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Transcriptomic Analysis of *Yarrowia lipolytica YlNTH1* and *YlTPS1* Genes Under Different Carbon Sources

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Abstract
The biosynthesis of trehalose is catalyzed by synthase enzyme complex and degraded by neutral trehalase enzyme. *Yarrowia lipolytica* is a dimorphic yeast and used as a model organism for understanding the lipid metabolism of higher eukaryotes. *YlTPS1* and *YlNTH1* genes are responsible from the recycling of trehalose in *Y. lipolytica*. The aim of this work is to determine the expression patterns of *YlTPS1* and *YlNTH1* genes under different carbon sources by using RNAseq study. The files used in this analysis were obtained from the EMBL-EBI data bank and Study ID is "PRJEB2863". The substrates used in the work are Alkane, Glucose, Glycerol, Oleic Acid, Tributyrin and Triolein. The run accession numbers of substrates are ERR073010, ERR073011, ERR073009, ERR073008, ERR073012 and ERR073007, respectively. "Trimmomatic" tool was used for croping the readings in the next generation sequencing files. Reference genome of *Y. lipolytica* were obtained from Ensembl genome data base. The files were converted to "ERR0730xx_tophat2.bam" files and used in the "FeatureCounts" tool. We found that the transcript level of *YlTPS1* gene was 2 to 4 times higher than *YlNTH1* gene. The rate of trehalose synthesis is greater than the rate of breakdown in *Y. lipolytica* yeast cells.

Keywords: *YlTPS1, YlNTH1,* RNAseq*, Yarrowia lipolytica*

INTRODUCTION

Trehalose is a non-reducing disaccharide composed of two glucose units and an essential component of the yeast metabolism. The one of the most important function of trehalose is to protect cell membrane components, proteins and lipids, from different types of stress factors such as heat stress [1, 2]. The cytoplasmic level of trehalose is strictly controlled by recycling of trehalose continouslyin yeast cells. This recycling process involves the actions of trehalose sythesis and breakdown enzymes. The biosynthesis of trehalose is catalyzed by TPS complex, composed of Tps1p, Tps2p, Tps3p and Tsl1p subunits. The breakdown of stress-accumulated trehalose is catalyzed by neutral trehalase enzyme (Nth1p) in *Saccharomyces cerevisiae* [3].

Yarrowia lipolytica is a non-pathogenic dimorphic yeast that can shift from yeast to hyphal form, or vice versa. *Y. lipolytica* is also used as a model organism for understanding of lipid metabolism in higher eukaryotes because of its ability to utilize and accumulate high levels of lipid [4, 5]. *Y. lipolytica* stores triacylglycerol (TAG), glycogen and trehalose as a source of carbon. Yeast cells can adapt themselves to environmental fluctuations by making some metabolic and genetic switches. The accumulation of trehalose is triggered as a response to nutrient starvation such as carbon-limited condition. *YlTPS1* (YALI0E14685) and *YlNTH1* (YALI0D15598) genes are responsible from the recycling of trehalose in *Y. lipolytica* [6]. In the absence of environmental stresses, the rate of trehalose synthesis and breakdown must be enzymatically balanced in exponentially growing *S. cerevisiae* yeast cells. Therefore, the main objective of this work was to determine the expression patterns of *YlTPS1* and *YlNTH1* genes under different carbon sources by using RNA sequencing study presented in the EMBL-EBI (European Molecular Biology Laboratory- European Bioinformatics Institute) data bank The exponentially growing *Y. lipolytica* yeast cells in different carbon sources (Alkane, Glucose, Glycerol, Oleic Acid, Tributyrin and Triolein) were used for callculating mRNA levels of *YlTPS1* and *YlNTH1* genes [7]. The transcript level of *YlTPS1* gene was 2 to 4 times higher than *YlNTH1* gene in *Y. lipolytica* yeast cells.

MATERIALS AND METHODS

The files used in this analysis were obtained from the EMBL-EBI (European Molecular Biology Laboratory-European Bioinformatics Institute; http://www.ebi.ac.uk) data bank. The name of the work being used is "Comparative transcriptomics of *Yarrowia lipolytica* on different substrates" and Study ID is "PRJEB2863". The substrates used in the work are Alkane, Glucose, Glycerol, Oleic Acid, Tributyrin and Triolein, and the full protocols were given in the web page [7]. The run accession numbers of substrates are ERR073010, ERR073011, ERR073009, ERR073008, ERR073012 and ERR073007, respectively. They were saved as "fastq.qz" files in our computer, and all "ERR0730xx.fastq.gz" compressed files extracted to uncompressed files such as "ERR0730xx.fastq". All the uncompressed files were converted to "ERR0730xx.fastqsanger" files via "fastqGroomer" tool. "Trimmomatic" tool was used for croping the readings in the next generation sequencing files that is*.fastqsanger files. The order of "Trimmomatic" tool parameters are TruSeq2-PE-fa:2:30:10 (Illuminaclip adapters), Crop: 36, Leading: 3, Trailling: 3 and Slidingwindow: 4:20. The cropped files obtained from "Trimmomatic" tool (i.e.: "ERR0730xx_trimmed.fastqsanger" files) were used in "Tophat2" mapping tool which is developed for RNAseq reads. Reference genome of *Y. lipolytica* were obtained from Ensembl genome data base [7]. As a result of "Tophat2" tool, the mapping rates are 92.3%, 66.1%, 74.8%, 81.9%, 69.8, and 67.2% for ERR073010, ERR073011, ERR073009, ERR073008, ERR073012 and ERR073007, respectively. And all the "ERR0730xx_trimmed.fastqsanger" files, obtained from "Trimmomatic" tool, are converted to "ER-

R0730xx_tophat2.bam" files by means of "Tophat2" tool. All the "*.bam" files were used in the "FeatureCounts" tool for counting the mapped readings for genomic regions. The gene annotations used in "FeatureCounts" tool were obtained from Ensembl genome data base [9]. The output files of the "FeatureCounts" software ("ERR0730xx.tabular") were opened with the application of "Openoffice - Calc" tool.

RESULTS AND DISCUSSION

The oleaginous yeast *Y. lipolytica* can use different types of hydrophobic substrates as a carbon source in order to growth and TAG accumulation [10]. The ability of *Y. lipolytica* to grow on alkanes, oleic acid, tributyrin and triolein make this yeast a good model for investigating lipid metabolism. In addition, the growth of *Y. lipolytica* on these hydrophobic carbon sources may effect the accumulation of other carbon sources such as glycogen and trehalose. From this point of view, we tried to determine expressions of *Y. lipolytica* genes involved in trehalose metabolism. RNA sequencing technology is used for monitoring mRNA levels, and thereby the gene expression levels. That's why we used RNA sequencing data base to monitoring the *YlTPS1* and *YlNTH1* gene expression patterns in different carbon sources including hydrophobic carbon sources also.

When *Y. lipolytica* yeast cells were grown in hydrophilic carbon sources such as glucose and glycerol, mRNA levels (given as read counts) were calculated as 185 read counts in glucose and 155 read counts in glycerol for *YlNTH1* gene; and 479 read counts in glucose and 480 read counts in glycerol for *YlTPS1* gene (Figure 1). As shown, *YlTPS1* and *YlNTH1* gene expressions in glucose and glycerol were nearly same, but the mRNA level of *YlTPS1* gene was 2-3 fold greater than that of *YlNTH1* gene.

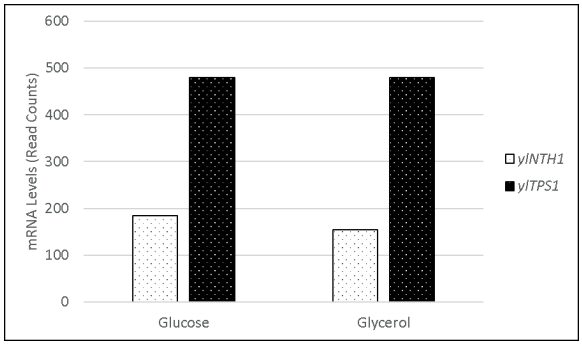

Figure 1. The mRNA levels of *YlTPS1* and *YlNTH1* genes in glucose and glycerol.

When *Y. lipolytica* yeast cells were grown in hydrophobic carbon sources such as alkane and oleic acid, *YlNTH1* and *YlTPS1* gene expression levels were given in figure 2. The gene expression levels of *YlNTH1* gene was calculated as 176 read counts in alkane and 306 read counts in oleic acid. The transcript level *YlTPS1* gene was calculated as 360 read counts in alkane and 557 read counts in oleic acid. As you can see the *YlTPS1* gene expression level was nearly 2 fold greater than *YlNTH1* gene expression. Also *YlNTH1* and *YlTPS1* gene expression levels increased when yeast cells were grown in oleic acid.

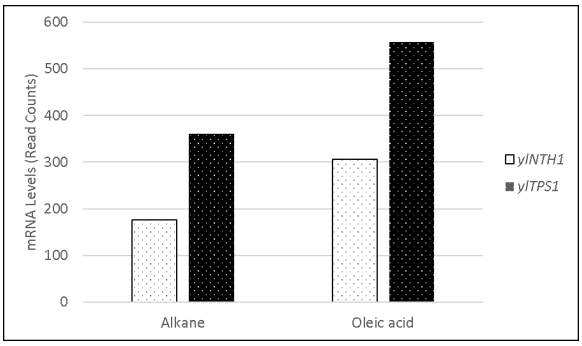

Figure 2. The mRNA levels of *YlTPS1* and *YlNTH1* genes in alkane and oleic acid.

Additionally, when yeast cells were grown in tributyrin and triolein hydrophilic carbon sources, the mRNA levels were calculated as 176 read counts in tributyrin and 158 read counts in triolein for *YlNTH1* gene; and 559 read counts in tributyrin and 525 read counts in triolein for *YlTPS1* gene (Figure 3). *YlTPS1* and *YlNTH1* gene expressions in tributyrin and triolein were nearly same, but the mRNA level of *YlTPS1* gene was nearly 4 times higher than *YlNTH1* mRNA level.

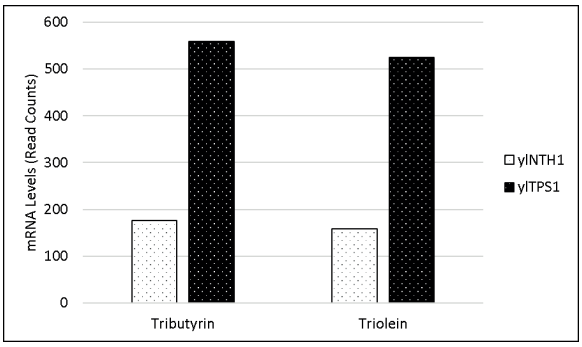

Figure 3. The mRNA levels of *YlTPS1* and *YlNTH1* genes in tributyrin and triolein.

Generally the transcript level of *YlTPS1* gene was 2 to 4 times higher than *YlNTH1* transcript level. There was no considerable effect of carbon sources -hydrophobic or hdrophilic- on *YlTPS1* and *YlNTH1* gene expressions. *YlNTH1* gene expression level of yeast cells grown in oleic acid was higher than other carbon sources. As a result in *Y. lipolytica*, the rate of trehalose synthesis is greater than the rate of trehalose breakdown.

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