Transcriptome analysis of high-temperature stress in yeast during industrial scale bioethanol production

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Currently there is a growing demand for renewable energy sources, since the traditional energy sources are becoming limited, such as petroleum oil. The production of bioethanol, an alternative energy source used in Brazil, is based on the fermentation of sucrose from sugarcane feedstock using highly adapted industrial strains of the yeast *Saccharomyces cerevisiae*. In the industrial environment, yeasts are usually under several stress conditions, such as high temperature, low pH, bacterial contamination and others. The high-temperature tolerance is a desirable phenotype because can decrease production costs and increase the productivity in the bioethanol industry. In this context, bioinformatics has a key role by enabling large-scale analysis of transcriptome in industrial strains, identifying genetic aspects involved in the stress tolerance process of these yeasts. Therefore, the aim of this study was identifying the transcriptomic profile of industrial yeasts (*Pedra II - PEII*) under two temperature conditions in industrial environment operating in fed batch fermentation. The samples were collected in triplicates direct from the industrial fermentation tanks after 4 and 8 hours of fermentation under 32°C (control group) and 38°C (test group). mRNA samples from yeasts were sequenced using Illumina HiSeq2000 and composition of fermentation broth was measure by HPLC. Reads from each RNA-seq library were aligned against a reference gene database constituted by all *Saccharomyces cerevisiae S288c* genes and 20 *PEII*-specific genes. In-house perl scripts were performed to calculate the number of aligned reads per gene (read counts) and gene expression levels (RPKM). For differentially expressed genes (DEG) analysis, read counts values were submitted to negative binomial statistical test using R/Bioconductor packages DESeq2 and edgeR and filtered by p-value < 0.05, |fold-change| >= 2 and RPKM >= 1. The DEG were submitted for Gene ontology (GO) enrichment analysis using SGD database (www.yeastgenome.org). As results, 447 DEG were found (181 up-regulated and 266 down-regulated) after 4 hours of fermentation and 1080 DEG (540 up-regulated and 540 down-regulated) after 8 hours. GO terms as response to heat, stress, and temperature stimulus were enriched in up-regulated genes on both fermentation time, but cell wall organization and protein folding processes appeared only after 4 hours. For down-regulated genes, ergosterol, sterol and lipid biosynthetic process were enriched after 4 hours while oxidative phosphorylation and aerobic respiration processes appeared after 8 hours of fermentation. Finally, a protein-protein interaction network of DEG were constructed for identifying hub proteins that can represent master regulators of this process.