Unveiling the Molecular Insights of Yeast Fermentation in Dough Through RNA-seq Analysis

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Abstract

Differential expression analysis illuminates the molecular mechanisms governing biological processes, crucial for understanding how organisms respond to environmental stimuli. In this study, we investigated the dynamics of gene expression during yeast fermentation, revealing 1647 downregulated and 1657 upregulated genes after 30 minutes compared to the control. Enrichment analysis highlighted upregulated genes associated with oxidoreductase activity, suggesting their role in shaping dough properties and enhancing baking quality, while downregulated genes implicated in ribonucleoprotein complex biogenesis and RNA processing reflected yeast adaptation to fermentation conditions, shedding light on metabolic shifts during dough fermentation. These findings offer insights into yeast physiology during fermentation, with implications for optimizing dough fermentation processes and enhancing bread quality.

Keywords: Differential expression analysis, *Saccharomyces cerevisiae*, Salmon, Functional analysis

1. Introduction

Differential expression analysis serves as a pivotal tool in deciphering the intricate dynamics of gene expression under varying conditions, shedding light on molecular mechanisms governing biological processes. This analysis aids in understanding how organisms respond to environmental cues or stimuli, unraveling key regulatory networks orchestrating cellular functions [1]. By comparing transcriptomic profiles between different experimental conditions, researchers can discern genes whose expression levels significantly alter, offering insights into biological adaptations, developmental processes, and disease states [2]. In the context of yeast fermentation, studying changes in RNA expression elucidates fundamental insights into the metabolic shifts occurring during this biotechnologically significant process. Yeasts, being versatile organisms capable of adapting their metabolism to fluctuating environmental conditions, offer a compelling model system for such investigations [3]. Differential expression analysis enables the identification of genes crucially involved in the transition from aerobic respiration to fermentation, providing a comprehensive understanding of the molecular mechanisms underpinning this metabolic switch [4]. In this study, we delve into the dynamics of gene expression during yeast fermentation, aiming to elucidate the molecular underpinnings of metabolic shifts occurring in *Saccharomyces cerevisiae*. Leveraging differential RNA expression analysis, we investigate alterations in gene expression profiles before and during fermentation, offering insights into the regulatory mechanisms driving this metabolic transition. Through the integration of advanced bioinformatics tools and techniques, we seek to uncover key genes involved in yeast adaptation to fermentation conditions, thus contributing to our understanding of cellular responses to environmental stimuli.

2. Materials and Methods

2.1. Data Availability

The RNA-seq data of *Saccharomyces cerevisiae* can be found in NCBI Genbank (accession numbers SRR941816, SRR941817, SRR941818, SRR941819). As the reference transcriptome we used the S288c transcriptome (assembly R64-1-1).

2.2. Used Tools

Raw reads were mapped to the S288c transcriptome with Salmon (v1.10.0, https://github.com/COMBINE-lab/salmon). The gene expression abundance was normalized by median of ratios using DESeq2 (v1.42.0) [5]. Differentially expressed genes (DEGs) were identified, with the threshold $|\log_2 \text{Foldchange}| > 0.58$ and adjusted $p{-}value < 0.05$ as the criteria of significant gene expression difference. Enrichment of GO terms was analyzed based on the identified DEGs. Enrichment of functional categories among DEGs was performed with clusterProfiler (v4.10.0) [6,7].

3. Results

To investigate the difference in transcriptomic profiles of yeast fermented 0 minutes and 30 minutes we performed transcriptome analysis in S. cerevisiae cells. Two high-quality

mRNA samples were selected for RNA-Seq analysis for each condition and were represented as two replicates.

To visualize variation between expression analysis samples we used principal component analysis. For each sample, RNA-Seq replicates are clustered together as shown in Figure 1A.

The DEGs were identified to show significant change in transcriptional expression with more than 1.5-fold change (adjusted $p-value < 0.05$). There were 3304 significantly expressed genes at a state of 30 minutes fermentation. Among them, 1657 genes exhibited increased expression, while 1647 genes showed decreased expression (Fig. 1B, 1C).

All the DEGs were annotated with GO terms. The functional categories are presented in Figure 1D. The most dominant subcategories of up-regulated genes were involved in different catabolic and metabolic processes, cellular and aerobic respiration in biological process (BP); mitochondria and peroxisome in cellular component (CC); oxidoreductase, peptidase activity etc. in molecular function (MF). The down-regulated genes were involved in processes related to rRNA processing in BP; nucleus and ribosome in CC; catalytic activity, binding on RNA, rRNA and snoRNA binding in MF.

4. Discussion

Our analysis revealed significant changes in gene expression and Gene Ontology (GO) terms before and after fermentation in bread dough. Specifically, we identified 1647 downregulated genes and 1657 upregulated genes in samples after 30 minutes of fermentation compared to the control. Exploring the GO results of upregulated genes, we observed an enrichment of genes associated with oxidoreductase activity. Previous studies have shown that investigating the effects of oxidoreductases, such as glucose oxidase (GOe) and pyranose oxidase (P2O), on bread dough allows for a better understanding of their impact on dough properties and baking quality. It was suggested that the addition of GOe or P2O to dough increases its resistance to extension during mixing, consistent with the expected effects of oxidizing agents. Therefore, oxidoreductases may play a key role in shaping dough structure and enhancing its quality during the baking process [8]. Furthermore, we observed a significant decrease in the expression of genes related to ribonucleoprotein complex biogenesis, ribosome biogenesis, ncRNA processing, RNA processing, and catalytic activity acting on nucleic acid and RNA. This downregulation can be interpreted as part of yeast cells' adaptation to the changing nutrient environment and metabolic demands during fermentation. It reflects a shift in cellular resources towards fermentation pathways for energy production, accompanied by changes in gene expression to prioritize osmoregulation mechanisms over RNA processing and ribosomal biogenesis [9,10]. Overall, our findings underscore the dynamic regulatory mechanisms underlying yeast physiology during dough fermentation. The observed changes in gene expression and GO term enrichment provide insights into the metabolic adaptations of yeast cells as they transition from respiration to fermentation in bread dough. These insights may inform future studies aiming to optimize dough fermentation processes and improve bread quality.

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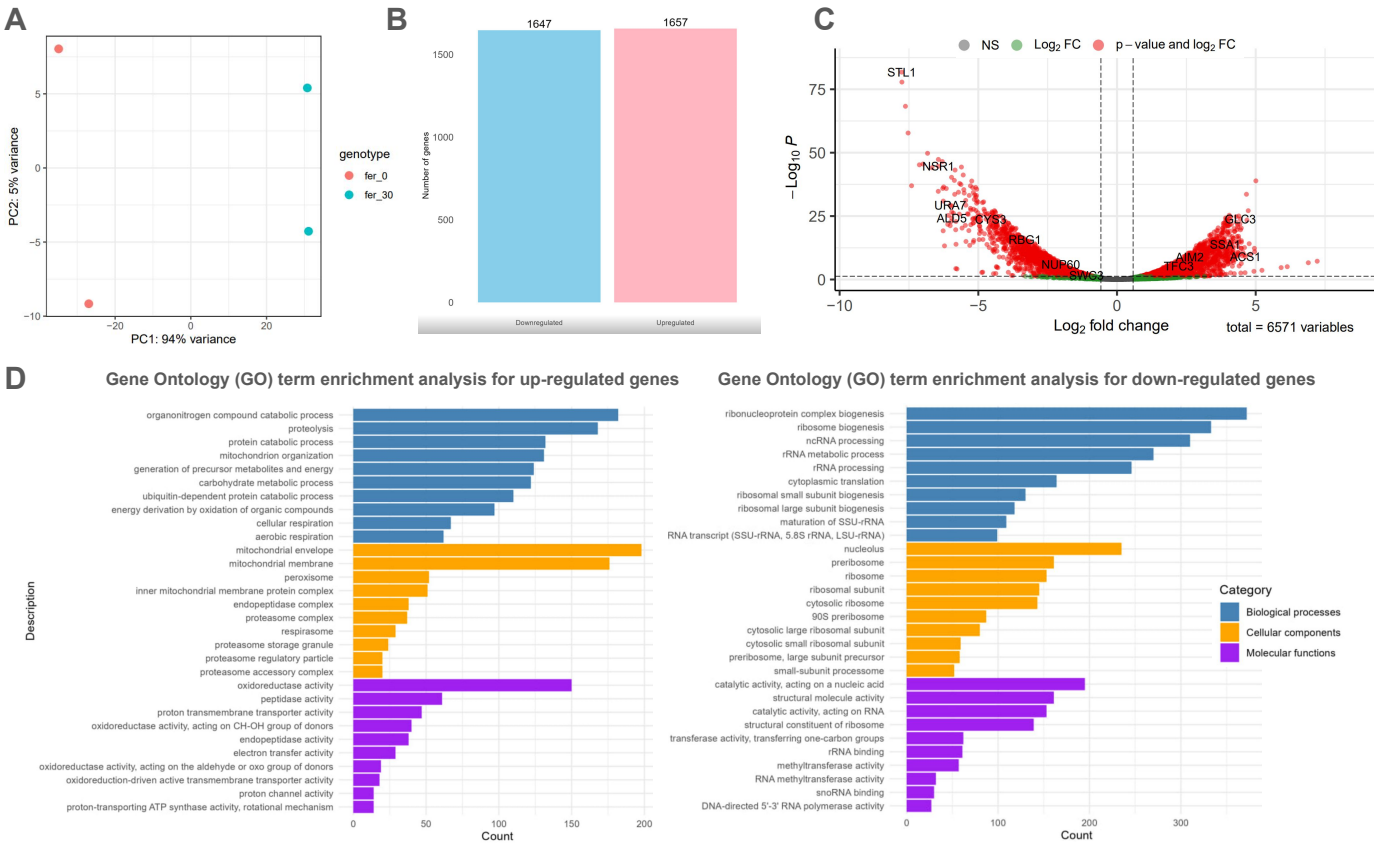

Figure 1: Transcriptomic profiles of *S. cerevisiae* cells fermented for 0 minutes and 30 minutes. (A) Principal component analysis (PCA) showing clustering of RNA-Seq replicates for each sample. (B) Number of significantly expressed genes with more than 1.5-fold change (adjusted $p-value < 0.05$) at 30 minutes fermentation. (C) Distribution of up-regulated and down-regulated genes. (D) Functional analysis of differentially expressed genes annotated with GO terms.