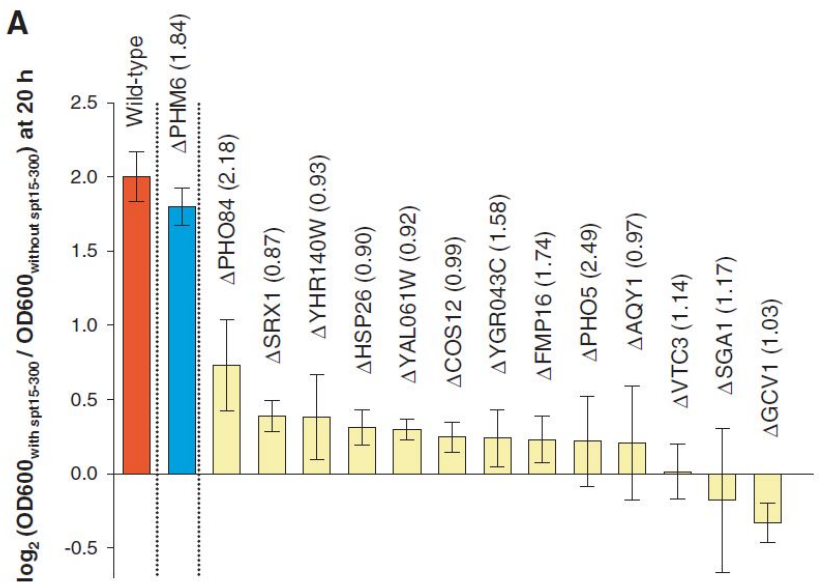
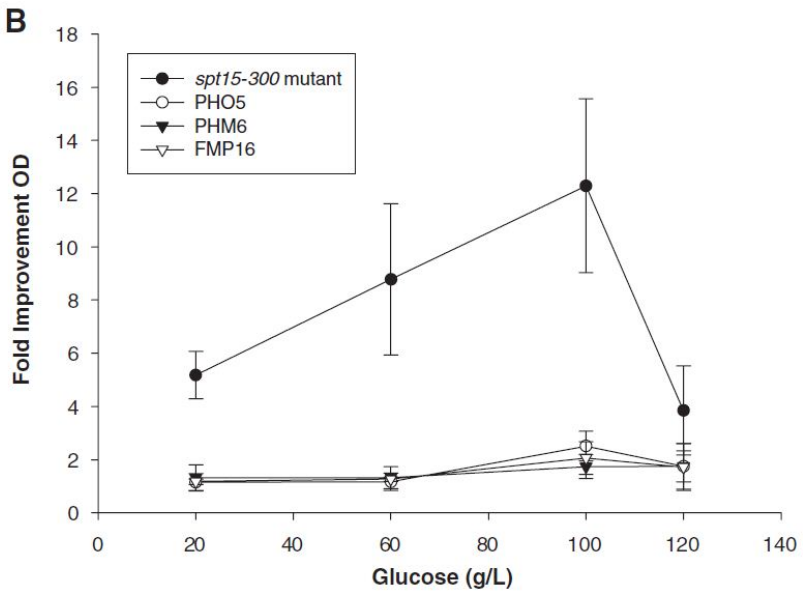


Figure: Gene-knockout analysis to probe the transcriptome-level response elicited by the mutant spt15.

Results

- Gene overexpression studies are provided for the top three candidate genes from the microarray (PHO5, PHM6, and FMP16) and assayed under 6% ethanol by volume as previously assayed. The overexpression of these genes failed to impart a tolerance phenotype



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- Furthermore, 7 of the 10 most highly expressed genes in the spt15-300 mutant are SPT3-dependent genes
- As a test of the link between Spt15p and Spt3p, it was found that an spt15-300 mutant gene was unable to impart its ethanol and glucose tolerance phenotype to an spt3 knockout strain

Results

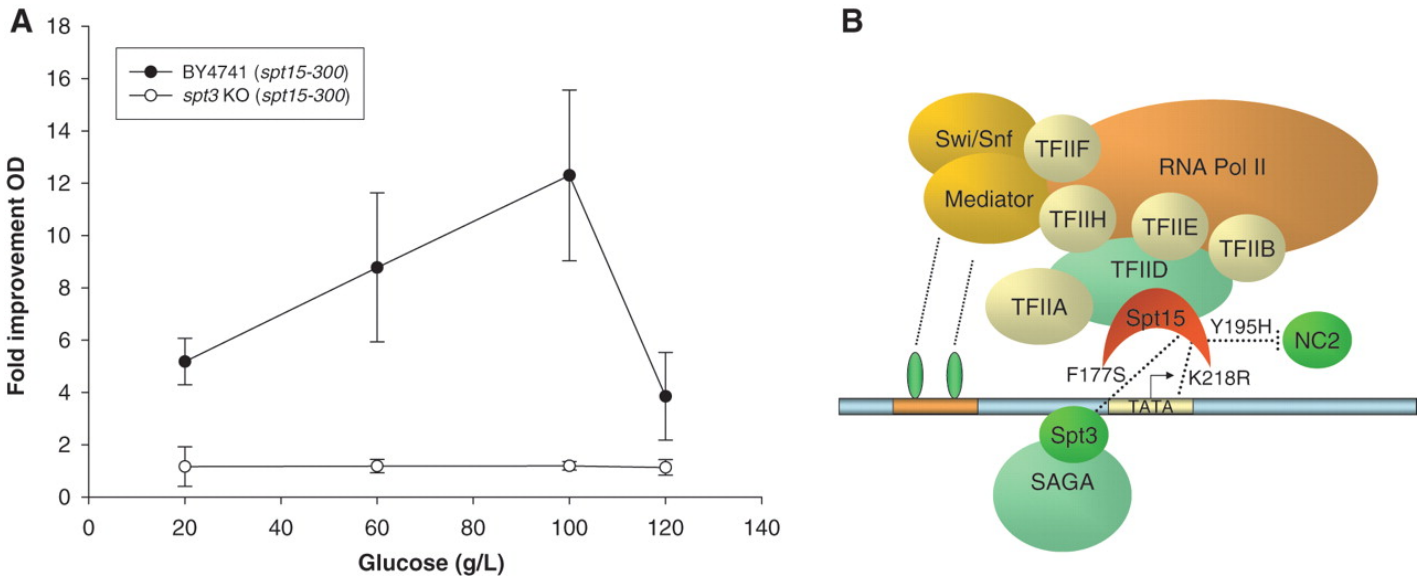

Figure: Elucidation and validation of a mechanism partially mediated by the SPT3-SAGA complex

Result

- The impact of an spt3 knockout was evaluated through the introduction of the spt15-300 mutant and assaying in the presence of 6of SPT3 as a part of the mechanism provided

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- Specifically, in highcell density fermentations, with an initial optical density at 600 nm(OD600) of 15, the mutants performance far exceeds that of the control, with more rapid utilization of glucose, improved biomass yield, and higher volumetric ethanol productivity (2 g/liter of ethanol per hour) relative to the control strain

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- In addition, sugars were rapidly and fully used at a yield that exceeds that of the control and approaches the theoretical value when taking into account the amount of glucose consumed for cell growth

Results

Parameter	*spt15-300* mutant	Control	Percent improvement
Initial DCW (g/liter)	4.06	4.10	—
Final DCW(g/liter)	6.46	5.39	+20%
Volumetric productivity (g/liter h^{-1})	2.03	1.20	+69%
Specific productivity (g/DCW h^{-1})	0.31	0.22	+41%
Conversion yield calculated between 6 and 21 hours	0.36	0.32	+14%
True EtOH yield accounting for biomass production (Percentage of 0.41 g/g, which represents the theoretical maximum)	0.40 (98%)	0.35 (86%)	+15%

$$\frac{\text{EtOH produced (g/liter)}}{\text{glucose used (g/liter)} - \left(\frac{1\text{ g glucose}}{0.5\text{ g DCW}}\right)\text{DCW produced (g/liter)}}$$

Figure: Fermentation results to evaluate the ethanol production potential of the spt15 mutant

Thank You
Questions and Comments