# Weakening Ethanol Synthesis Capacity of *Saccharomyces cerevisiae* with Randomly Mutated SPT15 Transcription Regulator

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## Introduction

With the global warming,over recent decades, the alcohol concentration of wines produced by many warm regions around the world has increased by approximately 2% (v/v). There is significant interest in the wine industry to develop methods to reduce the ethanol content of wine. Generally, microbiological strategies relating to the isolation and/or generation of the yeast strains used to make wine have proved to be the simplest and most economical methods, including *Saccharomyces cerevisiae* and non-conventional yeast species.

In this study, global transcriptional machinery engineering (gTME), was used to develop strains of *S. cerevisiae* with reduced ethanol-production ability. The gTME technology was carried out by mutating the general transcription factor Spt15p, the TATA-binding protein (Alper et al., 2006); this protein plays a key role in the action of RNA polymerase and is one of the main DNA binding proteins that regulate promoter specificity in yeast. The gTME technology is first used to improve the glucose/ethanol tolerance of *S. cerevisiae*, which shows the ability to re-program global gene transcription and change the complex phenotype of yeast strains. Since then, several research studies have used the gTME approach to optimize the ethanol tolerance and ethanol production capacity of *S. cerevisiae*, and successfully demonstrated that gTME is advantageous when attempting to regulate the ethanol metabolism of yeast strains.

In the present study, we used gTME technology to weaken the capacity of yeast to produce ethanol and ultimately created a strain of *S. cerevisiae* (YS59-409) with a low yield of ethanol production. RNA-Seq and metabolomic analysis were also conducted in an attempt to understand the metabolic mechanisms underlying the modified phenotype of YS59-409. This study highlighted the critical role of the SPT15 regulator in reducing ethanol production in yeast and provided comprehensive insights to understand the molecular mechanisms of a new low-ethanol yeast.

## Materials & Methods

Materials: *S. cerevisiae* YS59 (MATα; ura3-52, leu2-3, and his 5-519); pY16 vector; Triple M medium.

Methods:*SPT15* gene site-directed mutagenesis were carried out with a Mut Express II Fast Mutagenesis Kit(Vazyme, China). RNA-Seq Analysis were carried out by Beijing Genomics Institute (Shenzhen, China) . The RNA-Seq data generated in this study were submitted to NCBI Sequence Read Archive (SRA) under the accession number PRJNA548495.Metabolomics Analysis were analyzed by Shanghai Biocluster Biotech Co., Ltd. (China) using the Ultimate 3000 LC system coupled with an Orbitrap Elite mass spectrometer (Thermo, United States).

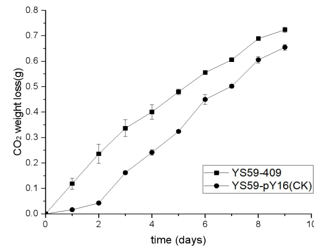



Fig. 1 $CO_2$ weight loss (g). Black square represents *S. cerevisiae* YS59-409 and black round represents *S. cerevisiae* YS59-pY16 (control strain).

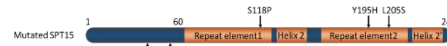
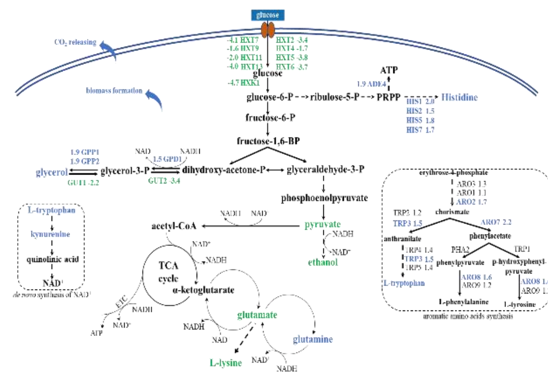



Fig. 2 Mutation sites in the SPT15 gene of mutant strain (arrows). The schematic of structural domain is referred to the previous study (Alper et al., 2006)



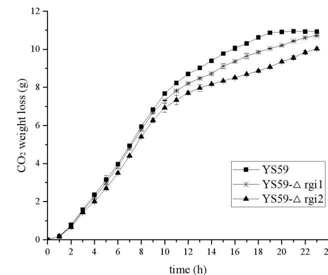




Fig. 4 $CO_2$ weight loss (g). Black asterisk and black triangle represent knockout strains (YS59-△rgi1 and YS59-△rgi2, respectively) and black square represents control strain (YS59).

Fig. 3 Changes in genes and metabolites of mutant strain YS59-409. Blue and green represent up- and down-regulation, respectively. The values in brackets represent the fold change of expression level of mutant strain compared to control strain.

Table 1. Fermentation characteristics of the mutant strain *S. cerevisiae* YS59-409

| Strains | $OD_{600}$ | Residul sugar (g/L) | Ethanol concentration(g/L) | Glycerol concentration(g/L) | Acetate concentration(g/L) | Ethanol yield[a] (g/g Sugar) | Change in ethanol yield |
|---|---|---|---|---|---|---|---|
| YS59-pY16 | 4.52±0.34 | 1.13±0.35 | 54.67±0.94 | 2.13±0.08 | 0. 493±0.109 | 0.367±0.007 | 0 |
| YS59-409 | 5.45±0.40* | 1.90±0.30 | 35.33±3.09* | 3.05±0.07* | 0. 487±0.066 | 0.239±0.021* | -34. 9% |

Table 2. KEGG pathway enrichment analysis ($p < 0.05$)

| Pathway | P-value | Number of genes | Gene match (genome match)[a] | Pathway | P-value | Number of genes | Gene match (genome match)[a] |
|---|---|---|---|---|---|---|---|
| Translation | | | | Nucleotide metabolism | | | |
| Ribosome | 1.00E-71 | 135 | 19.77(4.33) | Pyrimidine metabolism | 1.28E-04 | 24 | 3.51(1.65) |
| Transcription | | | | Purine metabolism | 3.23E-02 | 24 | 3.51(2.41) |
| RNA polymerase | 5.87E-05 | 14 | 2.05(0.69) | Amino acid metabolism | | | |
| Carbohydrate metabolism | | | | Histidine metabolism | 4.08E-03 | 6 | 0.88(0.26) |
| Starch and sucrose metabolism | 6.27E-10 | 22 | 3.22(0.84) | Arginine and proline metabolism | 4.58E-02 | 6 | 0.88(0.41) |
| Galactose metabolism | 6.11E-04 | 13 | 1.90(0.74) | | | | |

[a] The percentage of DEGs involved in individual pathway account for all DEGs with pathway annotation (683) and all genes with pathway annotation (4184).

Table 3. Fermentation characteristics of knockout strains

| Strains | Residual Sugar (g/L) | Ethanol (g/L) | Ethanol yield(g/g Sugar) | Change in ethanol yield |
|---|---|---|---|---|
| YS59 | 1.96±0.68 | 52.04±1.43[c] | 0.351±0.008[c] | 0 |
| YS59-△rgi1 | 0.99±0.19 | 40.59±0.96[a] | 0.272±0.006[a] | -22.0% |
| YS59-△rgi2 | 1.62±0.40 | 43.43±0.77[b] | 0.292±0.004[b] | -16.5% |

[a,b,c] Values followed by different letters within the same column are significantly different using Duncan's multiple range test at the level of 0.05. Data are mean±SD of independent triplicate.

## Conclusion

In this study, we used global transcriptional machinery engineering (gTME) technology, based on the mutation of the *SPT15* gene, to weaken the capacity of *S. cerevisiae* to produce ethanol and ultimately created a new strain of S. cerevisiae (YS59-409) , with ethanol-production capacity reduced by 34.9% compared to the control strain. Sequence analysis was performed on the mutated *SPT15* gene, demonstrating that the five mutation sites may work collectively, or at least partly, to create the specific characteristics of YS59-409, including a higher $CO_2$ release, biomass, and glycerol formation. The specific phenotype of the new mutant strain featured changes in ribosome biogenesis, nucleotide metabolism, glycolysis flux, the Crabtree effect, $NAD^+$/NADH homeostasis, and energy metabolism. Furthermore, two genes related to energy metabolism, *RGI1* and *RGI2*, were found to be associated with the weakened ethanol production capacity, although the precise mechanisms involved need to be further elucidated.