Early stage of dough fermentation - feast or disaster?

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Abstract

In this work, we tried to reveal differentially expressed yeast genes before and during fermentation. According to our results, under osmotic stress budding yeasts exhibit enhanced expression level of genes involved in glycerol accumulation, while expression of many genes involved in aerobic catabolism of carbohydrates and fatty acids (e.g. ferments of citrate cycle) are downregulated.

Introduction

Yeast has accompanied mankind throughout virtually all of our history. The earliest known records of yeast risen bread come from Ancient Egypt in 1300–1500 BCE [8]. Nowadays, yeast fermentation is widely used in a huge number of industrial sectors. It is used in production of alcoholic beverages, coffee and cocoa, bread and even in the chemical industry to produce biofuel and various chemicals. However, the molecular mechanisms of yeast adaptation to the changing environment during fermentation are still not fully understood. The study of these processes can help to design improved and more productive yeast strains. For example, in the early stages of fermentation, the yeast cells undergo considerable stress - they are exposed to anaerobic conditions and need to restructure their entire metabolism in order to switch from aerobic respiration to anaerobic alcoholic fermentation [12]. Moreover oxygen is essential not only for the aerobic respiration but also for the biosynthesis of biomass constituents like haem, unsaturated fatty acids and sterols [11]. Also yeast suffer from osmotic stress due to excess sugars and sodium in their environment especially during dough or soy sauce fermentation. That’s why induction of pathways associated with osmotic stress has been observed during early stages of yeast fermentation in some studies [9, 10].
In this study, we want to reveal what problems S. Cerevisiae face in the early stages of dough fermentation and how do they solve them? Differential analysis of gene expression levels will help us answer this question. This technique is based on RNA sequencing and analyzes differences in abundance of gene transcripts within a transcriptome. Due to the large number of genes to be tested, (e.g., >6000 in the S. Cerevisiae genome), multiple testing correction is usually applied after analysis.

Methods

Raw bulk RNA-seq reads from S. Cerevisiae strain S288c before and after 30 minutes of fermentation in dough were obtained from public data sources.
First, reads were aligned to the annotated reference genome (assembly version R64) with hisat2 v.2.1.0 [1]. After that, annotation format was changed from gff to gtf with GffRead [2]. Then we counted genes with the featureCounts v.2.0 [3] tool. Differentially expressed genes were found with Deseq2 [4]. Heatmap of gene expression generated using gplots R library. Finally, we used gene ontology terms - Generic GO Term Mapper [5] to get a mapping from the granular GO annotations for genes in a list to a set of broader, high-level GO parents terms.
Data about expression of genes involved in metabolic processes were used for construction and analysis of metabolic network using Shiny GATOM [7].

Results

After alignment with hisat2 and gene counting with featureCounts all reads were totally aligned 21638774 times to 6070 S.cerevisiae genes.

DESeq2 analysis

DESeq2 analysis revealed that after 30 minutes of fermentation expression for 1588 genes was significantly upregulated and for 1592 genes was significantly downregulated compared to expression levels before fermentation (FDR-adjusted p-value <.05). Heatmap visualization for gene expression clusterization shown in fig. 1.

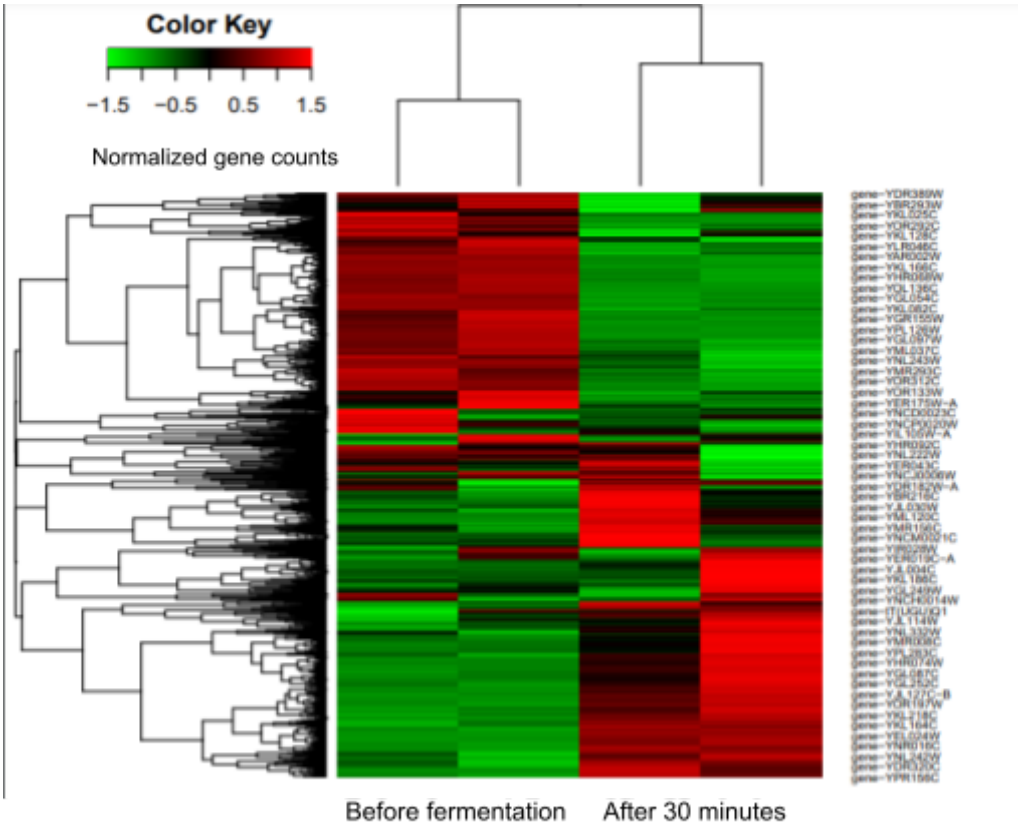

Fig 1. Heatmap of gene expression levels before fermentation and after 30 minutes of fermentation

GO-annotation

GO process annotation of top 50 significantly up- and downregulated genes showed that upregulated genes were involved in rRNA synthesis and ribosome assembly (GO:0006364, GO:0042274, GO:0042273, GO:0042255), aminoacid metabolism (GO:0006520) and membrane transport (GO:0055085). Top 50 significantly downregulated genes were commonly involved in carbohydrate and lipid metabolism (GO:0005975, GO:0006629), generation of energy and cellular respiration (GO:0006091,GO:0045333) and other cellular processes. Differential expression data for genes involved in membrane transport and cellular respiration shown in Supplementary table 1 and 2.

Metabolic network

Metabolic network was constructed in Shiny GATOM to further explore metabolic changes during early fermentation. Metabolic network consists of vertices corresponding to various cell metabolites and the edges corresponding to the up- or downregulated genes that are involved in the transformation of these metabolites. Resulting metabolic network is shown in the Supplementary figure S.1. This network revealed that genes involved in carbohydrate oxidation were downregulated and genes involved in synthesis of glycerol and various amino acids were upregulated.

Discussion

In this study we revealed transcriptomic changes during the early stage of dough fermentation in S.cerevisiae. Almost half of genes (>3000 genes) significantly change expression during this stage of fermentation.

Upregulated genes

Many genes involved in transcription, translation, amino acid synthesis and assembly of rRNA were upregulated probably because the cell needs to synthesize a large number of new proteins to adapt to the anaerobic conditions when the environment changes [14].
According to GO, one of the interesting upregulated processes is a transmembrane transport - the process in which a solute is transported across a lipid bilayer, from one side of a membrane to the other.
The AQR1 gene, which is related to transmembrane transport, was one of the most upregulated genes. This gene is a plasma membrane transporter of the major facilitator superfamily, involved in the excretion of excess amino acids under conditions in which, despite an abundant availability of carbon, cell growth is limited by a second factor such as the lack of an essential compound. In bread dough fermentation, osmotic stress is likely to be the growth-restrictive factor that leads to a striking upregulation of AQR1 [5].

Also, STL1, a gene involved in glycerol transport, is one of the most upregulated genes [15]. So, we can see that osmotic stress induces expression of genes involved in glycerol accumulation. This is because glycerol accumulation increases osmotic pressure in cytoplasm and protects cells from water loss in an aggressive hyperosmotic environment [13].

Downregulated genes

According to GO-analysis and metabolic network construction expression of many genes involved in aerobic catabolism of carbohydrates and fatty acids (e.g. ferments of citrate cycle) are downregulated - because these processes cannot go on in anaerobic conditions. Interestingly many genes involved in anaerobic part of carbohydrate metabolism (e.g. glycolysis) were downregulated too. This may be an overreaction to stress that requires further study.

Supplementary materials

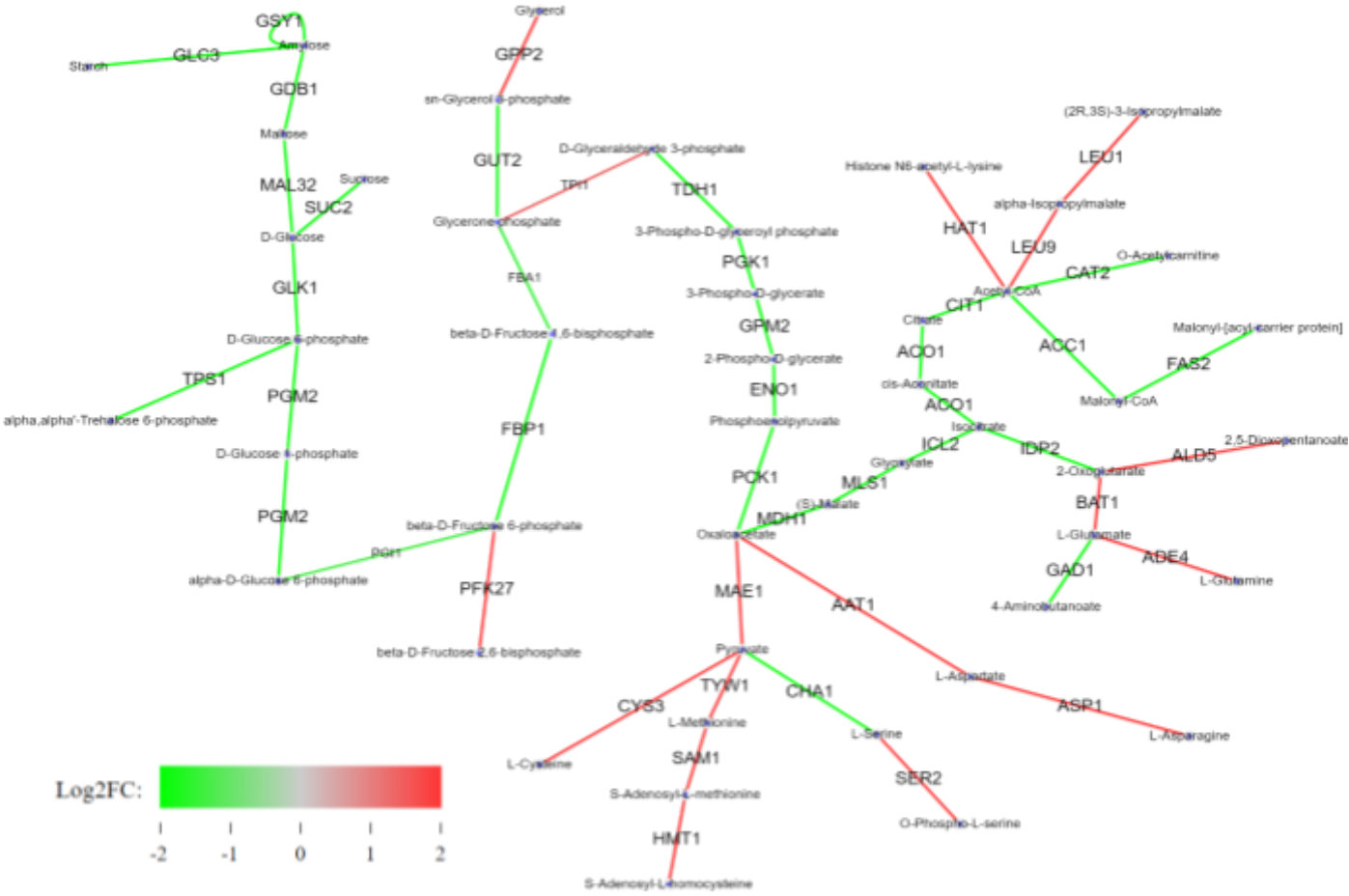

Fig.S1 Metabolic network of gene expression changes before and after 30 minutes of fermentation (red edges - genes upregulated during fermentation, green edges - genes downregulated during fermentation).

Supplementary table 1. Downregulated cellular respiration genes

Gene name	log2FoldChange	FDR corrected p-value	GO term	Gene description
ISF1	-4,79308	4,14E-27	cellular respiration	Serine-rich, hydrophilic protein
MLS1	-4,79028	1,45E-24	cellular respiration	Malate synthase, enzyme of the glyoxylate cycle
CIT1	-4,34657	3E-24	cellular respiration	Mitochondrial citrate synthase
IDP2	-4,43461	1,49E-21	cellular respiration	Cytosolic NADP-specific isocitrate dehydrogenase
ACO1	-3,98699	1,02E-20	cellular respiration	Aconitase
MDH1	-3,86591	1,98E-19	cellular respiration	Mitochondrial malate dehydrogenase
HAP4	-3,68647	2,33E-18	cellular respiration	Transcriptional activator of respiratory gene expression

Supplementary table 2. Upregulated membrane transport genes

Gene name	log2FoldChange	FDR corrected p-value	GO term	Gene description
STL1	7,874602	3,31E-78	transmembrane transport	Glycerol proton symporter (induced when cells are subjected to osmotic shock)
HXT1	7,881946	4,06E-71	transmembrane transport	Low-affinity glucose transporter
AQR1	7,78578	2,73E-59	transmembrane transport	Plasma membrane transporter of the major facilitator superfamily
OAC1	7,269787	2,89E-48	transmembrane transport	Mitochondrial inner membrane transporter
FSF1	6,844879	9,11E-45	transmembrane transport	Predicted to be an alpha-isopropylmal ate carrier
PRM10	5,99648	6,83E-42	transmembrane transport	Proposed to be involved in mating
YJL107C	6,172188	1,96E-41	transmembrane transport	Putative protein of unknown function

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