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DETERMINATION OF GENE EXPRESSION OF ADH1 IN *Zymomonas mobilis* IN RESPONSE TO ETHANOL STRESS

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ABSTRACT:

High tolerance to ethanol is a most desirable characteristic for ethanologenic strains. *Zymomonas mobilis* possess many desirable industrial characteristics because of its special Entner Doudoroff pathway, as a candidate microorganism for converting cellulosic biomass into ethanol. *Z. mobilis* is one of the best ethanol producers which produce ethanol with the help alcohol dehydrogenase (ADH) encoded by adh II gene. In this study ethanol stress was used to induce the ADH1 gene expression and determined by Real Time PCR. The ethanol tolerance of the strain was tested by supplementing the ethanol (5%) into the growth medium. RNA was isolated and Reverse transcription (RT) followed by the polymerase chain reaction (PCR) is used to analyze mRNA expression. The SYBR Green fluorescence dye is used for detection of newly synthesized PCR products in real-time PCR. The comparative Ct method is used to calculate changes in gene expression as a relative difference between experimental samples. Z. *mobilis* grown in ethanol stress caused an extensive reprogramming of ADH1 gene expression and metabolism. In our results sucrose consumption rate correlates with ADH1 expression.

KEYWORDS: Bioethanol, *Z. Mobilis*, ADH1, RT-PCR

[I] INTRODUCTION

At present most of the fuels are made from petroleum, which is irreversibly being depleted [1, 2]. The plant cellulose biomass conversion to ethanol by microbial fermentation is the significant area of research. The major challenge in this area is to use industrially suited microorganisms for the cost-effective biofuel production. Biomass conversion to ethanol requires development of microorganisms capable of fermenting a wide range of carbohydrates and capable of high ethanol tolerance [3]. Zymomonas mobilis possess many desirable industrial characteristics because of its special Entner-Doudoroff pathway, as a candidate microorganism for converting cellulosic biomass into ethanol [4]. High tolerance to ethanol is a most desirable characteristic for ethanologenic strains However; ethanol is generally toxic to microorganisms and inhibits cell growth and metabolism due to intracellular and extracellular accumulation so ethanol becomes major stress factor during fermentation [5, 6].

Ethanol acts as inhibitor of cell growth and metabolism in Z. mobilis, thus resulting in decrease in the rate of sugar conversion to ethanol [7]. Bacteria normally produce less

ethanol; because of their poor efficiency in converting pyruvate to ethanol hence ethanol producing bacteria could be developed by transferring genes that encode for the ethanol-fermenting enzymes [8]. Z. mobilis is one of the best ethanol producers which produce ethanol by two essential enzymes pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) encoded by pdc and adh II genes, respectively. During fermentation PDC catalyzes the nonoxidative decarboxylation of pyruvate to acetaldehyde and carbon dioxide, whereas ADH catalyzes the reduction of acetaldehyde to ethanol [9-11]. Alcohol dehydrogenases (ADHs) are family of oxidoreductases that catalyze the NAD(P)H-dependent inter conversion between alcohols and the corresponding aldehydes or ketones [12]. These enzymes PDC and ADH are adequate to convert intracellular pool of pyruvate and NADH to ethanol [13]. A deeper understanding of the molecular mechanisms underlying ethanologenic strains tolerance of ethanol stress may guide the design of rational strategies to increase process performance in industrial alcoholic fermentation. In this study ethanol stress was used to induce the ADH1 gene expression and determined by Real Time PCR.

[II] MATERIALS AND METHODS

2.1 Stress Conditions:

The *Z. mobilis* was ordered from MTCC. The ethanol tolerance of the strain was tested by supplementing the ethanol (5%) into the medium. Then ethanol production was estimated for different sucrose concentrations 5, 10 and 15 %.

2.2 RNA Extraction:

Overnight grown culture (2ml) was centrifuged at 3000 rpm for 5 min. To the pellet 1 ml of GuTC RNA extraction buffer was added and incubated at 60 °C for 30 min, then added phenol, chloroform: isoamyl alcohol (25:24:1) and centrifuged at 10000 rpm for 10 min. Then supernatant was collected and added equal volume of isopropanol and centrifuged at 12000 rpm for 10 min. To the pellet 100 µl of sterile water was added. The RNA was quantified by Nanodrop spectrophotometer.

2.3 RT-PCR

For the reverse transcriptase PCR (RT-PCR) cDNA was synthesized using a reverse transcriptase (M-MLV) 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.

[III] RESULTS AND DISCUSSION

First the ethanol production was estimated and then ADH1 gene expression was determined. Reverse transcription (RT) followed by the polymerase chain reaction (PCR) is the technique of choice to analyze mRNA expression derived from various sources. Real-time RT–PCR is highly sensitive and allows quantification of rare transcripts and small changes in gene expression. The simplest detection technique for newly synthesized PCR products in real-time PCR uses SYBR Green fluorescence dye that binds specifically to the minor groove double-stranded DNA. The fluorescence data was showed in figure 1 and 2.

Figure 1: Raw fluorescence data

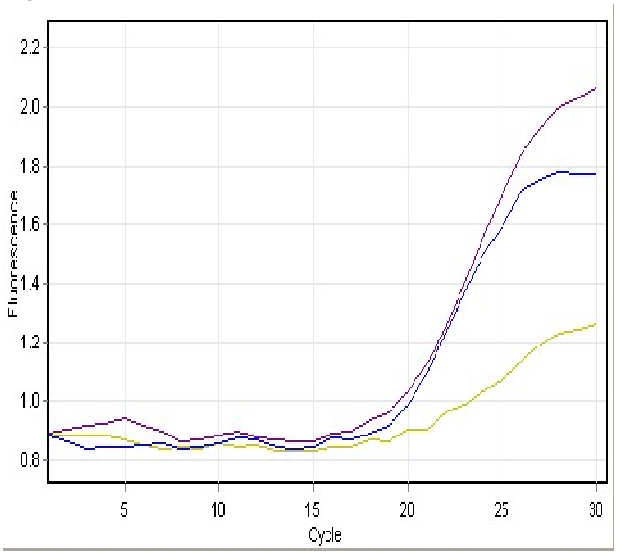
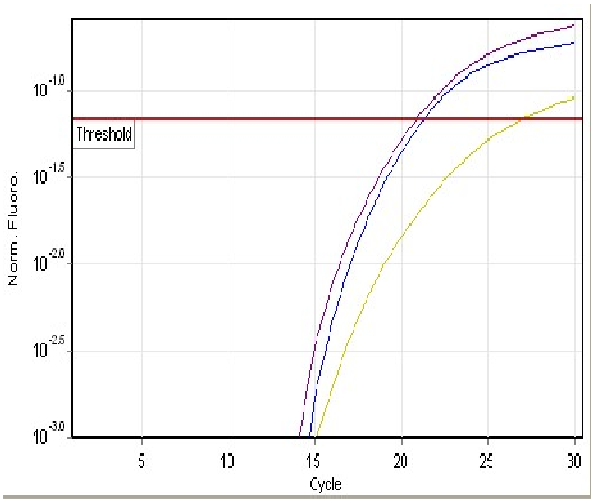


Figure 2: Fluorescence Quantification data



Generally two quantification types in real-time RT-PCR are possible. (i) A relative quantification based on the relative expression of a target gene versus a reference gene. The comparative Ct method is a mathematical model that calculates changes in gene expression as a relative fold difference between an experimental and calibrator sample. While this method includes a correction for non-ideal amplification efficiencies the amplification kinetics of the target gene and reference gene assays must be approximately equal because different efficiencies will generate errors when using this method. The standard curve was showed in figure 3 and the determined ct value was showed in table 1.

Figure 3: Standard curve

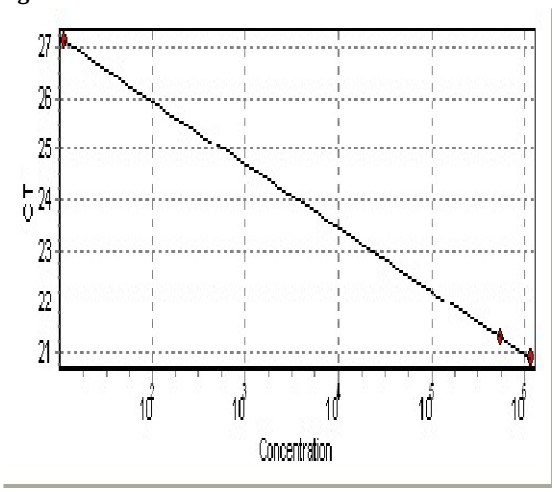


Table 1: Calculated ct value from standard curve

No.	Colour	Treatment	Ct	Calc Conc in ng
2		Sucrose 5	27.12	11.40
3		Sucrose 10	21.33	547166.28
4		Sucrose 15	20.83	1171979.024

[IV] CONCLUSION

The extraordinary sensitivity and virtually unlimited dynamic range of real-time PCR makes it the preferred technology for quantitative gene expression profiling. The *Z. mobilis* grown in ethanol causes an extensive reprogramming of gene expression and metabolism. In our study sucrose consumption rate correlates with ADH1 expression.

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