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Gene expression study of *Saccharomyces cerivisae* GPH1 gene in response to chemical modulators

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ABSTRACT

The aim of the present study was to evaluate the gene expression of GPH1 gene of *Saccharomyces cerivisae* after it was induced to different stress conditions. The *S. cerivisae* was procured from MTCC and grown in YPD medium with different modulators like NaCl, Glucose, Calcium chloride, Sodium chloride, Potassium Nitrate, Arabinose and Dipotassium hydrogen orthophosphate. The RNA was isolated and analyzed on formaldehyde gel electrophoresis and determined by Nanodrop spectrophotometer. The cDNA was synthesized and used for Real Time PCR. Quantification was based on a standard curve, prepared from samples of known template concentration. The concentration of unknown sample is then determined by Ct value. There is a varied response to glycogen metabolism in *S. cerivisae* to environmental stress conditions such as nutrient limitation and heat shock. This response is mediated, in part, by the regulation of the glycogen metabolic genes. The results indicated that gene expression was high in modulator 3 and lowest in 2. Environmental stress induces a number of glycogen metabolic genes, including GPH1, which encodes glycogen phosphorylase. This study confirmed induction patterns of gene encoding glycogen phosphorylase GPH1 after treatment with modulators. This study gives an insight to the transcriptional control of GPH1 after modulator treatment.

1. INTRODUCTION

Saccharomyces cerevisiae is an important organism in both fundamental and applied research. In fact, we probably know more about the biology of the yeast cell than any other eukaryote. This makes *S. cerevisiae* an ideal model organism for studies on environmental stress responses and stress tolerance. The yeast S. cerevisiae is of particular importance in fermentation industries for the role that it plays in fermenting sugars into ethanol. There is no microorganism more closely associated with human life, from ancient to contemporary times, than this single-celled fungus. Research into the causes of ethanol toxicity in yeast has identified a role for acetaldehyde in stimulating the rate of adaptation of yeast to ethanol stress [1-5]. Glycogen phosphorylase, which catalyzes the release of glucose 1-phosphate from glycogen, plays a vital role in this process in many cells and organisms. When yeast cells are grown in suboptimal conditions, they exhibit a complex stress response. This stress response is a reprogramming of cellular activities to ensure survival, protect essential cell components, and to drive a resumption of cellular activities during recovery [6-8].

Magnesium is involved in many physiological functions, including growth, cell division, and enzyme activity. Magnesium ions also decrease proton and anion permeability of the plasma membrane by interacting with membrane phospholipids, resulting in stabilization of the membrane bilayer. Hu *et al*. demonstrated that increasing the extracellular availability of magnesium ions, increases physiological protection against temperature and ethanol-stress [9, 10]. The general stress response is induced by a wide variety of stressing agents including heat, osmotic stress, oxidative stress, nitrogen starvation, ethanol, sorbate and low pH [11, 12]. This tolerance is presumably acquired by rapid molecular responses that protect against damage caused by ongoing exposure to the same or other forms of stress. These responses include changes in gene transcription, translational and post-translational modifications of stress-associated protein, and are triggered, at least in part, by stress-induced denaturation of proteins, disordering of membranes, DNA damage and metabolic disturbances [13-15]. For example, yeast cells exposed to a salt shock of 300 mM NaCl for 45 minutes accumulate glycerol and heat shocked cells accumulate trehalose; Hottiger *et al*. suggesting that the accumulation of trehalose and glycerol have important roles in stress tolerance [16-18]. Thus, mild stress conditions may trigger cellular responses that prepare cells to cope with severe stress. Such investigations suggest that yeast and other microorganisms have an inherent ability to improve the stress

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stress tolerance provided that the appropriate external and internal triggers are activated. A better understanding of these built-in molecular processes that underpin, and are a part of, the yeast stress response will greatly facilitate the development of strategies to improve yeast stress tolerance.

2. MATERIALS AND METHODS

2.1 Treatment Conditions

The *Saccharomyces cerivisae* was grown in YPD medium with different modulators such as NaCl, Glucose, Calcium chloride, Sodium chloride, Potassium Nitrate, Arabinose and Dipotassium hydrogen orthophosphate. The sample was incubated at room temperature for 24 hrs.

2.2 RNA Extraction

RNA was isolated with GuTC RNA extraction buffer. The samples were incubated at 60 °C for 30 min. Then Phenol: Chloroform: Isoamyl alcohol (25:24:1) mixture was added and centrifuged at 10000 rpm for 10 min. Then supernatant was collected and equal volume of isopropanol was added, incubated at 4 °C for 60 min and centrifuged at 12000 rpm for 10 min. To the pellet 100 µl of sterile water was added.

2.3 RT-PCR

For the reverse transcriptase PCR (RT-PCR) cDNA was synthesized using a reverse transcriptase (MMLV) according to the manufacturer's recommendations. Reverse transcription was carried out at 45°C for 40 min. Double-stranded DNA was synthesized by PCR using both reverse and forward primers. The PCR was performed as follows: denaturation for 15 sec at 94°C and annealing and extension for 30 sec at 60°C.

3. RESULTS AND DISCUSSION

Nucleic acids are traditionally quantified using UV absorption using a spectrophotometer. In its simplest form the absorbance is measured at 260 and 280 nm. The concentration of nucleic acid can be determined using the Beer-Lambert law, which predicts a linear change in absorbance with concentration. An A260 reading of 1.0 is equivalent to about 40 µg/ml of RNA and the OD at 260 nm is used to determine the RNA concentration in a solution. RNA has its absorption maximum at 260 nm and the ratio of the absorbance at 260 and 280 nm is used to assess the RNA purity of an RNA preparation. Pure RNA has an A260/A280 of 2.0. Real-time PCR technology has recently reached a level of sensitivity, accuracy and practical simplicity allowing its use as a routine bioinstrumentation for pathogen detection, single nucleotide polymorphism and gene expression analysis (VanGuilder 2008). Real-Time PCR can be used to quantify gene expression by two methods: relative and absolute quantification. The relative quantification method compares the gene expression of one sample to that of another sample: drug-treated samples to an untreated control, for example, using a reference gene for normalization. Absolute quantification is based on a standard curve, which is prepared from samples of known template concentration. Once the run is finished the software automatically opens the Analyze Data window and performs a basic analysis using auto-baseline and threshold settings (Figure 1).

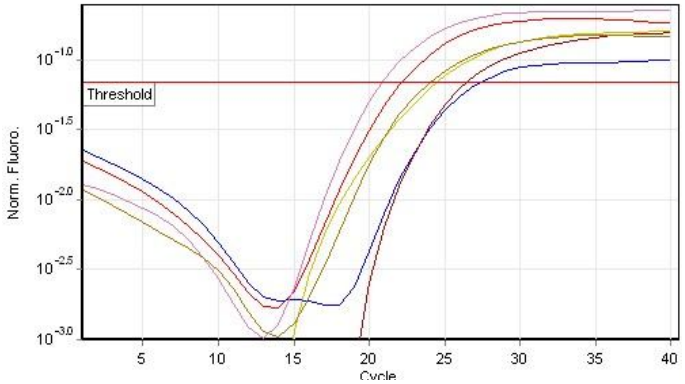

Fig. 1: Raw fluorescence data.

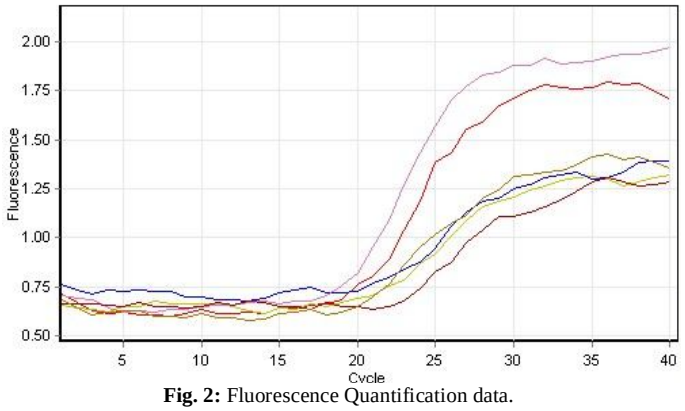

Fig. 2: Fluorescence Quantification data.

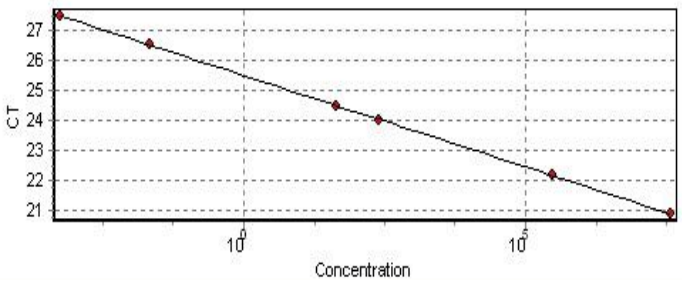

Fig. 3: Standard curve.

Table. 1: Calculated ct value from standard curve.

No.	Colour	Name	Type	Ct
1		Control	Calibrator (RQ)	22.17
2		Treatment 1	Unknown	24.49
3		Treatment 2	Unknown	27.45
4		Treatment 3	Unknown	20.90
5		Treatment 4	Unknown	26.48
6		Treatment 5	Unknown	24.02

The amplification plot with optimum threshold, standard curve, and results table are displayed (Figure 2). Individual windows can be enlarged. We can now see how the standard samples relate to

the standard curve after the regression. Each blue marker represents a standard (Figure 3). The concentration of any unknown sample can then be determined by simple interpolation of its PCR signal (Ct) into this standard curve (Table 1).

Environmental stress triggers multiple responses in the yeast *Saccharomyces cerevisiae*. One such response is the induction of the expression of GPH1, the gene encoding glycogen phosphorylase. GPH1 expression responds to a variety of stressful conditions, such as growth into stationary phase, heat shock and osmotic shock. Real time (RT-PCR) is the recommended method for quantitative gene expression analysis. A compulsory step is the selection of good reference for normalization. A few genes often referred to as Housekeeping genes (HSK), such as ACT1, RDN18 or PDA1 are the most commonly used as their expression is assumed to remain unchanged over a wide range of conditions. Since this assumption is very unlikely, a geometric averaging of multiple, carefully selected internal control genes are now strongly recommended for normalization to avoid this problem of expression variation of single reference genes. Yeast glycogen metabolism responds to environmental stressors such as nutrient limitation and heat shock. This response is mediated, in part, by the regulation of the glycogen metabolic genes. Environmental stressors induce a number of glycogen metabolic genes, including GPH1, which encodes glycogen phosphorylase.

4. CONCLUSION

In the present study the yeast *Saccharomyces cerevisiae* was subjected to different modulators for the induction of a limitation in the nutrients supplied. RNA was isolated and and was then analyzed through RT-PCR to quantify the gene expression. The amplification plot with optimum threshold was observed along with the standard curve. The standard samples related to the standard curve after the regression which is represented by the blue marker. The concentration of the unknown sample was determined by simple interpolation of PCR signal (Ct) into the standard curve. Response was seen as the induction of the expression of GPH1, the gene encoding glycogen phosphorylase which was in response to the stressful condition induced.

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